

SCIENTIFIC SECTION

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THE U. S. P. XI STANDARD FOR TINCTURE DIGITALIS.*

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The application of the best available form of standardization to preparations of a very important but also variable drug such as digitalis is now recognized as essential by the pharmaceutical and medical professions. It is now about forty years since a physiological method using the frog as the test animal was first proposed. The necessity of having some reference standard for comparison has been almost universally accepted and definite progress was made when the U. S. P. XI Revision Committee decided to adopt the International Digitalis Standard Powder or its equivalent in place of the Ouabain which had been the official digitalis standard in U. S. P. IX and X.

However, it is almost equally desirable in the case of a powerful drug such as digitalis that pronounced changes in the activity of official preparations should not be made for obvious reasons. The decision to make the U. S. P. XI standard for Tincture Digitalis conform to the International Standard seemed logical and it was generally felt by those in close touch with the situation that only about a 20% increase in potency would result, which probably would not be particularly noticed clinically.

The present digitalis reference standard No. 915921 of the U. S. P. XI first became available to us late in February of 1936, and since that time frequent extracts and tests of these extracts have been made. At the very outset particular attention should be called to the fact that the proper correction factor was always applied in preparing different extracts of the standards for test so that a *corrected* tincture was always used. The purpose has been to determine from the averages of a sufficient number of careful comparative tests the following facts which should be of much interest to the pharmacist and to the practicing physician: (1) Just how much more active is the present U. S. P. XI standard for Tr. Digitalis than was the U. S. P. X standard? and, (2) What is the activity relationship between the International Standard for Tr. Digitalis, the Canadian Standard and the U. S. P. XI standard?

In a recent and excellent article on this subject by Edmunds, Moyer and Shaw (1) results of a series of assays were given showing how the correction factor 0.745 was obtained for the present U. S. P. XI standard digitalis powder. Reference will frequently be made to points of similarity and contrast as developed in the two papers. It was also shown in a short paper (2) published in February 1934, that the International Standard for Tr. Digitalis averaged 23% higher in activity by the U. S. P. One-Hour Frog Method than the U. S. P. X standard for Tr. Digitalis in which Ouabain was used as the standard. The Canadian Standard Tincture averaged 28% higher in activity than the U. S. P. X Standard Tincture.

From the very beginning of our work with the present digitalis standard powder of the U. S. P. XI it was quite apparent that it was a highly active preparation. Extracts for test were always made with 75% or 80% ethyl alcohol according to the pharmacopoeial method, *i. e.*,

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contact in a glass-stoppered graduated cylinder with frequent shaking for 24 to 48 hours, at room temperature. The powder was quickly and accurately weighed and the correction factor applied so that the tests were always made on a corrected or exactly U. S. P. XI standard Tr. Digitalis. The frogs used were of the *Rana pipiens* variety weighing from 15 to 35 Gm. and the statistical method of Trevan was employed. Twenty to 30 frogs were used on each preparation. It is gratifying to learn that the U. S. P. statistical curve as determined by Edmunds, *et al.*, upon *Rana pipiens* using the U. S. P. XI standard digitalis powder and the one-hour frog method, is very nearly identical with that prepared by Trevan using *Rana temporaria*, the International Standard digitalis powder and a lethal dose frog method.

In 1934 it was found from an average of five direct comparisons between freshly made tinctures from International Standard digitalis powder and U. S. P. X Standard ouabain that the International Standard was 123% of the U. S. P. X standard for Tr. Digitalis. One additional assay was made on June 15, 1936, with the M. S. D. of the International Standard tincture at 0.0065 cc. per Gm. body weight of frog and that of U. S. P. X standard ouabain at 0.00000060 Gm. per Gm., giving a relationship of 115% in favor of the International Standard. One indirect comparison of the two standards gave a 117% figure. Averaging these in with the other five we get a composite figure of 121%. We had been told by others that the International Standard for digitalis was from 20% to 25% stronger than the U. S. P. X standard and our findings confirm that very nicely. All of the assays reported in this paper were supervised by the senior author (R) and about half of the direct comparisons of the various standards were personally carried out by him.

