contain, but the final results were practically identical with those first obtained.

The conclusions to be drawn are then, that the ninth revision method for the assay of Fluidextract of Hydrastis is satisfactory; and that the method for Hydrastis might well be reconsidered, and a larger proportion of ether used to extract the drug.

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WHAT IS THE BEST END-POINT OF THE REACTION IN THE FROG-HEART METHOD OF DIGITALIS ASSAY?

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While there are various methods in use for standardizing the digitalis series of heart tonics, the frog-heart method devised and introduced by Houghton,[1] in 1894, has perhaps been most widely used in more or less modified form.

These modifications are specifically due to differences of opinion, as to the proper length of time, after dosing, to note the end-point of the reaction, namely, the characteristic systolic stand-still of the heart or the death of the animal with its heart in systole.

The original method made use of the minimal lethal dose, or smallest dose capable of causing the death with heart in systole, of a majority of the frogs to which a certain amount of the preparation in question had been administered. In a somewhat amplified form[2] the method was presented before this Society in 1909.

In 1902, Famulener & Lyons[3] described a method which has been in use in the University of Michigan Pharmacology Department for some time, according to Edmunds.[4] This consists, in brief, in administering such a dose of a digitalis heart-tonic to a frog, as to cause paralysis of the heart in systole in one hour. Edmund's modification differs only in having complete stoppage of the heart—not only systolic but auricular as well.

Barger and Shaw[5] used the same method of injection, namely, into the dorsal lymph-sac, but the frogs were kept under observation until the heart stopped, which they found was within three hours, if at all.

Fraenkel[6] practically limited the time to one hour, although a range from thirty-five to one hundred minutes is allowable, in his modification.

Ziegenbein[7] used the modification originated by Hans and Arthur Meyer of fastening male frogs to a board and exposing the heart before injection. The solution is injected into the thigh lymph-sac and in such a quantity as to produce systolic standstill in two hours.

Gottlieb[8] used as his unit "The smallest amount of the solution which will call forth systolic standstill of the heart of a 30 gm. frog in exactly thirty minutes."

Focke first published his modification of the frog-heart method in 1902.[9] This has been changed somewhat, but is essentially to determine the minimum dose causing systolic standstill in seven to fifteen minutes.

His method is more complicated than the others because of his taking into account the time period. The value of a sample is the result obtained by dividing

the frog weight by the product of the dose multiplied by the time. This makes the element of time a very important factor. Delayed absorption or exceptional resistance will lower the apparent value greatly.

While at this time, we are not considering suggested methods for standardizing the heart tonics of the digitalis series, other than by the use of frogs, it is not inappropriate to refer to the use of the warm-blooded animals. For example, Hatcher's Cat Method,[10] Reed and Vanderkleed's Guinea Pig Method,[11] Heinz' Mouse Method,[12] and the use of rabbits or dogs to determine the blood pressure and heart action, are all valuable. But for obtaining a fairly accurate estimation of the relative values of two preparations they do not appear to offer any material advantage over the frog-heart method first suggested and used for this purpose. Not only this, but cost, convenience and lack of general adaptability have prevented any extended application of them.

Edmunds & Hale [13] concluded that because in most cases the toxic action is not on the heart but on the respiratory centres "Methods which employ as a standard the minimum lethal dose obtained from the higher animals are not applicable to the physiological assay of the digitalis series."

The Frog-Heart Method may be considered to have three distinct modifications or that there are two modifications of the original twelve-hour method of Houghton, namely, the so-called Short-Time Method of Focke, and the One-Hour Method of Famulener & Lyons.

The Twelve-Hour Method of Houghton, is, distinctly, one allowing the total toxic effect of the drug to take place. The animal dies or recovers. A more or less total paralysis of the whole heart or of the ventricles may have taken place in many of the test animals, but unless this occurred and resulted in the death of a majority of five or more frogs following the injection of a certain quantity of the drug, a larger quantity must be chosen as the minimal dose. Delayed absorption, therefore, due to the nature of the drug will not vitiate the results: even digitalis has every opportunity to exert its characteristic effect.

