10%. Using "pure principles" Hale has found the one hour method fully as accurate as this. In our own laboratory we have made our work, carried out independently, check within 10%.

There can be no question as to the economy of the different methods. Frogs for an assay cost us about 50 cents. Guinea pigs would cost us about $4.00. Cats could not be secured in Indianapolis in sufficient numbers for our use.

As regards simplicity, there is little to choose between Houghton's and Cushny's method. The guinea pig can not be handled by one man; while Hatcher's method is quite complicated.

The one hour frog method enables us to complete an assay in, at most, three hours. Houghton's method requires at least 24 hours, as does the guinea pig method. The actual time needed to run one cat, according to Hatcher's method, is 90 minutes. If, as seems necessary, three animals are used, the whole day is taken up, the preparation of the animals requiring some time.

Accuracy, cheapness, simplicity, speed. It would seem that in none of these points is Cushny's method excelled. Houghton's method is more time consuming, and it is conceivable that it may give erroneous results when other poisons besides the active glucosides are present in large amount.

It seems that the frog heart method is the only one that has been controlled clinically. Pratt, in this country, has shown how the therapeutic efficiency of digitalis leaves varied as did their strength as determined by this method. Focke, also, mentions similar comparisons. The worth of digipuratum, which is standardized by a modification of Cushny's method, has been shown by many clinical tests.

In conclusion, it may be said that in the one hour frog heart method is offered a means of standardizing digitalis which compares favorably with chemical assay methods when the test is carried out with due precautions by trained men.

It would probably be unwise to adopt as official any of the methods now used for the pharmacological assay of aconite, cannabis indica, or ergot. Further study is needed before it can be determined which are most suitable, but in the meantime it is very desirable that manufacturers use these methods, thereby insuring more nearly uniform preparations and also acquiring valuable data upon the methods used.

ELI LILLY & CO. PHARMACOLOGICAL LABORATORY, July 9, 1911.

VARIATION IN THE SUSCEPTIBILITY OF THE GUINEA PIG TO THE HEART TONIC GROUP.

CHAS. E. VANDERKLEED.

Pharmacologists are divided in their opinion as to the best method for determining the strength of preparations of the digitalis series by biologic means. Many papers have appeared during the last few years advocating the use of this or of that method, but a careful review of the literature shows that, in the opinion of

the majority of the workers, the question narrows down to a choice between one of the frog methods and the guinea pig method of Reed and Vanderkleed. Hatcher's proposed cat method has apparently gained no additional supporters, undoubtedly because of the complexity of its technique.

It is not the purpose of this short communication to discuss all of the many phases and problems of biologic standardization. Attention is called, however, to the fact that the frog method and the guinea pig method are both toxic or lethal does methods, and hence, to this extent at least, are amenable to comparison. The question of the effect of the heart tonic drugs on the respiration, in the case of guinea pigs, has been offered as one of the objections to the employment of these animals for the biologic assay of these drugs. This problem has been the subject of an extensive series of experiments during the past summer by Dr. L. T. De M. Sajous, consulting pharmacologist of the H. K. Mulford Company, who will report on this subject during the course of the next few months. He has authorized me to say, however, that in the course of his work, by means of artificial respiration, he was able at most only to prolong the life of a guinea pig to which had been administered a minimum lethal dose of tincture of digitalis for from 30 to 40 minutes. Such being the case, he believes that the effects of digitalis on the respiration in the case of guinea pigs does not materially affect the results obtained by the lethal dose method.