The U. S. P. XI standard for Tr. Digitalis was to be equal to the International Standard according to a preliminary decision made by the Revision Committee and confirmed on page 136 (3) of the U. S. P. XI as follows: "One U. S. P. Digitalis Unit is identical in potency with the International Digitalis Unit. . . . One International Digitalis Unit represents the activity of 0.10 Gm. of the International Standard Digitalis Powder." Consequently there was every reason to believe that the U. S. P. XI standard Tr. Digitalis would be found by actual test to be 120% or 125% of the U. S. P. X standard to which we were accustomed. Such was not the case, however, as the direct comparisons in Table I between Ouabain and corrected U. S. P. XI digitalis and the indirect comparisons in Table II, where digitalis preparations were assayed at the same time against both standards, will show.

TABLE I.—DIRECT COMPARISON BETWEEN U. S. P. X OUABAIN AND U. S. P. XI DIGITALIS.

Date.	U. S. P. XI Digitalis (1 Cc. = 0.0745 Gm.). Dose per Gm.	U. S. P. ONE-HOUR FROG METHOD.			U. S. P. X. Ouabain (U. S. P. X). Dose per Gm.	Potency (XI of X).	
		Stopped.	Curve No.	Stopped.			Curve No.
1 3/19/36	0.0070 cc.	80%	4.32	0.00000080 Gm.	80%	4.32	137%
2 3/24/36	0.0040 cc.	67%	4.18	0.00000060 "	67%	4.18	180%
3 4/24/36	0.0035 cc.	67%	4.18	0.00000055 "	67%	4.18	188%
4 5/11/36	0.0045 cc.	60%	4.10	0.00000065 "	60%	4.10	160%
5 5/22/36	0.0050 cc.	50%	4.00	0.00000045 "	70%	4.22	108%
6 6/1/36	0.0050 cc.	50%	4.00	0.00000050 "	30%	3.80	126%
7 6/13/36	0.0040 cc.	53%	4.03	0.00000060 "	40%	3.90	186%
8 7/1/36	0.0035 cc.	53%	4.03	0.00000050 "	67%	4.18	165%
9 7/20/36	0.0045 cc.	40%	3.90	0.00000050 "	53%	4.03	129%
10 8/21/36	0.0055 cc.	53%	4.03	0.00000060 "	47%	3.98	133%
11 9/4/36	0.0055 cc.	47%	3.98	0.00000065 "	60%	4.10	138%
12 10/21/36	0.0060 cc.	60%	4.10	0.00000060 "	60%	4.10	120%
13 11/4/36	0.0050 cc.	60%	4.10	0.00000060 "	68%	4.20	140%
14 11/14/36	0.0050 cc.	60%	4.10	0.00000050 "	40%	3.90	126%
15 12/9/36	0.0050 cc.	40%	3.90	0.00000070 "	80%	4.32	152%
16 12/22/36	0.0055 cc.	67%	4.18	0.00000075 "	33%	3.83	178%
17 1/19/37	0.0055 cc.	43%	3.93	0.00000080 "	47%	3.98	172%
18 2/10/37	0.0060 cc.	73%	4.25	0.00000090 "	50%	4.00	191%

Av. of 18 tests = U. S. P. XI standard for Tr. Digitalis is 151% of U. S. P. X standard.

TABLE II.—INDIRECT COMPARISON BETWEEN U. S. P. X AND XI STANDARDS FOR TR. DIGITALIS.

