THE STANDARDIZATION OF DIGITALIS PREPARATIONS.

BY E. E. SWANSON AND C. C. HARGRAVES.

1. REVIEW OF LITERATURE; 2. DATA; 3. DISCUSSION AND CONCLUSIONS.

The question of a suitable standardization method for the biological or chemical assay of digitalis has been a subject of considerable controversy and discussion for many years. Perhaps on no other drug has there been more variety or kinds of methods suggested for its standardization. The physiological assay of digitalis is determined on both cold-blooded and warm-blooded animals. Such methods have been introduced and discussed by Houghton (1), Hatcher and Brody (2), Houghton and Hamilton (3), Edmunds and Hale (4), Ziegenbein (5), Eckler (6), Holste (7), Reed and Vanderkleed (8), Famulener and Lyons (9), Locke (10), Heinz (11), Pittenger and Vanderkleed (12), Führer (13), Kobert (14), Pick and Wasicky (15), Heffter (16), Gottlieb (17), Storm Van Leeuwen (18), Wiechowski (19), Watanabe (20), and a host of others. The most important methods at present in use are the one-hour frog method U. S. P. or modification of this method, and Hatcher's and Brody's (2) cat method together with modifications and suggestions. Reed and Vanderkleed (8) suggested the use of guinea-pigs, a method which is still preferred by many workers; Berry (21) carefully worked out a heart perfusion test; Hale (22) has shown the adequacy of the one-hour frog method (U. S. P.); Roth (23) also finds the one-hour frog method (U. S. P.) favorable. Hatcher's cat method has been studied and modifications found and suggested by Macht and Colson (24), Rowntree and Macht (25), Eggleston (26), den Besten and de Lind van Wijngaarden (27), Haskell and Courtney (28), Kuroda (29), de Lind van Wijngaarden (30), and more recently that of Knaffl-Lenz (31) and McFarlane and Masson (32). The recent work of Straub (33) and his co-workers and Erik Knaffl-Lenz (31) on the standardization of digitalis on cats and guinea-pigs in connection with the Hygiene Committee of the League of Nations is very important. Hirschfelder (34) studied the effect of temperature on the action and toxicity of digitalis testing; Dooley and Higley (35) found an intramuscular injection in frogs to be accurate; Schneider (36) suggested the use of paramecia as a test method; Berardi (37) and Berardi, Canan and McGuigan (38) describe a method of assay on normal and anæsthetized dogs. Hanzlik and Shoemaker (39) suggested the use of pigeons as an index for the therapeutic potency. Bond (40) found that the M. L. D. to the heart weight will give a biological comparison. Knudson and Dresbach (41) reported a colorimetric method of testing digitalis, but Wible (42) and Rowe (43) found this to be unreliable. More recently Macht and Krantz (44) reported an interesting method of study and testing of digitalis called phytopharmacology or a plant method of standardization of digitalis preparations on the growth on the seedlings of Lupinus Albus.

2. DATA.

Considering the above brief review of the literature all of which have very valuable and interesting data, a more reliable biological and chemical method still remains to be studied. However, perhaps with a more careful study of some of the above methods by various workers and a standardization of technic, a method

might be found that will be satisfactory for an accurate indication of the therapeutic action of digitalis.

The purpose of this paper is not to suggest a new method of assay, but to study some of the more important or well-known methods of standardization of digitalis now in use and to compare them with the colorimetric method of Knudson and Dresbach. The writers had planned a series of experiments covering all samples of digitalis for a period of two years, but before the completion of this work, Wible and Rowe reported their results which so confirmed our findings, that a further study of this series did not seem practical.

The biological methods studied are, first, the one-hour frog method (U. S. P.), second, Houghton's twelve-hour frog method, third, guinea-pig method, and, fourth, Hatcher's cat method. The chemical method of Knudson and Dresbach is compared with the one-hour frog method (U. S. P.). Table I includes the comparative assay of four Digiglusin samples, four Fluidextract of Digitalis N. F. samples and four Tincture Digitalis U. S. P. samples by the above five methods, together with nine samples of Tincture Digitalis U. S. P. assayed by the one-hour frog method and the colorimetric method.

