SCIENTIFIC SECTION

BIOLOGICAL STANDARDIZATION OF THE HEART TONIC PREPARATIONS.*

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Up to the present time it has been generally recognized that the standardization of the Heart Tonic Preparations such as digitalis, strophanthus, squill and others of less importance, may best be accomplished by biological rather than by chemical methods of assay. The nature of the active principles in the various drugs with the possible exception of strophanthus, in which the strophanthin can comparatively easily be estimated chemically, is such that they cannot conveniently be isolated and estimated in a pure state. Further, since each of these drugs, as for instance digitalis, contains quite a number of active principles which are not always present in the drug in the same definite proportion to each other, it would be impossible to base the valuation of the drug on the estimation of only one active principle.

The value of the drug, *i. e.*, the estimation of its active principles, which may or may not act synergistically upon each other, can, however, be determined by biological assay.

The real problem which confronts investigators in this branch of science, resolves itself into determining the biological method of assay most suitable for the preparations in question, that is, the method which approaches in its accuracy, rapidity of execution and dependability under general laboratory conditions that of the best chemical analytical procedures.

Biological methods of estimation or standardization are in general inherently subject to certain variables which tend to affect the final results. This is true whether the object is to standardize a disinfectant by the Hygienic Laboratory Coefficient Method, or to assay digitalis preparations by the one-hour frog, 12-hour frog or the cat method.

In all cases, with the exception of the cat method, the factors of time, temperature at which the drug is allowed to act, relative concentration of test solution and rate of absorption (in the case of frog assays), or the type of organism employed (in the case of bacteriological standardizations), enter to modify, individually and collectively, the final result. The biological method which has the fewest number of variables and at the same time takes into account the action upon which its therapeutic usefulness depends, should be the one selected.

In my opinion, the cat method offers the best means for standardizing the heart tonic drugs of the digitalis series. Briefly stated, this method consists in

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ascertaining the minimum lethal dose per kilo cat, the drug being slowly injected into the femoral vein until death of the animal with typical digitalis poisoning occurs, and expressing the minimum lethal dose (M. L. D.) as percentage strength of an arbitrary standard, which has been adopted as 100 mgs. of digitalis leaves per kilo body weight.

The reasons for this opinion are based on the experience of this laboratory in the physiological assay of heart tonic preparations by both frog and cat methods.

1. *The One-Hour Frog Method of the U. S. P. IX.*—The one-hour frog method, as recommended by the U. S. P. IX, has, in my opinion, the following variables and constants upon which the final result depends, namely:

(1) Concentration of Test Solution, whereby all or only part of the solution may be absorbed within the time limit of one hour.

(2) Absorption Rate due to nature of Test Solution; for instance, digitalin is absorbed much more slowly than tincture of digitalis, etc.

(3) Seasonal variation of Test Animal.

(4) Degree of accuracy in weighing frogs and administering dose.

The factors in the assay which can be made constant if due care is taken are:

(*a*) Length of Test Period (1 hour).

(*b*) Temperature at which the drug is allowed to act (20° C.).

(*c*) Species of Test Animal (*Rana Pipiens*).

Furthermore, as the final calculation of the strength of a sample is dependent upon a secondary assay of a so-called standard substance, ouabain, which assay is likewise affected by the same experimental conditions as set forth above, it is readily seen that in reality at least eight variable factors are liable to enter into a single standardization by the one-hour frog method.

The Factor of Concentration.—The concentration of the test solution recommended for injection, *i. e.*, that the total average dose does not exceed 0.015 mil for each gramme of body weight of frog, or an average total of 0.5 mil, is not always attainable, and many times where the sample is low in toxicity it is necessary to give over 1 mil, which is equivalent to 0.030 mil per Gm. frog. Other things being equal, it is obvious that the larger the quantity of liquid necessary to represent a certain dose, the longer the time required for total absorption. The volume of the dose may be made smaller by evaporating the samples to give the required concentration, but no matter how careful the process of evaporation may be, it is more than probable that some loss in activity will take place. This applies especially to the digitalis series of preparations.

Rate of Absorption.—Experience has shown that some substances are absorbed more slowly than others; among these are, as already mentioned, solutions of digitalin; further solutions of digitoxin and those containing acetic acid. Other investigators have recorded similar observations.[1] The rate at which the dose is absorbed after injection into the lymph sac frequently depends upon the health of the frog. The so-called red-legged disease is very prevalent among frogs and without doubt such diseased frogs give unreliable results. However, it is equally true that apparently healthy frogs often have a relatively large amount of unabsorbed solution in the lymph sac at the end of the test period of one hour. This applies especially to fluidextract of digitalis, digitalin and acetic squill.

While therefore frogs are entirely unsuitable for assaying digitalin by the one-hour method very concordant and satisfactory results are obtained by the cat method as shown in Table I. Therefore the cat method has proved with me to be a very useful and satisfactory method of assaying hypodermic and tablet triturate preparations of digitalin.

TABLE I.—BIOLOGIC ASSAY OF DIGITALIN.

