ABSTRACT OF DISCUSSION.

J. C. Munch was particularly interested in the close agreement of the various physiological methods of assay; he recognized the amount of work involved in the report presented by the author, and referred briefly to the many methods of assay; the efforts of the laboratory were directed to find out which method of the animal assays corresponded most closely to results obtained upon humans. In his opinion, the fact that clinicians and general practitioners give repeated doses is no reason why market preparations should be permitted to vary in potency. His conclusions, based on samples of the same lot of tincture of digitalis given into the hands of 28 collaborators, were that there should be not more than 15% variance in assay results. Digitalis should, for obvious reasons, be represented in preparations uniform in strength; otherwise there will be a demand for a standardization method which will bring this about. He hoped it would be possible to standardize digitalis preparations within reasonable limits of the U. S. P.

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NOTES ON DIGITALIN STANDARDIZATION.*

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For many years prior to the world war there was available on the American market, a water soluble, highly active digitalis preparation known as *Digitalin*, German, which was reasonably uniform in potency. During the war this product became almost unobtainable and the quality of supplies available since the war has varied widely in potency, most of it being very inferior.

Though different lots of digitalin are not uniform in chemical composition as well as physiological potency and though it has never been made official in the U. S. P. there has been a large amount of it prescribed, both orally and hypodermically, because it does not deteriorate and is fairly well absorbed in the small dosage necessary.

Since no official standard was ever set for this important digitalis preparation and no method of assay recommended, it is obvious that each manufacturer must set his own standard and these evidently varied considerably. For many years we assayed digitalin by the Houghton M. L. D. Frog Method with reasonable success. True the irritant action of the solution injected into the frog often caused the lymph sac to become filled with fluid which delayed absorption, but the period of time allowed (12 to 18 hours) was long enough for most of the activity to be absorbed from the diluted contents of the lymph sac. With the officially recommended One-Hour Frog Method the case is entirely different since the time is so short that complete absorption cannot take place and indefinite results are to be expected.

As an example of the results obtained in testing digitalin by the One-Hour Frog Method take the following data on lot No. 309,390.

The M. S. D. in this case was reported to be 0.00020 Gm. per Gm. to 0.00030 Gm. but there was nothing definite or satisfactory about it. With U. S. P. Ouabain giving an M. S. D. at this time of 0.0000008 Gm. per Gm. it is found that 1 Gm. of this digitalin is about equivalent to 3.3 mg. of ouabain.

In the tests of four other samples of digitalin by the "One-Hour Method" results were just as indefinite and unsatisfactory.

* Scientific Section, A. PH. A., St. Louis meeting, 1927.

Frog.	Wt., Gm.	Dose.	Dil.	Total.	Result.	Remarks.
1	13	.00012 Gm.	1–100	.16 cc.	Beating	
2	13	15 Gm.		.20 cc.	Beating	
3	13	20 Gm.		.26 cc.	Stopped?	Indefinite
4	20.5	15 Gm.		.31 cc.	Beating	
5	20.5	18 Gm.		.37 cc.	Beating	
6	21	22 Gm.		.46 cc.	Beating	
7	22.5	15 Gm.	1–50	.17 cc.	Beating	
8	21.5	20 Gm.		.22 cc.	Stopped	Diastole not systole
9	23.5	25 Gm.		.29 cc.	Stopped	Diastole not systole
10	17	15 Gm.		.13 cc.	Beating	
11	20.5	20 Gm.		.21 cc.	Beating	
12	20	25 Gm.		.25 cc.	Beating?	Indefinite
13	17.5	15 Gm.		.13½ cc.	Beating	Almost stopped
14	21	20 Gm.		.21 cc.	Beating	Almost stopped
15	22.5	25 Gm.		.28 cc.	Beating	Almost stopped

Absorption was always incomplete in the time limit of one hour although the dose injected was very small and of a fairly dilute solution.

No. 1.			No. 2.	
Dose.	Result.		Dose.	Result.
.00002 Gm.	Beating		.00007 Gm.	Beating
3 Gm.	Beating?		8 Gm.	Beating
5 Gm.	Beating?		10 Gm.	Beating
6 Gm.	Stopped		10 Gm.	Beating
10 Gm.	Stopped		12 Gm.	Beating
15 Gm.	Stopped		15 Gm.	Beating
.00004 Gm.	Beating?		12 Gm.	Beating
5 Gm.	Beating?		15 Gm.	Stopped
6 Gm.	Beating?		20 Gm.	Stopped
5 Gm.	Beating		12 Gm.	Beating?
6 Gm.	Stopped.	Not systole	15 Gm.	Beating?
8 Gm.	Stopped.	Not systole	20 Gm.	Beating?

