EXPERIMENTS WITH THE CAT METHOD FOR TESTING DIGITALIS AND ITS ALLIES.

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There are at present four American methods in use for the physiological testing of the digitalis series, namely: The twelve-hour frog method, proposed by Houghton; the one-hour frog heart method, proposed by Famulener and Lyons; the guinea pig method, proposed by Reed and Vanderkleed, and the cat method, proposed by Hatcher and Brody.

In view of the large amount of work which is being carried out just now with these methods, with the hope that some one may be found sufficiently accurate and convenient to justify its insertion into the next Pharmacopœia, it seemed proper to report some results obtained with one of these—the cat method of Hatcher and Brody. They describe their method in the *American Journal of Pharmacy* for August, 1910, and state that it is an accurate one, and one that can be conveniently carried out by the retail pharmacist. The purpose of this study was to ascertain with what ease the method could be used, and what uniformity of results could be obtained. Since they have not given all the details of manipulation, I will describe the method as I used it, my endeavor being to carry it out in all respects just as they did.

Fully grown, apparently healthy cats were selected. In general these were stray cats of the city, and represented all common breeds and mixtures. They were accurately weighed, and then anaesthetized in the following manner: The animal was placed in a small box, just large enough to accommodate the body, with a small circular notch at the top of one end of a size which would just admit the neck. A cat in the box with the neck in place, the sliding lid was forced shut and held by a peg. Thus the animal was unable to withdraw its head. The anaesthetic was then given from a small copper cone carrying on a transverse screen a pad of cotton or gauze. A few drops of chloroform were placed on the cotton at the start in order to hasten this operation. As soon as the animal was unconscious this pad of cotton was replaced by another upon which only ether was dropped. The cat was then tied on an animal board (somewhat resembling the Harvard) with back down, legs outstretched, and head securely fastened in a holder. This board, supported on legs, was made so as to drain at a point near the lower (tail) end, under which a receiving vessel was placed. The animal in place, the femoral veins were dissected out and small glass cannulae inserted. The solutions were contained in burettes, the ouabain in one and the digitalis body in the other, and were conveyed to the cannulae by narrow catheter tubing. The injection extended, as near as could be arranged, over a period of ninety minutes.

THE OUABAIN SOLUTION.

Merck's crystalline ouabain was used. The weighing was done on an accurate chemical balance, and a stock solution 1:10,000 was made in a one-litre volumetric flask. For use, samples were drawn off with a pipette and diluted to the strength

1:100,000 in a narrow glass-stoppered 200 cc. cylinder. All dilutions were made with recently prepared physiological salt solution (0.75% NaCl). The stock solution was kept in a cool, dark cupboard, and in no case was used after two weeks old. The solution for use was made up as needed.

After running two preliminary experiments to become acquainted with the technique, a series of twenty-six experiments with ouabain was begun. The procedure and results were as follows:

The weight of the animal having been taken, the theoretical amount of solution required was calculated. Since the lethal dose of ouabain for cats, according to Dr. Hatcher, is .0001 gram per kilogram of body weight, the theoretical amount of solution would be the number of cubic centimeters required, for any given animal, to supply .0001 gm. per kgm. And since each cubic centimeter of a 1:100,000 solution would contain .00001 gm. a 3.2 kgm. cat, for example, would require 32 cc. Ninety minutes being the period of injection, the proportionate part of the theoretical amount necessary to be run in each minute or two minutes, was calculated. The operator seated at the table, continued the anaesthesia by placing a small pad of gauze over the nose and supplying only sufficient ether to just keep the animal quiet. The burette having been filled and the time noted, the injection was begun, running in slowly every minute or two minutes the amount proportioned. The cat was carefully watched particularly toward the end when the larger part of the theoretical amount had been injected. Death was usually preceded by very rapid respiration and decided convulsive movements after which the respiration ceased to be regular and was prolonged for a few minutes only by gasps. As soon as these symptoms of approaching death appeared, the injection was stopped. If, after waiting a few minutes, the animal did not die, the injection was continued very slowly and with great caution. When the respiration had ceased to be regular, the number of cubic centimeters of solution used and the time were noted, and before the gasping had entirely ceased the heart was exposed. In the majority of cases, rhythmic contractions of the heart had ceased. Sometimes the heart was in feeble delirum, but usually the left ventricle was still and the other chambers were feebly contracting. Out of twenty-six experiments with ouabain, sixteen with strophanthus, and twenty-seven with digitalis, only seven hearts were found beating rythmically, and in these the contractions were very feeble. Twelve hearts showed the left ventricle in quite complete systole. It should be remembered that regular respiration had ceased from one to three minutes previous to the exposure of these hearts. In one instance under ouabain, paraldehyde was used as the anaesthetic (1.8 cc. per kgm. Merck's).