(Merck's Digitalin.)

(Test Animal—Cat.)

Date of assay.	Sex and wt. (kilos) of T. A.	Value of sol. injected	Lethal Dose for test animal (mils).	L. D. per kilo (mils).	L. D. expressed as Gms. digitalin per kilo.
9/19/16	*2.49	1 mil = .001	14	5.63	.005,63
11/3/16	*2.47	1 mil = .001	14	5.67	.005,67
3/15/17	†2.78	1 mil = .001	16	5.75	.005,75
3/22/17	†3.40	1 mil = .001	19	5.88	.005,88
3/26/17	*3.22	1 mil = .001	19	5.90	.005,90

* = Female. † = Male.

NOTE.—The average M. L. D. expressed in Gm. digitalin per kilo cat is equal to .005,77.

The absorption of preparations of squill containing acetic acid is especially slow and results obtained by the one-hour frog method are generally especially unsatisfactory.

The apparent difference in toxicity of two preparations or substances may possibly be due largely to their different rates of absorption by the frogs. The more rapid the absorption, the greater the apparent toxicity. The one-hour frog method does not properly take care of this source of error, as the test period of one hour is too short and constitutes, in my opinion, the chief objection or criticism of this method of assay. The fact that absorption rates of individual healthy frogs are different and also that some members of the digitalis series are absorbed much more rapidly than others, should be taken into account in a biologic assay. The 12-hour frog method overcomes this objection and in this respect is to be preferred to the method recommended by the U. S. P.

Changes in Reaction of Frogs at Different Seasons.—That a seasonal variation exists in the reaction of frogs to the digitalis series has been generally claimed, but so far as my observations are concerned, there is nothing definite to be concluded on this point. A possible lessening in the vitality of the frogs has been noted during the summer months which may give an unwarranted increase in toxicity of samples. No positive evidence of this has been noted, but undoubtedly one lot of frogs differs from a succeeding lot in its reactions to drugs even at the same season of the year. Even the same lot of frogs may react differently to the same sample of ouabain when tested at different times. For example: 0.000,000,7 Gm. per Gm. frog was the M. S. D. by the one-hour method for a sample of ouabain, and when assayed the following day on the same lot of frogs a M. S. D. of 0.000,000,9 Gm. per Gm. frog was obtained.

TABLE II.—OUABAIN.

Biologic Assays by "One-Hour Frog."

Series No.	Date of assay.	Data on		M. S. D. Gm. per Gm. frog.
		Ouabain.	Frogs.	
1	7/3/17	Stock sol. A made Sept. 7, 1916	Held 1 month in storage tank previous to test	.000,001,1
	7/3/17	Stock sol. B made Aug. 29, 1917, from new sample ouabain	Held 1 month in storage tank previous to test	.000,000,7
2	7/3/17	Stock A	Held 2 days in storage tank previous to test	.000,000,7
	7/3/17	Stock B	Held 2 days in storage tank previous to test	.000,000,6

It is to be noticed that in both series of tests tabulated above, the new sample of ouabain was slightly more toxic than the other. These two series bring out not only the difference in reaction of two lots of frogs to the same sample of ouabain, but also the difference in toxicity between ouabain obtained from different sources of supply.

It will also be seen that both lots of frogs showed a slightly greater toxicity for the Stock B ouabain.

These two samples of ouabain were assayed by the cat method with the following results:

TABLE III.—OUABAIN.

Assayed by Cat Method.

Series No.	Date of assay.	Data on ouabain used.	Sex and wt. of test animal, kilos.	Value of solution injected.	Dose of dil.	Equiv. dose of original.	Gm. ouabain per kilo cat.
1	7/31/17	Stock sol. A made Sept. 7, 1916	†2.19	1 mil = .000,01 Gm.	29	15.22	.000,132,2
			†4.15	1 mil = .000,02 Gm.	30	7.23	.000,144,5
			*2.85	1 mil = .000,01 Gm.	36	12.63	.000,126,3
2	7/31/17	Stock sol. B made Aug. 29, 1917, from new sample of ouabain	*2.99	1 mil = .000,01 Gm.	28	9.37	.000,093,7
			*2.56	1 mil = .000,01 Gm.	20	7.82	.000,078,2
			†3.33	1 mil = .000,01 Gm.	26	7.81	.000,078,1

† = Male. * = Female.

The variation in toxicity of ouabain of different source therefore manifests itself also in the cat tests. For Stock A ouabain, the average M. L. D. is 0.000,129,3 Gm. per kilo and for Stock B it is 0.000,078,2 Gm. per kilo cat, hence the latter sample is decidedly more toxic than the other and in this respect the cat method corroborates the frog assay. The so-called "standard" of the U. S. P. one-hour frog assay method is therefore of doubtful value, a fact which has been verified by Rowe.[2]

TABLE IV.—ASSAYS MADE ON DIFFERENT DATES, SHOWING VARIATIONS IN TOXICITY OF OUABAIN AND DIGITALIS, AND EFFECT UPON PERCENT ACTIVITY AS DETERMINED BY U. S. P. IX.

