TABLE XI.

TINCTURE DIGITALIS.

Alcohol Removed.

Amt. of tincture in 500 mils.	Temperature.	Results after 3 hours.
2.5	22° C.	Recovered
2.6	22° C.	Recovered
2.7	22° C.	Recovered
2.8	22° C.	Recovered
2.85	22° C.	Died
2.9	22° C.	Died

M. L. D. = 2.85.

It will be noted that the above results confirm our former conclusions that *alcohol to the extent of that contained in the U. S. P. tincture does not affect the results.*

As a result of our experimental work to date we have arrived at the following conclusions:

1. Variations of less than 2 percent in the strength of tincture of digitalis can be accurately determined by the method outlined.

2. Variations due to difference in the rate of absorption appear to be practically eliminated by the use of these animals.

3. The weight of the fish may be disregraded when making tests by this method.

4. Variations in temperature markedly influence the resistance of gold fish to digitalis poisoning.

5. The individual variations in susceptibility of gold fish is much less than that in guinea pigs and frogs.

6. The gold fish method is unquestionably the simplest so far proposed and can easily be carried out by those not especially skilled in the pharmacodynamic art.

7. The inexpensiveness of the assay is decidedly in its favor. Gold fish of the proper size can be purchased wholesale for from 45 to 60 cents per dozen.

8. A sufficient number of animals can be procured at all seasons of the year.

9. Alcohol to the extent of that contained in the U. S. P. tincture does not affect the results.

10. A tincture of digitalis to be of standard strength should have a M. L. D. of 2.85 when assayed by this method.

Finally the author wishes to acknowledge his indebtedness to Mr. LeRoy Goinez for most of the laboratory work in connection with this paper.

PHARMACODYNAMIC LABORATORY,
H. K. MULFORD COMPANY,
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DIGITALIS STANDARDIZATION: A CONSIDERATION OF CERTAIN METHODS OF BIOLOGICAL ASSAY.*

BY L. W. ROWE.

The physiological standardization of the drugs comprising the digitalis series of heart tonics has received much consideration since Houghton[1] proposed the first method for the assay of Strophanthus preparations in 1898.

One of the more recent of the methods proposed for standardizing digitalis preparations and one which constantly appears to be receiving consideration is

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the cat method, which was suggested by Hatcher and Brody[2] in 1909. In this method the digitalis preparation, suitably diluted, is administered intravenously to an anesthetized cat. The degree of dilution and size of dose are such that when slowly injected the animal will be killed within 90 minutes. The result gives the M. L. D. based on the amount of the preparation necessary per kilogramme body weight of cat.

Several objections have been raised to the use of this method, the chief one being that the death of the cat is not always caused by the action of the digitalis on the heart since respiration often ceases before the heart stops beating. Other objections are the irregularity in time of death of the test animal and the difficulty in obtaining a sufficient number of cats and in handling them.

The experiments reported in this paper were carried out, first, to determine whether any relationship exists between the results of assays by the cat and frog methods; second, to determine the accuracy of the cat method, and third, to suggest certain modifications of the method, in order to make it more practical for commercial assay work.

A search of the literature reveals the fact that very little experimental work with the cat method has been reported except by Hatcher and his co-worker, Eggleston. Eckler's[3] work published in 1912 on this subject seems to prove that the heart of the cat stops beating before respiration ceases. The number of samples tested, however, is too small to prove whether the method is suitable for commercial testing. Rowntree and Macht[4] in their work by this method have recently suggested certain changes which seem to improve it. The rate of injection used by Rowntree and Macht is the most important of these changes and has been adopted in my experiments.

In a later publication, Hatcher[5] enumerates certain features which he claims as distinct advantages, namely, elimination of absorption, speed of obtaining results, ease of handling animals and small comparative cost of assay. The method of administration certainly eliminates the question of absorption which is an important factor in the U. S. P. frog method. The most important consideration, however, in selecting a method of assay is accuracy; this and cost involved in obtaining and handling cats is open to question.

While results can be obtained more rapidly than by the M. L. D. frog method, it is doubtful if it is shorter than the official frog method. Most pharmacologists will agree that cats are not as easy to work with as other animals but a suggestion will be made later which has seemed to facilitate the handling of the cats. As to the expense involved, the test animals cost 75 cents instead of 10 cents, as Hatcher states, and then cannot be obtained in sufficient numbers for ordinary experimental purposes. Raising cats is also very unprofitable and our experience confirms that of Eckler[3] on this phase of the subject.

Eggleston's[6] work on the comparison of clinical results apparently established a ratio between the M. L. D. for cats and the dosage for man by carrying out clinical experiments in conjunction with his cat assays. It does not seem necessary, however, that results be transferable from the test animal to man in choosing a physiological method of assay, but other things being equal it is a point in favor of the cat method.

In my first experiments with the cat method, I attempted to kill the animal

in as nearly 90 minutes as possible just as Eckler did in his experiments. Later experiments showed, however, that much more uniform results could be obtained if the end-point was reached in from 20 to 45 minutes with 30 minutes as a good average. Also, in the first experiments the solutions were injected at a uniform rate from the beginning to the death of the animal. Injecting rapidly at first and then giving 1 mil every two minutes thereafter until the death of the animal is a better procedure.