Immediately upon appearance of the gasping, without opening the thorax, artificial respiration was instituted. The heart seemed to improve, and continued to beat until at the end of ten minutes one cubic centimeter more of the solution was slowly injected, when it stopped. With cat No. 26, artificial respiration was supplied all through the experiment, still the animal died within the ninety minutes, having received almost the exact theoretical amount. To accurately determine the effect of artificial respiration upon the lethal dose would, of course,

require a large number of experiments, an interesting point, but one I have not been able to work out for lack of time and animals.

OUABAIN.

Date	Cat. No.	Sex	Wt. in kgs.	Cc. of Sol.	Ouabain in gms. per kgm.	Time in Min.
12-16-10	1	Male	2.10	25.0	.000,119	99
12-18-10	2	Male	4.70	32.0	.000,068	70
12-21-10	3	Male	2.80	25.0	.000,089	71
12-22-10	4*	Male	2.00	15.0	.000,075	75
12-24-10	5	Female	1.10	11.5	.000,104	118
12-24-10	6	Male	1.80	16.8	.000,093	87
1- 4-11	7	Male	1.70	16.0	.000,094	93
1- 5-11	8	Male	2.70	16.0	.000,060	61
1-30-11	9	Female	1.19	11.2	.000,094	88
1-31-11	10	Male	2.11	22.1	.000,105	94
2- 1-11	11	Female	1.77	17.4	.000,098	95
2- 2-11	12	Female	2.33	19.6	.000,084	82
2- 2-11	13	Female	0.97	13.0	.000,134	104
2- 3-11	14	Female	2.13	27.0	.000,126	106
2- 4-11	15	Male	3.42	46.0	.000,134	106
2- 4-11	16	Female	2.43	26.5	.000,109	97
2-10-11	17	Male	2.50	23.0	.000,092	82
2-11-11	18	Male	3.27	32.7	.000,100	91
2-13-11	19	Male	2.94	25.5	.000,086	77
2-13-11	20	Male	1.81	17.5	.000,096	86
2-14-11	21	Male	2.40	20.0	.000,083	76
2-14-11	22	Female	1.87	16.0	.000,085	83
2-15-11	23	Male	2.28	22.0	.000,096	90
2-15-11	24	Female	2.25	21.0	.000,093	87
2-15-11	25	Female	1.92	13.5	.000,070	65
6-14-11	26	Female	2.38	24.0	.000,100	87

* Received paraldehyde instead of ether.

Two samples of strophanthus seed (Kombe) were received for testing at this time. These were reduced to No. 60 powder. Ten gm. samples were placed in 150 cc. Erlenmeyer flasks, supplied with good, tightly-fitting corks, and macerated with 40 cc. of 75 per cent. alcohol for 72 hours with occasional agitation. The content of each flask was then poured into a small, narrow percolator fitted at the neck with a tight plug of cotton. The first portion of each percolate was returned and the percolation was then allowed to proceed at the rate of ten drops per minute. Seventy-five per cent. alcohol was added from time to time until 200 cc. of percolate were obtained, thus finishing a 5 per cent. tincture. For injection, 1:6000 solutions were used. These were made in the same manner as described under ouabain. The results from eight experiments on each of these samples were as follows:

STROPHANTHUS SEED No. B-565.

Dilution 1:6000. 1 cc.=.000,166 gm. drug.

Date	Cat No.	Sex	Wt. in kgm.	Cc. Sol.	Strophanthus in gm.	Time
2-16-11	1	Male	2.23	16.0	.001,19	69
2-16-11	2	Male	2.65	22.0	.001,37	100
2-16-11	3	Female	2.82	26.0	.001,53	98
2-17-11	4	Male	2.29	22.9	.001,66	107
2-17-11	5	Male	2.11	24.0	.001,88	100
2-18-11	6	Male	2.98	30.0	.001,66	95
2-18-11	7	Male	3.07	31.0	.001,67	100
2-18-11	8	Female	2.04	18.0	.001,46	66

STROPHANTHUS SEED No. B-566.

