# DIGITALIS ASSAY STANDARDS.*

BY L. W. ROWE.[1]

In spite of the wide-spread consideration which the bio-assay of digitalis and other members of this group of heart tonics has received in recent years as evidenced by the many published articles proposing slightly modified methods, the standards suggested for the different methods have seldom been compared. When one says that a certain Tincture of Digitalis has been standardized by the Houghton M. L. D. Frog-Heart Method to contain 6.6 Heart Tonic Units and is therefore of 100% activity, what relation has such a tincture to one which is 100% by the Cat Method?

It is the purpose of this short paper to present data giving average experiences over a period of years with comparative tests of representative samples by several of the more frequently used methods so as to give some basis for comparison between products standardized by different methods.

The methods chiefly concerned will be the M. L. D. Frog-Heart (Houghton), the One-Hour (Official, U. S. P.), the Cat (Hatcher), with occasional tests by the Four-Hour Frog (Geneva Conference) and the M. L. D. Guinea-Pig (Reed and Vanderkleed). The details of the technique of these methods will not be given here as they are easily available in other published articles and scarcely need repetition. The test data will be tabulated so as to compare results by each method with those by the Official One-Hour Frog Method. A few tests of Digitalin and Strophanthin are also included.

From the data in Tables I and II and the summarized percentages and ratios in Table III, it is seen that the four main standards for Tincture of Digitalis, namely (1) U. S. P. One-Hour Frog Method standard (1 cc. Tincture = 0.083 mg. U. S. P. Ouabain), (2) the M. L. D. Frog-Heart Method (Houghton) standard (1 cc. Tincture = 0.033 mg. U. S. P. Ouabain), (3) the Cat Method (Hatcher) standard (1 cc. Tincture = 0.1 mg. U. S. P. Ouabain), (4) the Four-Hour Frog Method (International) standard, (1 cc. Tincture = 1 cc. Tincture from standard leaf), are actually not very different as judged by the average results on a number of samples each tested by more than one method.

Roughly it seems that the One-Hour Method (U. S. P.) standard may be about 10% *more* active than the M. L. D. Frog Method standard and about 10% *less* active than the Cat Method standard thus making the latter about 20% higher than the standard of the M. L. D. Frog Method. As for the Four-Hour Frog Method (International) standard, this seems to be about equal to the One-Hour Method standard and consequently 10% more active than the M. L. D. Frog Method standard and 10% less active than the Cat Method standard. The Guinea-Pig Method standard is apparently about right, that is, equal to the One-Hour Method standard but an insufficient number of tests on Tincture of Digitalis, one of which was on a sample where the One-Hour Method result was apparently low, made the ratio seem to be in favor of the One-Hour Standard.

As for the actual standards, I do not favor Ouabain as the standard for Digitalis since its action on the various animals in the more frequently used methods

* Scientific Section, A. PH. A., Rapid City meeting, 1929.

[1] Research Laboratories of Parke, Davis & Co., Detroit, Michigan.

is much more rapid due to better absorption and a naturally faster toxic action on the heart. It is a suitable standard for Strophanthus activity, however. The international standard Digitalis leaf used as a freshly prepared tincture is no doubt the best standard which is available at present for Digitalis assay. The potency of this international standard Digitalis leaf is apparently about equal to the standard set in the present U. S. P. and consequently is a practical and easily attainable standard which will not place too potent a tincture of digitalis in the hands of the average physician. Thus the equivalent of the present international standard Digitalis leaf would seem from many considerations to be a better standard for the next U. S. P. than is our present Ouabain standard.

The Cat Method standard is apparently the most active of all the standards proposed for Tincture of Digitalis and the method itself is open to criticism from the standpoint of accuracy since in eliminating the absorption factor, it frequently fails to show deterioration in tinctures which have been shown both clinically and by other bio-assay methods to have lost potency very definitely.

While a consideration of the choice of a method of digitalis assay is a little beyond the scope of this paper, it seems logical to go on record on the basis of years of experience with the better known methods as being definitely opposed to the present One-Hour Method because of the indefiniteness of its end-point under actual conditions due to too short a period of absorption of a slowly acting drug, and to favor an M. L. D. method in which a long enough period of time is allowed to elapse for the complete absorption and toxic action of the dose injected. The Four-Hour Frog Method proposed by the International Conference is entirely satisfactory for the assay of Strophanthus preparations where the action of the drug is more rapid, but for Digitalis the four-hour period is scarcely long enough for complete absorption and action even at 20° C.; for Digitalis the time limit should be placed at six hours at least for definite and easily comparable results.

