Then by replacing the hydrogen electrode with the gold electrode and adding a small amount of quinhydrone to the metal organic solution the p_H can be readily determined with the same Hildebrand arrangement and in this way gives a combined hydrogen and quinhydrone apparatus.

From the foregoing we hope we have made it quite clear that at comparatively little expense an accurate hydrogen-ion apparatus may be built, entirely suitable for the needs of pharmaceutical, medicinal and biological chemists.

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STUDIES IN BIOASSAYS—"THE TINCTURE OF STROPHANTHUS."*[1]

BY CHARLES W. EDMUNDS, M.D.,[2] HAROLD W. LOVELL[3] AND SPENCER BRADEN.[3]

The U. S. Pharmacopœia directs that Tincture of Strophanthus shall be of such a strength that 0.0006 cc. of the tincture shall be equivalent in strength to 0.0005 mg. of ouabain when tested upon frogs by the official method. Burn and Trevan have pointed out that this is equivalent to saying that 1 cc. of the tincture must equal in activity 8.33 mg. of ouabain. These workers assayed six tinctures of strophanthus by the cat method, and found all of them to be considerably weaker than the U. S. P. requirement, averaging an ouabain equivalent of about 3.5 mg. instead of 8.33 mg.

Other writers at an earlier date have reported upon the activity of tinctures of strophanthus as they appear upon the market, but some of these papers reported conditions existing in this country before a biological standard was required, or in other instances conditions existing in localities to-day where such standards are still not demanded. For this reason it will hardly be necessary to discuss their findings at length. The Burn-Trevan paper will be discussed later, as it is an important criticism of the U. S. P. standard, which perhaps should be lowered in the next revision, if it is, as stated, unreasonably high. It was in an effort to shed further light upon the question that we examined a number of tinctures of strophanthus which were obtained in original, unopened bottles direct from the manufacturers. We employed the same methods and standards which we had used in our study of ouabain and strophanthin as outlined in the section of this paper which appeared in an earlier number of THIS JOURNAL.

The bioassay methods employed were as follows: the various frog methods, including the official one-hour method; the intravenous method; the four-hour method and in one or two instances the minimal lethal dose method. We used also the cat-assay method and finally the colorimetric method. In addition to using ouabain as a standard we employed also a special tincture prepared from Strophanthus Kombé seeds which we obtained through the courtesy of Dr. Paul S. Pittenger. These seeds which were obtained direct from a "Kombé" district in Africa and identified as being of that variety, were ground and made into a tincture according

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to U. S. P. directions. It is designated in the table as "Mich." The results of our assays together with the calculated ouabain equivalents for each tincture are given in Table I.

TABLE I.—RESULTS OF ASSAYS OF VARIOUS TINCTURES OF STROPHANTHUS BY DIFFERENT BIOLOGICAL METHODS.

Tincture number.	Official method.	Ouabain equiv. in mg.	Intravenous method.	Ouabain equiv. in mg.	Four-hour method.	Ouabain equiv. in mg.	Cat method.	Ouabain equiv. in mg.	Colorimetric method.
Ouabain control	0.00050	...	0.00035	...	0.00040	...	0.103	...	...
XV[1]	0.105	4.76	0.06	5.8	0.065	6.16	0.025	4.12	4.33
XVI[1]	0.100	5.00	0.05	7.0	0.055	7.28	0.021	4.90	5.65
XVII	0.070	7.14	.....	...	0.050	8.00	0.019	5.42	6.85
XVIII	0.130	3.84	.....	...	0.100	4.00	0.042	2.45	3.80
XIX	0.100	5.00	.....	...	0.065	6.16	0.028	3.68	...
Mich.	0.070	7.14	0.04	8.75	0.050	8.00	0.020	5.00	...

[1] Specimens XV and XVI were made by the same manufacturer.

A study of this table makes at least one point clear; *viz.*, that in this country, too, some of the tinctures of strophanthus on the market are certainly below the U. S. P. standard. Only one of the commercial tinctures (XVII) approaches it and it may be said to meet the official requirements, as it is within 15% of the standard. The "Michigan" tincture prepared from the Kombé seeds was of identical strength with commercial Tincture XVII.