2-21-11	1	Male	2.45	26.4	.001,78	102
2-21-11	2	Male	3.17	23.0	.001,20	60
2-21-11	3	Male	1.29	11.1	.001,42	71
2-22-11	4	Male	3.00	24.0	.001,32	60
2-22-11	5	Male	3.17	25.5	.001,33	87
2-22-11	6	Female	3.14	23.5	.001,23	87
2-23-11	7	Male	3.82	29.0	.001,24	95
2-23-11	8	Male	2.93	25.0	.001,41	111

Hatcher and Brody have found after many experiments that if digitalis and the other members of the series are injected, like ouabain and strophanthus, until the animal dies, the results will usually be too high—necessitating a correction of about 20 per cent. They have, therefore, devised a modification of the method which gives results comparable in accuracy, they believe, to those obtained with crystalline ouabain itself. This modification is as follows:

A measured quantity of the digitalis solution (I understand about 5 per cent. of the required amount) is injected during the first period of about ten minutes. After an interval of about twenty minutes the injection is continued, substituting ouabain solution for the digitalis, until the animal dies. The difference between the amount of ouabain actually used to complete the experiment, and the theoretical amount necessary to kill the animal in the absence of the digitalis body, represents the amount of ouabain to which the digitalis body is equivalent. The amount of digitalis body equivalent to .0001 gm., or one "cat unit," is then calculated.

EXAMPLE TO SHOW METHOD OF CALCULATION.

Digitalis solution=1:100 1 cc.=.010 gm.
Ouabain solution=1:100,000 1 cc.=.000.01 gm.

Cat weighing 3.21 kgms. received { 30.2 cc. digitalis sol. (.302 gm. drug) or, .0940 gm. drug per kgm. body weight. 5.5 cc. ouabain sol. (.000,055 gm. ouabain) or, .000,017 gm. ouabain per kgm. of cat.

The difference between .000,017 gm. the amount of ouabain (per kgm.) actually used to complete the experiment and .000,100 gm. the theoretical amount, or one "cat unit," which would have been required in the absence of the digitalis body, is .000,083 gm.

.094 gm. of the digitalis is therefore equivalent to .000,083 gm. ouabain, or .094 gm. of the digitalis=83% of one "cat unit."

.113 gm. of the digitalis would then be equivalent to one "cat unit."

F. E. DIGITALIS No. 405467.
Digitalis Dilution 1:100.
Ouabain Dilution 1:100,000.

Date	Cat No.	Sex	Wt. in kgms.	Cc. Dig. Sol.	Cc. Ouab. Sol.	Equiv in gm. of 1 Cat Unit	Time in Min.
2-27-11	1	Female	3.21	30.2	5.5	.113,5	95
2-27-11	2	Male	2.88	25.0	3.0	.096,8	61
2-27-11	3	Male	2.87	25.0	7.0	.115,2	100

F. E. DIGITALIS No. 416233.

5- 8-11	1	Male	2.33	14.0	5.2	.077,3	66
5- 8-11	2	Female	2.18	12.0	3.6	.065,8	65
5- 9-11	3	Male	2.07	11.0	4.6	.068,2	80
5- 9-11	4	Female	1.83	10.0	6.7	.086,1	73
5-10-11	5	Male	2.21	11.1	6.0	.068,8	97
5-10-11	6	Male	2.40	14.0	8.0	.087,4	82

F. E. DIGITALIS No. 335929.

5-11-11	1	Female	1.72	10.0	3.5	.072,8	79
5-11-11	2	Male	3.18	15.0	21.0	.138,7	114
5-11-11	3	Female	1.75	11.7	9.0	.137,5	100
5-12-11	4	Female	1.89	10.0	10.0	.112,3	90
5-13-11	5	Female[1]	2.15	10.0	15.0	.153,4	155
5-15-11	6	Female[1]	2.55	12.0	25.5	.522,7	114
5-15-11	7	Female[1]	1.82	12.0	12.0	.193,5	80
5-16-11	8	Male	3.00	16.0	17.5	.127,9	115
5-17-11	9	Female[1]	2.90	17.5	9.7	.090,6	75
5-18-11	10	Male	2.46	16.0	7.0	.090,8	75
5-18-11	11	Female	1.98	11.0	8.0	.093,2	85

[1]In varying stages of lactation.

