

CONCLUSIONS.

1. From the results obtained, it would appear that digitalis leaves containing from 4.8 per cent to 11.9 per cent moisture deteriorate on standing.
2. The percentage of deterioration which occurs during a storage period of 100 days does not appear to bear any relation to the moisture content within a range of 4.8 per cent to 11.9 per cent or to the temperature of storage within a range of 70° F. to 100° F.
3. No evidence was obtained which would appear to indicate that storage in air-tight containers enhances the keeping qualities of the drug during a 100-day storage period.
4. Further investigation should be carried out employing a longer storage period and moisture contents lower than 4.5 per cent.

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FURTHER EVIDENCE OF THE STRONG AND VARIABLE ACTION OF
THE U. S. P. XI DIGITALIS STANDARD.*BY L. W. ROWE.¹

In a previous report (1) a considerable amount of experimental data was presented showing that the U. S. P. XI standard for Tr. Digitalis is 50% stronger than the U. S. P. X standard and nearly 25% stronger than the International Standard.

Since that time the 150% figure has been confirmed by Munch and his Committee (2) and by Thompson (3). This seems to point to the fact that the present official standard is definitely higher in potency than it was intended to be by its sponsors (4), since the International Standard was never reported to be 50% stronger than the U. S. P. X standard for Tr. Digitalis.

During the past year further work with official extracts of this U. S. P. XI digitalis standard powder No. 915,921, by the official one-hour frog method, has given additional evidence of its variable action which may be attributed either to the unsuitability of the method itself or of the standard digitalis powder or both.

EXPERIMENTAL DATA.

Last year it was noted that the minimum systolic dose of the corrected standard digitalis tincture was running consistently from 0.0050 cc. to 0.0070 cc. per Gm. which seemed quite low. This was with regular 1 to 3 and 1 to 4 dilutions of the standard which are permissible since the

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alcohol content in such dilutions is less than 25%. The procedure outlined in the U. S. P. XI for the conduct of the official one-hour frog method also permits the careful removal of all or nearly all of the alcohol from the standard extract before dilution and injection into the frogs. When this was done by careful use of a fan without heat the M. S. D. of the standard immediately rose to from 0.0070 cc. to 0.010 cc. per Gm. Such a pronounced change was not to be expected as it had always been said that the use of less than 25% alcohol in the final dilutions had no perceptible effect upon the M. S. D. of any preparation. Table I gives the results obtained during the past year in the use of the U. S. P. XI standard both with and without alcohol.

TABLE I.—ONE-HOUR METHOD. U. S. P. XI STANDARD.