During a large part of the year this takes place in three or four hours, or even less, in the case of Strophanthus, but during the winter months, and especially if the water in which they are kept is very cold the final result may be delayed considerably more than twelve hours.

If, however, we limit the time to one hour, or to ten minutes, and expect the drug to show its whole range of action, from therapeutic through to toxic, it is probably demanding the impossible of such a complex mixture as a fluidextract of digitalis. Absorption could not be complete in ten minutes and possibly not in one hour.

If the sample were a pure principle to be tested in comparison with a standard of like properties, the results should be comparable, otherwise the uncertainties of a physiological assay are considerably increased. Edmunds & Hale[13] conclude "that between these two methods (the twelve-hour and one-hour) it is largely a question of personal preference or convenience as far as can be judged in the light of our present knowledge."

Focke's Short-Time Method gives such inaccurate results, is so complicated, and is open to such extreme variations, that no results, by this method, are included.

The following series of tests has been carried out to compare more directly the advantages of two of the frog-heart methods of digitalis assay previously mentioned. The minimum dose of each preparation was determined according to both methods under as nearly similar conditions as possible. By personal observation as to the definiteness of the end-point, we were able to form an opinion regarding the value of each method as a means of determining the activity of preparations of the digitalis series:

TABLE No. I

FLUID EXTRACT DIGITALIS.

Description		M/L. D. 12-Hr. Method	Ratio, dose by 12-Hr. Meth. to 1-Hr. Method	Dose 1-Hr. Method
	1	.0010	(1.42)	.0007
	2	.0011	(1.37)	.0008
	3	.0017	(1.13)	.0015
	4	.0014	(1.55)	.0009
	5	.0016	(1.07)	.0015
	6	.0008	(1.14)	.0007
	7	.0008	(1)	.0008
	8	.0010	(1)	.0010
	9	.0008	(1)	.0008
	10	.0007	(1)	.0007
	11	.0009	(1.12)	.0008
	12	.0020	(1.81)	.0011
	13	.0013	(1.3)	.0010
	14	.0009	(1.29)	.0007
		Average	(1.22)	

TABLE No. II

TINCTURE DIGITALIS.

		M. L. D. per gm. 12-Hr. Method	Ratio of Doses	1-Hr. Method Dose
U. S. P.	1	.014	(1.27)	.011
B. P.	2	.009 (.010)+	(1.5)	.006 (.007—)
		Figured to U. S. P. Strength		
B. P.	3	.010 (.011)+	(1.43)	.007 (008.)
U. S. P.	4	.011	(1.27)	.008
U. S. P.	5	.016	(1.23)	.013
U. S. P.	6	.012	(1.5)	.008
U. S. P.	7	.009	(1.28)	.007
U. S. P.	8	.009	(1.5)	.006
U. S. P.	9	.008	(1.6)	.005
U. S. P.	10	.016	(1.33)	.012
U. S. P.	11	.014	(1.4)	.010
U. S. P.	12	.006	(1.2)	.005
		Average	(1.36)	

TABLE No. III.

POWDERED EXTRACT.

1	.00024	(1.09)	.00022
2	.00014	(1.4)	.00010
3	.00030	(1.2)	.00030
4	.00025	(1.25)	.00020
	Average	(1.20)	

SOLID EXTRACT.

1	.00014	(1.27)	.00011
2	.00018	(1.28)	.00014
3	.00036	(1.28)	.00028
4	.00036	(1.20)	.00030
5	.00022	(1.22)	.00018
	Average	(1.24)	

DIGITALONE AND DIGITALIN.

1	.028	(1.33)	.021
2	.013	(1.41)	.008
3	.00009	(1)	.00009
4	.00004	(1)	.00004

TABLE No. IV.

STROPHANTHUS.