Sample.	Date examined.	One-Hour Frog Method.				M. L. D. Cat Method.		
		Temp. of medication.	M. S. D. Ouabain.	M. S. D. Sample.	Percent activity of standard.	M. L. D. per kilo.		Percent activity.
						Mils.	Mgs.	
Tr. Digitalis								
	11/6/'16	20° C.	.000.000,65	.006	130.0	1.56	156.0	64.2
(1)	12/4/'16	20° C.	.000.000,80	.009	106.0	1.56	156.0	64.0
	1/29/'17	20° C.	.000.000,70	.009	90.0	1.495	150.0	66.7
F. E. Digitalis	11/21/'16	20° C.	.000.000,80	.000,75	128.0	0.200	200.0	50.0
	12/4/'16	20° C.	.000.000,80	.000,88	109.6	...	...	...
	1/29/'17	20° C.	.000.000,60	.000,72	103.0	0.205	205.0	48.7
Tr. Digitalis	1/5/'17	20° C.	.000.000,7	.0070	120.0	0.975	97.5	102.5
(2)	1/6/'17	20° C.	.000.000,6	.0065	110.6	0.976	97.6	
	1/9/'17	20° C.	.000.000,7	.0065	129.2	...	...	...
	1/9/'17	20° C.	.000.000,7	.0070	120.2	...	...	...
Tr. Digitalis	4/30/'17	20° C.	.000.000,85	.0125	81.6	1.005	100.5	99.5
(3)	5/11/'17	20° C.	.000.001,1	.0110	120.0	...	...	...
Tr. Digitalis	5/1/'17	20° C.	.000.000,6	.008	90.0	1.284	128.4	77.8
(4)	5/11/'17	20° C.	.000.001,1	.008	165.0	...	...	...
Tr. Digitalis	8/15/'17	20° C.	.000.000,7	.0095	88.5	0.752	75.2	133.0
(5)	8/22/'17	20° C.	.000.001,0	.0090	133.4	0.768	76.8	130.0

NOTES:

(*a*) Three cats were use for each cat assay, and the average taken for above table.

(*b*) One-hour frog method of U. S. P. IX used.

The data in Table IV shows that when tested by the one-hour frog method, the variation in the toxicity of ouabain is proportionally greater than that of digitalis preparations tested at the same time. In other words, it is due to this variableness of the ouabain or so-called standard that the samples show such large differences in activity when tested on different dates. Much more constant results are obtained by M. L. D. cat method. While the data are not complete in all cases, still there is sufficient to bring out this point.

Degree of Accuracy Obtainable in Weighing Frogs and Administering Dose.—The U. S. P. recommends that the frogs for assay be weighed to 0.1 Gm. Whether it is possible to comply with this degree of accuracy is doubtful, owing to the large moisture content of the animals and the difficulty in wiping them completely dry. Frogs removed from the constant temperature bath, weighed, returned to the bath for one hour and then reweighed, invariably show a change in weight, generally a loss of approximately one gramme per frog, as shown by the following tables.

A lot of frogs which had been kept in running cold water for one month was weighed, then placed in a constant temperature bath for one and one-half hours,

three hours and twenty-four hours, and after the lapse of these periods was reweighed. The results are given in Table V, which shows that frogs lose considerable weight during the keeping and that the loss is to some extent in proportion to the length of time the frogs are kept, as is evident from the results in this table. This loss applied both to frogs which had been kept for one month in a storage tank and to frogs which were examined two days after their arrival. The loss in this latter lot of frogs was even greater than with the former (see Table VI). The conclusion is that frogs cannot be first weighed and then held at a special temperature an hour or two before injecting doses based on the initial weight if accuracy is desired; and also that frogs should be weighed to the nearest Gm. rather than 0.1 Gm. as specified by the U. S. P. IX.

TABLE V.—SHOWING VARIATION IN WEIGHTS OF FROGS PREVIOUSLY KEPT IN STORAGE TANK FOR ONE MONTH.