In practically all of the experiments chloretone[7] was used as the anesthetic. It is easily given, is rapid in its action and in every respect is very satisfactory. This is a very important point in the use of cats as the injection is given so easily and the action is so rapid and pronounced. For cats the solution used is only half as strong as that suggested for completely anesthetizing dogs since a dose of 0.15 Gm. to 0.20 Gm. of chloretone per Kg. body weight is sufficient, when injected intraperitoneally, to produce a satisfactory anesthesia which does not effect the heart or depress the respiratory center, and requires no further administration of anesthetic after the first dose. The use of chloretone entirely eliminates the trouble experienced in administering anesthetics to cats, with the attendant danger of giving too much, though of course, it does not overcome the other difficulty of working with these animals, namely, the insertion of a cannula into the small femoral vein. If chloretone is used as the anesthetic the only physical objections to the use of the cat as the test animal is the difficulty and even impossibility of obtaining them in sufficient numbers for assay purposes and the trouble of working with the small and delicate blood vessels of this animal.

Because of the greater convenience in using dogs rather than cats as well as the further advantages that dogs are more easily obtained and cheaper, I have made a series of tests of a number of samples, using the cat and the dog in the modified intravenous method, and comparing the activities thus obtained with the test of the sample by the minimum lethal dose frog method. The technique of the injection used in the later experiments which is preferable to that of Hatcher, is that of Rowntree and Macht, in which approximately one-half of the calculated amount is injected at the rate of 3 mils per minute and the rest at the rate of 1 mil every two minutes. In the case of digitalis, ouabain was not used to complete the reaction, as originally suggested by Hatcher. By using a larger dose, results equally as accurate can be obtained without the additional complication.

The dilutions chosen for injection should be such that the M. L. D. for cats is between 10 and 25 mils; for dogs between 20 and 50 mils, 18 mils being a good average total dose for cats and 25 to 30 mils being a good average total dose for dogs.

The following tables of data give the results of tests of 18 samples upon 61 cats and of 30 samples upon 132 dogs:

TABLE I.—EXPERIMENTS WITH CATS.

OUABAIN "A."

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
1	M	Good	2.12 Kg.	Deep	1 : 20000	3.9 mils	60 min.	0.092 mg.
2*	M	Good	2.72 Kg.	Deep	1 : 20000	6.4 mils	90 min.	0.1176 mg.
3	M	Good	2.47 Kg.	Deep	1 : 20000	4.7 mils	82 min.	0.095 mg.
5	F	Good	3.74 Kg.	Deep	1 : 20000	6.5 mils	75 min.	0.087 mg.
6*	M	Fair	1.73 Kg.	None given	1 : 20000	4.4 mils	50 min.	0.127 mg.

Average M. L. D. per Kg. = 0.091 mg.

TABLE I.—EXPERIMENTS WITH CATS (*Continued*).

OUABAIN "B."

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
10	F	Fair	1.36 Kg.	Deep	1 : 50000	8.75 mils	46 min.	0.128 mg.
11	M	Good	2.1 Kg.	Light	1 : 50000	11.5 mils	33 min.	0.109 mg.
12	F	Good	2.82 Kg.	Deep	1 : 50000	18.5 mils	41 min.	0.131 mg.
13*	F	Poor (kitten)	0.86 Kg.	Deep	1 : 50000	11.6 mils	38 min.	0.270 mg.
15*	M	Fair (kitten)	1.94 Kg.	Deep	1 : 50000	14.7 mils	36 min.	0.151 mg.
16*	F	Poor (kitten)	1.41 Kg.	Deep	1 : 50000	12.4 mils	48 min.	0.175 mg.

Average M. L. D. per Kg. = 0.123 mg.

OUABAIN "C."

17	F	Good	2.74 Kg.	Deep	1 : 50000	21.9 mils	58 min.	0.160 mg.
18	M	Good	2.84 Kg.	Deep	1 : 50000	19.2 mils	65 min.	0.135 mg.
19	F	Poor	0.88 Kg.	Deep	1 : 100000	12.8 mils	40 min.	0.145 mg.

Average M. L. D. per Kg. = 0.147 mg.

STROPHANTHIN (KOMBE) SAMPLE NO. 256490.

51	F	Good	2.40 Kg.	Fair	1 : 30000	15.0 mils	18 min.	0.228 mg.
52	F	Good	3.20 Kg.	Fair	1 : 30000	16.0 mils	16 min.	0.166 mg.
53	F	Good	2.70 Kg.	Fair	1 : 30000	15.0 mils	20 min.	0.185 mg.

Average M. L. D. per Kg. = 0.186 mg.

TINCTURE OF STROPHANTHUS U. S. P. 1890.

20	F	Good	2.28 Kg.	Deep	1 : 100	13.3 mils	87 min.	0.059 mil
21	F	Good	2.60 Kg.	Deep	1 : 200	25.3 mils	47 min.	0.049 mil
22	F	Good	2.50 Kg.	Deep	1 : 100	14.1 mils	30 min.	0.056 mil
23	M	Fair	1.24 Kg.	Deep	1 : 100	6.44 mils	27 min.	0.052 mil
44*	M	Good	3.66 Kg.	Fair	1 : 100	26.0 mils	36 min.	0.071 mil

Average M. L. D. per Kg. = 0.054 mil.

TINCTURE OF DIGITALIS FROM DRUG NO. 250139.

24*	M	Good	2.00 Kg.	Deep	1 : 10	27.5 mils	30 min.	1.38 mils
25	M	Fair	1.40 Kg.	Deep	1 : 10	13.5 mils	21 min.	0.96 mils
26	F	Good	1.94 Kg.	Deep	1 : 10	18.2 mils	30 min.	0.94 mils
27	F	Good	2.16 Kg.	Deep	1 : 10	22.0 mils	60 min.	1.02 mils

Average M. L. D. per Kg. = 0.97 mil.

TINCTURE OF DIGITALIS "A."

36	F	Good	2.92 Kg.	Deep	1 : 4	30.2 mils	30 min.	2.58 mils

TINCTURE OF DIGITALIS "B."