No.	Product.	Number.	U. S. P. One-Hour Frog Method.		Potency.
			M. S. D.	(Sample and Standard).	
1	Digifortis Amps.	883494	0.0045 cc. per Gm. at	0.00000050 Gm.	133% of X Std.
2	" "	"	0.0045 cc. " " "	0.0035 cc.	78% of XI "
3	" "	3106476	0.0055 cc. " " "	0.00000050 Gm.	109% of X "
4	" "	"	0.0055 cc. " " "	0.0040 cc.	74% of XI "
5	Digifortis	3090870	0.0040 cc. " " "	0.00000050 Gm.	150% of X "
6	" "	"	0.0040 cc. " " "	0.0040 cc.	100% of XI "
7	Tr. Digitalis	880608	0.0080 cc. " " "	0.00000060 Gm.	90% of X "
8	" "	"	0.0080 cc. " " "	0.0040 cc.	50% of XI "
9	" "	B. C. O.	0.0075 cc. " " "	0.00000060 Gm.	96% of X "
10	" "	"	0.0075 cc. " " "	0.0040 cc.	54% of XI "
11	" "	381509	0.0035 cc. " " "	0.00000060 Gm.	205% of X "
12	" "	"	0.0040 cc. " " "	0.0050 cc.	125% of XI "
13	" "	886032	0.0050 cc. " " "	0.00000075 Gm.	180% of X "
14	" "	"	0.0050 cc. " " "	0.0060 cc.	120% of XI "
15	Powd. Ext. Digitalis	873903	0.00018 Gm. " " "	0.00000055 Gm.	122% of X "
16	" " "	"	0.00018 Gm. " " "	0.0040 cc.	70% of XI "
17	" " "	891217	0.00020 Gm. " " "	0.00000055 Gm.	108% of X "
18	" " "	"	0.00020 Gm. " " "	0.0070 cc.	94% of XI "
19	" " "	891013	0.00025 Gm. " " "	0.00000050 Gm.	83% of X "
20	" " "	"	0.00025 Gm. " " "	0.0055 cc.	72% of XI "
21	" " "	895728	0.00030 Gm. " " "	0.00000090 Gm.	119% of X "
22	" " "	"	0.00030 Gm. " " "	0.0060 cc.	72% of XI "
23	Solid " "	888718	0.00020 Gm. " " "	0.00000055 Gm.	110% of X "
24	" " "	"	0.00018 Gm. " " "	0.0035 cc.	63% of XI "
25	Digitalone	3108679	0.009 cc. " " "	0.0000008 Gm.	103% of X "
26	" "	"	0.009 cc. " " "	0.0065 cc.	72% of XI "
27	" "	031911	0.008 cc. " " "	0.00000060 Gm.	86% of X "
28	" "	"	0.008 cc. " " "	0.0060 cc.	74% of XI "
29	Digifortis	884477	0.0065 cc. " " "	0.00000090 Gm.	166% of X "
30	" "	"	0.0040 cc. " " "	0.0050 cc.	119% of XI "
31	Tr. Digitalis	2946033	0.0080 cc. " " "	0.00000080 Gm.	113% of X "
32	" "	"	0.0080 cc. " " "	0.0055 cc.	68% of XI "

Assays 1 and 2 show the U. S. P. XI standard is 170% of U. S. P. X ($133\% \div 78\%$).

3 and 4 = 147%	5 and 6 = 150%	7 and 8 = 180%	9 and 10 = 177%	11 and 12 = 164%
13 and 14 = 150%	15 and 16 = 174%	17 and 18 = 116%	18 and 20 = 115%	21 and 22 = 165%
23 and 24 = 174%	25 and 26 = 143%	27 and 28 = 116%	29 and 30 = 139%	31 and 32 = 166%

Average of these 16 comparative tests of the same product against the two standards shows the U. S. P. XI standard for Tr. Digitalis to be 153% of the U. S. P. X standard.

Thus by the direct and indirect methods of comparison very similar averages are obtained and the present standard for Tr. Digitalis is shown to be roughly 50% stronger than the U. S. P. X standard instead of the 20% or 25% stronger which we had reason to expect. No data on U. S. P. X standard ouabain are presented in the recent paper by Edmunds so that no comparison can be made from this standpoint.

