

## THE U. S. P. STROPHANTHUS STANDARD.\*

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About the time the U. S. P. X became official there was a period of several years during which for some unknown reason it was difficult, in fact almost impossible, to obtain Kombé Strophanthus Seed containing the usual high degree of activity. This fact worked a hardship upon the manufacturers and was probably partly responsible for a severe criticism of the U. S. P. standard by Burn and Trevan (1). Much of the weight of this criticism was lost, when it was pointed out by Edmunds, Lovell and Braden (2) that Burn and Trevan did not use the official One-Hour Frog Method or even the kind of frogs specified in the official method. There can be no direct relationship assumed between Ouabain (the standard) and Tr. Strophanthus when tested by different methods, even though the methods are related.

The official Strophanthus standard, in which, with the One-Hour technique, 1 cc. of Tr. Strophanthus should be equivalent to 8.3 mg. of U. S. P. Ouabain, is without doubt a high standard but a large part of the trouble is with the method itself. One hour is an insufficient period of time for the complete absorption and action of either Strophanthus or Digitalis and the systolic standstill is only achieved by an excess of drug. Even then the results are very erratic due to the variable rate of absorption and action which is inherent in the individual frogs. Where an M. L. D. method is employed with a period of six hours at 20° C. between dosing and reading results, the end-point in the reaction is much more definite and the time factor provides for complete absorption and *action* of the drug in the great majority of the test animals. Longer periods of time than six hours were only necessary when temperature was not controlled and absorption was very slow at the low water temperatures which were common in the winter. The adoption by the International Conference in 1928 of a Four-Hour M. L. D. Frog Method as one of several methods for Strophanthus and Digitalis assay was a real advance, but for complete action of *Digitalis* even at 20° C. a six-hour period is much better than four and scarcely gives time enough for a frog to die when not dosed, which was one of the objections and criticisms raised against the Twelve to Twenty-Four Hour M. L. D. time period.

It has been stated that according to the U. S. P. One-Hour Frog Method 1 cc. of standard Tr. Strophanthus should be equivalent to 8.3 mg. of standard Ouabain. I have conducted a great many tests of U. S. P. Ouabain and of Tinctures of Strophanthus by the official method and always have difficulty in interpreting results as well as getting the M. S. D. of Ouabain anywhere near the figure (0.0000005 Gm.) set as a standard in the Pharmacopœia. In four years of testing, only once or twice have I observed the M. S. D. of Ouabain at the standard figure and more often it is near 7 or 8. Very recently in testing a Tr. Strophanthus, which was 100% by the M. L. D. Frog Method, I called the M. S. D. of standard Ouabain 0.0000070 Gm. per Gm. on results like these: 2 fives beating and 1 questionable, 2 sixes beating, 2 questionable and 1 stopped; 3 sevens stopped and one questionable; 1 eight stopped, 1 beating and 1 questionable, and

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the M. S. D. of the Tincture was 0.00008 cc. to 0.00009 cc. on these results; 1 five stopped; 2 sixes beating and 1 questionable; 3 sevens beating and 1 stopped; 2 eights stopped, 2 questionable and 1 beating; 2 nines stopped. This test gave an equivalence such that 1 cc. of the 100% Tincture corresponded to about 8 mg. of Ouabain but it was a very unsatisfactory test and is merely one illustration of the difficulty one meets in applying the official test even to *Strophanthus* preparations in spite of considerable experience with it.

By the M. L. D. Frog Heart Method, 1 cc. of standard Tr. *Strophanthus* should be equivalent to only 6.67 mg. of standard Ouabain. This figure is derived from the fact that U. S. P. Ouabain in frequent tests shows an activity of 200,000 Heart Tonic Units (3) per Gm., while standard Tr. *Strophanthus* U. S. P. contains 1333 H. T. U. per cc. One gram of Ouabain is therefore equivalent to 150 cc. of Tr. *Strophanthus* and 1 cc. of the latter is equivalent to 6.67 mg. of Ouabain. Thus due to the differences of the two methods there is a relative difference of 20% less or 25% more between the two Ouabain equivalents.