37*	M	Good	4.48 Kg.	Fair	1 : 4	27.0 mils	32 min.	1.50 mils
38	M	Good	3.00 Kg.	Light	1 : 5	21.0 mils	28 min.	1.40 mils
42	M	Small O. K.	1.60 Kg.	Light	1 : 5	11.0 mils	12 min.	1.38 mils

Average M. L. D. per Kg. = 1.40 mils.

TINCTURE OF DIGITALIS "C."

39	F	Good	2.76 Kg.	Fair	1 : 5	15.0 mils	15 min.	1.09 mils
40	M	Good	2.75 Kg.	Fair	1 : 5	19.0 mils	25 min.	1.38 mils
41	M	Good	3.00 Kg.	Fair	1 : 5	19.0 mils	24 min.	1.27 mils
43	F	Good	3.35 Kg.	Light	1 : 5	23.0 mils	22 min.	1.37 mils

Average M. L. D. per Kg. = 1.28 mils.

TABLE I.—EXPERIMENTS WITH CATS (*Continued*).

DIGITALONE No. 046798.

Cat No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
34	F	Good	2.00 Kg.	Deep	1 : 10	17.9 mils	27 min.	0.90 mil

FLUIDEXTRACT OF DIGITALIS, ℞ No. 665561.

48	F	Good	2.00 Kg.	Light	1 : 50	14.0 mils	14 min.	0.140 mil
49	M	Good	3.86 Kg.	Light	1 : 50	33.0 mils	37 min.	0.171 mil
50	F	Good	2.30 Kg.	Fair	1 : 50	15.0 mils	15 min.	0.130 mil

Average M. L. D. per Kg. = 0.147 mil.

FLUIDEXTRACT OF SQUILL, ℞ No. 681685.

45	M	Good	2.25 Kg.	Fair	1 : 50	13.0 mils	15 min.	0.115 mil
46	F	Good	2.35 Kg.	Fair	1 : 60	16.0 mils	20 min.	0.113 mil
47	F	Good	2.50 Kg.	Very light	1 : 60	16.0 mils	18 min.	0.107 mil

Average M. L. D. per Kg. = 0.112 mil.

Asterisk after number of experimental animal means that result was not used in determining average.

TABLE II.—EXPERIMENTS WITH DOGS.

OUABAIN "A."

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
1	F	Good*	7.05 Kg.	Deep	1 : 20000	16.5 mils	97 min.	0.117 mg.
2*	F	Good*	10.5 Kg.	Deep	1 : 40000	50.0 mils	Not fatal	
3	M	Good*	18.5 Kg.	Deep	1 : 20000	37.5 mils	85 min.	0.101 mg.

Average M. L. D. per Kg. = 0.109 mg.

OUABAIN "B."

4	F	Good*	9.1 Kg.	Deep	1 : 25000	33.4 mils	57 min.	0.147 mg.
5	F	Good*	11.9 Kg.	Deep	1 : 20000	26.25 mils	60 min.	0.110 mg.
6*	M	Good*	12.05 Kg.	Deep	1 : 25000	48.2 mils	40 min.	0.160 mg.
7	F	Good	16.4 Kg.	Deep	1 : 20000	39.9 mils	38 min.	0.121 mg.

Average M. L. D. per Kg. = 0.126 mg.

OUABAIN "C."

11	M	Good*	9.0 Kg.	Deep	1 : 25000	28.6 mils	35 min.	0.127 mg.
12*	M	Fair*	7.0 Kg.	Deep	1 : 25000	28.8 mils	65 min.	0.165 mg.
13	M	Good*	9.5 Kg.	Deep	1 : 25000	33.0 mils	45 min.	0.139 mg.
14	F	Good*	17.2 Kg.	Deep	1 : 20000	50.0 mils	45 min.	0.145 mg.
15	F	Fair	6.0 Kg.	Deep	1 : 25000	30.4 mils	50 min.	0.136 mg.
16	M	Good*	9.0 Kg.	Deep	1 : 25000	33.5 mils	30 min.	0.149 mg.

Average M. L. D. per Kg. = 0.139 mg.

STROPHANTHIN (KOMBE) No. 183774.

21*	M	Good*	8.5 Kg.	Deep	1 : 10000	29.1 mils	45 min.	0.343 mg.
22	F	Good*	10.8 Kg.	Deep	1 : 10000	29.4 mils	20 min.	0.273 mg.
24*	M	Good*	13.4 Kg.	Deep	1 : 10000	50.0 mils	Not fatal	
25	F	Good*	15.0 Kg.	Deep	1 : 10000	36.4 mils	26 min.	0.242 mg.
30	F	Good*	8.2 Kg.	Deep	1 : 10000	20.5 mils	36 min.	0.250 mg.

Average M. L. D. per Kg. = 0.255 mg.

STROPHANTHIN (KOMBE) No. 256490.

98	M	Good	9.2 Kg.	Light	1 : 15000	35.0 mils	25 min.	0.253 mg.
99	F	Good*	12.8 Kg.	Fair	1 : 15000	45.0 mils	12 min.	0.234 mg.
100	M	Good	10.0 Kg.	Fair	1 : 15000	37.0 mils	28 min.	0.247 mg.
101*	M	Good*	14.4 Kg.	Deep	1 : 10000	41.0 mils	18 min.	0.284 mg.

Average M. L. D. per Kg. = 0.245 mg.

TABLE II.—EXPERIMENTS WITH DOGS (*Continued*).

STROPHANTHIN (KOMBE) NO. 256491.

