THE VARIABILITY OF STROPHANTHIN WITH PARTICULAR REFERENCE TO OUABAIN.*

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The importance of Strophanthus in therapeutics has led to much original investigation of its activity both chemical and physiological. Although it has been many years since Fraser [1] first obtained an active principle from strophanthus seed which he called Strophanthin, the preparation of a chemically pure Strophanthin, *i.e.*, one of constant chemical and physiological properties, has not been consistently accomplished. It is true that Fraser's method has been improved and new methods worked out by which a more active strophanthin has been obtained but commercial preparations from the same kind of seed still show a surprising lack of uniformity in physiological activity and in chemical purity as well.[3] A large part of this variability can no doubt be attributed to the difficulty encountered in obtaining seed of one particular kind. It is now known that there are more than 20 different species of Strophanthus Seed on the market [2] and it is a relatively common occurrence to find upon close examination that a given lot of seed purchased as *Kombe* contains some seed of other varieties.

There are only three different strophanthins (*Kombe-strophanthin, hispidus-strophanthin* and *gratus-strophanthin* or *ouabain*) of which any chemistry or pharmacology is known and these differ considerably in the degree of their physiological activity. Consequently, if the strophanthin is not obtained from seeds of the same species, its activity will be greater or less than the average for that kind of seed.

Another reason for the variability of strophanthin lies in the fact that some species of seed such as *Kombe* contain a crystalline and an amorphous acid strophanthin which are closely related but which differ markedly in quantitative physiological activity. If the product is very carefully crystallized and labeled " Crystalline Strophanthin," this cause of non-uniformity should disappear, but it is sometimes difficult to obtain a definite separation of the crystalline from the amorphous variety.

The chemistry and pharmacology of the strophanthins derived from *Strophanthus Kombe* have been carefully studied, but Ouabain, the strophanthin from *Strophanthus Gratus,* which seems to be identical with the Ouabain from some species of *Acocanthera,* has not been so thoroughly investigated. It is known to be a nicely crystalline body which may contain variable amounts of water of crystallization, but, since it does not yield a crystalline strophanthidin as *Kombe strophanthin* does, the study of its chemistry is under a disadvantage and the identity of the glucoside is to that extent uncertain. The fact that different samples of Ouabain may contain variable amounts of water of crystallization is a source of serious disagreement in the activity of various samples.

In the quantitative testing of heart tonics of the digitalis series such as Digitalis, Strophanthus, and Squill, physiological methods have been found to be the most feasible. A number of methods have been proposed but most of them agree on one thing, namely, that the frog is best suited for use as the test animal. The

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new Pharmacopœia (ninth revision) recommends that heart tonics be assayed by the use of one of these frog methods, the "One-Hour Method."[6] This method consists in brief of determining the smallest dose of the heart tonic preparation per unit of weight of the test animal which will just stop the heart of a frog in systole one hour after the dose is administered and is, therefore, the minimum systolic dose per gram weight of frog for one hour. This dose must be compared with that of a standard preparation to be tested at the same time in order to take into consideration the variation in resistance of the frogs from season to season and to a lesser degree from day to day.

The standard recommended for use is Ouabain, the strophanthin obtained from *Strophanthus Gratus*. In the selection of a standard, the chief considerations should be the purity of the preparation, its stability, its universal availability and its qualitative and quantitative activity. It is true that Ouabain is a crystalline compound, a fact which should point to its purity and stability. However, different samples contain variable amounts of water of crystallization so that the activity is not uniform. Even *Kombe Strophanthin*, which has been more thoroughly studied chemically and which seems to be a constant chemical compound, is not found to be of uniform physiological activity when various samples on the market are purchased and tested. The following tests of samples of *Kombe Strophanthin* made during the last few years serve to show the variable activity of the commercial preparation.

TABLE I.

