medulla and that aconitine does stimulate the other medullary centers, this would seem to further substantiate the theory that the emetic action of aconitine is central.

All the pigeons upon injection reacted in the same characteristic manner whether death followed, or whether emesis followed or whether there were no other results. Immediately after injection the bird appeared to be very ill, there was a downward drooping of the wings, dizziness and very rapid breathing. The bird's sense of equilibrium appeared to be upset for it had no control of balance and would either squat clear down until its body rested on the floor of the cage or else lean against the side of the cage.

If emesis followed it occurred in a characteristic manner as before described. If death followed it too occurred in a characteristic manner. Just preceding death there would be a violent or convulsive flapping of the wings, the bird would fall over with its neck bent far backward, and gasping for breath. Death followed in a very few seconds from what appeared to be respiratory paralysis. Occasionally death occurred before the bird could be released from the operating table, and a few times even before the needle was withdrawn.

It would appear that the chief disadvantage of the pigeon emesis method of assay is that the emetic dose is entirely too close to the fatal dose, for too many times the same dose would one time cause emesis and the next time cause death. This is further seen in the fact that in each case, regardless of the method of administration, an increase of approximately 0.001 mg./Kg. over the M. E. D. would be fatal, indicating that the emetic effect is obtained only when the near lethal dose is administered.

It was noticed that the birds which were able to vomit appeared to recover from the effects much more rapidly than did the ones that did not vomit. And also if the bird did vomit it was more likely to recover from the larger doses than if it did not vomit, for in no instance did a bird die after emesis, regardless of the size of the dose.

The results of this work indicate that the pigeon emesis method of assay is applicable to aconite and offers a possible method of standardization. It is, of course, realized that this work is for the most part only preliminary and that fruther research is necessary before any definite conclusions can be drawn.

It should also be kept in mind that this work was pursued with the intent of finding a method of standardizing the *analgesic* agent in aconite and not with any reference to the cardiac or blood pressure effects.

The pigeon emesis method of assay is simple, rapid, economical, has a definite and unmistakable end-point and gives a reasonable degree of accuracy.

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Assay of Digitalis

The Use of Dogs in, and a Comparison of the International Standard (1936) and the U. S. P. Reference Powder

*By Philip Blickensdorfer and H. A. McGuigan**

In this work we present evidence to show that dogs are quite suitable animals for the standardization of digitalis; that the U. S. P. Digitalis Reference Powder is stronger than it is labeled; and to correct a measurable error in the data of a previous paper.[1]

EXPERIMENTAL

Method.—The method used is that described by McGuigan and McGuigan (1). Dogs are anes-

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[1] Paper by McGuigan and McGuigan, *J. Pharm. and Exp. Therap.*, 63 (1938), 76.

thetized by intraperitoneal injection of 35 mg. per Kg. of pentobarbital in water solution. In about 15 minutes, they are ready for digitalis injections. The tincture of digitalis (0.1 cc. per Kg.) is injected slowly (at least 1 minute for each injection) into the femoral vein. Injections are repeated every five minutes until the animal dies. If the tincture of digitalis is of International Standard strength, twelve injections will stop the heart.

Test of the Method on Commercial Products.—To compare our results on the dog with those obtained by using the frog, we asked five leading manufacturers of digitalis preparations to furnish us with samples and their data on standardization. All gladly complied with our request. We found all the samples submitted to comply with the U. S. P. standards, but with considerable variations. One house sent us an old sample (I), which they had retested, and our results agreed exactly with their work. The average results from closely agreeing individual experiments are as follows:

Table I.—Results of Test of Method on Commercial Products

Sample	Our Findings	Manufacturer's Statement
I	86%	85%
II	83%	100%
III	111%	111%
IV	120%	110%
V	92.5%	100%
Average	98.5%	101%

We believe these results show the method to be quite dependable, since the slight differences are well within the limit of experimental error.

DISCUSSION

Unsolved Questions.—While we believe that the use of the dog as we have outlined in the standardization of digitalis gives consistent and reliable results, there are some factors that future work may improve. The first of these is the anesthetic. Pentobarbital in our experience does not always give the same depth of anesthesia. When the anesthesia varies, as sometimes happens, the blood pressure, after a few injections of digitalis, may rise to enormous heights, occasionally to 260 or even 300 mm. of mercury. It has been shown that the kind of anesthesia, which we think means the depth of anesthesia, changes the fatal dose (2).

The effect on the vasomotor center especially needs investigation. When the pressure is high, digitalis may kill with smaller doses. This is a factor concerned in many of our lower variations from the average. Again, in cases where the blood pressure remains low throughout the experiment, more digitalis is required to stop the heart. This accounts for some of the highest doses we have recorded.