Having determined experimentally by two groups of independent data a relationship between the two latest U. S. P. standards for Tr. Digitalis, assays will be submitted in Table III bearing on the second objective of this paper, *i. e.*, What is the activity relationship of the International, the Canadian and the U. S. P. XI standards for Tr. Digitalis?

Comparative data by the official One-Hour Frog Method on the International Standards of 1926 and 1928 as regular tinctures and the Canadian, U. S. P. XI and International 1936 as *corrected* tinctures will be submitted though it now seems apparent that the International Standard of 1928 was a British Standard for the B. P. 1932 and is identical with the International Standard 1936 which, however, bears a correction factor of 0.80 to make it conform to the 1926 International Standard.

TABLE III.—COMPARISON OF FOUR DIGITALIS STANDARDS.

1 International 1926 (No Factor)	3 Canadian Corr. (0.85 and 1.05 Factor)
2 U. S. P. XI Corrected (0.745 Factor)	4 British 1928 or Inter. 1936 (0.80 Factor)

Date.	Products.	U. S. P. One-Hour Frog Method. M. S. D. and Curve Number.	Potency.
6/15/36	2 vs. 1	0.0040 cc. (4.03) at 0.0065 cc. (4.03)	163% of Inter. Std. 1926
8/12/36	2 vs. 1	0.0055 cc. (4.03) at 0.0060 cc. (4.22)	104% " " " "
11/25/36	2 vs. 1	0.0045 cc. (4.03) at 0.0065 cc. (4.03)	145% " " " "
4/24/37	2 vs. 1	0.0070 cc. (4.25) at 0.0075 cc. (3.90)	117% " " " "
5/25/37	2 vs. 1	0.0070 cc. (4.10) at 0.011 cc. (3.95)	164% " " " "
5/22/37	2 vs. 1	0.0085 cc. (3.90) at 0.010 cc. (3.90)	118% " " " "

Av. of 6 tests is 135% (U. S. P. XI is of Inter. Std. 1926) or the 1926 Inter. Std. is 76% of U. S. P. XI Standard.

6/15/36	3 vs. 1	0.0050 cc. (4.10) at 0.0065 cc. (4.03)	132% of Inter. Std. 1926
6/13/36	3 vs. 1	0.0050 cc. (3.76) at 0.0065 cc. (4.03)	121% " " " "
4/24/37	3 vs. 1	0.0080 cc. (3.98) at 0.0075 cc. (3.90)	96% " " " "
5/25/37	3 vs. 1	0.0090 cc. (3.95) at 0.011 cc. (3.95)	122% " " " "

Av. of 4 tests is 118% (Canadian Std. is of Inter. Std. 1926) or the 1926 Inter. Std. is 85% of Canadian Standard.

8/5/36	4 vs. 1	0.0065 cc. (4.32) at 0.0050 cc. (3.90)	85% of Inter. Std. 1926
8/13/36	4 vs. 1	0.0060 cc. (4.00) at 0.0060 cc. (4.22)	95% " " " "
11/28/36	4 vs. 1	0.0055 cc. (4.00) at 0.0065 cc. (4.03)	177% " " " "
4/24/37	4 vs. 1	0.0075 cc. (4.18) at 0.0075 cc. (3.90)	108% " " " "
5/25/37	4 vs. 1	0.0080 cc. (4.00) at 0.011 cc. (3.95)	139% " " " "

Av. of 5 tests is 109% (Inter. Std. 1936 is of 1926) or the 1926 Inter. Std. is 92% of the 1936 Inter. Standard.