Test.	Date.	Alcohol.	M. S. D.	% Stopped.	
1	6/9/37	With	0.0045 cc. per Gm.	60.0	
2	6/15/37	"	0.0040 " " "	66.7	
3	6/25/37	"	0.0045 " " "	73.3	
4	"	"	0.0055 " " "	60.0	
5	7/13/37	"	0.0060 " " "	60.0	
6	7/21/37	"	0.0050 " " "	70.0	
7	7/27/37	"	0.0050 " " "	73.3	
8	8/9/37	"	0.0050 " " "	50.0	
9	8/18/37	"	0.0055 " " "	66.7	
10	8/25/37	"	0.0055 " " "	80.0	
11	9/2/37	"	0.0050 " " "	60.0	
12	9/8/37	"	0.0055 " " "	53.3	
13	9/15/37	"	0.0075 " " "	66.7	
14	10/6/37	"	0.0065 " " "	53.3	
15	10/14/37	"	0.0070 " " "	66.7	
16	10/20/37	"	0.0055 " " "	86.7	
17	10/26/37	"	0.0050 " " "	40.0	99.3%
18	"	"	0.0055 " " "	80.0	100.0%
19	11/1/37	"	0.0055 " " "	53.3	120.0%
20	"	Without	0.0060 " " "	20.0	100.0%
21	11/4/37	With	0.0065 " " "	30.0	127.0%
22	"	Without	0.0085 " " "	40.0	100.0%
23	11/17/37	"	0.0070 " " "	46.7	
24	11/26/37	"	0.0070 " " "	50.0	
25	12/8/37	"	0.0070 " " "	20.0	103.7%
26	12/9/37	"	0.0075 " " "	30.0	100.0%
27	12/14/37	"	0.0080 " " "	66.7	95.6%
28	12/20/37	"	0.0075 " " "	60.0	100.0%
29	12/31/37	"	0.0100 " " "	60.0	94.6%
30	1/5/38	"	0.0090 " " "	40.0	100.0%
31	1/12/38	"	0.0085 " " "	60.0	
32	1/20/38	"	0.0077 " " "	60.0	
33	1/27/38	"	0.0080 " " "	45.0	100.0%
34	"	With	0.0055 " " "	65.0	152.8%
35	2/4/38	Without	0.0080 " " "	53.3	
36	2/10/38	"	0.0085 " " "	60.0	100.0%
37	2/11/38	With	0.0050 " " "	60.0	170.0%
38	2/21/38	Without	0.0085 " " "	60.0	100.0%
39	2/23/38	"	0.0085 " " "	66.7	100.0%
40	2/25/38	With	0.0055 " " "	60.0	151.6%
41	3/3/38	Without	0.0080 " " "	46.7	
42	3/16/38	"	0.0085 " " "	66.7	100.0%
43	"	With	0.0045 " " "	73.3	192.0%
44	3/25/38	"	0.0045 " " "	66.7	107.0%
45	3/31/38	"	0.0050 " " "	80.0	153.6%

TABLE I.—(Continued from page 845.)

46	4/2/38	Without	0.0080	cc. per Gm.	90.0	100.0%	104.0%
47	4/6/38	"	0.0075	" "	53.3		100.0%
48	4/13/38	With	0.0044	" "	60.0	179.2%	
49	4/16/38	Without	0.0075	" "	40.0	100.0%	
50	4/22/38	With	0.0048	" "	53.3		104.0%
51	4/27/38	"	0.0048	" "	37.5	141.2%	100.0%
52	4/29/38	Without	0.0072	" "	62.5	100.0%	
53	5/5/38	With	0.0065	" "	53.3	111.6%	
54	5/6/38	Without	0.0072	" "	50.0	100.0%	
55	5/12/38	With	0.0075	" "	66.7		
56	5/20/38	Without	0.0085	" "	53.3		
57	5/26/38	With	0.0072	" "	66.7	125.0%	
58	5/21/38	Without	0.0090	" "	66.7	100.0%	
59	6/15/38	With	0.0080	" "	66.7	150.0%	
60	6/14/38	Without	0.0110	" "	33.3	100.0%	

Thus in 33 tests of the U. S. P. XI standard tincture *with* alcohol over a period of just one year the M. S. D. averaged 0.0055 cc. In 27 tests of the same standard *without* alcohol over the same period the M. S. D. averaged 0.0080 cc. per Gm. The year's average M. S. D. of the standard tested without alcohol is, therefore, 45% higher than for the same standard if tested with alcohol.

In 11 *direct* comparisons of the standard with and without alcohol the results averaged 48% higher for the standard with alcohol. In the four winter months, January to April, inclusive, the average increase was much higher (63%) than for the other months (21%), thus showing in addition a surprising seasonal variation in action of this U. S. P. standard.

Two tests of the standard at about the same time on the same lot of frogs agree very well if both tests are made either with alcohol or both without. Thus test No. 17 is 99.3% of No. 18; No. 25 is 103.7% of No. 26; No. 27 is 95.6% of No. 28; No. 29 is 94.6% of No. 30; No. 39 is 102% of No. 38; No. 44 is 107% of No. 45; No. 46 is 104% of No. 47; No. 50 is 104% of No. 51.

In Table II are given results obtained with certain digitalis preparations when both the sample and standard are tested with alcohol; when both are tested without alcohol; and when one has alcohol and the other little if any. Since most digitalis preparations do not show the pronounced difference in M. S. D. under these two conditions that the U. S. P. standard digitalis does, it is interesting to see what a difference in relative activity is thus obtained.