Date	M. F. D. Sample	Activity M. F. D. Std.	Activity Percent of Std.
(1) May 8, 1906	.00000050	.00016	192
(2) November 10, 1906	.00000040	.00016	240
(3) February 22, 1907	.00000045	.00013	173
(4) April 4, 1907	.00000050	.00015	180
(5) May 18, 1907	.00000060	.00016	160
(6) July 7, 1907	.00000050	.00016	192
(7) June 10, 1908	.00000050	.00015	180
(8) October 9, 1909	.00000070	.00011	94
(9) July 18, 1911	.00000040	.00009	135
(10) December 7, 1911	.00000100	.00013	78
(11) December 11, 1911	.00000100	.00013	78
(12) January 16, 1912	.00000086	.00010	67
(13) January 13, 1913	.00000055	.00011	120
(14) October 8, 1913	.00000045	.00011	145
(15) April 10, 1914	.00000065	.00011	102
(16) August 21, 1914	.00000055	.00011	120
(17) September 25, 1914	.00000070	.00011	93
(18) April 28, 1915	.00000055	.00011	120
(19) June 15, 1915	.00000060	.00012	120
(20) October 9, 1915	.00000075	.00012	96
(21) March 4, 1916	.00000055	.00010	110
(22) August 8, 1916	.00000055	.00010	110

From this table it can be seen that the least active sample was 67 percent of standard while the most active was 240 percent or approximately 3.5 times as active. The ten samples tested since January 1, 1913, have shown a much

greater degree of uniformity in activity. In this later period the most active sample was but 1.5 times as strong as the least active sample. It has also been shown experimentally that a crystalline Strophanthin can be prepared from *Kombe* seed (the official drug) which is constant in chemical composition and physiological activity.[3] In order to do this, great care must be exercised in the selection of the seed and in following a definite method of preparation and purification.*

The facts favoring the selection of Ouabain as a standard for the testing of heart tonics of the digitalis series might be advanced as follows:

First, that Ouabain is the only active principle of *Strophanthus Gratus* whereas the *Kombe* seed contains two or more.

Second, that it is a nicely crystalline body.

Third, that it is the most active strophanthin yet obtained.

Fourth, that its absorption is comparatively rapid with very slight if any tendency to cumulative action.

These advantages appear to be more than offset by the disadvantages previously mentioned such as the variable content of water of crystallization and the consequent variation in physiological activity of samples of Ouabain, and the fact that its chemistry and pharmacology have not been so thoroughly investigated as has that of *Kombe Strophanthin*. The fact that *Kombe* seed rather than *Gratus* seed is official should make the crystalline *Kombe Strophanthin* the logical choice as a standard. The greater activity of Ouabain can scarcely be considered of any advantage and the *Kombe Strophanthin* is readily absorbed, even though not quite so rapidly as Ouabain. In fact the greater toxicity of Ouabain may be due in part to the greater rapidity with which it is absorbed.

In order to determine whether the physiological activity of Ouabain varies, three samples were purchased at different times and submitted to physiological assay. These assays were made by two methods and at several different times. The following table shows the results obtained in the various tests of these three samples of Ouabain.

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* *Method of Preparation.*—1.5 Kg. ground, fat-free Strophanthus Seed was percolated with 12 liters 70 percent alcohol and the percolate was distilled off in *vacuo* until about 1 liter fluid remained. To this fluid sufficient lead subacetate solution (Liquor plumbi subacetatis, U. S. P.) was added, to obtain an easily filtering mixture. The filtrate is a clear yellow fluid. The excess of lead was removed by hydrogen sulphide and the clear filtrate was evaporated at 40°–45° with constant stirring. Until the fluid becomes concentrated it is important that it be kept alcoholic by frequent addition of a little alcohol. When the fluid has become a thin extract, the alcohol must be evaporated as much as possible. It will then crystallize readily. The crystals are separated on a hardened filter of large surface by suction. The recrystallization is made in the following manner to avoid conversion into the amorphous body: The crystals are dissolved by placing them in a dish with a small amount of 9 percent alcohol and heating to 40°–45° and stirring occasionally. After having filtered the solution, the alcohol is now evaporated to a thick extract at this temperature and water is added until a thin extract is obtained. With constant slow stirring with a motor, the extract is again evaporated to remove the rest of the alcohol. The extract will then crystallize readily.

Crystalline strophanthin as we have shown can be obtained without any chemical purification from the alcoholic extract. It is thus possible to determine whether a certain chemical method of preparation of strophanthin from Strophanthus Kombe seed, gives a yield of naturally occurring crystalline strophanthin or of a derivative of strophanthin.[3]

TABLE II.

TESTS OF THREE SAMPLES OF OUABAIN BY TWO METHODS.

M.L.D. METHOD.

SAMPLE "A," 1 OZ.