There is some relationship between these wide variations and oxygen supply, though we do not think this explains all. However, we have been able to revive animals, apparently dead, with artificial respiration, so that one or two more injections were required to kill the heart. Quite often this "asphyxia (?)" occurs at the point where the toxic action on the heart begins, especially if it occurs rapidly. In such cases, apparently, depression of the respiratory center is a complicating factor. A study of these factors will improve the method, but biological standardization will never have the accuracy of a chemical balance.

The Lag of Digitalis Action.—The question has arisen, how much, if at all, the last one or two injections are active in causing death, because they may not have time to act. We believe that this factor is not so important because:

1. The conditions are the same in the standardization of any sample as they are in the control.

2. We are able to show that even the first injection has an unseen action within five minutes. If, before injecting digitalis, one tests the rise given by an injection of adrenalin, and then injects digitalis, a second injection of adrenalin within a five-minute period gives a more marked rise. That an action on the heart occurs after the first injection is shown by the fact that after two or three injections of digitalis an injection of adrenalin immediately elicits the slowing effect of digitalis on the heart, which would not arise normally until more digitalis is given. However, when the heart is damaged to such an extent that the blood pressure becomes very low, as happens sometimes near the end of the experiment, there may be some lag in the last one or two doses. Consequently, if the blood pressure becomes so low that circulation is impeded, it may be hard to decide whether the last dose is effective.

Again the selection of dogs may be important. We have used dogs as they were

brought in from the pound. The only requirement was that they should be in a "fair" state of health. No "conditioning" was attempted though we believe by more critical selection more closely agreeing results may be obtained. Chows, in our experience, are unreliable. To avoid bias, we include all our results, although some, quite wide from the average, might justifiably be omitted.

EXPERIMENTAL

A tincture was prepared by macerating the equivalent of 8 Gm. of the powder in 100 cc. alcohol (4 volumes of alcohol and 1 volume of water), as directed by the U. S. P. XI. One cubic centimeter of this tincture is equal to one International Unit. The results obtained are shown in Table II.

Table II.—Results with a Tincture Prepared with the International Standard (1936) Powder

Weight of Dog, Kg.	Number of Injections	Dose, mg. per Kg. (Corrected)	International Units per Kg.
6.4	15	150	1.5
8.6	13	130	1.3
10.0	12	120	1.2
6.4	15	150	1.5
8.6	15	150	1.5
10.9	13	130	1.3
7.7	10	100	1.0
4.9	12.5	125	1.25
13.2	12	120	1.2
9.6	12	120	1.2
14.5	10.5	105	1.05
12.0	11	110	1.1
8.2	11	110	1.1
8.2	13	130	1.3
Average	12.5	125	1.25

Table III.—Results with Tincture Prepared with U. S. P. Reference Powder (0.745 Gm. = 1 Gm. International Strength) in Dogs

Weight of Dog, Kg.	Number of Injections	Dose, mg. per Kg. (Corrected)	Units per Kg.
11.4	9	90	0.9
9.6	13	130	1.3
18.2	9	90	0.9
10.0	9	90	0.9
7.7	10	100	1.0
6.4	11	110	1.1
14.0	8	80	0.8
14.5	9.5	95	0.95
14.5	9	90	0.9
12.7	10	100	1.0
9.3	10	100	1.0
12.8	10	100	1.0
Average	9.8	98	0.98

Table III shows the results obtained when a tincture was prepared by macerating the equivalent of 7.45 Gm. of the powder in 100 cc. of alcohol for 24 hours, and the supernatant liquid obtained by centrifugation used; 0.1 cc. per Kg. was injected every 5 minutes.

Table IV.—Results with Tincture Prepared with U. S. P. Reference Powder (0.745 Gm. = 1 Gm. International Strength) in Cats

Weight of Cat, Kg.	Number of Injections	Fatal Dose, mg. per Kg. (Corrected)	Units per Kg.
1.8	11	110	1.1
2.6	6	60	0.6
3.9	13	130	1.3
2.1	14	140	1.4
2.6	10	100	1.0
2.3	11	110	1.1
2.5	7	70	0.7
2.6	9	90	0.9
3.4	10	100	1.0
1.7	7	70	0.7
Average	9.8	98	0.98

While the average is 98 mg. per Kg. in both dogs and cats, the outstanding figure is 100 mg. per Kg. No artificial respiration was used on these cats, and we think some of the low figures in cats may be complicated with respiratory failure. The average dose for cats is practically the same as for dogs, *i. e.*, 0.1 cc. per Kg., but the variation is wider. However, from the work below and the results of other workers, some doubt exists as to this dosage for cats. The work of Bone, Elam and Blickensdorfer in the following paper (see page 105) justifies this opinion.