TR. DIGITALIS No. 2-B.

5-22-11	1	Female	2.11	13.0	3.5	.073,2	61
5-23-11	2	Male	2.89	14.0	16.5	.112,6	95
5-23-11	3	Female[1]	2.35	13.0	15.0	.154,2	85
5-24-11	4	Male	2.83	17.0	3.0	.067,1	111
5-24-11	5	Female[2]	2.46	15.0	14.0	.141,4	106
5-24-11	6	Male	2.30	16.4	8.0	.109,3	81
5-25-11	7	Female	3.00	16.0	7.5	.071,1	73

[1]Lactating.

[2]Apparently in period immediately following lactation. Glands were still enlarged, but not functionating.

ASSAYS ON FOREGOING PREPARATIONS BY OTHER METHODS.

One hour frog heart method. Variety Rana pipiens. Temperature 20° C.

Strophanthus Seed B-565.

Weight in grams.	Dose per gram.	Result
36.5	.000,006,0	Stopped
40.8	.000,005,0	"
15.1	.000,005,0	"
28.1	.000,004,0	"
39.6	.000,004,0	"
23.0	.000,004,0	"
43.8	.000,003,5	"
36.4	.000,003,5	"
48.7	.000,003,5	Beating
35.2	.000,003,0	"
19.4	.000,003,0	"

Strophanthus Seed B-566.

18.2	.000,006,0	Stopped
20.6	.000,005,0	"
23.6	.000,005,0	"
15.4	.000,005,0	"
43.7	.000,005,0	"
18.1	.000,004,0	"
18.5	.000,004,0	"
25.8	.000,004,0	"
28.0	.000,004,0	Beating
28.8	.000,004,0	Stopped
34.4	.000,004,0	"
37.2	.000,003,5	"
45.3	.000,003,5	Beating
49.0	.000,003,5	"
37.6	.000,003,0	"

GUINEA PIG METHOD.

F. E. Digitalis No. 416233.

Weight in grams.	Dose per gram.	Result
709	.000,5	Recovered
786	.000,5	"
825	.000,5	"
467	.000,5	Died
524	.000,5	"
694	.000,6	Recovered
701	.000,6	"
814	.000,6	Died
835	.000,6	"
744	.000,7	"
517	.000,7	"
481	.000,8	"

F. E. Digitalis No. 335929.

750	.000,4	Recovered
340	.000,4	"
736	.000,4	"
680	.000,4	"
815	.000,5	"
630	.000,5	"
737	.000,6	"
772	.000,6	"
737	.000,7	Died
725	.000,7	"
531	.000,7	"
552	.000,7	Recovered
731	.000,8	"
538	.000,8	Died
375	.000,8	"

ONE HOUR FROG HEART METHOD.

Variety Rana pipiens. Temperature 20° C.

F. E. Digitalis No. 416233.

Weight in grams.	Dose per gram.	Result
40.9	.000,50	Beating
42.9	.000,60	"
39.1	.000,60	"
34.0	.000,70	"
46.5	.000,70	"
42.2	.000,75	"
50.8	.000,80	"
38.8	.000,80	"
31.2	.000,90	"
38.2	.000,90	Stopped
31.4	.001,00	"
35.5	.001,00	"

F. E. Digitalis No. 335929.

28.9	.000,90	Stopped
22.0	.000,80	"
21.0	.000,70	"
27.6	.000,70	"
30.5	.000,70	"
23.0	.000,70	"
42.9	.000,70	"
19.3	.000,65	Beating
20.2	.000,60	Stopped
23.5	.000,60	"
36.5	.000,60	"
37.4	.000,60	Beating
36.5	.000,50	"

The assays of these preparations by other methods have been inserted here, with the belief that they will be of some interest to the reader if closely analyzed,

although no decided conclusions can be drawn from so small a number. Considering F. E. Digitalis No. 416233 and No. 335929 by the guinea pig and frog heart methods, it will be seen that while they show almost the same result on the guinea pig, there is a decided difference on the frog's heart. A lack of relationship in the results obtained by these two methods has been observed by others. Remembering that these fluids test the same on the guinea pig, consider the assays by the cat method where No. 416233 is decidedly more active than No. 335929, the reverse of what was found by the frog heart method.

