A Note on the Biological Assay of Tincture Digitalis.

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Abstract.

The strength of several samples of Tr. Digitalis obtained at random on the local drug market is determined by the method of biological assay outlined at the Geneva Conference and now adopted as in accordance with the requirements of the Canadian Government. The samples are compared in strength with the present U.S.P.X. standard and with the International standard powder of Digitalis. The results obtained indicate that for the tinctures examined the maximum deviation from the U. S. P. X. standard is much less than that previously found, and also that the strength of the International powder is considerably above that of the present U. S. P. X. standard.

In the case of certain potent pharmaceutical preparations the usual chemical method of assay is impossible due to the complexity of the active principles and the difficulty involved in their separation. It is customary in such cases to assay the preparations biologically by their effect on experimental animals. Tincture of Digitalis belongs to this group of biologically standardized preparations.

Our attention was directed to this investigation for several reasons. Rusby[1] found that the reports of the biological assay of another drug, ergot, gave extremely discordant results; assays of the same lot of ergot referred to different analysts and to the same analysts at different times yielded results varying from 90.9 to 167 percent. Equally discordant results were obtained on a sample of the fluid extract, the same sample was reported anywhere from 128-250 percent. These findings indicate the present U. S.P. X. biological assay for ergot to be of questionable value. In regard to Digitalis preparations Wokes[2] found amongst 80 samples of tincture that a variation of activity of nearly 400 percent occurred—25 samples differing by more than the permitted 25 percent deviation from

1. Rusby, *J. Am. Pharm. Assoc.*, **18**, 1125 (1928).
2. Wokes, *Quart. J. Pharm. and Pharmacol.*, **2**, 48, (1929).

the International standard powder. Rowe[3] reported a series of results for various tinctures, the deviation found here being about 130 percent; while Ward[4] investigating a series of tinctures purchased on the open Canadian market found a variation in potency of 120 percent. Samples were found as low as 30 percent and as high as 150 percent if the U. S. P. standard, a mean lethal dose of 0.006 cc. per gram weight of frog be taken as 100 percent. Since the investigations of Ward the International standard powder of Digitalis has been adopted as its standard by the Canadian Government.

Experiments.

The following results represent the potency found upon the assay of a few samples of Tincture of Digitalis obtained at random on the local open market. They have been assayed in accordance with the U. S. P. X. requirements and the results compared with the International standard powder, the latter supplied through the courtesy of the Department of Health at Ottawa. The samples assayed were obtained in their original containers. Healthy male frogs, procured from American Biological supply houses, were used for the assay. The four hour frog method of the Geneva Conference was used in preference to the one hour method of the U. S. P. X. The required dose of the diluted tincture was injected into the breast lymph sac of the frog through the floor of the mouth by means of a Luer Tuberculin syringe graduated in hundredths of a cubic centimeter. At the end of the four hours the frog was pithed, if necessary, and the heart examined; systolic stoppage of the ventricle being indicative of a positive result provided no unabsorbed tincture was found present in the lymph. A first approximation of the mean lethal dose was obtained from a series of several frogs injected with doses varying by 20 percent per gram weight of frog. The final tests consisted of the injection of frogs with doses differing by 10 percent per gram weight of frog from the approximate mean lethal dose found above. The determination is completed when of two

3. Rowe, *J. Am. Pharm. Assoc.*, **18**, 1138 (1928).
4. Ward, *Can. Med. Assoc. J.*, **16**, 409-12 (1926).

doses the higher kills a majority of the frogs injected, the lower, a smaller number.

TABLE 1.

	cc. Tincture per gm. frog.	Frogs injected.	Positive results	Mean lethal dose cc. per gm. frog.
1. TINCTURE A.	.0080	1	1	.0072
Age of tincture	.0075	3	3	
3 months	.0070	3	2	
	.0065	3	0	
	.0060	1	0	
2. TINCTURE B.	.0065	5	5	.0052
Dated: To be used	.0055	5	5	
before Dec. 16, 1930.	.0050	5	4	
	.0045	5	3	
3. TINCTURE C.	.0075	3	3	.0070
Dated: Made	.0070	3	2	
June 4, 1929.	.0065	3	2	
	.0060	3	1	
	.0055	3	0	
4. TINCTURE D.	.0072	6	4	.0065
No date	.0065	6	2	
	.0058	6	3	
	.0050	2	0	
5. TINCTURE INTER-	.0055	1	1	.0038
NATIONAL POWDER:	.0050	4	4	
Extracted by U. S. P. X.	.0045	4	4	
Process. Tested	.0040	4	4	
immediately.	.0035	6	3	
	.0025	6	2	
6. TINCTURE INTER-	.0048	1	1	.0032
NATIONAL POWDER:	.0043	3	3	
Extracted by the method	.0038	3	3	
of the International	.0035	3	3	
Conference.	.0030	3	1	

CONCLUSIONS.

Considering the present U. S. P. X. definition of Tincture of Digitalis as one which has a mean lethal dose of .006 c. c. per gram weight of frog, the following tables of comparison are obtained.

TABLE 2.

TINCTURE	I	Percent	II
A	83		48
B	115		67
C	86		50
D	92		54
International Powder	171		100

I. U. S. P. X. Requirements: Mean Lethal Dose 0.006 cc. per gm. frog —100 percent activity.
II. Requirements: 1 cc. Tincture =1 cc. International tincture (within 25 per cent).

The maximum deviation of Tinctures examined was 32 percent except for the International powder which gave a figure about 70 percent over U. S. P. X. activity when measured by method I while in method II the maximum deviation was 52 percent.

The authors regret being unable to procure in time for this report a sample of the U. S. P. standard ouabain to complete the comparison with the International standard. The results of Edmunds, Lovell and Branden[5] however, with an ouabain standard for comparison indicate the International standard powder used in their assays to be about 30 percent stronger than the present U. S. P. X. standard. The results here obtained indicate that for the tinctures examined the maximum deviation from the U. S. P. X. standard is much less than that previously found; they also seem to indicate that the International standard sample used in this investigation is apparently considerably stronger than the present U. S. P. strength. It would seem advisable [illegible] in the near future all tinctures be standardized to conform to the Canadian Government requirements, which are those originated and adopted by the International Conference. We take this opportunity of thanking Dean Burbidge for his interest in making this investigation possible.

5. Edmunds, Lovell and Branden, *J. Am. Pharm. Assoc.*, **18**, 778 (1928).