No. 3.		No. 4.	
Dose.	Result.	Dose.	Result.
.00015 Gm.	Stopped	.00010 Gm.	Stopped
20 Gm.	Stopped	15 Gm.	Beating
25 Gm.	Stopped	20 Gm.	Stopped
12 Gm.	Beating	10 Gm.	Beating
15 Gm.	Beating?	15 Gm.	Stopped
18 Gm.	Stopped	20 Gm.	Beating
12 Gm.	Stopped	10 Gm.	Stopped
15 Gm.	Stopped?	15 Gm.	Stopped
18 Gm.	Beating	20 Gm.	Stopped
12 Gm.	Beating	10 Gm.	Beating
15 Gm.	Beating	15 Gm.	Beating
18 Gm.	Stopped	20 Gm.	Beating
		10 Gm.	Beating
		15 Gm.	Stopped?
		20 Gm.	Stopped

Because of these very indefinite results with the "One-Hour Method" on Digitalin and because the M. L. D. "Frog Method" is not entirely satisfactory, due to the frogs sometimes being nearer dead than alive and yet not actually dead, other methods were tried such as the Cat Method, the Guinea-Pig Method and finally the "mouse" intraperitoneally. One sample (No. 309,390) required six

cats and of the four successful experiments the average M. L. D. was 6.23 mg. per Kg. The variation between the lowest and highest M. L. D. in this series of 4 was 74% which is quite extreme. Two experiments were unsuccessful because the animals did not die within a reasonable time from comparatively large doses.

Guinea-pigs injected subcutaneously give satisfactory results but an average of 10 or 12 pigs is necessary for an assay and this makes the cost very high. Three such tests were made and will be included in the table summarizing the tests by the various methods.

The white mouse injected intraperitoneally seemed logical as a test animal for digitalin since it absorbs the drug readily and can be used in large numbers because of its relative cost. Most important, however, was its satisfactory use in standardizing aconite as previously reported. Several tests were made by this method and the results will appear in the following general table of data where tests by all the methods are grouped.

Sample.	M. L. D. Frog Method.	U. S. P. Frog Method.	Guinea-Pig.	White Mouse.
No. 295460	.000060 Gm. per Gm. 1100 H. T. U.	About .00006 Gm.	.000015 Gm. per Gm.	.....
No. 49929	.00012 Gm. per Gm. 300 H. T. U.	.00010 to .00020 Gm.	.000025 Gm. per Gm.	.....
No. 309390	.00030 Gm. per Gm. 120 H. T. U.	.00020 to 30 Gm. 8 for ouabain		
DCC	.00014 Gm. per Gm. 400 H. T. U.	.00015 to 25 Gm.	.000040 Gm.	.00018 Gm.
No. 313823	.00005 Gm. per Gm. 1200 H. T. U.	.00010 to 20 Gm.		.00012 Gm.

This table shows, first of all, the impossibility of using the official U. S. P. digitalis method for assaying digitalin. The M. L. D. Frog Method can be used but it often shows some indefinite results. The Cat Method was very unpromising in the assay of one sample. The Guinea-Pig Method could be used but is quite expensive. The white mouse injected intraperitoneally seemed very promising in the limited experiments because results can be read in 5 or 6 hours and the animals are reasonable in price, so that a number could be used for one test. Further comparative data would be necessary, however, before a definite standard could be set.

ABSTRACT OF DISCUSSION.

The author stated that his paper was presented as a preliminary report, that standardization of digitalin is in its infancy. He hoped that the report would stimulate further interest so that a suitable method for assay and a reliable standard would result.

J. C. Munch said he had found assay of digitalin rather unsatisfactory; in his opinion the white mouse, as a test animal, might prove best.

Paul S. Pittenger stated that the guinea-pig had proven satisfactory; relative to the expense, the surviving animals could be used after recovery and, in estimating the cost, this should be taken account of.

Albert Schneider said that daphnia had been suggested as a test organism and Chairman Krantz referred to the possibility of using paramecium. Both of these, however, are very sensitive to alcohol.

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