THE BIOLOGIC STANDARDIZATION OF DIGITALIS BASED ON THE RELATIONSHIP OF THE M. L. D. TO THE HEART WEIGHT.*

BY W. R. BOND.

The misconception that even one or two animals suffice to determine with accuracy the potency of a digitalis preparation, when assayed by the Cat Method of Hatcher and Brody, may often lead to serious error. Attention has been called to the fact that in a large number of series of assays there occur wide variations in the values obtained when the M. L. D. is calculated per unit of cat weight. While it has been recognized that two cats in the same series may be abnormal in opposite directions, and their average approximate the true potency of the preparation, it is also possible, and with equal frequency, for the two animals to be abnormal in the same direction and lead to an error in many instances amounting to as much as fifty per cent of the average M. L. D. Experiments have shown that it is only possible by employing a large number of animals that accuracy can be attained, not only in the standardization of digitalis, but in every biologic assay method as well.

The question arises—Does the total body weight accurately represent the amount of animal tissue accessible to the action of the drug? One frequently encounters in the same series two cats requiring approximately the same amounts of digitalis to produce death, yet there is quite a difference in their body weights and hence in unit doses. Autopsies upon these animals often reveal that whereas one may show a considerable amount of subcutaneous and visceral fat, the organs of each are very nearly of the same size and weight.

Obviously such difficulty might in part be overcome by a careful selection of animals, or by basing one's calculations of M. L. D. upon some portion of the body which bears a constant ratio to the body weight in normal cats. Since with intravenous injections it seems logical to suppose that the degree of manifestation of action would depend largely upon the concentration of the drug in the blood stream, blood volume might offer a suitable basis for these calculations. The determination of blood volume, however, does not seem practical for this purpose, aside from the uncertainty in the results obtained. The question of accuracy alone is sufficient to render its use unwarranted.

The relationship of certain organs of the body to the total body weight has been thoroughly worked out in the case of the white rat, *Mus Norvegicus*, and it has been shown that after the early period of development of the animal the heart weight bears a constant ratio to the body weight. Information of this nature concerning the cat has not been obtained on account of the difficulty in securing sufficient material of one species for making such observations. It is reasonable, however, to suppose that a similar ratio exists. When the increase in body weight is due to an accumulation of fat, there may be a slight compensatory hypertrophy of the heart, but there is little reason to believe that the ratio of heart to total weight is maintained under these circumstances. On the other hand it seems fair to assume that an increase in blood volume might result in a compensatory enlargement of the heart which would more closely preserve the relationship of heart to blood volume.

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On the above assumption, and aside from the specific cardiac action of digitalis, a series of assays has been conducted to compare the results obtained when the M. L. D. of the drug is calculated per Kg.-body weight, and per Gm.-heart weight. No attempt has been made to select animals for these experiments, the material received in the laboratory being rather cosmopolitan in its nature. The assay is conducted essentially as described by Hatcher and Brody. After recording the total weight, the thorax is opened, the heart stripped from the pericardium and the vessels cut close to their proximal ends. Longitudinal incisions are made in each chamber and the heart washed in running water until free of blood, after which the excess moisture is removed by means of a dry towel and the heart weighed.

Table I shows the results obtained on Tincture No. 45.

TABLE I.

ASSAY OF TINCTURE OF DIGITALIS No. 45.

No.	Wt. Kg.	Cc.	Mg./Kg.-body wt.	Gm.-heart wt.	Mg./Gm.-heart wt.
1	2.15	20	93.0	8.3	24.05
2	2.6	29	111.6	11.4	25.4
3	2.05	20.5	100.0	8.1	25.3
4	2.27	23	101.3	9.8	23.4
Averages			101.4		24.5
Greatest percentage deviation			9.97		4.48

This table shows no marked deviation for either method of basing the calculations of M. L. D.; however, the greatest deviation from the average is 9.97% when the unit dose of digitalis is expressed in mg. leaf per Kg. cat, and 4.48% on the base Gm.-heart, giving 45% more accuracy in favor of the latter method of assay.

In the second series cats Nos. 1 and 2 with weights of 3.5 and 2.27 Kg., respectively, required about the same amounts of digitalis. The M. L. D. calculated on the basis of total weight gives for the former 63.8 mg., for the latter 119.0 mg. This is a typical example of abnormal variability in opposite directions. Attention is also called to the fact that whereas there was a variation of 1.23 Kg.-body weight, the difference in heart weight amounted to only .7 Gm., giving 21.4 mg. as the M. L. D. for the first and 24.7 for the second. The combined results of the series showed maximum deviations of 39.3% and 13%, respectively, or 62% greater when the total weight is used as the basis.

TABLE II.

ASSAY OF TINCTURE OF DIGITALIS No. 45.

No.	Wt. Kg.	Cc.	Mg./Kg.-body wt.	Gm.-heart wt.	Mg./Gm.-heart wt.
1	3.5	24	63.8	11.2	21.4
2	2.27	26	119.4	10.5	24.7
3	3	35.5	116.7	12.7	27.9
4	2.93	27.5	93.8	12.3	22.6
5	2.4	29	120.7	10.5	27.6
Averages			103.1		24.8
Greatest percentage deviation			38.1	13.7	

The result of series III run on a freshly prepared tincture showed a maximum deviation 33% greater when the M. L. D. was calculated per Kg.-body weight.

TABLE III.
TINCTURE P. D. LEAF—1925.