TABLE II.

Test.	Date.	Product.	M. S. D.	% Stopped.	Alcohol.	Result.
1	10/14/37	Tr. Dig. \mathbb{R} 906087	0.0075 cc./Gm.	46.7	With	89%
	"	U. S. P. XI Std. Tr.	0.0070 "	66.7	"	100%
2	11/4/37	Tr. Dig. \mathbb{R} 906087	0.0085 "	40.0	Without	100%
	"	U. S. P. XI Std. Tr.	0.0085 "	40.0	"	100%
3	10/20/37	Tr. Dig. Int. Std.	0.0090 "	80.0	With	60%
	"	U. S. P. XI Std. Tr.	0.0055 "	86.7	"	100%
4	11/17/37	Tr. Dig. Int. Std.	0.0110 "	33.3	Without	70%
	"	U. S. P. XI Std. Tr.	0.0080 "	40.0	"	100%
5	2/18/38	Tr. Dig. 3184092	0.0056 "	60.0	With	78%
	"	U. S. P. XI Std. Tr.	0.0045 "	73.0	"	100%
6	2/18/38	Tr. Dig. 3184092	0.0070 "	53.0	Without	119%
	"	U. S. P. XI Std. Tr.	0.0085 "	60.0	"	100%
7	2/18/38	Tr. Dig. 3184092	0.0056 "	60.0	With	150%
	"	U. S. P. XI Std. Tr.	0.0085 "	60.0	Without	100%
8	3/18/38	Tr. Dig. 3159081	0.0048 "	60.0	With	90%
	"	U. S. P. XI Std. Tr.	0.0045 "	73.3	"	100%
9	3/18/38	Tr. Dig. 3159081	0.0075 "	60.0	Without	111%
	"	U. S. P. XI Std. Tr.	0.0085 "	66.7	"	100%
10	3/18/38	Tr. Dig. 3159081	0.0048 "	60.0	With	170%

	3/18/38	U. S. P. XI Std. Tr.	0.0085 cc./Gm.	66.7	Without	100%
11	3/16/38	Tr. Dig. No. 3	0.0050 "	66.7	With	88%
	"	U. S. P. XI Std. Tr.	0.0045 "	73.3	"	100%
12	3/16/38	Tr. Dig. No. 3	0.0075 "	50.0	Without	109%
	"	U. S. P. XI Std. Tr.	0.0085 "	66.7	"	100%
13	3/16/38	Tr. Dig. No. 3	0.0050 "	66.7	With	170%
	"	U. S. P. XI Std. Tr.	0.0085 "	66.7	Without	100%
14	3/18/38	Tr. Dig. No. 4	0.0055 "	80.0	With	85%
	"	U. S. P. XI Std. Tr.	0.0045 "	73.3	"	100%
15	3/18/38	Tr. Dig. No. 4	0.0045 "	46.7	Without	180%
	"	U. S. P. XI Std. Tr.	0.0085 "	66.7	"	100%
16	3/18/38	Tr. Dig. No. 4	0.0055 "	80.0	With	160%
	"	U. S. P. XI Std. Tr.	0.0085 "	66.7	Without	100%
17	3/31/38	Tr. Dig. R̄ 909836	0.0052 "	60.0	With	91%
	"	U. S. P. XI Std. Tr.	0.0050 "	80.0	"	100%
18	3/31/38	Tr. Dig. R̄ 909836	0.0065 "	40.0	Without	107%
	"	U. S. P. XI Std. Tr.	0.0080 "	90.0	"	100%
19	3/31/38	Tr. Dig. R̄ 909836	0.0052 "	60.0	With	140%
	"	U. S. P. XI Std. Tr.	0.0080 "	90.0	Without	100%

This table shows clearly that when the sample is tested against the U. S. P. XI standard both with alcohol, the result is definitely and consistently lower for the sample than when both are tested without alcohol. This should not be so since either procedure is permitted by the Pharmacopœia and both should yield practically the same results.