Dog. No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
86*	F	Good	7.56 Kg.	Fair	1 : 15000	32.0 mils	33 min.	0.282 mg.
89*	M	Good*	7.6 Kg.	Deep	1 : 15000	37.0 mils	35 min.	0.324 mg.
90*	F	Good	7.1 Kg.	Fair	1 : 15000	30.0 mils	20 min.	0.282 mg.
91	F	Good	8.4 Kg.	Deep	1 : 15000	30.0 mils	17 min.	0.238 mg.
92	M	Good	9.2 Kg.	Deep	1 : 15000	33.0 mils	24 min.	0.239 mg.
93	F	Good	10.5 Kg.	Light	1 : 15000	36.0 mils	20 min.	0.228 mg.

Average M. L. D. per Kg. = 0.235 mg.

TINCTURE OF STROPHANTHUS U. S. P. 1890.

17	M	Good	7.94 Kg.	Deep	1 : 50	27.0 mils	35 min.	0.068 mil
18*	F	Good	*19.5 Kg.	Deep	1 : 25	19.2 mils	28 min.	0.039 mil
19	F	Good*	10.45 Kg.	Deep	1 : 50	26.5 mils	38 min.	0.051 mil
20*	M	Good*	14.10 Kg.	Deep	1 : 50	26.8 mils	40 min.	0.038 mil
23*	M	Good*	16.7 Kg.	Deep	1 : 50	35.0 mils	60 min.	0.042 mil
74	M	Good*	9.8 Kg.	Fair	1 : 50	27.0 mils	32 min.	0.055 mil
75	M	Good*	13.1 Kg.	Deep	1 : 50	46.0 mils	53 min.	0.070 mil
76	F	Good*	10.7 Kg.	Deep	1 : 50	34.0 mils	36 min.	0.064 mil
77	F	Good*	8.3 Kg.	Deep	1 : 50	21.0 mils	37 min.	0.051 mil
78	M	Good*	17.5 Kg.	Light	1 : 50	47.0 mils	42 min.	0.054 mil
79	F	Good*	11.1 Kg.	Light	1 : 50	34.0 mils	31 min.	0.061 mil
83	M	Good*	19.0 Kg.	Deep	1 : 40	44.0 mils	45 min.	0.058 mil
84	M	Good*	15.2 Kg.	Deep	1 : 50	44.0 mils	38 min.	0.058 mil
106	M	Good*	10.4 Kg.	Deep	1 : 50	32.0 mils	31 min.	0.062 mil

Average M. L. D. per Kg. = 0.059 mil.

TINCTURE OF STROPHANTHUS, U. S. P. 1910, ℞ 86068.

63	M	Good*	20.7 Kg.	Deep	1 : 50	32.0 mils	31 min.	0.031 mil
64	M	Good	13.6 Kg.	Fair	1 : 50	21.0 mils	32 min.	0.031 mil
65	M	Good	15.0 Kg.	Fair	1 : 50	22.0 mils	20 min.	0.030 mil
66	M	Fair	14.2 Kg.	Light	1 : 50	23.0 mils	36 min.	0.032 mil

Average M. L. D. per Kg. = 0.031 mil.

TINCTURE OF STROPHANTHUS, 1910, ℞ 683866

118	M	Good	14.0 Kg.	Fair	1 : 50	15.0 mils	18 min.	0.021 mil
119	M	Good	10.2 Kg.	Fair	1 : 75	20.0 mils	25 min.	0.026 mil
120*	M	Fair*	6.75 Kg.	Deep	1 : 75	14.0 mils	14 min.	0.0276 mil
121	F	Good*	12.0 Kg.	Fair	1 : 100	25.0 mils	20 min.	0.021 mil

Average M. L. D. per Kg. = 0.0227 mil.

TINCTURE OF DIGITALIS FROM DRUG NO. 250139.

26	M	Good*	9.75 Kg.	Deep	1 : 2	35.0 mils	86 min.	1.14 mils

TINCTURE OF DIGITALIS, ℞ 674678.

31	F	Good*	9.75 Kg.	Deep	1 : 2	37.8 mils	42 min.	1.94 mils
32	M	Good*	7.07 Kg.	Light	1 : 25	34.5 mils	30 min.	1.95 mils
33*	F	Poor	4.75 Kg.	Light	1 : 3	31.0 mils	57 min.	2.18 mils

Average M. L. D. per Kg. = 1.95 mils.

TINCTURE OF DIGITALIS "A."

35*	M	Good*	10.5 Kg.	Deep	1 : 2	42.0 mils	50 min.	2.0 mils
36	M	Good*	7.2 Kg.	Deep	1 : 3	59.0 mils	80 min.	2.73 mils
37	F	Good	7.75 Kg.	Fair	1 : 3	67.5 mils	85 min.	2.90 mils
38	M	Good	7.1 Kg.	Fair	1 : 2	44.0 mils	68 min.	3.09 mils

Average M. L. D. per Kg. = 2.91 mils.

TABLE II.—EXPERIMENTS WITH DOGS (*Continued*).

TINCTURE OF DIGITALIS, ℞ 676593.

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
49	F	Good*	8.8 Kg.	Fair	1 : 3	32.0 mils	50 min.	1.21 mils
50	M	Good*	10.7 Kg.	Deep	1 : 3	36.3 mils	50 min.	1.13 mils
	Average M. L. D. per Kg. = 1.17 mils.							

TINCTURE OF DIGITALIS, ℞ 676593 (ADJUSTED).