Frog No.	Original weight in Gm.	Weight after 1½ hrs. in 21° C. bath.	Weight after 3 hrs. in 21° C. bath.	Weight after 24 hrs. in 21° C. bath.	Change in weight at end of		
					1½ hrs.	3 hrs.	24 hrs.
1	20.3	21.0	20.6	17.9	+0.7	+0.3	—2.4
2	16.1	15.9	15.9	15.8	—0.2	—0.2	—0.3
3	15.1	15.3	15.4	15.3	+0.2	+0.3	+0.2
4	15.2	14.8	14.8	14.1	—0.4	—0.4	—1.1
5	19.5	19.4	19.8	18.0	—0.1	—0.1	—1.5
6	20.3	21.0	20.7	19.1	+0.7	+0.4	—1.2
7	30.4	29.2	28.2	25.2	—1.2	—2.2	—5.2
8	21.1	20.2	20.3	20.2	—0.9	—0.8	—0.9
9	29.1	29.2	29.1	24.8	+0.1	0.0	—4.3
10	21.1	21.4	21.7	20.0	+0.3	+0.6	—1.1
11	20.8	20.8	20.5	20.0	0.0	—0.3	—0.8
12	18.6	18.4	18.3	17.2	—0.2	—0.3	—1.4
13	25.8	25.1	25.0	24.3	—0.7	—0.8	—1.5
14	34.0	33.3	33.2	33.0	—0.7	—0.8	—1.0
15	27.5	28.6	28.8	28.1	+1.1	+1.3	+0.6
16	26.7	26.2	26.3	25.9	—0.5	—0.4	—0.8
17	18.8	18.9	17.5	17.5	+0.1	—1.3	—1.3
18	17.6	17.5	17.7	16.2	—0.1	+0.1	—1.4
19	21.3	21.7	21.9	20.5	+0.4	+0.6	—0.8
20	22.7	22.8	23.5	21.0	+0.1	+0.8	—1.7
Averages,					—0.07	—0.17	—1.39

Granted that a frog is accurately weighed, which in my opinion would be the nearest Gm. or half Gm., and also that the exact dose is administered, there is still the probability that some of the drug may leak out during the test. This may not take place through the point of injection but the fact that the injected fluid distends the tissues, which are readily permeable to fluids, furnishes the basis of this criticism of the one-hour frog method.

The Factor of Length of the Test Period.—In regard to the time factor, it may be said that this can doubtless be made practically a constant if due care is exercised. A few minutes plus or minus one hour frequently have been shown to be highly important, a minus result, *i. e.*, frog heart still beating or in diastole at the end of one hour, being changed to plus result, *i. e.*, systolic standstill of frog heart,

TABLE VI.—SHOWING VARIATION IN WEIGHTS OF FROGS PREVIOUSLY KEPT IN STORAGE TANK 2 DAYS.

Frog No.	Original weight in Gm.	Weight after 1½ hrs. in 21° C. bath.	Weight after 3 hrs. in 21° C. bath.	Weight after 24 hrs. in 21° C. bath.	Change in weight at end of		
					1½ hrs.	3 hrs.	24 hrs.
1	34.5	33.1	32.8	32.0	—1.4	—1.7	—2.5
2	19.3	18.2	18.6	18.3	—1.1	—0.7	—1.0
3	23.8	23.5	23.5	23.0	—0.3	—0.3	—0.8
4	20.8	19.7	19.7	19.1	—1.1	—1.1	—1.7
5	40.9	40.3	40.0	36.1	—0.6	—0.9	—4.8
6	36.9	36.2	36.2	30.8	—0.7	—0.7	—6.1
7	45.7	44.7	45.1	41.3	—1.0	—0.6	—4.4
8	29.8	29.5	28.4	27.2	—0.3	—1.4	—2.6
9	28.8	28.1	28.1	26.8	—0.7	—0.7	—2.0
10	30.6	30.4	30.5	28.8	—0.2	—0.1	—1.8
11	28.8	26.9	26.9	25.2	—1.9	—1.9	—3.6
12	25.7	24.2	22.9	22.9	—1.5	—2.8	—2.8
13	32.6	32.6	32.5	31.0	0.0	—0.1	—1.6
14	18.7	18.2	18.4	17.7	—0.5	—0.3	—1.0
15	38.6	36.3	34.7	34.9	—2.3	—3.9	—3.7
16	27.7	26.0	25.2	25.5	—1.7	—2.5	—2.2
17	32.2	30.1	29.8	28.7	—2.1	—2.4	—3.5
18	15.2	15.0	14.7	14.6	—0.2	—0.5	—0.6
19	37.8	36.6	36.8	35.3	—1.2	—1.0	—2.5
20	32.7	32.5	32.5	28.3	—0.2	—0.2	—4.4
21	32.3	31.4	32.3	29.5	—0.9	0.0	—2.8
Average,					—0.995	—1.14	—2.69

a few minutes after the hour. It has been noted many times in the one-hour frog assay that a positive at the end of exactly one hour is changed in two minutes thereafter to a negative result. In the case of digitalis the administration of sublethal doses may have a paralytic action on the heart from which the frog may and does recover, hence this also accounts for some of the discrepancies in this method of assay.

The Importance of Temperature at Which Test Is Made.—The temperature at which the frogs are maintained during the experiment has been repeatedly shown to have an important bearing on the result.[1,3]

In most laboratories it is feasible to maintain the 20° C. bath recommended by the U. S. P. and the factor of the temperature at which the medicated frog is kept for one hour cannot be overestimated because dependent upon it in large measure is the rate of absorption and *vice versa*.

The Species of the Test Animal.—That the species of frogs used may affect the assay is generally admitted, but by using only one species of frogs, preferably *Rana Pipiens* as recommended by the U. S. P., this factor may also be rendered a constant.

Before taking into consideration the cat method of biochemic assay, other weak points of the frog assay may be mentioned.