Such large inconsistencies should not exist even though it is intended that similar amounts of alcohol be present in dilutions of standard and sample when direct comparison is made. Such discrepancies are indications that something is materially wrong with the standard digitalis powder or with the U. S. P. XI one-hour frog method or both. In view of the results presented below which show that these discrepancies do not exist when the M. L. D. frog method is used but only that the U. S. P. XI standard is relatively higher, it is logical to assume that the present official method is chiefly at fault, and that the glucosides of the present standard powder are relatively more rapidly absorbed than are those of average drug.

In Table III are given a smaller number of M. L. D. frog method tests both with and without alcohol which show that the presence of alcohol makes little difference in the M. L. D. and even the U. S. P. XI digitalis standard is much more consistent.

TABLE III.—M. L. D. FROG METHOD.

Test.	Date.	Product.	M. L. D.	% Died.	Alcohol.	Result.
1	1/31/38	U. S. P. XI Std. Tr.	0.0036 cc.	40.0	With	107.5%
	"	"	0.0040 cc.	53.3	Without	100.0%
2	1/28/38	Int. Std. Tr.	0.0040 cc.	50.0	With	104.0%
	"	"	0.0040 cc.	33.3	Without	100.0%
3	1/28/38	Canadian Std. Tr.	0.0058 cc.	66.7	With	93.0%
	"	"	0.0050 cc.	40.0	Without	100.0%
4	2/1/38	U. S. P. XI Std. Tr.	0.0040 cc.	93.3	With	105.0%
	"	"	0.0036 cc.	40.0	"	100.0%
5	2/1/38	U. S. P. XI Std. Tr.	0.0036 cc.	40.0	With	108.0%
	"	Int. Std. Tr.	0.0040 cc.	50.0	"	100.0%
6	1/28/38	U. S. P. XI Std. Tr.	0.0040 cc.	53.3	Without	107.0%
	"	Int. Std. Tr.	0.0040 cc.	26.7	"	100.0%
7	1/31/38	U. S. P. XI Std. Tr.	0.0036 cc.	40.0	With	150.0%
	"	Canadian Std. Tr.	0.0058 cc.	66.7	"	100.0%
8	1/28/38	U. S. P. XI Std. Tr.	0.0040 cc.	53.3	Without	130.0%
	"	Canadian Std. Tr.	0.0050 cc.	40.0	"	100.0%
9	3/23/38	U. S. P. XI Std. Tr.	0.0036 cc.	53.3	With	103.4%
	"	"	0.0036 cc.	40.0	Without	100.0%

TABLE III.—(Continued from page 847.)

10	3/23/38	U. S. P. XI Std. Tr.	0.0036 cc.	40.0	Without	102.0%
	"	"	0.0040 cc.	73.3	"	100.0%
11	3/22/38	Tr. Dig. No. 4	0.0032 cc.	73.3	With	119.0%
	"	U. S. P. XI Std. Tr.	0.0036 cc.	53.3	"	100.0%
12	3/22/38	Tr. Dig. No. 4	0.0032 cc.	73.3	With	125.0%
	"	U. S. P. XI Std. Tr.	0.0040 cc.	73.3	Without	100.0%
13	3/22/38	Tr. Dig. 3159081	0.0048 cc.	66.7	With	78.0%
	"	U. S. P. XI Std. Tr.	0.0036 cc.	53.3	"	100.0%
14	3/23/38	Tr. Dig. 3159081	0.0055 cc.	26.7	Without	63.0%
	"	U. S. P. XI Std. Tr.	0.0036 cc.	40.0	"	100.0%

This table shows that by the M. L. D. frog method whether the sample is tested with alcohol in the dilution and the standard without alcohol or vice versa, the experimental relationship is found to vary from 3% to 15% which is well within the recognized limits of bioassay.

Some results will now be combined from Tables II and III where they concern the same sample to show how difficult it is to decide which one of two or three direct comparisons should be used as the correct U. S. P. XI potency.

TABLE IV.—U. S. P. XI POTENCY.