52*	F	Good*	9.0 Kg.	Deep	1 : 3	45.0 mils	40 min.	1.66 mils
53	F	Good	6.1 Kg.	Light	1 : 3	33.0 mils	62 min.	1.80 mils
54*	F	Good*	10.0 Kg.	Deep	1 : 3	47.6 mils	27 min.	1.59 mils
56	F	Good	8.3 Kg.	Fair	1 : 3	49.0 mils	50 min.	1.97 mils
57	F	Good	9.65 Kg.	Fair	1 : 3	57.0 mils	58 min.	2.00 mils
	Average M. L. D. per Kg. = 1.92 mils.							

TINCTURE OF DIGITALIS "B".

59	F	Good	6.9 Kg.	Fair	1 : 3	29.0 mils	33 min.	1.40 mils
85*	F	Very Poor	5.0 Kg.	Light	1 : 3	30.0 mils	42 min.	2.00 mils
87	F	Good	11.4 Kg.	Fair	1 : 3	50.0 mils	48 min.	1.46 mils
88*	F	Poor	5.8 Kg.	Fair	1 : 3	34.0 mils	36 min.	1.95 mils
	No. 85 had distemper and No. 88 was mangy and emaciated.							
	Average M. L. D. per Kg. = 1.43 mils.							

TINCTURE OF DIGITALIS "C".

60	M	Good*	12.1 Kg.	Deep	1 : 3	45.0 mils	40 min.	1.24 mils
61	M	Good	9.55 Kg.	Light	1 : 3	37.0 mils	37 min.	1.29 mils
62	F	Small*	7.9 Kg.	Deep	1 : 3	27.0 mils	20 min.	1.14 mils
	Average M. L. D. per Kg. = 1.22 mils.							

TINCTURE OF DIGITALIS, ℞ C136053.

114*	M	Good*	11.0 Kg.	Deep	Undil.	14.0 mils	24 min.	1.27 mils
115	M	Good	13.6 Kg.	Deep	Undil.	21.0 mils	30 min.	1.54 mils
116	M	Good	7.75 Kg.	Fair	1 : 2	32.0 mils	46 min.	1.83 mils
117	M	Good*	14.6 Kg.	Deep	Undil.	22.0 mils	28 min.	1.50 mils
	Average M. L. D. per Kg. = 1.62 mils.							

DIGITALONE, No. 046798.

27	M	Good*	6.8 Kg.	Deep	1 : 3	31.5 mils	105 min.	1.54 mils
28	M	Good*	8.8 Kg.	Fair	1 : 2	29.5 mils	70 min.	1.67 mils
29	M	Good*	9.4 Kg.	Deep	1 : 2	26.0 mils	30 min.	1.38 mils
	Average M. L. D. per Kg. = 1.53 mils.							

DIGITALONE, No. 049780.

107	F	Good*	9.7 Kg.	Deep	Undil.	17.0 mils	20 min.	1.75 mils
108	M	Good*	13.35 Kg.	Fair	Undil.	23.0 mils	30 min.	1.72 mils
110	M	Good	8.8 Kg.	Light	1 : 2	32.0 mils	27 min.	1.81 mils
	Average M. L. D. per Kg. = 1.76 mils.							

FLUIDEXTRACT OF DIGITALIS, ℞ 665561.

68	F	Good*	12.9 Kg.	Fair	1 : 20	47.0 mils	50 min.	0.186 mil
69	M	Good*	16.5 Kg.	Deep	1 : 10	29.0 mils	25 min.	0.176 mil
70*	M	Good	8.45 Kg.	Light	1 : 20	40.0 mils	52 min.	0.236 mil
71	M	Good	17.8 Kg.	Deep	1 : 10	31.0 mils	30 min.	0.174 mil
72	F	Small	6.25 Kg.	Light	1 : 20	25.0 mils	29 min.	0.200 mil
	Average M. L. D. per Kg. = 0.183 mil.							

SOLID EXTRACT OF DIGITALIS, ℞ 661579.

80	M	Good*	20.0 Kg.	Fair	1 : 30	34.0 mils	48 min.	0.057 mil
81	F	Good	10.0 Kg.	Deep	1 : 50	35.0 mils	50 min.	0.070 mil
82	M	Good*	14.7 Kg.	Deep	1 : 30	26.0 mils	35 min.	0.059 mil
	Average M. L. D. per Kg. = 0.062 Gm.							

TABLE II.—EXPERIMENTS WITH DOGS (*Continued*).

FLUIDEXTRACT OF SQUILL, ℞ 675384.

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
39*	F	Good*	13.0 Kg.	Deep	1 : 10	24.0 mils	65 min.	0.185 mil
40	M	Good	9.0 Kg.	Deep	1 : 15	21.2 mils	30 min.	0.156 mil
41*	F	Good*	11.2 Kg.	Deep	1 : 15	31.1 mils	48 min.	0.184 mil
42	F	Good	10.7 Kg.	Deep	1 : 15	25.8 mils	41 min.	0.160 mil
43*	F	Good*	13.5 Kg.	Deep	1 : 15	30.0 mils	34 min.	0.148 mil
44	M	Good	7.3 Kg.	Very light	1 : 15	17.0 mils	32 min.	0.156 mil
45	F	Good	9.0 Kg.	Very light	1 : 15	22.0 mils	60 min.	0.163 mil
48*	F	Good*	12.8 Kg.	Deep	1 : 15	36.3 mils	35 min.	0.190 mil

Average M. L. D. per Kg. = 0.159 mil.

FLUIDEXTRACT OF SQUILL, ℞ 681685.

102	F	Good	15.9 Kg.	Deep	1 : 10	28.0 mils	20 min.	0.176 mil
103*	M	Poor	14.25 Kg.	Deep	1 : 10	20.0 mils	12 min.	0.140 mil
104	F	Fair	8.7 Kg.	Fair	1 : 20	28.0 mils	32 min.	0.161 mil
105	M	Good	10.0 Kg.	Deep	1 : 15	24.0 mils	19 min.	0.160 mil
109	F	Good	7.8 Kg.	Light	1 : 20	24.0 mils	27 min.	0.154 mil

Average M. L. D. per Kg. = 0.163 mil.