4/24/36	3 vs. 2	0.0045 cc. (4.10) at 0.0035 cc. (4.18)	77% of U. S. P. XI Std.
6/13/36	3 vs. 2	0.0050 cc. (4.10) at 0.0040 cc. (4.03)	81% " " " "
6/15/36	3 vs. 2	0.0050 cc. (3.76) at 0.0040 cc. (4.03)	74% " " " "
4/24/37	3 vs. 2	0.0080 cc. (3.98) at 0.0070 cc. (4.25)	82% " " " "
5/25/37	3 vs. 2	0.0090 cc. (3.95) at 0.0070 cc. (4.10)	75% " " " "

Av. of 5 tests is 78% (Canadian Std. is of U. S. P. XI Std.) or the U. S. P. XI Std. is 128% of the Canadian Standard.

8/12/36	4 vs. 2	0.0060 cc. (4.00) at 0.0055 cc. (4.03)	90% of U. S. P. XI Std.
11/4/36	4 vs. 2	0.0055 cc. (4.00) at 0.0045 cc. (4.03)	81% " " " "
11/25/36	4 vs. 2	0.0060 cc. (4.18) at 0.0050 cc. (4.10)	85% " " " "
3/25/37	4 vs. 2	0.0055 cc. (4.50) at 0.0060 cc. (4.22)	116% " " " "
4/24/37	4 vs. 2	0.0075 cc. (4.18) at 0.0070 cc. (4.25)	91% " " " "
5/25/37	4 vs. 2	0.0080 cc. (4.00) at 0.0070 cc. (4.10)	85% " " " "

Av. of 6 tests is 91% (Inter. Std. 1936 is of U. S. P. XI Std.) or U. S. P. XI Std. is 110% of the 1936 Inter. Standard.

TABLE III.—Continued from page 185.

4/24/37	4 vs. 3	0.0075 cc. (4.18) at 0.0080 cc. (3.98)	112% of Canad. Std.
5/25/37	4 vs. 3	0.0080 cc. (4.00) at 0.0090 cc. (3.95)	112% " " "

Av. of 2 tests is 112% (Inter. Std. 1936 is of Canadian Std.) or the Canadian Standard is 89% of the 1936 Inter. Standard.

From these 28 tests covering a period of about 15 months the following averages were obtained as a direct comparison with the 11 tests covering a period of 10 days as reported by Dr. Edmunds in his Table I.

International standard powder 1926 considered as	100%
U. S. P. XI standard powder No. 915921, <i>corrected</i> (0.745)	135%
International 1928 and 1936 standard, <i>corrected</i>	109%
Canadian standards, <i>corrected</i> (0.85 and 1.05)	118%

Thus about the same figure is obtained for the U. S. P. XI powder, even after using a high correction factor in preparing the extract for test, as was obtained by Dr. Edmunds on the same powder but uncorrected.

Since the Four-Hour Frog Method is quite similar to the Lethal Dose Frog method only data on the various standards by the latter method are submitted in Table IV.

TABLE IV.—COMPARISON OF FOUR DIGITALIS STANDARDS.