	M. L. D. 12-Hr. Meth.	Ratio of dose by 12-Hr. Meth. to 1-Hr. Method	1-Hr. Method
1	.00005	(.83)	.00006
2	.00004	(.80)	.00005
3	.00006	(.75)	.00008
4	.000045	(.32)	.00014
5	.00012	(.54)	.00022
6	.00011	(.64)	.00017
7	.00009	(.45)	.00020
8	.00007	(.43)	.00016
9	.00011	(.68)	.00016
10	.000075	(.53)	.00014
	Average	(.543)	

TABLE No. V.

SQUILL.

1	.0006	(.75)	.00008
2	.0007	(.87)	.0008
	Average	(.81)	

TABLE No. VI.

CONVALLARIA.

1	.00016	(1.23)	.00013
2	.00024	(1.09)	.00022
3	.00010	(.91)	.00011
	Average	(1.08)	

The fact which stands out most prominently from a superficial examination of this date is that the minimum dose of digitalis preparations is in most cases less by the one-hour method than it is by the twelve-hour method. The opposite is true in the case of Strophanthus preparations. This seems more logical since one would naturally expect it to require more of the active substance to cause systolic stoppage of the heart in one hour than to cause the death of the frog. Digitalis in sub-lethal doses must, therefore, produce an early paralysis of the heart from which the frog recovers. This fact in itself would seem to point to a possible cause for discrepancies in the one-hour method.

It is only with samples of Tincture Digitalis that we are able to obtain a clearly defined and uniform end-point by the one-hour method. In most of the tests of other members of the digitalis series the end-point is either difficult to determine because of inability to check the minimal dose or the heart does not stop in definite systole. It would seem that there should be some very nearly constant ratio between the minimum dose obtained by each method with the same preparation, but as stated above this has not been found true except approximately in the case of Tincture Digitalis.

Our observations would lead to the conclusion that the variability in the individual resistance of the frogs to digitalis plays a more important part in the one-hour method of assay than it does in the twelve-hour method and consequently adds to the indefiniteness and inaccuracy of results by the latter method. The

time element also has an important bearing on the comparative results by the two methods. Where the time which elapses between the injection of the active material and the observation of the result is relatively short the effect of the same dose of the same preparation (and by the same does we mean in proportion to weight) upon frogs of different resistance may be sufficient to produce conflicting results. On the other hand in a method involving longer period of observation where the death of the animal rather than a paralysis of the heart is the final end-point this difference of resistance does not play so important a part. It is true that even in the twelve-hour method the variation in the resistance of the test animals is an important factor, but it can be more easily and completely eliminated by this method than could be done in the case in the one-hour method, even if a similar procedure were applied, i. e., elimination of the factor of resistance variation by the use of a large number of test animals, and of a standard for comparison.

The point that we wish to emphasize, however, is that while variation in resistance can apparently be offset in both methods by the carrying along of a standard preparation of known strength, yet there seems to be varying degrees of paralysis of the heart; and that this paralysis has no uniform relationship to the death of the test animal. In the data on F. E. Digitalis in some cases, the minimum dose was the same by both methods, but the average lethal dose exceeded the one-hour dose by 22%, with a maximum variation of 81%. In the case of the Tincture Digitalis the variation ranges from 10% to 60%, the average excess required to kill the frogs over that necessary to cause systolic stand-still being 33%. Comparison of Strophanthus tinctures by the two methods shows that 54% of the one-hour dose will kill the frog, Squill 80%, Convallaria 108%.

From our observations we, therefore, summarize as follows: First, that the end-point in the one-hour method is more indefinite and consequently more difficult to determine than that of the twelve-hour method; second, that the variation in resistance of the test animal is a source of much greater error in the accuracy of the shorter method than it is in the other; third, that an absolute end-point such as death is more satisfactory than one which may show so many degrees of variability.

Our conclusion is that the death of the frog with heart in systole is a more accurate and dependable end-point in the reaction than a similar stoppage of the heart observed at any time previous to the absolute death of the animal.

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