FLUIDEXTRACT OF SQUILL, ℞ C134342.

55	M	Good*	14.5 Kg.	Deep	1 : 20	31.5 mils	27 min.	0.108 mil
58	M	Good*	12.6 Kg.	Deep	1 : 20	31.0 mils	36 min.	0.123 mil

Average M. L. D. per Kg. = 0.115 mil.

Asterisk after number of experimental animal means that result was not used in determining average.

In Table II asterisk after condition of dog means that animal had been used prior to the test of the heart tonic.

Tables I and II give in as concise a form as possible all of the data which is necessary to a critical analysis of the results reported. An attempt was made to test a variety of preparations and yet to test several different samples of each type. The results are summarized in the following Tables III and IV:

TABLE III.—COMPARISON OF AVERAGE M. L. D. TO CATS AND DOGS.

Sample.	Cats. M. L. D. per Kg.	Dogs. M. L. D. per Kg.	Ratio.
Ouabain "A"	0.092 mg.	0.109 mg.	1 to 1.2
Ouabain "B"	0.123 mg.	0.126 mg.	1 to 1.02
Ouabain "C"	0.147 mg.	0.139 mg.	1 to 0.96
Ouabain "D"	0.134 mg.	0.151 mg.	1 to 1.12
Strophanthin			
No. 256490	0.186 mg.	0.245 mg.	1 to 1.31
No. 256491		0.235 mg.	
No. 183774		0.255 mg.	
Tr. Strophanthus			
U. S. P. 1890	0.054 mil	0.059 mil	1 to 1.09
℞ 86068		0.031 mil	
℞ 681309	0.0179 mil	0.0263 mil	1 to 1.47
℞ 683866	0.0179 mil	0.0227 mil	

Sample.	Cats. M. L. D. per Kg.	Dogs. M. L. D. per Kg.	Ratio.
Tr. Digitalis			
No. 250139	0.97 mil	1.14 mils	1 to 1.17
℞ 674678		1.95 mils	
"A"	2.58 mils	2.91 mils	1 to 1.12
℞ 676593		1.17 mils	
℞ 676593 (Adj.)		1.92 mils	
"B"	1.40 mils	1.43 mils	1 to 1.02
"C"	1.28 mils	1.22 mils	1 to 0.95
"D"	1.56 mils	1.82 mils	1 to 1.17
℞ C136053		1.62 mils	
Digitalone			
No. 046798	0.90 mil	1.53 mils	1 to 1.7
No. 049780		1.76 mils	
F. E. Digitalis			
℞ 665561	0.147 mil	0.183 mil	1 to 1.24
S. E. Digitalis			
℞ 661579		0.062 Gm.	
F. E. Squill			
℞ 673584		0.159 mil	
℞ 681685	0.112 mil	0.163 mil	1 to 1.45
℞ 134342		0.115 mil	
Tr. Digitalis Unknown Activity	0.98 mil	0.134 mil	1 to 1.37
Tr. Strophanthus Unknown activity	0.0286 mil	0.0326 mil	1 to 1.14
Ouabain Unknown activity	0.202 mg.	0.191 mg.	1 to 0.95

TABLE IV.

Sample.	Cat units.	Dog units.	M. L. D. frog method. Heart tonic units.
Ouabain "A"	10,989	9174	201,500 (101%)
Ouabain "B"	8,130	7936	185,200 (93%)
Ouabain "C"	6,802	7194	197,600 (98%)
Ouabain "D"	7,462	6622	177,800 (89%)
Strophanthin			
No. 256490	5,376	4081	150,000 (150%)
No. 256491		4255	171,000 (171%)
Tr. Stroph.			
U. S. P. 1890	18.5	17.0	650 (50%)
℞ 86068		32.2	815 (62.7%)
℞ 681309	55.86	41.1	1500 (115%)
℞ 683866		44.0	1978 (136%)
Tr. Digitalis			
No. 250139	1.03	0.88	10.0 (166%)
℞ 674678		0.51	5.0 (83%)
"A"	0.387	0.343	2.0 (33%)
℞ 676593		0.854	8.25 (137.5%)
℞ 676593 (Adj.)		0.52	6.67 (111%)
"B"	0.71	0.70	9.52 (158%)
"C"	0.78	0.82	6.06 (101%)
"D"	0.64	0.55	5.0 (83%)
℞ 136053	0.64	0.62	9.17 (153)

Sample.	Cat units.	Dog units.	M. L. D. frog method Heart tonic units.
Digitalone			
046798	1.1	0.65	5.9 (98%)
049780		0.57	6.6 (110%)
F. E. Digitalis			
℞ 665561	6.8	5.5	72.0 (110%)
S. E. Digitalis			
℞ 661579		16.1	140.0 (70%)
F. E. Squill			
℞ 675384		6.3	120.0 (150%)
℞ 681685		6.1	100.0 (125%)
℞ 134342		8.7	140.0 (175%)
Tr. Digitalis			
Unknown activity	1.02	0.746	8.0 (133%)
Tr. Stroph.			
Unknown activity	35.0	30.4	1000 (77%)
Ouabain			
Unknown activity	4950	5235	114300 (57%)

Table III gives the average M. L. D. decided upon from the data obtained in the test of each sample upon either cats or dogs. Whenever the sample was tested upon both cats and dogs the ratio of the M. L. D. for the cat to that for the dog is placed in the fourth column. In this table it is plainly shown that there is no constant relation between the M. L. D. of a sample to cats and that of the same sample to dogs. In general, it can be stated, however, that the M. L. D. per Kg. body weight is slightly greater for the dog than for the cat.