No.	Wt. Kg.	Cc.	Mg./Kg.-body wt.	Gm.-heart wt.	Mg./Gm.-heart wt.
1	2.1	14.8	70.5	7.45	19.9
2	2.31	14.2	61.5	7.88	18.0
3	3.35	26.8	80.0	12.7	21.1
4	3.54	25.0	70.6	14.8	16.9
5	2.215	22.1	99.8	9.2	24.0
Averages			76.5		20.0
Greatest percentage deviation			30.5		20.0

In series number IV decerebrate cats were used. It will be observed that cat No. 3 required nearly twice as much tincture as No. 12. The difference in weight, however, is not proportionate, there being about 16% difference in weight against 47% difference in quantity of digitalis required. Attention is also called to the heart weights amounting to 13.4 and 7.3 Gm., respectively, a variation of about 45%, which is in excellent keeping with the total quantity of digitalis required for each animal. The average of this series of thirteen animals shows 63.1 mg. of leaf per Kg. cat and 14.45 mg. per Gm.-heart. In this series the percentage deviation from the average has been calculated in each case; while but eight of the thirteen determinations showed less deviation when calculated per Gm.-heart, the average percentage deviation of the series showed 12.9% for body weight and 9.7% for heart weight. As might be expected these averages will not only approach each other, but both in turn approach zero as a limit, as the result of increasing the number of animals in the series.

TABLE IV.
DECEREBRATE CATS—TINCTURE P-D LEAF—1925.

No.	Wt. Kg.	Cc.	Mg./Kg.-body wt.	Gm.-heart wt.	Mg./Gm.-heart wt.	Percentage deviations. Body wt.	Heart wt.
1	2.54	19.3	76.0	11.2	17.2	25.2	19.0
2	1.95	15.6	81.4	10.2	15.3	28.9	5.90
3	2.67	17.0	64.0	13.4	12.8	1.42	11.4
4	1.715	13.8	80.2	8.7	15.8	11.25	9.35
5	2.65	20.0	75.4	12.7	15.7	19.45	8.64
6	4.06	25.0	61.3	19.4	12.8	3.05	11.4
7	2.05	12.0	58.5	9.2	13.0	7.26	10.0
8	1.99	11.6	58.3	8.0	14.5	7.59	.346
9	2.595	17.0	65.5	10.3	16.3	3.95	12.8
10	2.27	14.5	63.8	9.3	15.5	.9	7.25
11	2.965	17.0	57.3	12.5	13.6	9.17	5.87
12	2.25	9.0	40.0	7.3	12.3	36.6	14.9
13	2.02	11.2	54.6	8.55	13.1	13.45	9.35
Averages			63.1		14.45	12.9%	9.7%
Greatest percentage deviation						36.6%	19.0%

CONCLUSION.

In the Cat Method of Hatcher and Brody for the assay of digitalis, the error due to individual variability seems to be lessened from 30% to 50% when the M. L. D. is calculated on the basis of the heart weight.

Thanks are due to Parke, Davis & Co. for the digitalis leaf used in making the tinctures assayed in Tables III and IV. I am also indebted to Mr. H. B. Haag for much of the data on decerebrate cats.

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ANALYSIS OF ORGANIC SILVER COMPOUNDS.

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It is not always an easy procedure to determine the metallic part of a substance when the latter is in organic combination or associated with a large amount of organic matter. When the element is easily reduced to the metallic state the simplest method is to burn the substance. When the element forms volatile compounds on ignition, a more complicated procedure is required.

In order to have a method applicable to both classes of elements the author, several years ago, adopted and modified the Lehman method,[1] which was originally used for the determination of arsenic in arsphenamine. This method is of special interest to pharmaceutical chemists, for the analysis of Argentum Proteinicum Mite and Fortior. The latter is a true silver protein salt (of acids similar to protalbinic and lysalbinic acids) dissolved in excess of the same protein substance, while the former is true *colloidal* silver with a protective colloid.

The method has the following advantages:

1.   In the case of a volumetric determination, the analysis is finished in the same Erlenmeyer flask in which it is started.

2.   A light-colored solution is always obtained for titration.

3.   There are no interfering elements to be removed when finishing the assay gravimetrically.

Method for Argentum Proteinicum Mite and Fortior:

Weigh a one-Gm. sample into a 300-ml. Erlenmeyer flask; dissolve in 10 mls. of water and add 10 mls. of concentrated H_2SO_4; cover the neck of the flask with a small watch glass. Begin to add, immediately, small portions of finely powdered $KMnO_4$. Keep the solution hot. The addition of $KMnO_4$ is discontinued when a deep red solution is obtained; the red color remains for more than five minutes. About two Gm. of $KMnO_4$ are required.

Heat the solution to boiling, wash down the sides of the flask and add finely powdered oxalic acid until the red color disappears, and the oxides of manganese are dissolved. Quite often, the last traces of these oxides are not dissolved until the solution is boiled; an excess of oxalic acid is to be avoided. Again heat solution, and boil for a few minutes; add 5 mls. concentrated nitric acid, and heat on steam-bath for 15 minutes. Cool the flask and contents, and add 50 mls. of water, and 2 mls. of 10% solution of ferric alum. Titrate with $N/10$ potassium thiocyanate V. S., to the regular end-point.

The number of mls. of $N/10$ potassium thiocyanate V. S. used multiplied by 1.08 and divided by weight of sample equals the per cent of silver.

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[1] Lehman, "Ueber die Bestimmung des Arsens in Salvarsen und Neo-Salvarsen," *Apoth. Ztg.*, 27, 545 (1912).