By the Cat Method less data is available but the M. L. D. of U. S. P. X Ouabain in a good test on 5 cats was found to be 0.097 mg. per Kg. with an extreme variation between the highest and lowest result of only 28%. In a second test more than a year later an average M. L. D. of 0.105 mg. per Kg. was found. This agrees very well with Dr. Edmunds' result of 0.103 mg. per Kg.; (2) and both these results are quite different from that reported by Dr. Burn of 0.061 mg. per Kg. (1). Such great variations in the test of the same substance by experienced men can only reflect great discredit upon the Cat Method as a quantitative assay method of digitalis and *strophanthus*. For a U. S. P. standard Tr. *Strophanthus* one careful test on four animals gave an M. L. D. of 0.027 cc. per Kg. Using 0.027 cc. per Kg. as the M. L. D. of Tr. *Strophanthus* and 0.097 mg. per Kg. as the M. L. D. of standard Ouabain, then 1 cc. of Tr. *Strophanthus* is equivalent to 3.6 mg. of Ouabain.

By the three methods we thus have the following equivalents for two standard products based on experimental averages and adopted standards.

One-Hour Frog Method (U. S. P.)	1 cc. Tr. <i>Strophanthus</i> equivalent to 8.3 mg. Ouabain
M. L. D. Frog Heart Method (Houghton)	1 cc. Tr. <i>Strophanthus</i> equivalent to 6.7 mg. Ouabain
Cat Method (Modified Hatcher)	1 cc. Tr. <i>Strophanthus</i> equivalent to 3.6 mg. Ouabain

The figure for the Cat Method may be low due to insufficient data but Hatcher found that 1 cc. Tr. *Strophanthus* should be equivalent to 3.0 mg. Ouabain and Edmunds found about 5.0 mg. Dr. Burn found an average of 3.46 mg. for six tinctures and on that basis criticized the U. S. P. standard as being abnormally high. Actually a result of 4.0 mg. by the Cat Method probably corresponds very closely to the 8.3 mg. by the U. S. P. One-Hour Method and Dr. Burn's results indicated a good quality of Tr. *Strophanthus* on the British market rather than a 50% quality. As for Dr. Trevan's low result on two tinctures of Dr. Burn's series employing an M. L. D. Frog Method, the use of a different species of frogs must have been partly responsible.

Having shown that the Ouabain equivalents of a U. S. P. Tr. *Strophanthus* will vary widely by the three types of methods as represented by the One-Hour Frog Method, the M. L. D. Frog Method and the Cat Method, and having given

the equivalents for the several methods, data will now be submitted to show that the present U. S. P. standard is high and that a reduction is advisable in the next Pharmacopœia. Also a change in the official method of testing is recommended.

In 1928 we tested seven different samples of Kombé Strophanthus Seed as U. S. P. tinctures, which represented drug available for purchase. Of these, five were equal to or better than the U. S. P. requirement which had been impossible to meet for several years previous to 1928, except by the use of an excess of sub-standard drug.

Sample.	Ouabain equivalent, M. L. D. Frog Method.	U. S. P. standard.
No. 800921	1 cc. Tr. = 2.0 mg.	30%
No. 317912	1 cc. Tr. = 3.0 mg.	45%
No. 915	1 cc. Tr. = 6.7 mg.	100%
"B"	1 cc. Tr. = 6.7 mg.	100%
No. 320580	1 cc. Tr. = 7.5 mg.	110%
No. 808882	1 cc. Tr. = 6.7 mg.	100%
No. 1506	1 cc. Tr. = 6.7 mg.	100%

In 1929 we also tested seven lots of crude drug and results were uniformly lower although not so low as to be alarming except in one case, which, of course, it was unnecessary to use.