## CONCLUSIONS.

1. Comparisons of the various standards proposed in the more important methods for the assay of Tincture of Digitalis, by means of tests of a number of samples, each by more than one method, show that the standard set for the Cat Method is fully 10% higher than that set for the One-Hour Method and the Four-Hour Frog Method (these two seem about equal) while the standard originally set 30 years ago for the M. L. D. Frog Method is not more than 10% lower than the present U. S. P. standard.

2. Very few comparative results were obtained with the guinea-pig method but the data pointed to the standard suggested being about right, that is, equal to that of the One-Hour and Four-Hour Methods for both Tr. Digitalis and Ouabain.

3. The present U. S. P. standard for Tincture of Digitalis, namely, Ouabain, is not satisfactory and it is suggested that the international standard Digitalis leaf or its equivalent be adopted as the standard for Tincture of Digitalis in the next U. S. Pharmacopœia.

4. The official method for the assay of Digitalis preparations (the One-Hour Frog Method) as recommended in the present U. S. P. is not satisfactory and it is suggested that an M. L. D. Method with a time limit of at least six hours be considered for recommendation in the next U. S. Pharmacopœia.

TABLE I.

| Sample. | One-Hour M. S. D. (per Gm.). | In terms of U. S. P. Ouab. | Frog Heart M. L. D. (per Gm.). | In terms of U. S. P. Ouab. | Cat intrav. M. L. D. per Kg. | In terms of U. S. P. Ouab. |
|---|---|---|---|---|---|---|
| Tr. Digitalis A, 1 | 0.010 cc. | 1 cc. = 0.070 mg. | 0.012 cc. | 1 cc. = 0.030 mg. | | |
| Tr. Digitalis A, 2 | 0.011 cc. | 1 cc. = 0.064 mg. | 0.010 cc. | 1 cc. = 0.036 mg. | 1.14 cc. | 1 cc. = 0.088 mg. |
| Tr. Digitalis A, 3 | 0.011 cc. | 1 cc. = 0.064 mg. | 0.011 cc. | 1 cc. = 0.033 mg. | | |
| Tr. Digitalis B, 1 | 0.009 to 0.010 cc. | 1 cc. = 0.074 mg. | 0.012 cc. | 1 cc. = 0.030 mg. | | |
| Tr. Digitalis B, 2 | 0.010 cc. | 1 cc. = 0.070 mg. | 0.012 cc. | 1 cc. = 0.030 mg. | 1.42 cc. | 1 cc. = 0.070 mg. |
| Tr. Digitalis B, 3 | 0.009 cc. | 1 cc. = 0.078 mg. | 0.010 cc. | 1 cc. = 0.036 mg. | | |
| Tr. Digitalis No. 1–22 | 0.008 cc. | 1 cc. = 0.075 mg. | 0.014 cc. | 1 cc. = 0.021 mg. | 1.80 cc. | 1 cc. = 0.055 mg. |
| Tr. Digitalis No. 2–22 | 0.012 cc. | 1 cc. = 0.050 mg. | 0.016 cc. | 1 cc. = 0.019 mg. | 1.95 cc. | 1 cc. = 0.051 mg. |
| Tr. Digitalis No. 3–22 | 0.014 cc. | 1 cc. = 0.043 mg. | 0.022 cc. | 1 cc. = 0.014 mg. | 2.36 cc. | 1 cc. = 0.042 mg. |
| Tr. Digitalis S-22 | 0.006 cc. | 1 cc. = 0.080 mg. | 0.005 cc. | 1 cc. = 0.060 mg. | 0.86 cc. | 1 cc. = 0.116 mg. |
| Digitalin No. 309390 | 0.00025 Gm. | 1 Gm. = 3.3 mg. | 0.00012 Gm. | 1 Gm. = 2.5 mg. | 6.23 mg. | 1 Gm. = 16 mg. |
| Ouabain U. S. P., 1927 | 0.0000008 Gm. | | 0.00000033 Gm. | | 0.097 mg. | |
| Ouabain U. S. P., A. D. M. A. | 0.0000006 Gm. | | 0.00000036 Gm. | | 0.105 mg. | |
| Ouabain U. S. P., 1928 | 0.0000005 Gm. | | 0.0000003 Gm. | | | |
| Digitalin A. D. M. A. | 0.00010 Gm. | 1 Gm. = 6 mg. | 0.00011 Gm. | 1 Gm. = 2.45 mg. | 6.15 mg. | 1 Gm. = 16 mg. |
| Digitalin D. C. C. | 0.00020 Gm. | 1 Gm. = 3 mg. | 0.00014 Gm. | 1 Gm. = 2.2 mg. | 10 mg. | 1 Gm. = 10 mg. |
| Tr. Digitalis No. 2791741 | | | 0.022 cc. | 1 cc. = 0.016 mg. | 1.46 cc. | 1 cc. = 0.068 mg. |
| Tr. Digitalis Intern't'l St'd | 0.008 cc. | 1 cc. = 0.083 mg. | 0.006 cc. | 1 cc. = 0.045 mg. | 1.05 cc. | 1 cc. = 0.095 mg. |
| Tr. Digitalis "S" | 0.005 cc. | 1 cc. = 0.10 mg. | 0.006 cc. | 1 cc. = 0.050 mg. | 0.76 cc. | 1 cc. = 0.132 mg. |
| Tr. Digitalis A | 0.012 cc. | 1 cc. = 0.05 mg. | 0.010 cc. | 1 cc. = 0.030 mg. | | |
| Tr. Digitalis B | 0.009 cc. | 1 cc. = 0.067 mg. | 0.0085 cc. | 1 cc. = 0.038 mg. | | |
| Tr. Digitalis C | 0.010 cc. | 1 cc. = 0.060 mg. | 0.014 cc. | 1 cc. = 0.023 mg. | | |
| Strophanthin Kombé | 0.0000005 Gm. | 1 Gm. = 1 Gm. | 0.00000055 Gm. | 1 Gm. = 0.55 Gm. | | |
| Digitalin No. 295460 | 0.00006 Gm. | 1 Gm. = 10 mg. | 0.00006 Gm. | 1 Gm. = 6 mg. | | |
| Digitalin No. 49929 | 0.00015 Gm. | 1 Gm. = 4 mg. | 0.00012 Gm. | 1 Gm. = 3 mg. | | |