If we take the values found for dogs, 120 mg. per Kg. (1.2 units) for the International Standard, and 100 mg. per Kg. for the U. S. P. Reference Powder, then the reference powder is about 20 per cent stronger than it is labeled. The label states that 0.745 Gm. = 1 Gm. of International Powder. If 0.745 = 120 per cent, 100 per cent = 0.62 Gm. We, therefore, made a tincture using 0.62 Gm. to 10 cc. alcohol and tested it on dogs with the following results:

Table V.—Results with Reference Powder in Dogs (Factor 0.62 Instead of 0.745)

Weight of Dog, Kg.	Number of Injections	Fatal Dose, mg. per Kg. (Corrected)	Units per Kg.
8.8	13	130	1.3
13.6	15.5	155	1.55
7.7	10	100	1.0
10.2	12.5	125	1.25
9.6	11	110	1.1
10.0	12.5	125	1.25
12.0	13	130	1.3
Average	12.5	125	1.25

The tincture referred to above (made by using 0.62 Gm. of the U. S. P. Reference Powder to 10 cc. of alcohol) was also tested on cats with the results shown in Table VI.

Table VI.—Results with Reference Powder in Cats (Factor 0.62 Instead of 0.745); Artificial Respiration Used

Weight of Cat, Kg.	Number of Injections	Fatal Dose, mg. per Kg. (Corrected)	Units per Kg.
3.3	10	100	1.0
3.4	13	130	1.3
3.0	8	80	0.8
3.4	12	120	1.2
3.2	10	100	1.0
Average	10.6	106	1.06

With five cats with the International Powder, we found an average of 1.02 cc. of the tincture per kilo.

DISCUSSION

These results show that when we use dogs in the standardization of digitalis, the reference powder is 20 per cent stronger than it is labeled. By using cats, less consistent results are found, the reason for this being that cats are more variable in their reaction.

From work on frogs, both by the one-hour method and the minimum lethal dose method, Rowe (3) concludes that the U. S. P. XI Reference Powder is about 25 per cent stronger than labeled.

Spruth and Olsen (4), also using frogs, found that the U. S. P. Reference Powder varied from 110 to 130 per cent of the International Standard.

Edmunds and co-workers (5) found the U. S. P. Reference Powder, in terms of the International Standard (100 per cent) to be by the cat method, 141.65%; one-hour frog method, 134.22%; four-hour frog method, 157.37%; twelve-hour frog method, 190.40%. The average of all frog methods is 160.66, or exactly as we have found in the dog. In our terms, 100/62 = 161 per cent. Our results are also practically the same as his results on the four-hour frog method.

The lower strength shown by the one-hour method seems due to less absorption. Lessened absorption or irregularities in absorption lessen the value of this method.

Supposing we have two samples of digitalis, exactly 80 and 100 per cent, to show this difference by the frog method, it will be necessary that each be absorbed to the same degree. If 50 per cent of each is absorbed in one hour, 50 per cent of 100 = 50, and 50 per cent of 80 = 40, the difference is 20 per cent. However, if 50 per cent of the stronger is absorbed (50), and 25 per cent of the weaker (20), the difference is 60 per cent. Again, if 50 per cent of the weaker (40) and 25 per cent of the stronger (25) is absorbed, the difference is 37.5 per cent; but the stronger sample is to be only 62.5 per cent that of the weaker. Consequently irregularities of absorption are potential factors of enormous error. The four-hour method or longer would lessen this error.

In the work of McGuigan and McGuigan, while they found that an average of 12 injections (120 mg. per Kg.) of the reference powder kills dogs, they prepared their solutions by making them up to volume by washing the residue. The U. S. P. method uses only the supernatant liquid. This error is measurable because it is quite difficult to wash the powder free from the active ingredient. Their results are correct for the International Standard, and for what the reference powder should be.

CONCLUSIONS

1. Dogs are reliable animals for the standardization of digitalis.

2. The dose of International Powder in the form of the tincture, prepared as directed by the U. S. P. XI and using only the supernatant fluid, is (corrected) 120 mg. (1.2 units) per Kg., or an average of 12 injections of 0.1 cc. per Kg., given intravenously.

3. The U. S. P. Reference Powder is 20 per cent stronger than labeled. Instead of 0.745, the factor should be 0.62.

To determine the dose for cats more satisfactorily we refer to the accompanying paper by Bone, Elam and Blickensdorfer.

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Titles of papers to be presented at the Richmond meeting must reach Secretary E. F. Kelly by March 18th to be included in the printed program.