Sample.	Ouabain equivalent, M. L. D. Frog Method.	U. S. P. standard.
S. & N.	1 cc. = 5.5 mg.	85%
O. S. C	1 cc. = 5.5 mg.	85%
B.	1 cc. = 4.5 mg.	70%
B. & S.	1 cc. = 5.0 mg.	75%
W. & H.	1 cc. = 5.5 mg.	85%
M. & D.	1 cc. = 0.6 mg.	10%
326624	1 cc. = 4.5 mg.	70%

The results in 1928 show an average activity of 84%, and for 1929, excluding the very low 10%, an average of 78% of standard.

It would seem possible, therefore, to meet a standard 25% lower than the present one with drug available during the past two years. Furthermore, a 25% reduction would then make 1 cc. of standard Tr. Strophanthus equivalent to 5.0 mg. of U. S. P. Ouabain by the M. L. D. Frog Method, to about 3.0 mg. by the Cat Method and to 6.25 mg. by the One-Hour Frog Method.

#### SUMMARY AND CONCLUSIONS.

1. It is shown by summarized experimental data covering many years that the Ouabain equivalents of a standard U. S. P. Tr. Strophanthus should be quite different by different bio-assay methods. One cc. of Tincture should probably be equivalent to 8.3 mg. Ouabain by the official One-Hour Method; to 6.7 mg. Ouabain by the M. L. D. Frog Heart Method; and to only 4.0 mg. Ouabain by the Cat Method.

2. The present U. S. P. standard is high and it is suggested that it be reduced 25% in the next revision so that 1 cc. of Tr. is equivalent to 5.0 mg. Ouabain by the M. L. D. Frog Method; to 3.0 mg. Ouabain by the Cat Method; and to 6.25 mg. Ouabain by the present One-Hour Frog Method.

3. Ouabain appears to be a suitable standard for *Strophanthus* assay because it has the same rate of absorption and type of action. It is, however, definitely unsuitable as a standard for *Digitalis* (4) and *Squill* assay.

4. The present U. S. P. method (One-Hour Frog) is unsatisfactory even for *Strophanthus*. One hour is possibly sufficient time for absorption of a small dose of a *Strophanthus* dilution, but is not sufficient for complete *action* and the necessary excess causes erratic results due to individual variations in reaction. An M. L. D. Frog Method overcomes these objections and at a temperature of about 20° C., six hours is a suitable time limit for both *Strophanthus* and *Digitalis*. A Six-Hour M. L. D. Frog Method is therefore suggested for consideration for the next U. S. P. in place of the present One-Hour Frog Method.

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### PHARMACOLOGICAL NOTE ON PARAFFIN, LANOLIN, BEESWAX, PETROLATUM AND CETACEUM.\*

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#### INTRODUCTORY.

It is well known to all those having acquaintance with pharmacology that slight changes in the chemical composition of various substances, and more particularly of powerful drugs such as many of the alkaloids, hormones, etc., can be more easily detected by means of pharmacodynamic examination than by any ordinary physicochemical method; indeed, in some cases no physicochemical method is at present available for distinguishing between various substances closely related, and yet such substances may exhibit marked differences in their biological effects. Thus, for instance, Abel and his co-workers have been able to detect extremely minute quantities of an active pituitary principle in dilutions which could not be measured by ordinary chemical means (1). Again, Hatcher and his collaborators have found that they could detect traces of strychnine very readily by biological methods, whereas a quantitative chemical determination with the minute amounts present was out of the question (2). In the same way, Macht and Anderson were able to distinguish changes produced in certain drugs by polarized light by biological methods very much more readily than by physicochemical means (3).

With the development of phytopharmacological methods in studying drugs and chemicals, introduced by the present writer (4) it has been repeatedly shown that by means of living plant seedlings or tests on living plant protoplasm, minute quantities of powerful pharmacological principles can be detected now which could not hitherto be detected by older zoöpharmacological tests. This was found to be especially true for active principles elaborated by animals. Thus, Macht

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