Attention should be called to the lot of animals used for No. 335929, which was perhaps the least suitable of any. It may be noticed that the greater number were females, varying considerably in size, some being in different stages of lactation. (No. 6 was in the early stage and had exceptionally large glands). The males were all large, and the results, perhaps a coincidence, varied somewhat in relation to the weight:

No. 2	Weight.....	3.18 kgms.	Result.....	.140,3
No. 8	"	3.00 "	"	.127,8
No. 10	"	2.46 "	"	.090,8

The assays on the two samples of strophanthus seed are almost identical by the frog heart method, and show but a small difference by the cat method.

Animals. Hatcher and Brody selected cats in preference to dogs, and I believe rabbits, for several reasons, namely: "Accuracy afforded, facility with which they may be obtained, ease with which they may be handled, * * *, cheapness, and the fact that their use does not affect the sensibilities of the sentimental portion of the community to the same extent that the employment of the dog does." Having used no other animals for this particular method, I cannot remark on the point of accuracy. My experience has been that there is little in their favor regarding cost, all things considered. Cats are easily handled, though to my mind are no more so than dogs, or rabbits, except that in the latter greater care is necessary in regard to any dissection or the giving of anaesthetics. I have found them far more difficult to obtain than rabbits and hardly less so than dogs. Whether their use affects the sensibilities of the sentimental portion of the community less than that of the dog, seems questionable. At any rate, the use of cats certainly does affect the sensibilities of many people, and the procuring of a sufficient number of animals for this piece of work has been the source of considerable trouble. And for a manufacturing plant of this size, to secure enough cats to carry out the routine assays on the several members of the digitalis series, would be a practical impossibility. If some easily procurable animal, such as the rabbit, could be used for this work, then one great difficulty would be removed. This point is of immense importance to the manufacturer, by whom nearly all of the practical physiological assaying will always be done.

Having experienced difficulty in buying cats, an attempt was made at this laboratory to raise them, but this met with poor success. It has seemed that only under the very best conditions can cats be kept well for any considerable length of time. It has been our not infrequent experience that cats will refuse sweet milk and raw beef for some time after having been received, and while an abundance of food has been supplied, our cats have usually lost in weight.

Lactating animals cannot be depended upon, as they seem to possess a greater tolerance for the drug, the degree depending on the stage of lactation.

The period and rate of injection. The lethal dose of any of the digitalis bodies cannot, of course, be told at the outset. This is indeed the figure sought. Therefore, "50 per cent. of the lethal dose" is a quantity which can only be widely approximated by one's experience with the given preparation. Whether this point in itself is a matter of great importance, within certain wide limits, I am unable to say. It would seem to be of importance, however, that the injection of all of these drugs be proportioned as evenly as possible over the ninety minutes. After one has injected an amount of digitalis, for example, and has waited the twenty minutes, he is ready to proceed with the ouabain solution. Since he does not know the value of the digitalis, he does not know, consequently, how much ouabain solution it will be necessary to inject during the following period of one hour. And not knowing this point, he is unable to judge how rapidly to inject. If he calcurates on 5 cc. when 10 cc. would actually be required, then he will come to the end of the ninety minute period with the animal still alive, and he must cautiously proceed with the probable result that one hundred and five minutes or so will be covered in completing the experiment. And having injected at a slower rate, possibly a larger amount of ouabain may have been required. On the other hand, if he calculates on 10 cc. when only 5 cc. are necessary, he may kill the animal before the end of the period—perhaps in seventy-five minutes. And having injected at a more rapid rate, possibly less ouabain may have been used than would have been under normal conditions.

It might be remarked that the first experiment would furnish these points. This might be true; still, it might happen that the results from number one would be exceptional. Then the operator would be thrown off on number two, and when he found the results from number two quite different, number three would be necessary in order to tell which was more nearly correct.

If these points are of no importance, then it would seem that the time limit of ninety minutes would be of no importance.