A study of the other results also shows certain relationships which seem to hold good, but the picture is so confusing as to make it almost impossible to draw any general conclusions. To aid such a study the results were all refigured to the same basis with Tincture XVII considered as 100%. The results given in Table II are most interesting.

TABLE II.

	Methods.			
Tincture.	Official.	Four-hour frog.	Cat.	Colorimetric.
XV	66	77	76	63
XVI	69	91	90	86
XVII	100	100	100	100
XVIII	54	50	45	56
XIX	69	77	68	..

It will be seen at once that by all the methods of assay, Tincture XVIII is 50% strength as compared with No. XVII, and that No. XIX is 70–75% as strong as No. XVII. Such a uniformity in assay results is very encouraging. Considering Nos. XV and XVI which were manufactured by the same firm, it is evident by all the methods that they are both below U. S. P. strength and that No. XV is distinctly weaker than No. XVI. However, by the four-hour frog and by the cat method the relationship to XVII is very different from what it is by the official method. The values obtained for each of these tinctures is practically the same by the two unofficial methods named and in each there is a much closer approach to No. XVII than in the official method. The results of the colorimetric method, erratic as usual, show an agreement of XV with the results from XV and XVI by the frog one-hour method and with XVI an agreement with the results obtained with the other two methods.

It is evident from these figures that for some reason the one-hour frog method did not evaluate the true strength of these two preparations (XV and XVI) although it did evaluate XVIII and XIX correctly where the agreement between the results obtained by all the methods are in close agreement. There was probably some variation in the process of manufacture of XV and XVI which made a difference in the product which interfered with its absorption from the frog's lymph sac. On the other hand, with the four-hour period of observation, absorption is complete and we have a close agreement with the results obtained by the cat method in which, of course, there is no problem of absorption. The remaining results obtained on the tinctures agree very closely in so far as the relative values of these five specimens is concerned.

A further study of Table I discloses the fact that the actual ouabain equivalents of the tinctures as calculated from figures obtained by the four-hour frog method are much higher than are those obtained by the cat method—the former figure being about 50% greater than the latter. The values obtained by the official method occupy an intermediate position. The figures given by the intravenous method are practically the same as those obtained by the four-hour method, demonstrating the completeness of absorption in the four-hour period. It is noteworthy that the average ouabain value obtained by the cat method is lower than that given by any other method—as is shown in Table III.

TABLE III.—AVERAGE OUABAIN EQUIVALENTS OBTAINED BY THE VARIOUS METHODS OF ASSAY FOR THE DIFFERENT TINCTURES OF STROPHANTHUS.

	Mg.
Cat method	4.26
One-hour frog method	5.15
Four-hour frog method	6.60

This relationship is shown still more clearly in the graph prepared from the figures of ouabain equivalents given in Table I. Several points in this graph should be noted. In the first place, the practical parallelism which exists between all the lines is especially striking. There is only one exception, *viz.*, in the one-hour frog dose of Tincture XVI. This dose is evidently too low, but with this value[1] corrected a complete parallelism would exist, demonstrating the accuracy of the methods and the correctness of the findings. What is hard to explain is that the cat method gives such low figures as compared with those obtained by the other methods. The values obtained by the four-hour and by the intravenous method agree closely. The actual figures shown in the graph are not so important as is the fact of the difference in ouabain values obtained by the different methods. That these are essentially correct is shown by the parallelism of the curves.

To return to the results obtained on Tinctures XV and XVI by the one-hour method, the values obtained were reported to the makers who reassayed both preparations and reported that they found them both to be of standard strength—*viz.*,

[1] An examination of Table I shows that a change in the one-hour frog dose on Tincture XVI, from 0.100 cc. to 0.090 cc., would give an ouabain value of 5.5 mg., thus making all the lines parallel. Examination of the experimental data shows that such a dose given in the assay killed 14 frogs out of 33, therefore less than 50% of those injected, while a dose 0.100 killed 23 out of 30, so that by only a very slight margin was it necessary to take the latter figure as the correct M. S. D. instead of 0.090 cc.

with an M. S. D. of 0.063 cc. with ouabain at 0.0005 mg. The discrepancy between the manufacturer's results and ours is probably to be explained by the fact that for assay purposes they diluted their tinctures with 50% alcohol and this with distilled water, so that the doses injected contained about 25% alcohol. In our assays we use no alcohol, diluting the tinctures with saline solution. This difference in technic will probably explain the discrepancy in the results, and it further emphasizes the great need for standardization of the assay methods themselves.