My experience has shown that the average frog assay requires oft-repeated trial assays before the M. S. D. can be found. After reaching the approximate M. S. D. it is necessary to obtain the minimum dose which gives two positives

out of three, or better three out of five. What renders the preliminary assays so uncertain at times in their value for subsequent tests, is the fact that frogs very often show systolic standstill "out of order." For example, a preliminary series of three frogs at doses .006, .009 and .012 mil per Gm. body weight, respectively, gives results, —, +, +. Instead of a M. S. D. between .006 and .009 as indicated by the first series, subsequent trials show the M. S. D. around .012 or even above. In other words, frog with dose .009 and heart in systole was "out of order."

Another example frequently found in practice is a preliminary assay, where doses of .000,000,5, .000,000,7, .000,000,9 and .000,001,1 Gm. ouabain per Gm. body weight of frog give —, —, + and + results, respectively. This trial assay indicates a M. S. D. of between .000,000,7 and .000,000,9, but the completed assay indicates a M. S. D. of .000,000,7, or even less. The result is that the preliminary tests are often entirely misleading, a circumstance which often calls for a greatly increased number of trial assays.

The above considerations lead to the question of relative costs of the two methods. It has been my experience that a frog assay costs on the average $1.15 for animals compared with $0.70 for a cat assay. No difficulty has been experienced in obtaining test animals, either cats or frogs, as has been reported by some writers.

Finally, the following very important fact must be mentioned, namely, that the end point in one-hour frog-heart assay is many times far from satisfactory, and its interpretation one way or the other depends in large measure on the operator.

It has been my observation that the end point for the one-hour frog is frequently very indefinite as the heart does not stop in well-defined systole, in other words, there is a varying degree of paralysis of the heart which is interpreted according to the operators' judgment.

II. *Biologic Standardization—By the Cat Method.*—The cat method employed in this laboratory is a modification of the Hatcher procedure.[4] The suitably diluted sample is slowly injected into the femoral vein until the death of the animal with typical digitalis poisoning occurs. A solution of ouabain is not used, as nothing is gained thereby, either in time or in the accuracy of the test. As to my preference of the cat method over the one-hour method, the following may be pointed out:

The M. L. D. method for the physiologic standardization of the digitalis series using the cat as the test animal has, in the writer's opinion, many advantages for the following reasons:

(1) A shorter time is required for making an average assay, *viz.*, two hours (cat) as compared to three hours (frog).

(2) Cost of animals cost per assay less, $0.70 *vs.* $1.15.

(3) Assay capable of greater absolute as well as relative accuracy, since

(*a*) Cat assays are not affected by seasonal variation in test animal which is clearly shown in Tables I and X.

(*b*) Age or sex of T. A.: immaterial, as shown in the same tables and also in Table VII.

(*c*) Definite end point, *i. e.*, death.

(*d*) Quantity of drug injected is accurately measured.

(*e*) Calculation of activity or strength is not dependent upon a secondary assay (ouabain) which would tend to lower the degree of accuracy by introducing more variables.

(4) According to the U. S. P. the application of the digitalis series in therapeutics is proportional to its toxicity.

Time Necessary to Complete an Assay by Cat Method.—Compared with the one-hour frog, a much shorter time is needed to make an average assay. The assay can be completed in two hours, and usually the first two cats will be sufficient to obtain concordant results, and it is seldom necessary to use three animals.

Relative Cost of Two Methods.—The average cost of the two methods of assay has already been stated and is self-explanatory. This result is not, however, in agreement with the findings of some laboratories, as, for example, the Hygienic Laboratory.[5]

REASONS WHY CAT ASSAY IS MORE ACCURATE AND DEPENDABLE.

(*a*) In regard to the reaction of the cat to the active principles of the digitalis series, it has been my observation that it does not change from season to season, a fact borne out by data in Tables I and X. In this respect it seems to be an entirely satisfactory test animal and is very constant in its susceptibilities to the action of the digitalis series. The cats which have been used for standardization tests in this laboratory have been typical of the common stray cats of any large city which would ordinarily in time be destroyed by the duly authorized city officials. It has been my experience that such cats are seldom diseased.

(*b*) Age and sex of the cat are immaterial according to my experience. Results obtained by using female cats of 1.5 kg. body weight will check those of male cats weighing 3.0 kg.

(*c*) Cats can be weighed much more accurately than frogs and, furthermore, they do not change weight, at least during the period of the assay, as is the case with frogs. Roughly, the average error in weighing cats is ±0.5 percent and for frogs it amounts to ±2.0 percent. No leakage of the test solution during injection into the femoral vein of the cat is possible and since the total amount of drug injected is accurately measured by a burette, the calculation of the physiologic strength of the sample can approach in accuracy that of a quantitative chemical analysis.