M.L.D.	.00000045	June 21, 1915. (Std. .00013)
M.L.D.	.00000035	April 29, 1916. (Std. .00011)
M.L.D.	.00000030	August 21, 1916. (Std. .00011)
Aver.	215500 H.T.U. per Gm.	

ONE-HOUR METHOD.

SAMPLE "A," 1 OZ.

M.S.D.	.00000080	June 21, 1915.
M.S.D.	.00000080	February 15, 1916.
M.S.D.	.00000080	March 3, 1916.
M.S.D.	.00000110	April 12, 1916.
M.S.D.	.00000080	May 4, 1916.
M.S.D.	.00000090	May 9, 1916.
M.S.D.	.00000090	May 9, 1916.
M.S.D.	.00000080	August 16, 1916.
Aver. M.S.D.	.00000086	

M.L.D. METHOD.

SAMPLE "B," 5 GM.

M.L.D.	.00000045	June 21, 1915. (Std. .00013)
M.L.D.	.00000045	August 25, 1916. (Std. .00012)
Aver.	185200 H.T.U. per Gm.	

ONE-HOUR METHOD.

SAMPLE "B," 5 GM.

M.S.D.	.00000065	June 21, 1915.
M.S.D.	.00000080	February 15, 1916.
M.S.D.	.00000090	August 21, 1916.
M.S.D.	.00000080	August 16, 1916.
Aver. M.S.D.	.000000787	

M.L.D. METHOD.

SAMPLE "C."

M.L.D.	.00000040	April 22, 1916. (Std. .00011)
M.L.D.	.00000035	April 29, 1916. (Std. .00011)
M.L.D.	.00000040	August 25, 1916. (Std. .00012)
Aver.	197600 H.T.U. per Gm.	

ONE-HOUR METHOD.

SAMPLE "C."

M.S.D.	.0000010	April 7, 1916.
M.S.D.	.0000009	March 21, 1916.
M.S.D.	.0000010	May 4, 1916.
M.S.D.	.0000010	May 9, 1916.
M.S.D.	.0000008	August 22, 1916.
Aver. M.S.D.	.00000094	

From this table it can be seen first, that the three samples are more nearly uniform when tested by the "M.L.D. method"[7] than when tested by the "one-hour method," and, second, that the average M.S.D. for any of the samples is much larger than that chosen as the standard for the "one-hour method" by the Pharmacopœial Revision Committee.

In order to bring out these points more clearly, the results should be considered from a number of viewpoints. By the "M.L.D. method" the variation in activity in the three samples is 14 percent, while the average activity of the three is almost exactly double that of the average *Kombe Strophanthin*. However, by the "one-hour method," which is the method officially recommended, the variation in activity in the three samples is somewhat greater but the greatest discrepancy is the variation of each of the samples from average M.S.D. (.00000050 Gm. per Gm. wt. of frog) which is officially proposed for comparison. Since the M.S.D. of these three samples was determined in the different seasons and an average taken and since in no test was the dose found to be as small as that mentioned in the Pharmacopœia as being standard for Ouabain, it is very evident that all of these samples of Ouabain are considerably below standard. In fact, sample "C" which was expressly obtained for use as a standard is but 53 percent as active as it should be. Sample "B" which was the most active according to the "one-hour method" was only 63 percent as active as the standard proposed.

It is difficult to believe that three different samples obtained at different times from reliable sources should have an M.S.D. so much greater than the average proposed by the revision committee. The three samples were assayed so many times in different seasons and with such universally high results that any experimental error must have been eliminated. It seems quite probable therefore that the M.S.D. chosen by the committee is smaller than it should be since it is generally admitted that Ouabain is approximately twice as active as crystalline *Kombe Strophanthin*[4,5] and the M.S.D. of the latter preparation has been found experimentally to be approximately .0000015 Gm. per Gm. wt. of frog. The logical deduction from the foregoing data is that the average M.S.D. of active Ouabain is about .00000075 Gm. per Gm. body weight which is approximately the average found in our work.

From the data submitted it seems reasonable to conclude that the variation in activity of different samples of Ouabain is too great to admit of its use as a satisfactory standard in the testing of heart tonic preparations by the "one-hour method" and that the average minimum systolic dose obtained in a number of assays of three different samples is much larger than that proposed by the committee.

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