Again, our results showing that No. XVIII was about 50% under strength were also reported to the manufacturers of this preparation. It, in turn, was reassayed by two of their workers, one of whom reported it to be 40% U. S. P. and the other 53%. In this instance the manufacturer's figures agreed exactly with ours. They indicate a deterioration of 50% between February 1927, when the preparation was originally assayed by them, and May 1928, when these tests were made. The apparent great loss of strength in this tincture is important, as the tincture of strophanthus is generally believed to be a fairly permanent preparation. The tincture made locally was practically of the same strength as No. XVII —in other words, it was within 15% of standard strength.

Finally, the question naturally comes up as to whether the U. S. P. standard for the tincture of strophanthus is too high as claimed by Burn and Trevan. In this connection it is to be noted in their paper that, by the cat method of assay, they found a value for U. S. P. ouabain of only 0.06115 mg. per Kg., whereas (as judged by previous work) it should have been approximately 0.1 mg. per Kg. This latter value was originally found by Hatcher and Brody and it has been repeatedly confirmed since their figures appeared. In the present research our value for U. S. P. ouabain was 0.103 mg. per Kg. and the average of the six ouabains studied was 0.108 mg. The reason for the discrepancy between the Burn-Trevan figures and the Hatcher-Brody value is probably to be found in the fact that Burn took a different "end-point" in his experiments from that recommended by van Wijngaarden and by the Geneva Conference. Burn took the fall of blood pressure to zero as his end-point while the Hatcher-Magnus method directs stoppage of the heart as recognized by inspection and palpation of the thorax. This difference in technic may make quite a little difference in final readings as has been pointed out in the earlier section of this paper and as recognized by Trevan in his report and by Burn in his book on "Bioassays."

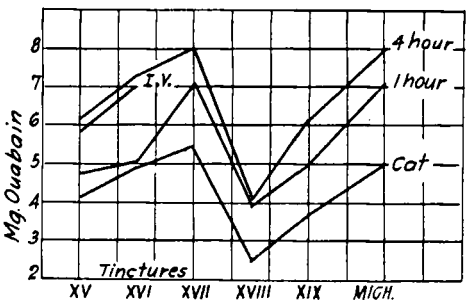
Much more important, however, in this connection is the fact that in their study of this problem and in the gaining of the data which formed the basis of their criticism, they did not employ the U. S. P. official method at all, but a lethal dose (eighteen-hour) method. In fact, in all of their experiments which were carried out in this particular research, so far as can be told by their papers, they did not use the U. S. P. method once, and yet from data so gained they draw conclusions as to the correctness of the U. S. P. standard. The U. S. P. standard holds good only so long as the methods of carrying out the test as outlined in the U. S. Pharmacopœia are followed. The M. L. D. obtained by an eighteen-hour period of observation is by no means the same as the M. S. D. found by a one-hour method.

In the next place the U. S. P. prescribes that a certain variety of frogs shall be used, *viz.*, *Rana pipiens*. Burn and Trevan used *Rana Temporaria*. What the relative degree of toxicity is upon the two species of frogs we do not know and until

we do and it can be shown that it is the same for both, the U. S. P. standard cannot be judged by results obtained on *Rana Temporaria*.

The U. S. P. standard strength of tincture of strophanthus, *viz.*, that 1 cc. equal 8.33 mg. ouabain, only holds for the one-hour frog (*Rana pipiens*) method. What the equivalent in terms of ouabain would be by a lethal dose method or by the cat method the Pharmacopœia does not state. That these figures differ widely is shown by the results given in Tables I and III and also in the curves here reproduced (Graph I). These curves representing the ouabain equivalents of six tinctures of strophanthus illustrate in a striking manner the difference in ouabain values of these tinctures when measured by different methods of assay. The explanation for these interesting differences is by no means clear, but the curves illustrate the impossibility of taking a standard obtained by one method and applying it to results obtained under other conditions and upon an entirely different variety of frogs.