Applicability of Cat Method to Biologic Standardization of Heart Tonics.—The cat assay method, while inherently subject to a certain limit of accuracy due to the fact that all physiologic standardizations are reactions between active chemical principles and healthy living tissues, has practically no variables to deal with, such as temperature, time or rate of absorption, etc., and hence in my opinion the percentage error is much less than in the assay recommended by the U. S. P. IX. The cat method of assay, as in all applications of biologic reactions to the purposes of quantitative standardization, requires that at least two out of three tests on the same sample shall be concordant. This is necessary because of the fact that individuals of the same species, whether man or the lower animals, show differences in their reaction to drugs which may be both qualitative and quantitative. The differences are generally quantitative and hence in all biologic assays one must obtain a majority number of concordant results in each series of tests. In this way the peculiar or exceptional reactions of a drug upon the test animal, *i. e.*, its idiosyncrasy, can be taken care of.

The argument is often made by those unfamiliar with physiologic testing, that a biologic test on cats, etc., does not furnish a reliable guide to the action or activity of a drug so tested, when it is applied to man. It is probably true that what constitutes a therapeutic or toxic dose of digitalis for a cat would not proportionally be the same for a man, but it is true that in both instances the typical actions of a potent digitalis preparation would be present.

The real purpose of biologic testing is to measure the strength or activity of a preparation, so that it may be adjusted to a uniform or standard strength,

i. e., standardized. The method of assay which accomplishes this object with the greatest degree of accuracy is the logical one to employ. The value of such a standardized product to the practicing physician is obvious.

The U. S. P. states that preparations of digitalis after being assayed by the "One-Hour Frog" (a toxic method) should be corrected so as to conform to the standard adopted. In my experience, the cat method, which also belongs to the toxic type of assay, has been more satisfactory in all respects for purposes of estimating or standardizing. Because in the cat method fewer variables enter to lower the limit of accuracy, the ratio between the toxicity of samples and their therapeutic efficiency must be more constant when determined by this method.

In this connection the following tables giving the results of biologic assays upon a series of digitalis samples will be of interest.

TABLE VII.—A COMPARISON OF BIOCHEMIC ASSAYS ON SAMPLES OF TINCTURE OF DIGITALIS BY THE CAT AND "ONE-HOUR FROG" METHODS.

Sample No.	One-Hour Frog.			M. L. D. for Cat.			
	M. S. D. of ouabain Gm./Gm.	M. S. D. of sample mils/Gm.	Percent strength by U. S. P. IX.	Wt. (kilos) and sex.	Mils per kilo.	Percent strength (100 mgs. lvs = 100%).	Ratio of percentage strengths Cat: Frog.
1	.000,000,8	.007	137.0	3.11*	1.156	1.123	89.0	1 : 1.540
				2.57*	1.090			
2	.000,001,0	.020	60.0	3.41†	1.114	1.124	89.0	1 : 0.67
				2.91*	1.139			
3	.000,000,9	.011	98.2	2.20*	1.18	1.18	84.8	1 : 1.158
				2.88*	1.18			
4	.000,000,8	.009	106.5	3.52†	1.193	1.184	84.5	1 : 1.260
				3.40†	1.176			
5	.000,001,2	.010	144.0	3.66†	1.147	1.217	82.2	1 : 1.753
				2.17*	1.290			
6	.000,001,0	.010	120.0	2.86†	1.05	1.07	93.5	1 : 1.284
				3.21†	1.09			
7	.000,000,8	.0065	147.5	2.88†	0.903	0.934	107.1	1 : 1.376
				3.11†	0.965			
8	.000,000,7	.0080	105.	1.70*	0.882	0.859	116.4	1 : 0.93
	.000,000,6	.0065	110.8	2.63*	0.837			
			107.9					
9	.000,000,7	.0070	120.	2.77*	0.975	0.975	102.5	1 : 0.85
	.000,000,6	.0065	110.6	3.89†	0.976			
	.000,000,7	.0065	129.2	2.94*				
			119.7					
10	.000,000,9	.007	154.3	2.98*	0.885	0.912	109.6	1 : 1.495
					0.940			
11	.000,000,9	.006	180.0	3.85†	0.727	0.727	137.8	1 : 1.305
				3.17*	0.726			
12	.000,000,9	.010	108.0	4.29†	1.024	1.03	97.1	1 : 1.112
				3.35†	1.372			
				2.41*	1.036			

NOTES:

The average ouabain M. S. D. obtained from above table is .000,000,83.

The average ratio of percentage strength equals Cat : Frog = 1 : 1.228.

† = Male. * = Female.

TABLE VIII.—REARRANGEMENT OF TABLE VII IN ORDER OF PERCENTAGE STRENGTHS AS DETERMINED BY EACH ASSAY METHOD.

Sample No.	Percentage strength by frog assay.	Sample No.	Percentage strength by cat assay.
11*	180.0	11*	137.8
10	154.3	8	116.4
7	147.5	10	109.6
5	144.0	7	107.1
1	137.0	9	102.5
6	120.0	12	97.1
9	119.7	6	93.5
12	108.0	1	89.0
8	107.9	2	89.0
4	106.5	3	84.8
3	98.2	4	84.5
2	60.0	5	82.2

*It will be seen from the above table that in only one instance, namely, sample of digitalis No. 11, are the samples arranged in the same order of strength by both methods of assay.