TABLE II.

| Sample. | One-Hour Frog M. S. D. | In terms of U. S. P. Ouab. | Four-Hour Frog M. L. D. | In terms of U. S. P. Ouab. | Guinea-pig M. L. D. | In terms of U. S. P. Ouab. |
|---|---|---|---|---|---|---|
| Ouabain U. S. P. | 0.0000006 Gm. | | 0.0000003 Gm. | | 0.0000002 Gm. | |
| Tr. Digitalis Intern't'l St'd | 0.008 cc. | 1 cc. = 0.075 mg. | 0.005 cc. | 1 cc. = 0.05 mg. | 0.003 cc. | 1 cc. = 0.067 mg. |
| Tr. Digitalis S-22 | 0.006 cc. | 1 cc. = 0.08 mg. | | | 0.0015 cc. | 1 cc. = 0.13 mg. |
| Tr. Digitalis No. 2465655 | | | 0.015 cc. | 1 cc. = 0.027 mg. | 0.006 cc. | 1 cc. = 0.033 mg. |
| Strophanthin Kombé | 0.0000005 Gm. | 1 Gm. = 1 Gm. | 0.00000055 Gm. | 1 Gm. = 0.55 Gm. | | |
| Digitalin A. D. M. A. | 0.00010 Gm. | 1 Gm. = 6 mg. | 0.00015 Gm. | 1 Gm. = 2.0 mg. | 0.000035 Gm. | 1 Gm. = 5.7 mg. |
| Digitalin No. 295460 | 0.00006 Gm. | 1 Gm. = 10 mg. | | | 0.000015 Gm. | 1 Gm. = 13 mg. |
| Digitalin No. 49929 | 0.00015 Gm. | 1 Gm. = 4 mg. | | | 0.000025 Gm. | 1 Gm. = 8 mg. |
| Digitalin DCC | 0.00020 Gm. | 1 Gm. = 3 mg. | | | 0.000040 Gm. | 1 Gm. = 5 mg. |