Test.	Product.	Both with Alcohol.	Both without Alcohol.	With and without.
1	Tr. R 906087	89% of std.	100%	...
2	Tr. 3184092	78% of std.	119%	150%
3	Tr. 3159081	90% of std.	111%	170%
4	Tr. No. 3	88% of std.	109%	170%
5	Tr. No. 4	85% of std.	180%	160%
	Tr. No. 4	119% of std. <i>M. L. D.</i>		125% <i>M. L. D.</i>
6	Tr. 909836	91% of std.	107%	140%
7	U. S. P. XI Std. Tr.	101% of std.	100%	149%
8	Tr. 912201	123% and 129%	...	220% and 209%

This table shows that while different tests of the U. S. P. XI standard Tr. Digitalis both with alcohol in the test dilution or both without alcohol check each other almost perfectly, the relationship of six different *commercial* lots of U. S. P. tincture to the U. S. P. XI standard is distinctly different depending on which of two permissible comparisons is made. In the six cases the activity averaged 25% less for the sample when both sample and standard were compared with alcohol in the test dilutions, than when most of the alcohol was removed from both. Either the glucosides of the standard leaf are relatively more rapidly absorbed in the presence of alcohol than are those of commercial drug or relatively more slowly absorbed in the absence of alcohol or both.

In Table V are tabulated the further experimental comparisons that have been made during the past year of the five digitalis standards, namely, the International for 1936 and 1926, the Canadian, the U. S. P. XI and X by the two best known frog methods (the One-Hour of the U. S. P. XI and the M. L. D. of the B. P. 1932) with and without alcohol in the final test dilutions.

TABLE V.—COMPARISON OF DIGITALIS STANDARDS.

Test.	Date.	Method.	Standard. vs. Standard.	Alcohol.	Result.
1	10/20/37	One-hour	U. S. P. XI Int. 1936	Both with	167% of Int. Std.
2	10/26/37	"	U. S. P. XI U. S. P. XI	Both with	99% of each other
3	11/1/37	"	U. S. P. XI U. S. P. XI	With and W/O	With is 118% of W/O
4	11/4/37	"	U. S. P. XI U. S. P. XI	" " "	With is 126% of W/O
5	11/17/37	"	U. S. P. XI Int. 1936 (fresh)	Both without	163% of Int. Std.
6	11/17/37	"	U. S. P. XI Int. 1936 (3 wks.)	" "	140% of Int. Std.
7	12/9/37	"	U. S. P. XI U. S. P. XI	" "	104% of each other