Table VII shows that excellent check results can be obtained on the same sample regardless of the weight and sex of the cat. This fact is also brought out by data in Table I.

TABLE IX.—OUABAIN.
Biologic Assays.

Date.	One-Hour Frog M. S. D.	Date.	One-Hour Frog M. S. D.
9/26/16	.000,000,5	3/27/17	.000,000,8
10/19/16	.000,000,6	3/30/17	.000,001,1
10/24/16	.000,000,6	3/31/17	.000,000,8
11/1/16	.000,000,5	3/17/17	.000,000,7
11/2/16	.000,000,5	3/22/17	.000,000,8
11/6/16	.000,000,65	4/3/17	.000,001,1
11/21/16	.000,000,8	4/7/17	.000,000,8
11/23/16	.000,000,55	4/10/17	.000,000,8
12/1/16	.000,000,8	4/14/17	.000,000,85
12/18/16	.000,000,85	4/23/17	.000,000,6
1/5/17	.000,000,7	4/24/17	.000,000,55
1/6/17	.000,000,6	4/26/17	.000,000,8
1/9/17	.000,000,7	5/4/17	.000,001,1
1/10/17	.000,000,7	5/10/17	.000,001,0
1/15/17	.000,001,2	5/12/17	.000,001,2
1/19/17	.000,000,6	5/17/17	.000,001,2
1/22/17	.000,000,55	5/17/17	.000,001,3
1/23/17	.000,000,7	5/18/17	.000,000,6
1/25/17	.000,000,65	5/21/17	.000,001,0
1/29/17	.000,000,6	5/22/17	.000,001,1
2/12/17	.000,000,8	5/24/17	.000,000,9
2/14/17	.000,000,85	5/25/17	.000,000,9
2/21/17	.000,000,7	5/28/17	.000,000,9
3/13/17	.000,001,1	6/4/17	.000,000,8
3/15/17	.000,001,1	6/7/17	.000,000,9
3/19/17	.000,000,8	6/12/17	.000,000,7
3/19/17	.000,000,7	6/13/17	.000,001,0
3/26/17	.000,001,1	6/21/17	.000,001,0

The preceding table, IX, gives the results of assay of ouabain by the one-hour frog method extending over the last nine months. The average of the 56 assays is .000,000,82 Gm. ouabain per Gm. body weight of frogs as against the .000,000,5 Gm. standard given by the U. S. P.

The lowest M. S. D. (minimum systolic dose) was .000,000,5 and the highest .000,001,3 Gm. ouabain per Gm. body weight of frog, thus showing a variation of 160 percent. The average of all results over the nine-month period using the same stock sample of ouabain, but many different lots of frogs, is .000,000,82 Gm. ouabain per Gm. body weight. This is much larger than the standard used in the "one-hour method" specified by the Pharmacopoeia, but agrees very closely with that obtained by Rowe,[2] namely, .000,000,86 Gm. per Gm.

A maximum variation in toxicity of ouabain of only 30 per cent is obtained when cats are used as the test animal as may be seen from Table X.

TABLE X.—BIOLOGIC ASSAY OF OUABAIN.
Test Animal—Cat.

Date of assay.	Sex and wt. (kilos) of T. A.	Value of sol. injected.	Lethal Dose for Test Animal (mils).	L. D. per kilo (mils).	L. D. expressed as Gm. ouabin per kilo.
11/28/16	†2.96	1 mil = .000,01	34.5	11.66	.000,116,6
11/28/16	†2.52	1 mil = .000,01	33.0	13.10	.000,131,0
12/13/16	*2.45	1 mil = .000,01	35.0	14.29	.000,142,9
12/14/16	*1.80	1 mil = .000,01	21.0	11.66	.000,116,6
12/21/16	†3.10	1 mil = .000,01	40.0	12.90	.000,129,0
4/4/17	†2.67	1 mil = .000,02	19.0	7.12	.000,142,4
4/4/17	*2.34	1 mil = .000,02	15.0	6.72	.000,134,4
4/6/17	†3.23	1 mil = .000,02	18.0	5.57	.000,111,4
8/1/17	†2.19	1 mil = .000,01	29.0	13.22	.000,132,2
8/1/17	†4.15	1 mil = .000,02	30.0	7.23	.000,144,5
8/8/17	*2.85	1 mil = .000,01	36.0	12.63	.000,126,3

† = Male. * = Female.

NOTE: Ouabain solution made up September 7, 1916.

1 mil = .001 Gm. Ouabain.

The above table shows that the minimum lethal dose varies from .000,111,4 Gm. to .000,144,5 Gm. per kilo cat, or, in other words, a variation of 29 percent as compared with 160 percent obtained by assaying ouabain with the frog as the test animal. It may readily be seen from a comparison of Tables IX and X that the cat method eliminates the factors of time, temperature at which drug is administered, age and sex of the animal.