Number of animals and time. In general it would seem that at least three experiments would be necessary in order to determine with confidence the strength of a preparation. If two out of three results checked quite closely, as under F. E. Digitalis No. 405467 (.113,5—.115,2), that number might be sufficient. Under strophanthus seed No. B-565, however, the results show a gradual increase up to the sixth experiment (.001,19; .001,37; .001,53; .001,66; .001,88), and under F. E. Digitalis No. 416233, results Nos. 1, 4 and 6 check each other rather closely (.077,3; .086,1; .087.4), and Nos. 2, 3 and 5 at a different figure check each other even more closely (.065,8; .068,2; .068,8).

If three or four experiments were sufficient, then an assay could be made in one day, a point in favor of the method. This would require one person's entire time and attention for the four and a half or six hours, besides part of the time of an assistant. At that, more actual time would be required than for any of the other methods.

Ease of manipulation and accuracy. The method seems simple, and still, all points considered, it is the most difficult of all with which I am acquainted.

My results have been quite disappointing. They show variations for the different preparations, as follows:

Ouabain	123.3%
Excluding results No. 2, 4, 8, and 25	61.4%
Strophanthus Seed No. B-565	57.9%
Strophanthus Seed No. B-566	48.3%
F. E. Digitalis No. 405467	19.0%
F. E. Digitalis No. 416233	32.8%
F. E. Digitalis No. 335929 (excluding lactating animals)	90.5%
Excluding lacating animals and No. 1	53.0%
Tr. Digitalis No. 2-B (excluding Nos. 3 and 5)	67.8%

My results with crystalline ouabain would indicate that the lethal dose of this substance varies considerably with different animals. It seems, then, irrational to estimate the value of a preparation of digitalis, from its supposed equivalent of a body which is in itself, for any given animal, an unknown quantity. The authors of this method claim that crystalline ouabain will exactly replace digitalis in regard to its toxicity on the cat. It seems to me, however, that there might be some variance in its power to exactly replace different samples of digitalis, depending on the proportion of active principles present and the condition of these principles, whether or not decomposed. Since the amount of digitalis to be injected which will represent 50.75 per cent. of the required amount, is an unknown quantity, it necessarily follows that the amount of ouabain required to complete the experiment, even if its toxicity could be exactly known, is an unknown quantity. Therefore, not knowing the amount of ouabain required, the rate of injection, which probably plays an important part, cannot be known. Lastly, the time required to kill, being dependent on the rate of injection, constitutes another unknown factor. So, when testing a sample of digitalis, one has to deal with six or more unknown factors. This requires an operator of considerable experience and skill.

SUMMARY.

Considering the results of this work, together with my experience with the other methods, I am lead to make the following statements in conclusion:

The cat method of Hatcher and Brody is unquestionably the most complicated and difficult of all the American methods, requiring an operator of considerable experience in animal experimentation.

It is *not* a method that will be found convenient and generally serviceable by the retail pharmacist.

It is more time-consuming than the other methods, requiring constant attention when started.

The item of expense, like that of the guinea pig method, is decidedly in its disfavor.

The procuring of a sufficient number of *suitable* animals is a practical impos-

sibility for the manufacturing pharmacist having a large number of preparations to test. This may also be the source of much unpleasantness and trouble.

Lactating animals cannot be depended upon, as they seem to possess a greater tolerance for the drug, the degree depending on the stage of lactation.

While the individual results will not infrequently check each other very closely, considering the results of an entire assay, great variations will often be observed, amounting in some cases to more than 100 per cent.

When testing a preparation one has to consider six or more unknown factors, namely:

1. Toxicity of ouabain.
2. Power of ouabain to exactly replace the digitalis bodies.
3. Amount of digitalis to be injected.
4. Amount of ouabain to be injected.
5. Rate of injection.
6. Time.

This method has perhaps one point of superiority over all others in that the matter of absorption is entirely eliminated.

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THE DRUGGIST AND THE LOCAL ORGANIZATION.

While times among retail pharmacists have improved when we compare them with the past, we believe that the progress is by no means completed. The few years of experience in organization work which pharmacists have had is only a beginning. The future must continue to bring improvement. The time will come when no pharmacist will think of trying to do business without belonging to his local organization any more than he now tries to get along without a bank account and credit with manufacturers and jobbers.—*Meyer Brothers Druggist.*