8	12/20/37	One-hour	U. S. P. XI	U. S. P. XI	Both without	96% of each other
9	2/11/38	"	U. S. P. XI	U. S. P. X	With and W/O	163% of U. S. P. X
10	2/10/38	"	U. S. P. XI	U. S. P. X	Both without	96% of U. S. P. X
11	2/23/38	"	U. S. P. XI	U. S. P. X	" "	138% of U. S. P. X
12	3/5/38	"	U. S. P. XI	U. S. P. X	With and W/O	170% of U. S. P. X
13	3/7/38	"	U. S. P. XI	U. S. P. X	Both without	110% of U. S. P. X
14	3/16/38	"	U. S. P. XI	U. S. P. X	" "	130% of U. S. P. X
15	2/23/38	"	U. S. P. XI	U. S. P. X	With and W/O	210% of U. S. P. X
16	5/26/38	"	U. S. P. XI	U. S. P. X	" " "	130% of U. S. P. X
17	5/26/38	"	U. S. P. XI	U. S. P. X	Both without	104% of U. S. P. X
18	5/18/38	"	Canadian	U. S. P. X	With and W/O	131% of U. S. P. X
19	5/18/38	"	Int. 1936	U. S. P. X	" " "	119% of U. S. P. X
20	5/18/38	"	U. S. P. XI	U. S. P. X	" " "	134% of U. S. P. X
21	5/18/38	"	Int. 1926	U. S. P. X	" " "	131% of U. S. P. X
22	1/27/38	"	Int. 1936	Canadian	Both without	81% of Canadian
23	1/26/38	"	Canadian	Int. 1936	Both with	98% of Int. 1936
24	1/27/38	"	U. S. P. XI	Canadian	" "	97% of Canadian
25	1/27/38	"	U. S. P. XI	Int. 1936	W/O and with	62% of Int. 1936
26	1/27/38	"	U. S. P. XI	Int. 1936	Both with	104% of Int. 1936
27	1/27/38	"	U. S. P. XI	Int. 1936	Both without	104% of Int. 1936
28	1/27/38	"	U. S. P. XI	Int. 1936	With and W/O	159% of Int. 1936
29	1/27/38	"	U. S. P. XI (1-3 dil.)	U. S. P. XI (1-2 dil.)	Both without	101% of each other
30	1/27/38	"	U. S. P. XI (1-4 dil.)	U. S. P. XI (1-3 dil.)	Both with	98% of each other
31	1/28/38	"	U. S. P. XI	U. S. P. XI	With and W/O	With is 157% of W/O
32	1/27/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 153% of W/O
33	1/28/38	"	Int. 1936 (1-3 dil.)	Int. 1936 (1-2 dil.)	Both without	95% of each other
34	1/27/38	"	Int. 1936	Int. 1936	With and W/O	With is 168% of W/O
35	1/27/38	"	Int. 1936 (1-3 dil.)	Int. 1936 (1-4 dil.)	Both with	105% of each other
36	1/28/38	"	Int. 1936	Int. 1936	With and W/O	With is 152% of W/O
37	1/26/38	"	Canadian	Canadian	" " "	With is 133% of W/O
38	2/9/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 170% of W/O
39	2/9/38	"	U. S. P. XI	Int. 1936	W/O and with	58% of Int. with
40	2/11/38	"	U. S. P. XI	Int. 1936	Both with	98% of Int.
41	2/23/38	"	U. S. P. XI	U. S. P. XI	W/O and with	W/O is 66% of with
42	2/25/38	"	U. S. P. XI	U. S. P. XI	Both with	102% of each other
43	3/5/38	"	U. S. P. XI	U. S. P. XI	With and W/O	With is 158% of W/O
44	3/16/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 192% of W/O
45	3/31/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 154% of W/O
46	4/16/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 179% of W/O
47	4/22/38	"	U. S. P. XI	U. S. P. XI	Both with	104% of each other
48	4/27/38	"	U. S. P. XI	U. S. P. XI	With and W/O	With is 144% of W/O
49	5/5/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 112% of W/O
50	5/25/38	"	U. S. P. XI	U. S. P. XI	" " "	With is 125% of W/O
51	5/12/38	"	Canadian	Int. 1936	Both with	110% of Int. Std.
52	5/12/38	"	Canadian	Int. 1926	" "	100% or equal
53	5/12/38	"	U. S. P. XI	Int. 1936	" "	112% of Int. 1936
54	5/12/38	"	U. S. P. XI	Int. 1926	" "	102% of Int. 1926
55	5/12/38	"	U. S. P. XI	Canadian	" "	102% of Canadian
56	5/12/38	"	Int. 1936	Int. 1926	" "	91% of Int. 1926
57	6/15/38	"	U. S. P. XI	U. S. P. XI	With and W/O	With is 150% of W/O
58	9/14/37	M. L. D.	U. S. P. XI	Int. 1936	Both with	126% of Int. 1936
59	9/22/37	"	U. S. P. XI	Canadian	" "	124% of Canadian

TABLE V.—(Continued from page 849.)