Table IV gives the comparative results, in units, of the tests of samples upon the cat, dog and frog. The cat unit is defined by Hatcher as the amount of drug which is just sufficient to kill one kilogramme of cat when slowly and continuously injected into the vein. The number of units per gramme of the pure principles or per mil of tinctures or fluidextracts is, therefore, one divided by the average M. L. D. per Kg. as determined in the test. This exact procedure was also used in determining the number of dog units in each preparation. In the case of the M. L. D. frog test the Heart Tonic Unit is ten times the minimum lethal dose per gramme body weight of standard test frogs kept under proper test conditions. The number of heart tonic units per gram or per mil of a preparation is, therefore, one divided by 10 times the normal M. L. D. per gramme body weight of frog. The percentage which is placed in parentheses after the number of heart tonic units found for each sample is the strength of the sample in terms of the standard which has been adopted for that particular preparation.

This table (IV) shows that there is no definite relation between either the M. L. D. of a sample to the cat and M. L. D. to the frog or between the M. L. D. to the dog and that to the frog. In the case of the samples of ouabain, Sample "C" was a close second in activity to Sample "A" by the frog test while by the cat and dog tests it was a poor third. The third sample of Tr. Strophanthus was three times as active as the first sample by the *cat* test and but 2.3 times as active by the frog test. The second sample of Tincture of Strophanthus was nearly twice

as active as the first by the *dog* test while it was but $1^1/_4$ times as active by the frog test. Particularly in the tests of samples of Tincture of Digitalis are the inconsistencies of the M. L. D. to dogs plainly shown. One sample, ℞ 676593, was diluted on the basis of the original frog assay to 80 percent of its original strength and the assay of the diluted product by the M. L. D. frog test checked the dilution almost exactly while the assay of these samples upon dogs showed the diluted product to be but 60 percent as strong as the original. Several other instances of inconsistency between the results obtained upon dogs and frogs might be pointed out but they can be readily seen upon close examination of the results.

In order to arrive at the relative accuracy of the three methods in as nearly an unprejudiced a manner as possible, and to check the results reported in the preceding tables, three samples were submitted for test. They were prepared by diluting or concentrating certain lots which had been tested by all three methods but the degree of dilution or concentration was entirely unknown to the writer until after the tests were completed and results reported. Tables V and VI give the detailed reports of the tests as well as the comparison of the results obtained with the actual change which was made.

TABLE V.—ORIGINAL SAMPLES FROM WHICH UNKNOWNS WERE MADE.

TINCTURE OF STROPHANTHUS.

Test on Dogs.

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
94*	F	Good	12.8 Kg.	Fair	1 : 100	30.0 mils	35 min.	0.234 mil
95	M	Fair	7.2 Kg.	Fair	1 : 100	10.0 mils	26 min.	0.0277 mil
96	F	Good*	9.8 Kg.	Deep	1 : 100	26.0 mils	24 min.	0.0265 mil
97	M	Good	11.5 Kg.	Fair	1 : 100	29.0 mils	32 min.	0.0252 mil
112	F	Good*	11.6 Kg.	Deep	1 : 50	15.0 mils	14 min.	0.0259 mil
	Average M. L. D. per Kg. = 0.0263 mil.							

Test on Cats.

54	F	Good	2.7 Kg.	Light	1 : 250	12.0 mils	12 min.	0.0180 mil
55	F	Good	2.9 Kg.	Fair	1 : 250	13.0 mils	35 min.	0.0179 mil
56*	M	Good	3.3 Kg.	Fair	1 : 250	20.0 mils	38 min.	0.0242 mil
57	M	Good	3.75 Kg.	Fair	1 : 250	17.0 mils	25 min.	0.0180 mil
61	F	Good	2.45 Kg.	Fair	1 : 250	13.0 mils	21 min.	0.0177 mil
	Average M. L. D. per Kg. = 0.0179 mil.							

TINCTURE OF DIGITALIS "D."

Test on Dogs.

122*	F	Poor	8.5 Kg.	Fair	1 : 2	24 mils	30 min.	1.41 mils
123	M	Good	15.0 Kg.	Fair	1 : 2	50 mils	54 min.	1.66 mils
124	F	Good	6.1 Kg.	Fair	1 : 2	22 mils	37 min.	1.80 mils
125	M	Good	10.6 Kg.	Fair	1 : 2	41 mils	52 min.	1.93 mils
132	F	Good	9.6 Kg.	Fair	1 : 2	18 mils	35 min.	1.88 mils
	Average M. L. D. per Kg. = 1.82 mils.							

Tests on Cats.

64	M	Fair	1.6 Kg.	Fair	1 : 6	15 mils	36 min.	1.56 mils
65	F	Fair	2.4 Kg.	Fair	1 : 6	20 mils	40 min.	1.39 mils
66	M	Fair	3.65 Kg.	Fair	1 : 6	38 mils	60 min.	1.73 mils
	Average M. L. D. per Kg. = 1.56 mils.							

TABLE V.—ORIGINAL SAMPLES FROM WHICH UNKNOWNS WERE MADE (*Continued*).

OUABAIN "D."

Test on Dogs.

Dog No.	Sex.	Cond.	Weight.	Anes.	Dil. of sample.	Total dose.	Time to kill.	M. L. D. per Kg.
126	M	Good	9.9 Kg.	Fair	1 : 20000	30.0 mils	42 min.	0.151 mg.
127	F	Good	14.65 Kg.	Fair	1 : 20000	40.0 mils	35 min.	0.137 mg.
128	M	Good	17.1 Kg.	Fair	1 : 10000	28.0 mils	37 min.	0.164 mg.
129*	M	Good	12.4 Kg.	Light	1 : 10000	23.0 mils	34 min.	0.185 mg.