TABLE III.

| Sample. | % of std.* One-Hour Method, per cent. | % of std.* M. L. D. Frog Method, per cent. | % of std.* Cat Method, per cent. | % of std.* Guinea-Pig M. L. D., per cent. | % of std. Four-Hour Frog, per cent. | Ratios. |
|---|---|---|---|---|---|---|
| Tr. Digitalis A, 1 | 84 | 90 | | | | 1 to 1.07 |
| Tr. Digitalis A, 2 | 77 | 108 | 88 | | | 1 to 1.40 to 1.14 |
| Tr. Digitalis A, 3 | 77 | 100 | | | | 1 to 1.30 |
| Tr. Digitalis B, 1 | 89 | 90 | | | | 1 to 1.01 |
| Tr. Digitalis B, 2 | 84 | 90 | 70 | | | 1 to 1.07 to 0.83 |
| Tr. Digitalis B, 3 | 94 | 108 | | | | 1 to 1.14 |
| Tr. Digitalis 1–22 | 90 | 63 | 55 | | | 1 to 0.70 to 0.61 |
| Tr. Digitalis 2–22 | 60 | 56 | 51 | | | 1 to 0.93 to 0.85 |
| Tr. Digitalis 3–22 | 52 | 42 | 42 | | | 1 to 0.81 to 0.80 |
| Tr. Digitalis S–22 | 96 | 180 | 116 | 165 | | 1 to 1.87 to 1.2 to 1.72 |
| Tr. Digitalis No. 2791741 | | 48 | 68 | | | 0 to 1 to 1.40 |
| Tr. Digitalis Inter. Std. | 100 | 135 | 95 | 83 | 100 | 1 to 1.35 to 0.95 to 0.83 to 1 |
| Tr. Digitalis Spec. | 120 | 150 | 132 | | | 1 to 1.25 to 1.1 |
| Tr. Digitalis A | 60 | 90 | | | | 1 to 1.50 |
| Tr. Digitalis B | 80 | 112 | | | | 1 to 1.40 |
| Tr. Digitalis C | 72 | 68 | | | | 1 to 0.94 |
| Tr. Digitalis No. 2465655 | | 72 | | 42 | 54 | 0 to 1 to 0 to 0.58 to 0.75 |
| Ouabain U. S. P. | 100 | 90 | 97 | 100 | 100 | 1 to 0.9 to 0.97 to 1 to 1 |
| Strophanthin Kombé | 200 | 100 | | | 110 | 1 to 0.50 to 0 to 0 to 0.55 |
| Digitalin A. D. M. A. | No std. | 45 | 58 | 46 | No std. | 0 to 1 to 1.30 to 1.02 |
| Digitalin No. 309390 | No std. | 45 | 58 | | | 0 to 1 to 1.30 |
| Digitalin D. C. C. | No std. | 36 | 36 | 40 | | 0 to 1 to 1 to 1.1 |
| Digitalin No. 295460 | No std. | 112 | | 100 | | 0 to 1 to 0 to 0.9 |
| Digitalin No. 49929 | No std. | 56 | | 64 | | 0 to 1 to 0 to 1.14 |

Footnotes referred to by asterisks—One-Hour standard, 1 cc. Tincture of Digitalis = 0.083 mg. U. S. P. Ouabain. M. L. D. Frog standard, 1 cc. Tincture of Digitalis = 0.033 mg. U. S. P. Ouabain.

Cat Method standard, 1 cc. Tincture of Digitalis = 0.1 mg. U. S. P. Ouabain. Guinea-Pig Method standard, 1 cc. Digitalis = 0.08 mg. U. S. P. Ouabain.

Average ratio One-Hour Method standard to M. L. D. Frog Method standard is 1 to 1.12.

Average ratio One-Hour Method standard to Cat Method (Hatcher) standard is 1 to 0.94.

Average ratio One-Hour Method standard to Guinea-Pig Method standard is 1 to 1.18 (3 comp.).

Average ratio One-Hour Method standard to Four-Hour Method standard is 1 to 1.0 (2 comp.).

Average ratio M. L. D. Frog Method standard to Cat Method standard is 1 to 0.83 (8 comp.), Cat standard is 20% higher.