60	9/14/37	M. L. D.	Canadian	Int. 1936	Both with	102% of Int. 1936
61	10/22/37	"	U. S. P. XI	Int. 1936	" "	125% of Int. 1936
62	11/22/37	"	U. S. P. XI	Int. 1936	Both without	180% of Int. 1936
63	11/17/37	"	U. S. P. XI	U. S. P. XI	" "	102% of each other
64	12/16/37	"	Canadian	Canadian	Both with	102% of each other
65	1/20/38	"	Canadian	Int. 1936	With and W/O	153% of Int. 1936
66	1/28/38	"	Canadian	U. S. P. XI	W/O and with	70% of U. S. P. XI
67	1/31/38	"	Canadian	U. S. P. XI	Both with	67% of U. S. P. XI
68	1/28/38	"	Canadian	Int. 1936	W/O and with	80% of Int. 1936
69	1/28/38	"	Canadian	Int. 1936	Both without	83% of Int. 1936
70	1/28/38	"	Canadian	U. S. P. XI	" "	77% of U. S. P. XI
71	1/28/38	"	Int. 1936	Int. 1936	W/O and with	W/O is 95% of with
72	2/1/38	"	U. S. P. XI	U. S. P. XI	With and W/O	With is 110% of W/O
73	2/1/38	"	U. S. P. XI	U. S. P. XI	Both with	105% of each other
74	2/1/38	"	U. S. P. XI	Int. 1936	With and W/O	118% of Int. 1936 W/O
75	2/1/38	"	U. S. P. XI	Int. 1936	Both with	112% of Int. 1936 with
76	2/1/38	"	U. S. P. XI	Int. 1936	W/O and with	102% of Int. 1936 "
77	2/1/38	"	Int. 1936	Int. 1936	Both with	98% of each other
78	3/23/38	"	U. S. P. XI	U. S. P. XI	With and W/O	With is 103% of W/O
79	3/23/38	"	U. S. P. XI	U. S. P. XI	Both without	102% of U. S. P. XI W/O
80	3/23/38	"	U. S. P. XI	Canadian	Both with	121% of Canadian
81	3/23/38	"	U. S. P. XI	Canadian	Both without	148% of Canadian
82	3/25/38	"	Canadian	Canadian	With and W/O	With is 125% of W/O
83	5/17/38	"	U. S. P. XI	Int. 1936	Both with	175% of Int. 1936
84	5/17/38	"	U. S. P. XI	Int. 1926	" "	162% of Int. 1926
85	5/17/38	"	Int. 1936	Int. 1926	" "	93% of Int. 1926
86	5/17/38	"	Canadian	U. S. P. XI	" "	83% of U. S. P. XI
87	5/13/38	"	Canadian	Int. 1936	" "	146% of Int. 1936
88	5/13/38	"	Canadian	Int. 1926	" "	135% of Int. 1926

Analysis of this data will show that results obtained by the one-hour frog method are somewhat more erratic than those obtained by the M. L. D. frog method and that the presence or absence of alcohol in the final test dilutions is not such a disturbing factor in the latter method as it is in the former. Wherever check tests were made of the same preparation with the same technique the agreement in results was quite satisfactory.

A small table can now be given comparing averages obtained in this year's work with the several standards with that reported in Table V last year (1).

TABLE VI.

Standard.	One-Hour Frog.			M. L. D. Frog.		
International 1936 Corr.	Taken as 100%			Taken as 100%		
U. S. P. XI Corrected	131%			138%		
Canadian Corrected	103%			116%		
U. S. P. XI vs. X	140%					
U. S. P. XI vs. Canadian				131%		
	With Alcohol.	W/O Alcohol.	W. & W/O Alcohol.	With Alcohol.	W/O Alcohol.	W. & W/O Alcohol.
U. S. P. XI vs. U. S. P. XI	101%	100%	149%	105%	102%	106%
Int. 1936 vs. Int. 1936	105%	95%	160%	98%	...	105%

Last year we showed (1) that the U. S. P. XI standard for Tr. Digitalis was higher than had been intended, since direct and indirect comparisons with the U. S. P. X standard showed the former to be about 50% stronger. This has been

largely confirmed this year by an experimental average of 40% higher for the U. S. P. XI.

Also last year the U. S. P. XI was found to be 35% more active than the International by the official one-hour frog method and this year it is 31% higher.