The most important contrast between frogs and guinea pigs as test animals lies in the claim by advocates of the latter that the susceptibility of the guinea pig, unlike that of the frog, does not vary or does not vary so greatly with climate, temperature, food, season, weight and sex. That frogs do so vary is admitted by the advocates for their employment, as shown by the suggestion by Houghton that crystallized strophanthin be employed as a standard for checking the susceptibility of each lot of frogs employed in the standardization of a preparation of unknown strength. (See also Hygienic Laboratory Bulletins Nos. 48 and 74, by Edmunds and Hale.) On the other hand, Haskell[1] has recently claimed that the advocates of the guinea pig method have only half-heartedly claimed that guinea pigs do not show the same variations. Thus he quotes Reed as saying that the guinea pig "does not *appear* to offer so wide a variation"; Githins as saying that the guinea pig "*shows* no such variation"; and the Philadelphia committee on pharmacologic assay as stating that the susceptibility of guinea pigs to digitalis does not vary under ordinary conditions, "*so far as is known.*" The effect of Haskell's quotations is to create the impression that these advocates of the guinea pig as a test animal were not all *convinced* of the superiority of the guinea pig over the frog in this respect, and he goes on to show the possibility of a great variation in the susceptibility of guinea pigs, *to digitalis,* by mentioning an article by Arms[2] entitled "Some Freak Results from Animal Inoculation," in which that author reported on the effects of inoculations of guinea pigs with glanders and with emulsion of nervous tissue from rabid dogs! The irrelevancy of such experiments to the question at issue only

[1]American Journal of Pharmacy, May, 1911, p. 201.
[2]Journal of Public Hygiene, XIX No.3.

seems to indicate an *a priori* prejudice against the employment of the guinea pig. Haskell's further observation that the advocates of the guinea pig method have put forth unusual efforts to discover defects from the unfitness of the frog, seems to be paralleled by his implied unfitness of the former animal.

Taking up the objections to lethal dose methods in general, Haskell further states that "the active glucosides of digitalis may become decomposed into such bodies as digitalresin and toxiresin, which, resembling picrotoxin, have a depressant action on the heart, and a preparation containing a large amount of such decomposition products, while testing high by lethal dose methods, might not only be below standard, but capable of causing dangerous poisoning." In support of this possibility he quotes Edmunds and Hale in their Bulletin No. 48 of the U. S. Public Health and Marine Hospital Service, as follows: "One solution might be very weak in its action upon the heart and yet contain decomposition products of digitalis whose typical action is upon the medulla, and it would, therefore, appear unduly strong when judged by such a standard. For this reason, we think that methods which employ as a standard the minimum lethal dose upon the higher animals are not applicable to the physiological assay of the digitalis series."

In this bulletin, however, these authors offer no evidence to show that such a condition ever obtains; on the contrary, a study of their experiments shows that they observed cases in which preparations containing large amounts of decomposition products and producing but a small or even negative rise in blood pressure, were administered in doses four times as great as the minimum lethal dose of an active preparation without causing any symptoms whatever in guinea pigs, and they observed other cases in which such preparations were injected in doses eleven times as great as the minimum lethal dose of an active preparation without causing death. This objection to lethal dose methods, therefore, does not seem to be sustained, or at least remains to be proved. Moreover, if the minimum lethal dose method be checked by a chemical assay for digitoxin, an additional safeguard against the possibility of wrong interpretation of the physiological results is provided.

Haskell, however, goes on to say, "Doubtless, numerous investigations have been carried out to show that guinea pigs do not vary in their resistance to digitalis intoxication, but I have been unable to find the report of a single series of experiments performed with the object of showing that guinea pigs are not fully as much influenced by adventitious circumstances as are frogs." This, being a perfectly rational and legitimate challenge, I shall endeavor to answer it, first, from a review of records of some hundreds of experiments startel in July, 1911, and so planned as to cover one complete revolution of the seasons. The complete report of this series of experiments can, of course, only be given twelve months hence—but some preliminary data have already been collected and may be of interest here.

Reverting to the records of guinea pig injections above referred to, I would state that the conclusions as guardedly expressed, and properly so as becomes scientific investigators, by Reed, Githens, and the Philadelphia Committee, were based upon the fact that, in the course of hundreds of injections, *apparent*