The frog assays, on the other hand, are vitally affected by the factors of time and temperature, which in turn have an important relation to the absorption rate of the test solution.

The conclusion, offered by Edmunds and Hale, that in most cases the toxic action is not on the heart but on the respiratory centers, hence, "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," is not corroborated by my experience. In practically every assay by the cat method which I have made the toxic action is not upon the respiratory centers, but upon the heart. This is shown by the fact that after the heart has stopped with typical digitalis poisoning the respiration continues for a number of minutes.

It is not the purpose of this paper to discuss the twelve-hour frog method at any length, chiefly because the experience of the writer with this method does not permit of any authoritative conclusions regarding it. Logically a larger dose should be required to cause systolic stoppage of the heart of the frog in one hour than is necessary to cause death in twelve hours. Of the heart tonic series, only strophanthus and squill comply with this. In the case of digitalis and convallaria the dose is less by the one-hour than by the twelve-hour method. Of the two frog assay methods, the twelve-hour is the writer's preference, since it takes into account the difference in the rate of absorption, and allows time for the reaction to be completed, and finally the end point (death) is absolutely unmistakable.

According to the experience of this laboratory with the twelve-hour method, more constant results are obtained than with the one-hour frog. This is especially noted in the tests on ouabain where the M. L. D. is always close to .000,000,45 Gm. per Gm. body weight, a fact which has already been verified by other investigators.[2]

Conclusions.—Conclusions relative to the two methods of biologic assay, which have been discussed in this paper, are for emphasis and conciseness tabulated as follows:

SUMMARIZING COMPARISON OF FACTORS IN BIOLOGIC ASSAYS BY THE ONE-HOUR FROG AND CAT METHODS OF ASSAYING THE HEART TONICS.

Variable or Constant affecting the result of assay.		Effect, if any, upon the	
		One-hour frog assay.	M. L. D. cat assay.
Variable	(1) Concentration of the test solution	Has important effect	Not especially important and negligible
	(2) Absorption rate	Has important effect	Not important and negligible
	(3) Seasonal variation	May or may not be important	Not important and negligible
	(4) Accuracy of administering dose	Low degree of accuracy, hence important effect	Accuracy high, therefore negligible factor
	(5) Sex and weight of test animal	Does not affect assay	Does not affect assay
	(6) So-called "standard" ouabain	Ouabain from different sources variable; important effect	No secondary "standard" used
Constants	(1) Length of test period	Effect vital to result	Time factor does not enter
	(2) Temperature at which drug acts	Effect is very important	Temperature factor does not enter
	(3) Species of test animal	Has been shown to be important by other workers	Species is not a factor

The above table summarizes the basis for the author's conclusion arrived at after extensive use of both assay methods, namely, that the cat method is capable of greater absolute as well as relative accuracy in biologic assays of the digitalis

series. It has also been pointed out in this paper that the considerations of relative cost, definiteness of the end point and time necessary for making an assay are all in favor of the M. L. D. cat method.

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BIOLOGICAL LABORATORIES OF SHARP AND DOHME,
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THE CHEMICAL INVESTIGATION OF THE RESINS FROM DATURA METELLOIDES.*

BY CHARLES H. ROGERS.

Comparative amounts of alkaloids, glucosides, neutral principles and other medicinally active plant constituents have to a large degree been the bases upon which the value of vegetable drugs or drug preparations have been established. Tschirch and others have pointed out that vegetable drugs are far from simples as indicated by Galen, but rather that they are exceedingly complex. Pharmacological investigation has shown that the action of isolated principles does not always correspond with the action of the drug itself. Therefore, the pharmaceutical chemist and pharmacologist must pay more attention to the complex ingredients of drugs, or, as they are usually called, "extractives." The term "extractives" used in this connection refers to those supposedly inert materials that are soluble in the menstruum used to dissolve the more important constituents of the drug. Before it is possible to determine the part that these extractives play in the production of the total or composite action of drugs it is necessary to have a clearer chemical and pharmacological understanding of them *per se*. Not only is it of importance to understand their chemistry and pharmacology but it is also necessary that their physical constants in respect to temperature, their susceptibility to enzymic action, etc., must be studied.

It has been shown[1] that preparations made from digitalis which has been dried at a uniformly high temperature (70–90° C.) has an absorption value from 25 to 30 percent greater than preparations made from the commercially prepared article. Knowing that the active constituents of this drug are not vitally affected by ordinary temperatures, it is reasonable to conclude that the extractives have in some way been injured by careless manipulation, and, furthermore, that their alteration may have a decided deleterious effect on the rate of absorption of preparations made from the drug. These same conclusions may have a very important bearing on other vegetable drugs. With these fundamental facts in view, the chemical investigation of the resins (extractives) of *Datura metelloides* was undertaken.

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*Read before meeting of Northwestern Branch A. Ph. A., December 5, 1917.

[1] E. L. Newcomb, *St. Paul Medical Journal*, August 1914.