TABLE I.

Kind of preparation. Digiglusin no.	Frog (U. S. P.) per cent activity.	Frog (Houghton) per cent activity.	Guinea-pig (Vanderkleed) per cent activity.	Cat (Hatcher) per cent activity.	Colorimetric (Knudson) per cent activity.	Color error between U. S. P. frog and colorimetric. Low %.
1	100	90	95	92	85	17
2	106	95	109	90	70	51
3	136	126	125	120	100	36
4	302	280	300	285	230	31
F. E. digit. no.						High %.
1	85	92	90	80	120	35
2	100	95	110	90	182	44
3	100	110	115	95	153	34
4	90	85	92	80	140	35
Tr. digit. U. S. P. no.						High %.
1	100	91	85	92	175	50
2	100	97	90	90	282	64
3	75	70	60	50	137	45
4	100	95	90	82	158	36
5	100	..	..	..	152	34
6	70	..	..	..	80	12
7	160	..	..	..	182	12
8	120	..	..	..	250	53
9	73	..	..	..	178	60
10	120	..	..	..	250	53
11	100	..	..	..	140	28
12	100	..	..	..	158	36
13	72	..	..	..	154	36

Sample No. 10 is the same as Sample No. 8, but tested a month later.

Table II includes a comparison of the one-hour frog method and the colorimetric method with ouabain as a color standard.

TABLE II.

Digiglusin sample.	(U. S. P. frog) per cent activity, ouabain standard.	(Colorimetric) per cent activity, ouabain standard.	Color error.
No. 5	127%	67%	89% low
No. 6	86%	82%	4.8% low
No. 7	33%	30%	10% low
No. 8	123%	55%	120% low
No. 9	55.2%	48%	50% low
No. 10	44.2%	....	....

Table III includes a comparison of the one-hour frog method and colorimetric method with a standard digiglusin as a standard color reaction.

TABLE III.

Digiglusin, sample.	U. S. P. frog per cent activity, digiglusin standard.	Colorimetric per cent activity digiglusin, standard.	Color error.
No. 5	127%	117%	9% low
No. 6	78%	71%	10% low
No. 7	33.4%	46%	19% high
No. 8	111%	133%	16% high
No. 9	55%	80%	31% high
No. 10	44%	70%	37% high

In Table I the assay of the digiglusin, tincture and fluidextract samples by the four biological methods seems to correlate rather consistently. The colorimetric method assays low for digiglusin samples, and high for tinctures and fluidextracts.

In Tables II and III six samples of digiglusin were compared with ouabain as a color standard and a standard digiglusin as a color standard. The colorimetric method with ouabain as a color standard gave low results as compared with the biological method. The colorimetric method with digiglusin as a color standard gave two low figures and four high figures. These variable figures leaves a rather questionable answer in regard to the accuracy of the colorimetric method.

DISCUSSION AND CONCLUSIONS.

Considering the review of the above literature and data on the biological and chemical methods, the comparative relationship is still more complex, which leads one to conclude that it is not possible to determine the absolute content of the various glucosides. However, as Erik Knaffl-Lenz states, if the conditions of technic are carefully controlled, it is possible by some of the biological methods on either warm-blooded or cold-blooded animals to obtain relative values of some consistency. The value of the biological methods is dependent on the clinical or therapeutic index, and inasmuch as digitalis is given by clinicians in repeated doses until the therapeutic effect is obtained—the amount given to obtain this therapeutic effect being irregular for individual patients—a more accurate method of assay than the ones now in use is not absolutely essential. An accurate biological or chemical method could more easily be developed if the various glucosides were isolated, purified and their chemical, physiological and therapeutic properties studied, for we know of no accurate relationship between the M. L. D. as determined by an animal experiment and the amount of the drug required to produce a therapeutic effect. If a biological method could be worked out that would indicate a minimum effective dose, or

therapeutic dose and the minimum lethal dose or a definite safety margin, a greater relationship would exist between biological methods and therapeutic effect. Therefore, as long as biochemists are not in a position to determine accurately the chemical, physiological and therapeutic properties of the pure isolated glucosides, the biological methods now in use do not justify a greater increase in accuracy.

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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.