Average M. L. D. per Kg. = 0.151 mg.

Test on Cats.

67*	F	Kitten	1.3 Kg.	Fair	1 : 75000	17 mils	38 min.	0.174 mg.
68	F	Good	2.5 Kg.	Fair	1 : 50000	17 mils	26 min.	0.136 mg.
69*	F	Kitten	1.0 Kg.	Fair	1 : 50000	11 mils	30 min.	0.220 mg.
70	M	Good	3.05 Kg.	Fair	1 : 50000	20 mils	34 min.	0.131 mg.

Average M. L. D. per Kg. = 0.134 mg.

Samples (activity unknown at time of test).

TINCTURE OF STROPHANTHUS FROM ℞ 681309.

Test on Dogs.

135	F	14.4 Kg.	Light	1 to 50	18 mils	24 min.	0.0250 mil
136	M	11.85 Kg.	Light	1 to 50	20 mils	38 min.	0.0337 mil
139*	M	8.2 Kg.	Fair	1 to 50	18 mils	31 min.	0.0440 mil
140*	M	10.6 Kg.	Fair	1 to 50	23 mils	35 min.	0.0434 mil
141	M	17.4 Kg.	Fair	1 to 50	29 mils	40 min.	0.0330 mil
142	M	10.6 Kg.	Fair	1 to 50	17 mils	20 min.	0.0320 mil
148	M	11.0 Kg.	Fair	1 to 75	26 mils	35 min.	0.0315 mil

Average M. L. D. per Kg. = 0.0326 mil.

Test on Cats.

58	F	2.5 Kg.	Fair	1 to 250	18 mils	34 min.	0.0288 mil
59	M	3.75 Kg.	Fair	1 to 200	19 mils	24 min.	0.0253 mil
60	F	2.9 Kg.	Fair	1 to 250	23 mils	37 min.	0.0319 mil

Average M. L. D. per Kg. = 0.0287 mil.

TINCTURE OF DIGITALIS FROM "D."

Test on Dogs.

133*	M	10.0 Kg.	Fair	1 to 2	22 mils	38 min.	1.1 mils
134	M	9.5 Kg.	Fair	1 to 2	23 mils	48 min.	1.31 mils
138	M	8.9 Kg.	Fair	1 to 2	23 mils	45 min.	1.29 mils
144	M	5.9 Kg.	Fair	1 to 3	25 mils	36 min.	1.41 mils

Average M. L. D. per Kg. = 1.34 mils.

Test on Cats.

62	M	Good	3.2 Kg.	Fair	1 to 5	15 mils	20 min.	0.94 mil
63	M	Good	2.6 Kg.	Light	1 to 6	16 mils	30 min.	1.02 mils

Average M. L. D. per Kg. = 0.98 mil.

OUABAIN FROM "D."

Test on Dogs.

143	F	11.4 Kg.	Light	1 to 15	34 mils	48 min.	0.198 mil
146	M	9.0 Kg.	Fair	1 to 15	25 mils	33 min.	0.185 mil
147	F	7.7 Kg.	Fair	1 to 15	22 mils	27 min.	0.190 mil

Average M. L. D. per Kg. = 0.191 mg.

Test on Cats.

71*	F	Kitten	1.2 Kg.	Fair	1 to 50000	15 mils	40 min.	0.250 mg.
72	M	Good	2.94 Kg.	Good	1 to 40000	25 mils	45 min.	0.213 mg.
73	F	Good	2.5 Kg.	Fair	1 to 40000	19 mils	34 min.	0.190 mil

Average M. L. D. per Kg. = 2.20 mg.

TABLE VI.—RESULTS OF TEST UNKNOWNS.

Sample.	% of Original. Dogs.	Cats.	Frogs.	Correct percent.
Tr. Stroph	74.5%	62.6%	66.7%	66.7%
Tr. Digitalis	135%	159%	160%	150%
Ouabain	79%	63%	64%	60%

From the results of the test of the unknowns reported in Table VI, it can be seen that the frog assay is the more reliable since in one case the report was exactly right, and in the other two, slightly high (a matter of about 7 percent). Two of the results on the dogs were considerably high while the third was about as much too low, showing no consistency toward either high or low results. The results on the cats were much better than those on the dogs but not quite as good as those obtained by the frog method. Because of the fact that we have used the M. L. D. frog method for so many years to check up dilutions (based on original assays) of commercial lots and have with *very* few exceptions found it to be accurate, it seems that the results obtained from this small series of unknowns is entirely representative of the relative merits of the method.

Enough data was not obtained with cats to absolutely prove that they are as unsatisfactory as dogs and in fact I scarcely believe that they are but the data reported in Table IV indicates that there is no real consistency between the results obtained when using the cat and those obtained with the frog. Since the frog method vindicated itself so satisfactorily in the test of the unknowns it seems hardly possible that the cat method can be considered to possess the same degree of accuracy.

It, therefore, seems most logical to conclude from the results reported that no relationship exists between the M. L. D.'s of heart tonic preparations to cats, dogs and frogs and that consequently, since the frog method has shown itself to be the most accurate by tests of samples of unknown activity, the M. L. D. frog method is the most accurate of the three. This being true, there should certainly be no hesitancy as to the choice of the method for use in quantitative assay work even though it might possibly be proved that the cat or dog method is a little the less expensive and that results can be obtained in a somewhat shorter time than with the frog method.

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