		1 International 1926 (No Factor)		3 Canadian Corr. (0.85 and 1.05 Factor)	
		2 U. S. P. XI, Corrected (0.745 Factor)		4 British 1928 or Inter. 1936 (0.80 Factor)	
Date.	Products.	Lethal Dose or Twelve-Hour Frog Method. L. D. and Curve Number.		Potency.	
3/12/36	2 vs. 1	0.0050 cc. (4.20) at 0.0060 cc. (4.02)	125% of Inter. Std. 1926		
5/14/36	2 vs. 1	0.0050 cc. (3.80) at 0.0070 cc. (3.90)	136% " " " "		
6/18/36	2 vs. 1	0.0040 cc. (3.90) at 0.0070 cc. (4.00)	170% " " " "		
7/23/36	2 vs. 1	0.0045 cc. (4.00) at 0.0070 cc. (4.50)	138% " " " "		
8/14/36	2 vs. 1	0.0055 cc. (4.32) at 0.0060 cc. (4.18)	113% " " " "		
11/6/36	2 vs. 1	0.0040 cc. (4.25) at 0.0070 cc. (3.98)	186% " " " "		
12/3/36	2 vs. 1	0.0030 cc. (3.68) at 0.0040 cc. (3.68)	133% " " " "		
4/20/37	2 vs. 1	0.0036 cc. (4.22) at 0.0045 cc. (4.22)	125% " " " "		
6/4/37	2 vs. 1	0.0040 cc. (3.83) at 0.0065 cc. (3.98)	156% " " " "		
Av. of 9 tests is 142% (U. S. P. XI is of Inter. Std. 1926) or Inter. Std. 1926 is 72% of U. S. P. XI Standard.					
6/18/36	3 vs. 1	0.0060 cc. (3.80) at 0.0070 cc. (4.00)	111% of Inter. Std. 1926		
7/23/36	3 vs. 1	0.0050 cc. (4.10) at 0.0070 cc. (4.50)	127% " " " "		
4/19/37	3 vs. 1	0.0036 cc. (3.90) at 0.0045 cc. (4.10)	119% " " " "		
4/20/37	3 vs. 1	0.0036 cc. (3.80) at 0.0045 cc. (4.22)	112% " " " "		
6/4/37	3 vs. 1	0.0060 cc. (4.25) at 0.0065 cc. (3.98)	115% " " " "		
Av. of 5 tests is 117% (Canadian Standard is of Inter. Std. 1926) or 1926 is 85% of Canadian Standard.					
8/14/36	4 vs. 1	0.0050 cc. (4.32) at 0.0060 cc. (4.18)	125% of Inter. Std. 1926		
12/3/36	4 vs. 1	0.0035 cc. (4.32) at 0.0040 cc. (3.68)	134% " " " "		
4/20/37	4 vs. 1	0.0045 cc. (4.22) at 0.0045 cc. (4.22)	100% " " " "		
6/4/37	4 vs. 1	0.0055 cc. (3.98) at 0.0065 cc. (3.98)	118% " " " "		

Av. of 4 tests is 119% (Inter. Std. 1936 is of 1926) or 1926 Inter. Std. is 84% of the 1936 Inter. Std.

3/2/36	3 vs. 2	0.0050 cc. (3.87) at 0.0035 cc. (3.78)	71% of U. S. P. XI Std.
6/18/36	3 vs. 2	0.0060 cc. (3.80) at 0.0040 cc. (3.90)	65% " " "
7/23/36	3 vs. 2	0.0050 cc. (4.10) at 0.0045 cc. (4.00)	92% " " "
8/14/36	3 vs. 2	0.0065 cc. (4.18) at 0.0055 cc. (4.32)	82% " " "
4/19/37	3 vs. 2	0.0036 cc. (3.90) at 0.0036 cc. (4.22)	92% " " "
4/20/37	3 vs. 2	0.0036 cc. (3.80) at 0.0036 cc. (4.22)	90% " " "
6/4/37	3 vs. 2	0.0060 cc. (4.25) at 0.0040 cc. (3.83)	74% " " "

Av. of 7 tests is 81% (Canadian Std. is of U. S. P. XI Std.) or the U. S. P. XI Std. is 123% of the Canadian Standard.

8/14/36	4 vs. 2	0.0050 cc. (4.32) at 0.0055 cc. (4.32)	110% of U. S. P. XI Std.
12/3/36	4 vs. 2	0.0035 cc. (4.32) at 0.0030 cc. (3.68)	99% " " "
4/20/37	4 vs. 2	0.0045 cc. (4.22) at 0.0036 cc. (4.22)	80% " " "
6/4/37	4 vs. 2	0.0055 cc. (3.98) at 0.0040 cc. (3.83)	76% " " "

Av. of 4 tests is 91% (Inter. Std. 1936 is of U. S. P. XI Std.) or the U. S. P. XI Std. is 110% of the 1936 Inter. Standard.

