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NATIONAL STANDARDS FOR TINCTURE DIGITALIS* WITH SPECIAL REFERENCE TO U. S. P. X AND B. P. 1932 STANDARDS.[1]

BY L. W. ROWE.

A few years ago (1) experimental data were submitted showing the relation among five of the better known methods of bioassay of digitalis and the various standards proposed for these methods. At that time the powdered digitalis leaf standard of the Geneva Conference had not been available long enough to permit of more than a very few comparative tests and it was only then being seriously considered by various foreign countries as a national standard for digitalis assay. Since then Canada, in the Food and Drugs Act Regulations of 1928, and England, in the 1932 revision of the British Pharmacopœia, have definitely made the international powdered digitalis leaves their standard for the bioassay of digitalis. Meanwhile Ouabain has continued as the U. S. Pharmacopœia standard for digitalis and in spite of its being illogical from the standpoint of relative degree of absorption in the short time period (one hour), as previously pointed out, the majority opinion favors its retention in the U. S. P. XI. Very recently Defandorf (2) has advocated revision of the present U. S. P. "One-Hour Frog Method" to permit of a longer time period for more complete absorption and action of the diluted digitalis preparation which would be a valuable step forward. He failed, however, to observe that a difference in degree of absorption between Ouabain and digitalis in any given time and particularly in different frogs may also influence the accuracy of the assay method.

The assay methods which are official in the present U. S. P. and B. P. are sufficiently different to make a comparison of the official tinctures quite difficult and added to this, the marked difference between the standards, Ouabain and International Standard Digitalis Leaves, makes a series of experimental tests the only basis of comparison. This short paper presents such data accumulated since 1930 and permits a conclusion based on averages which should be approximately correct. It should be stated before the assay results are tabulated that both the official B. P. 1932 tincture of digitalis and the standard extract of the international powdered digitalis leaves are made without defatting the drug while the U. S. P. X tincture is made from defatted drug. For some unknown reason the assay results

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* Scientific Section, A. PH. A., Madison meeting, 1933.

[1] From the Research Laboratories of Parke, Davis & Company, Detroit, Michigan.

are more definite and in general the drug is found to be somewhat more active when not defatted, though tests have failed to show any appreciable activity in the fats after removal. It is to be hoped that the U. S. P. XI will not require the drug to be defatted, as it has been clearly shown that the nausea frequently caused by active digitalis preparations is of central origin, and also the presence of the fats does not contribute to the instability of the tincture with any degree of uniformity.

The details of the methods of assay used in this series are available in the U. S. P. X and the B. P. 1932. The Ouabain standard was the official No. 626 obtained from the Food and Drug Administration in Washington. The Canadian Standard digitalis leaves No. 428 was obtained from Ottawa and certified that 0.85 Gm. is equivalent to 1.0 Gm. of International Standard digitalis leaves. This correction was always made in preparing the standard extract. The lot of International Standard used in this series was labeled "Standard Digitalis 1928" and was obtained from the National Institute for Medical Research, London, N. W. 3.

SUMMARY OF ASSAYS.