variations in susceptibility were so few as to be, on the whole, negligible. In these experiments, guinea pigs bred and raised by no less than a dozen different breeders were employed. The pigs, once aggregated from these various sources, were, of course, subjected to approximately the same general conditions, but no unusual means of preserving uniformity were employed. Seasonable food was given them, principally oats and hay, together with greens, such as lettuce, carrot tops, corn stalks, cabbage, etc., in season. The temperature change to which they were subjected was that of Philadelphia, which, as is well known, is a considerable one. In Winter, the general guinea pig quarters are heated to 65 or 70° F., while the rooms into which they are transferred during the time of testing are heated to about 75° F. Thus, no *particular* attention is paid to the question of source, food, or temperature, nor, in the hundreds of injections made in our laboratories, are any selections made as to sex. The weight of the individual animals employed has ranged from 225 to 500 gm.—the dose given being always calculated on the basis of 250 gm. weight. In spite of the lack of attempting to systematize the conditions under which the animals are kept, and tested, the precentage of non-concordant results obtained has been well within 5%. By this is meant that, in finding the minimum lethal dose of any preparation, down to a variation of 10%, and in most cases much less than 10%, a series of pigs, taken at random, and given injections of progressively larger doses, *all* receiving a certain dose or more will die, and *all* receiving a smaller dose will recover. A second smaller series is always injected to check the results of the first series, and, as stated above, not five pigs in one hundred have been found to die with a smaller dose than that found as the m.l.d. in the first series, or to recover when given the same or a larger dose—the doses being increased successively in tenths.

It was upon this evidence that the guarded opinions expressed by Reed, Githens and the Philadelphia Committee were based. In addition to the above variations, another variation not heretofore brought out has been noted. As is well known, the guinea pig is the official test animal employed in the standardization of sera such as diphtheria antitoxin. That its use for this purpose leads to unquestioned uniformity of product is universally acknowledged, and officially sanctioned by the U. S. P. H. and M. H. Service. In the course of standardizing sera, large numbers of pigs survive, but can not be used again for testing sera. The question naturally arose as to whether such pigs could be used for the standardization of the heart tonics. Series of such pigs have been repeatedly used along with previously unused pigs and no change in the susceptibility to digitalis and the other heart tonics noted. It is only essential that they may be in good physical condition and fully recovered from the physical injury inflicted by the prior injections of toxins and antitoxin.

Taking up now the experiments started in July, I would state that the principal advantage of the guinea pig over the frog lies in the claimed non-necessity for employing and keeping on hand a "standard" against which the susceptibility of the animals must be checked. If this advantage can not be sustained, the guinea pig method loses one of its more important claims to superiority, although it possesses some other advantages over the frog which in turn are met with

certain minor disadvantages, such, for example, as that of cost. Confining ourselves, however, to the main question at issue, I will outline the nature of the experiment being conducted, and give a summary of results so far obtained.

The experiment has been undertaken to show what effect, if any, season (and, incidentally, temperature), food, weight and sex has upon the susceptibility of the guinea pig to digitalis intoxication. Recognizing the difficulty and uncertainty of keeping a standard digitalis absolutely unchanged throughout one year (and any whatever would, of course, nullify the value of the experiment), I have adopted as the standard preparation to be employed, crystallized ouabain, which has been selected by the advocates of the frog methods for the purpose of standardizing their test animals.

The experimental pigs have been divided first into two classes, as regards sex—male and female. Each of these classes has been further subdivided into two classes as regards weight—those ranging from 225 to 275 gm., and those ranging from 350 to 500 gm.

Each of these sub-classes was at first further subdivided into two classes as regards food—one class receiving for two weeks prior to the test, nothing but oats—the other class receiving during the same time nothing but greens. It was soon discovered, however, that the pigs receiving nothing but greens easily succumbed to the unusually torrid weather which prevailed in Philadelphia and in many other parts of the country during July. Greens alone appeared to possess an insufficient amount of nourishment to maintain the animals in healthy physical condition—several deaths occurring in the cages.

The differentiation as regards food was, therefore, discontinued, the fact having been proved to us that test pigs must be fed upon grain (oats) as well as upon greens in season, and that the grain is the more important. This fact, however, does not in itself discredit the guinea pig as a test animal, since we are limited very much in any case in the variety of foods which this animal will eat.