8/14/36	4 vs. 3	0.0050 cc. (4.32) at 0.0065 cc. (4.18)	134% of Canad. Std.
4/19/37	4 vs. 3	0.0045 cc. (4.10) at 0.0036 cc. (3.90)	84% " " "
4/20/37	4 vs. 3	0.0045 cc. (4.22) at 0.0036 cc. (3.80)	89% " " "
6/4/37	4 vs. 3	0.0055 cc. (3.98) at 0.0060 cc. (4.25)	102% " " "

Av. of 4 tests is 102% (Inter. Std. 1936 is of Canadian Std.) or the Canadian Std. is 98% of the 1936 Inter. Standard.

From these 33 tests covering a period of 15 months the following averages may be tabulated for direct comparison with the 10 tests reported by Dr. Edmunds in his Table III and covering only a winter period of two and one-half months:

International standard powder 1926 considered as	100%
U. S. P. XI standard powder No. 915921, corrected (0.745)	142%
International 1928 and 1936 standard, corrected	119%
Canadian standards, corrected (0.85 and 1.05)	117%

In this series of tests by the lethal dose frog method there is a marked discrepancy between our 142% and Dr. Edmunds' 191.4% average for the relation of the U. S. P. XI standard to the International standard of 1926, but if the correction factor, 0.745 is applied to the 191.4% a result of 142.6% is obtained which is surprisingly close to the average actually obtained in our tests.

Comparison can now be made between average potencies determined by the two frog methods on the various digitalis standards. Table V gives all the relationships established experimentally.

TABLE V.

Digitalis Standard.	One-Hour Frog. Taken as 100%	Twelve-Hour Frog. Taken as 100%
International 1926		
U. S. P. XI, corrected	135%	142%
Canadian, corrected	118%	117%
International 1936 corrected	109%	119%
U. S. P. X	83%
U. S. P. XI vs. Canadian	128%	123%
U. S. P. XI vs. Inter. 1936	110%	110%
Inter. 1936 vs. Canadian	112%	102%

Throughout this table there is a very remarkable correlation of results on the same standards by the two frog methods. This was to be expected as it is a fundamental principle of physiological assay that a relationship between a standard and an unknown or between two standards

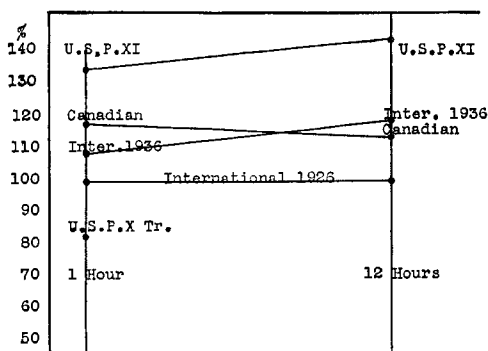


Fig. 1.—Potency of the various standards in terms of the International 1926 by the one-hour and twelve-hour frog methods.

as determined by two different but highly analogous methods should agree within the limits of experimental error of the methods. This agreement was not found by Dr. Edmunds and no very satisfactory explanation for the discrepancy was advanced.

Graphically our results may be placed in one figure which would be comparable to No. 2 in the Edmunds article.

This shows the relation of five digitalis standards by the one-hour frog method and four of them by the twelve-hour method. The figure also shows graphically the small variation in potency of the same standard by the different methods.

The mean L. D. 50 in mg. per Kg. was also calculated for a series of three comparable tests by both methods and this shows a

decrease in the twelve-hour dose over the one-hour dose but it is not as great as might be expected. The reduction is nothing like as great as that reported by Edmunds and is greatest for the 1936 International standard and least for the U. S. P. XI standard.

TABLE VI.—MEAN L. D. 50 IN MG. PER KG. OF FROG.

Standard.	M. S. D. Method.	M. L. D. Method.	Decrease.
International 1926	619 mg.	551 mg.	10.98%
U. S. P. XI	463 mg.	423 mg.	8.64%
Canadian	577 mg.	522 mg.	9.53%
International 1936	575 mg.	481 mg.	16.35%

Some years ago we found an average decrease for the M. L. D. dose over the M. S. D. dose of about 20% so that the present decrease ranging from about 9% to 16% is not so very different.