Average ratio M. L. D. Frog Method standard to Guinea-Pig standard is 1 to 0.92 (8 comp.), Guinea-Pig standard is 10% higher.

Average ratio M. L. D. Frog Method standard to Four-Hour Frog standard is 1 to 0.70 (2 comp.).

## ABSTRACT OF DISCUSSION.

**E. E. Swanson** stated that an interesting study of methods and standards had been presented, and indicates that the methods should be studied with the view of harmonizing or selecting a method for adoption. He thought that the Hanzlik Pigeon Emesis Method was deserving of consideration because of the amount of work that had been done. He said that digitalin is very slowly absorbed by the frog, whereas the pigeons are quickly responsive. The point he wanted to make, however, was that a decision should be reached on method to be adopted.

**James C. Munch** said that the Committee on Physiological Assays A. PH. A. is now engaged in a somewhat similar study. Approximately fifty gallons of tincture of digitalis had been prepared and submitted to bio-assayists of the ASSOCIATION for comparative assays and to clinicians for study. He had been collecting various methods for the assay of digitalis, and now had about fifty-seven methods; so far none of the studies by clinicians have been completed, but a study is under way. He stated that Rowe's study represented about a year's hard work. He conducted work of this kind several years ago with the view of determining the accuracy of the 1-Hour Frog Method. In writing to the assayists he had requested that they use the 1-Hour Method

and report on other methods which they had employed; also information was requested relative to the experience of the assayists.

In discussing the subject with Dr. Hanzlik in Boston, he had been informed that Dr. Hanzlik would publish the results he had obtained in several kindred clinical cases compared with the "pigeon emesis" method.

**W. H. Zeigler** stated that he had studied one of the assays and he was looking forward with interest to the clinical reports.

**E. Fullerton Cook** referred to the efforts being made to establish international standards. He stated that there will be an International Secretariat on Pharmacopœias under the auspices of the League of Nations where every nation may obtain definite standards.

**James C. Munch** stated that he had quite a lengthy discussion with bio-assayists of Canada while in Boston. They have been using "International Standard" leaf and obtained quite uniform results. They pointed out to him that it was necessary to specify in greater detail the method of preparing the test solution because slight variations in technic will give results varying from 25 to 100 per cent.

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## $p_H$ STUDIES OF NEOARSPHENAMINE.*

BY RALPH B. SMITH, A. E. JURIST AND W. G. CHRISTIANSEN.

Work done in this laboratory by Jurist and Christiansen[1] indicated that it would be desirable to determine the $p_H$ of neoarsphenamine solutions, since the very rough results obtainable with indicators had given considerable evidence that the stability of the product was related to the $p_H$ of its solution. Furthermore the $p_H$ could be used to indicate the presence of free acid because both sodium formaldehyde sulphoxylate and 3,3′-diamino-4,4′ dihydroxyarsenobenzene-*N*-methylene-sodium-sulphinate have a $p_H$ of more than 8.0. Williams and Swett[2] state that they have determined the $p_H$ of solutions of a number of commercial neoarsphenamines but give no details as to the method employed. Elvove and Clark[3] and Hunter and Patrick[4] have investigated the $p_H$ of arsphenamine solutions with the hydrogen electrode and found that true and reproducible values could be obtained and it was found in our Laboratory that the method of Elvove and Clark could be adapted to neoarsphenamine.

In this investigation a bubbling type of electrode was used with a saturated half cell of the type described by Clark and Cohn.[5] The electrodes were prepared according to the method of Elvove and Clark[3] by first plating them with a thin layer of bright gold and then with a thin layer of palladium black. Electrodes of both the coil and point type were used but as far as could be noted there was no difference between the behavior of the two types; in all cases a fresh pair of electrodes was used for each determination.

The general method was to put 13.5 cc. of double distilled water which had been cooled under nitrogen into the electrode vessel, place two electrodes in position and pass hydrogen through the bubbling tubes for about fifteen minutes,

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* Scientific Section, A. PH. A., Portland meeting, 1928.

[1] *J. A. C. S.*, 50 (1928), 191.

[2] "Proc. Soc. Expt. Biol. Med.," 19 (1922), 266.

[3] Hygienic Laboratory—Bulletin No. 135.

[4] *J. Lab. & Clin. Med.*, 10 (1925), 343.

[5] "Public Health Reports," 38 (1923), 933.