The most important fact reported this year is the marked influence which the presence or absence of alcohol in the test dilutions of U. S. P. XI standard tincture has upon the experimental M. S. D. of the standard—the difference being relatively greater than is found to be true for extracts of commercial drug. The explanation suggested is that either the glucosides of this particular lot of standard leaf are absorbed relatively faster in the presence of alcohol than are those of average commercial drug or relatively slower in the absence of alcohol in both, or possibly some of each. This factor does not appear to be important in the M. L. D. frog method and seems to point to a much greater dependability for this method over the present official one-hour frog method where the reaction is stopped short of completion and the relative rate of absorption of two different digitalis preparations is consequently so much more important. Also since the International authorities (Health Committee of the League of Nations) (5) have indorsed the M. L. D. Frog Method with no recognition whatever of the One-Hour Frog Method and the value of the former method has been further confirmed by inclusion in the B. P. 1932 (6) and the Canadian Regulations of 1934, it seems that the results reported here are in direct agreement with this international bioassay trend favoring a lethal dose frog method.

This fact, if verified by others, should practically prove that an uncompleted physiological action, which is so dependent upon the relative rates of absorption of different glucosides of digitalis as is the case in the present one-hour frog method of the U. S. P. XI, is definitely less satisfactory and accurate than the use of a completed action as is the case in the M. L. D. frog method.

This variable action of the present digitalis standard in the hands of different, experienced workers is probably due more to slight individual variations in the application of the present short time frog method, which is based on incomplete absorption and action of the digitalis glucosides, than to the standard powder itself though this also appears to be more active than was intended by its sponsors.

SUMMARY.

1. Further experimental tests confirm the previously published finding that the U. S. P. XI standard for Tr. Digitalis is definitely stronger than the International Standard by both the One-Hour and the M. L. D. frog methods and consequently much more active than the U. S. P. X standard, although some increase was intended in order to bring the U. S. P. standard into conformity with the International.

2. A variable action of the U. S. P. XI standard digitalis tincture by the official one-hour frog method has been found experimentally to depend upon the presence or absence of alcohol in dilutions used for test and consequently to indicate the importance of relative rates of absorption in such a short time or incomplete reaction comparison. This variable action is not apparent in similar tests by the M. L. D. frog method.

3. The preference of International authorities for a lethal dose frog method for the bioassay of digitalis preparations and the adoption of such a method by the B. P. 1932 and the 1934 Canadian Regulations are important indications which are substantiated by the experimental data here presented.

4. Both the official method and the correction factor of the U. S. P. XI digitalis standard powder should be changed by interim revision so that the U. S. P. tincture of digitalis shall conform in potency to the International Standard for Tr. Digitalis.

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DRUG EXTRACTION. XIX. THE EFFECT OF PRESSURE AND VACUUM ON EFFICIENCY OF EXTRACTION.*¹

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For more than one hundred and twenty years various types of drug extraction processes have been used in which pressure and vacuum have been employed. In the present paper the methods used in the past are summarized and classified and a report is given covering the experimental work of the present investigation in which a study was made of the effect of vacuum on the efficiency of extraction of belladonna root.

HISTORICAL REVIEW.

Hydrostatic Pressure of Liquid.—In 1816 Count Real devised an extraction apparatus in which a long pipe, frequently 50 or 60 feet in length, extended straight upward from the vessel containing the material to be extracted (1). The hydrostatic pressure of the liquid was presumed to be favorable to extraction. To shorten the column of liquid, Real devised a means of using a column of mercury to exert its hydrostatic pressure on the menstruum above the drug. Various modifications of the Real apparatus were made by Geiger (2), Dobereiner (3), Brandes (4), Wurzer (5) and Beindorf (6).

Air Pressure Applied on the Surface of the Menstruum.—In 1817 Semmelbauer applied pressure to the surface of the menstruum by use of an air compression machine (7). Pressure pumps were also used in percolation by Schubart (8), Payen (9) and Beral (10). In later years compressed air was used in percolation by Signoret (11), McPherson (12), Hinsdale (13), Phillips (14), Hoseason (15) and Lenz (16). Romershausen used steam pressure to force hot water through the drug

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