Our experimental data indicate strongly from four different standpoints that the U. S. P. XI standard digitalis powder is definitely more active than it is claimed to be and consequently the U. S. P. XI standard for Tr. Digitalis is a relatively high standard. Our averages show it to be 151% of the U. S. P. X standard by direct comparison, 153% of the U. S. P. X standard by indirect comparison, 135% of the International standard by direct comparison using the one-hour frog method and 142% when using the twelve-hour frog method.

It has also been shown that comparable results can be obtained within the limits of experimental error of the methods on tests of the same standards by both the one-hour and the twelve-hour frog methods.

SUMMARY.

1. The U. S. P. XI standard for Tr. Digitalis has been found experimentally both by direct and indirect comparison to be about 50% stronger than the U. S. P. X standard rather than 20% or 25% stronger as was expected.

2. The U. S. P. XI digitalis standard actually is not just equal to the International digitalis standard but is apparently nearly 25% stronger as shown by direct comparisons using two analogous methods.

3. The Canadian standard is slightly stronger than the International standard but definitely less active than the U. S. P. XI digitalis standard.

4. Very comparable results were obtained by the one-hour and the twelve-hour frog methods for the activity relationship between any two of the several official standards examined.

REFERENCES.

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- (3) United States Pharmacopœia, XI Revision, page 136.

THE SIGNIFICANCE OF SUGAR COMPONENT IN THE MOLECULE OF CARDIAC GLYCOSIDES.*

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According to literature, the aglycone appears to have a lower activity than its parent glycoside. Thus, digitoxigenin in frogs is about one-half, and "cymarigenin" about one-third, as potent as digitoxin and cymarin, respectively, as shown by Straub (1). Although Rothlin (2) indicated that the ratio of activity between scillaren A and scillaridin A is 10:1, Stoll (3) recently admitted, "Scillaridin A is so sparingly water soluble that, up to the present time, it has not been possible to test its physiological activity." Digoxigenin is almost one-eleventh as active as digoxin, as reported by Smith (4). Oleandrigenin, however, is only slightly less effective than folinerin (25:24) according to Flury and Neumann (5).

The purpose of this paper is to present data obtained with five aglycones, strophanthidin, digoxigenin, digitoxigenin, scillaridin A and calotropagenin, and to compare them with those of their parent glycosides, cymarin, digoxin, digitoxin, scillaren A and calotropin, respectively. Our sample of strophanthidin, isolated from *Strophanthus Kombé*, was generously supplied by Dr. Walter A. Jacobs, the Rockefeller Institute for Medical Research, New York City; that of digoxigenin by Dr. Sidney Smith of London, England, through the kindness of Howard B. Fonda, Experimental Research Laboratories, the Burroughs Wellcome and Company, Tuckahoe, New York; that of digitoxigenin by Dr. Rudolf Tschesche, Berlin, Germany; that of scillaridin A by Dr. Arthur Stoll of Basel, Switzerland, through the kindness of E. W. Marti, Sandoz Chemical Works, Inc., New York City; and that of calotropagenin by Dr. Gerhard Hesse, München, Germany.

The experimental procedures were the same as those previously reported (6). Since success depends upon the solubility of these substances in an inert medium, considerable care was taken in making the solutions—needless to say, the aglycones are comparatively less soluble in water than the glycosides. For a 1:1000 concentration, strophanthidin and calotropagenin required 19 per cent ethyl alcohol by volume, digoxigenin 27.5 per cent, and digitoxigenin 38 per cent. It was necessary to increase the alcoholic content of a 1:500 concentration—38 per cent for digoxigenin and 47.5 per cent for digitoxigenin. Much difficulty was encountered with scillaridin A, for in absolute alcohol it settled out in the concentration of 1:1000. It was finally decided to employ a 1:2000 solution in methanol. For the determination of the cat unit, intravenous injections were

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