A further important observation was made during this exceedingly hot month of July. We discovered that a factor of more importance than temperature on the health of the guinea pigs is ventilation—fresh air. Our main supply of pigs is kept under conditions already described in the country. For the purpose of making these and other tests, the pigs are brought into the city, where the problem of housing and ventilation is a more difficult one. During the July fourth vacation several deaths occurred in the cages, particularly among the pigs fed on greens, and it was found that these were in fact caused by the partial lowering of the windows in their quarters by the attendant during this period, as a precaution against fire from rockets, etc. However, all this only goes to show what all pharmacologists concede, that in any biologic assay whatever, normal, healthy test animals are the first requisite.

The seasonal variations will, of course, be shown by any differences in results noted during the year. Tests are to be made and a new series of pigs in each of the four classes selected for the tests each month.

Thus, at the end of the year, we shall have 12 sets of experiments showing the m.l.d. or resistance to crystallized ouabain, of 4 different kinds of guinea pigs, or 48 tests, covering an entire year's variation in season and, to a certain

extent, temperature. Moreover, if found practicable, we shall have from time to time lots of pigs shipped directly to us from various sections of the country—thus introducing the factor of climate.

Up to the present time, only one set of tests has been made, the results obtained being shown in the following tables. The doses given are in grams per 250 grams body weight:

Small Males, Weighing 140 to 210 gm.

Dose	Weight	Result
0.000040	195	— Recovered
0.000044	200	— Recovered
0.000047	175	— Recovered
0.000050	155	— Recovered
0.000050	210	— Recovered
x 0.0000525	205	+ Died
0.000055	195	+ Died
0.0000575	190	+ Died
0.000060	140	+ Died
0.000069	170	+ Died
0.000072	185	+ Died

M. L. D. = 0.0000525.

Large Males, weighing 270 to 410 gm.

Dose	Weight	Result
0.0000375	285	— Recovered
0.0000400	310	— Recovered
0.0000440	410	— Recovered
0.0000470	320	— Recovered
0.0000470	305	+ Died
0.0000500	270	— Recovered
0.0000500	370	— Recovered
x 0.0000525	315	+ Died
0.0000550	310	+ Died
0.0000600	345	+ Died

M. L. D. = 0.0000525.

Small Females, weighing 160 to 210 gm.

Dose	Weight	Result
0.00004	170	— Recovered
0.000044	210	— Recovered
0.000044	190	— Recovered
0.000047	160	— Recovered
0.000047	170	— Recovered
0.00005	180	— Recovered
0.00005	180	+ Died
x 0.0000525	195	+ Died
0.000055	175	+ Died
0.0000575	180	+ Died
0.00006	160	+ Died

M. L. D. = 0.0000525.

Large Females, weighing 260 to 350 gm.

Dose	Weight	Result
0.0000375	260	— Recovered
0.00004	265	— Recovered
0.00004	335	— Recovered
0.000044	295	— Recovered
0.00044	350	— Recovered
0.000047	260	— Recovered
0.000047	295	+ Died
x 0.00005	350	+ Died
0.00005	285	+ Died
0.0000525	300	+ Died
0.00006	275	+ Died

M. L. D. = 0.0000500.

Thus, it may be seen that male and female pigs ranging in weight from 140 to 410 gm. have shown a minimum lethal dose of about 0.0000525 per 250 gm. body weight, in the first month's tests. Out of 43 pigs in the series only one (the large male which was killed by 0.000047 gm. per 250 gm. body weight, while two other pigs receiving 0.00005 gm. per 250 gm. body weight recovered) died "out of order."

The M. L. D. for small females was considered to be 0.0000525, because, of two pigs receiving 0.00005 gm., one died and one recovered. The M. L. D. for large females was considered to be 0.00005 because, of two pigs receiving 0.000047 gm., one died and one recovered.

The variation in results obtained from month to month will in due season be published, and I trust that they may go far toward establishing the degree of variation in the susceptibility of these little animals to the heart tonic drugs which is to be expected.

As a matter of possible interest, the minimum lethal dose of the ouabain used in the guinea-pig experiments was determined by Houghton's "one-hour" method for three classes of frogs as follows:

MALE LEOPARD FROGS (RANA PIPIENS) FROM ILLINOIS.