Method.	Date.	M. L. D. or M. S. D.	Relative Potency.
		Sample: International Standard Digitalis.	
Lethal Frog	Sept. 1932	0.0075 cc. at 0.0075 cc.	100% of Canad. Std.
Lethal Frog	Jan. 1933	0.0040 cc. at 0.0055 cc.	137.5% of Canad. Std.
4-Hr. Frog	Nov. 1931	0.0060 cc. at 0.0050 cc.	83% of Canad. Std.
4-Hr. Frog	Feb. 1932	0.0040 cc. at 0.0045 cc.	112% of Canad. Std.
4-Hr. Frog	Apr. 1932	0.0040 cc. at 0.0045 cc.	112% of Canad. Std.
4-Hr. Frog	Dec. 1932	0.0060 cc. at 0.0060 cc.	100% of Canad. Std.
M. S. D. Frog	Mar. 1929	0.0080 cc. at 0.0000006 Gm.	110% of U. S. P. Std.
M. S. D. Frog	Oct. 1931	0.0050 cc. at 0.0000005 Gm.	120% of U. S. P. Std.
M. S. D. Frog	Oct. 1932	0.0060 cc. at 0.00000070 Gm.	140% of U. S. P. Std.
M. S. D. Frog	Jan. 1933	0.0060 cc. at 0.00000070 Gm.	140% of U. S. P. Std.
M. S. D. Frog	July 1933	0.0070 cc. at 0.00000060 Gm.	103% of U. S. P. Std.
Cat Method	1929	Av. M. L. D. 1.05 cc./Kg.	95% Hatcher Std.
Cat Method	1933	Av. M. L. D. 1.19 cc./Kg.	86% Hatcher Std.
		Sample: Ouabain U. S. P.	
Cat Method	1929	Av. M. L. D. 0.105 mg./Kg.	95% Hatcher Std.
		Sample: 1932 Digitalis.	
Lethal Frog	Oct. 1932	0.007 cc. at 0.00000033 Gm.	160% for Ouabain U. S. P.
M. S. D. Frog	Oct. 1932	0.006 cc. at 0.00000070 Gm.	140% of U. S. P. Std.
M. S. D. Frog	Oct. 1932	0.006 cc. at 0.006 (Inter. Std.)	100% of Inter. Std.
		Sample: Canadian Standard Digitalis.	
Lethal Frog	Aug. 1928	0.0088 cc. at 0.00000039 Gm.	115% for Ouabain
Lethal Frog	Sept. 1932	0.0075 cc. at 0.0075 cc.	100% of B. P.
Lethal Frog	Jan. 1933	0.0055 cc. at 0.0040 cc.	73% of B. P.
4-Hr. Frog	Nov. 1931	0.0050 cc. at 0.0060 cc.	120% of Inter. Std.
4-Hr. Frog	Feb. 1932	0.0045 cc. at 0.0040 cc.	90% of Inter. Std.
4-Hr. Frog	Apr. 1932	0.0045 cc. at 0.0040 cc.	90% of Inter. Std.
4-Hr. Frog	Dec. 1932	0.0060 cc. at 0.0060 cc.	100% of Inter. Std.
M. S. D. Frog	Apr. 1932	0.0060 cc. at 0.0000006 Gm.	120% of U. S. P. Std.
M. S. D. Frog	Apr. 1932	0.0060 cc. at 0.0000006 Gm.	120% of U. S. P. Std.
M. S. D. Frog	Jan. 1933	0.0055 cc. at 0.0000007 Gm.	152% of U. S. P. Std.
M. S. D. Frog	July 1933	0.0060 cc. at 0.0000006 Gm.	120% of U. S. P. Std.

DISCUSSION.

Assay results by the lethal frog method, which includes also the four-hour method, show that the International Standard digitalis leaves and the Canadian Standard leaves are very nearly equal to each other in activity, as they should be. An average of six tests shows the International Standard to be 107% of the Canadian Standard or that the latter is 93.5% of the former. This is a close enough agreement experimentally to consider them equal.

By the U. S. P. X one-hour method and against the official Ouabain standard the International Standard tincture averages 123% of the U. S. P. in five tests and the Canadian Standard tincture averages 128% of the U. S. P. or nearly the same as the B. P. Since each of these averages contains one or two high results obtained on winter frogs (Jan. 1933) it would seem that the B. P. and Canadian Standard tinctures of digitalis are from 20% to 25% more active than the U. S. P. Standard tincture and that conservatively the difference might be considered as being fully 20%.

By the cat method (two tests) the International Standard seems to be fully 90% of the standard proposed by Hatcher for Tincture of Digitalis but this series of tests is not large enough to prove much except that the B. P. 1932 Standard Tincture of Digitalis is about equal to the Hatcher Standard.

CONCLUSIONS.

1. The standard tincture of digitalis of the British Pharmacopœia, 1932 revision, is fully 20% more active than the U. S. P. X Standard. This difference is significant and is difficult to determine because of the recommended use of different standards and methods of bioassay.

2. The B. P. 1932 Standard tincture and the Canadian Standard, 1928 regulations, Food and Drugs Act are equal, within the limits of experimental error of the methods of assay, as they should be.

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PHYTOCHEMICAL NOTES.*

NO. 110. THE STEROLS FROM STRAMONIUM SEED.[1]

BY OLE GISVOLD.

The unsaponifiable material obtained by Ralph Clark in his study of the fatty acids of stramonium seed (1) was heated under a vacuum on a water-bath for a half day. It was then allowed to stand for several days in a vacuum desiccator in order to remove any traces of moisture present. Ten grams of this material (2) were dissolved in sufficient alcohol to make 150 cc. of solution. Two and one-half grams of digitonin, Merck, were dissolved in 250 cc. 90 per cent alcohol. Both

* From the laboratory of Edward Kremers.

[1] Scientific Section, A. PH. A., Toronto meeting, 1932.