The same error is made by Trevan, Boock, Burn and Gaddum in discussing the relative value of the international standard powder of digitalis to ouabain. They state that the tincture made from this powder is, for practical purposes, of U. S. P. standard strength inasmuch as 1 cc. of the international tincture is equivalent to 0.0804 mg. ouabain as compared to the U. S. P. standard of 0.083 mg. But here again figures obtained by two entirely different methods of assay are brought together and an effort made to compare them. The Trevan value of 0.0804 mg. was obtained by intravenous injection into cats; the U. S. P. equivalent of 0.083 mg. is to be obtained by the injection of the drug into the lymph sac of frogs—(*Rana pipiens*)—systolic standstill to occur in exactly one hour. The close values obtained prove nothing. In the paper above quoted it is also stated in this connection that the "evidence clearly demonstrates the unsuitability of ouabain as a standard for digitalis." In the light of what has been said above it will be seen that on the basis of the evidence presented this statement has no justification.



This graph shows ouabain values for six tinctures of strophanthus as obtained by four bioassay methods. Note the general parallelism of the lines and the different values given by the various methods.

It may be possible to work out a definite ratio or relationship so that when a standard dose is given for one method of assay the corresponding dose may be calculated for the other methods, but at present sufficient figures are hardly available, although the curves given above, if confirmed by others, are very suggestive.

Finally, it is a question deserving serious consideration as to whether the U. S. P. biological standard for tincture of strophanthus is not too high. The standard was set in the Ninth Revision which appeared in 1916 and there does not seem to have been any complaint made. When the Tenth Revision was under way and manufacturers were freely consulted, this point was never raised by them. In fact, none of the standards for the digitalis series were modified, which would

certainly have been done had experience with U. S. P. IX shown that it was desirable.

At first glance our figures would seem to justify and even to demand such a revision. However, a closer study does not make it seem quite so certain. For instance, Tincture XVII is practically of standard strength as was also the tincture made locally. Tincture XVIII is 50% strength, but the assayist (one of the most experienced in this country) reports that in February 1927, it was of standard strength.

One manufacturer when asked his opinion as to the standard said that while they have no trouble in meeting it, they feel that it is about the upper limit. They find many samples of drugs below standard, few above it. Another manufacturer reports that very few samples of Kombé seeds come up to the U. S. P. standard; that for two years it has been practically impossible to obtain strophanthus of more than 50% strength; and, in fact, in the past ten years they have received only one lot of seed of U. S. P. activity. A shipment of strophanthus seed recently received by them and carefully identified as being "Kombé" gave an activity of only 45% U. S. P. strength. The experience we had with the Kombé seeds is interesting in this connection.

In the light of these reports, it would seem that in the next pharmacopœial revision the standard for this tincture might well be taken under consideration and possibly a lower standard of strength be set. After all, strength of such a preparation is not so important as uniformity.

A PRELIMINARY REPORT ON THE CHEMISTRY OF PHYTOLACCA.*

BY GLENN L. JENKINS.†

Poke root was first officially recognized by the United States Pharmacopœia in the edition of 1820. It was retained in all subsequent revisions until the ninth when it was dropped and became official in the fourth edition of the National Formulary and was retained in the fifth edition, where it is defined as "the dried root of *Phytolacca Americana* Linné (*Fam. Phytolaccaceæ*)." It does not appear to have been included in any other pharmacopœia.

Tarwell[1] proposed that the proper designation of the source of poke root is *Phytolacca Americana* L. rather than *Phytolacca decandra* L. and this designation was adopted in the N. F. V. As is well known various popular names have been given to the plant.

Numerous claims of therapeutic action produced by the drug have been made. Hawkins and Sayre[2] mention the irritant action of the root on mucous surfaces, inhalation of the powder producing pain in the lungs for two weeks. Hammer[3] writes "Phytolacca has a wonderful action on the skin and usually relaxes it so that it is able to get rid of any irritable substance." It is also said to be useful in

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

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[1] *Drug. Circ.*, 61 (1917), 23.

[2] *Ibid.*, 36 (1902), 244.

[3] *Med. World*, 33 (1915), 35.