Weights ranged from 38.5 to 57.5 gm. Temperature of water in frog tank 26.5 to 29.5° C. Temperature of room 25.5 to 28.5° C. The doses given are in grams per gram body weight.

Dose	Weight	Result
0.000,000,30	42.0	— Beats.
0.000,000,31	40.0	— Occasional Beat.
x 0.000,000,32	45.5	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,32	55.0	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,32	56.5	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,32	57.5	+ Stopped. Extra Contraction on Stimulation.
0.000,000,33	42.5	— Non-absorption.
0.000,000,34	38.4	+ Stopped. No extra Contraction on Stimulation.
0.000,000,34	44.0	+ Stopped. No extra Contraction on Stimulation.
0.000,000,36	45.0	+ Stopped. No extra Contraction on Stimulation.
0.000,000,39	40.0	+ Stopped. No extra Contraction on Stimulation.

M. L D. considered to be 0.000,000,32.

FEMALE LEOPARD FROGS (RANA PIPIENS) FROM ILLINOIS.

Weights ranged from 30 to 62.3 gm. Temperature of water in frog tank 26.5 to 29.5° C. Temperature of room 25.5 to 28.5° C.

Dose	Weight	Result
0.000,000,36	40.0	— Beats.
0.000,000,36	34.0	+ Stopped.
0.000,000,37	34.0	— Slight beat in Auricle.
0.000,000,37	43.5	— Slight beat in Auricle.
0.000,000,37	36.0	— Beats.

0.000,000,38	37.5	— Beats.
0.000,000,38	34.0	— Beats.
x 0.000,000,38	30.0	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,38	37.5	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,38	62.3	+ Stopped. No extra Contraction on Stimulation.
0.000,000,39	50.5	— Beats.
0.000,000,39	66.6	— Auricles still Contracting.
0.000,000,39	37.0	+ Stopped. Extra Contraction on Stimulation.
0.000,000,39	46.0	+ Stopped. No extra Contraction on Stimulation.
0.000,000,39	48.0	+ Stopped. No extra Contraction on Stimulation.
0.000,000,40	40.0	+ Stopped. No extra Contraction on Stimulation.

M. L. D. considered to be 0.000,000,38.

FEMALE BULLFROGS (RANA CATESBIANA) FROM PENNSYLVANIA.

Weights ranged from 38.5 to 54 gm. Temperature of water in frog tank 24.5 to 26.5° C. Temperature of room 24 to 25.5° C.

Dose	Weight	Result
0.000,000,36	48.2	— Beats.
0.000,000,40	41.0	— Beats.
0.000,000,45	41.0	— Beats.
0.000,000,47	43.0	— Beats.
0.000,000,50	42.0	— Auricles still Contracting.
0.000,000,51	38.5	— Auricles still Contracting.
x 0.000,000,52	39.6	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,52	40.0	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,52	40.5	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,52	38.5	+ Stopped. Extra Contraction on Stimulation.
x 0.000,000,52	48.5	+ Stopped. Extra Contraction on Stimulation.
0.000,000,53	40.5	— Non-absorption.
0.000,000,53	48.0	+ Stopped. No extra Contraction on Stimulation.
0.000,000,53	54.0	+ Stopped. No extra Contraction on Stimulation.

M. L. D. considered to be 0.000,000,52.

It appears therefore that in the above experiments the minimum lethal dose for the three classes of frogs varied as follows:

Male Frogs (Rana pipiens) from Illinois.....	0.000,000,32
Female Frogs (Rana pipiens) from Illinois.....	0.000,000,38
Female Bull-frogs (Rana catesbiana) from Pennsylvania.....	0.000,000,52

or, the lethal dose for female frogs from Illinois was about 19% greater than for male frogs from the same locality, while the lethal dose for female bull-frogs from Pennsylvania was 62.5% greater.

In conclusion I wish to acknowledge my indebtedness to Dr. P. S. Pittenger and Mr. Leo Glickman for assistance in carrying out the experimental work.

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