CONCLUSIONS.

1. The physiological activity of the first-year leaves compared favorably with the second-year leaves.

2. The activity of native digitalis plants under favorable climatic conditions would probably be above U. S. P. standard.

3. One-hour frog method unsatisfactory chiefly because of the time element which has a tendency toward erratic results.

4. Observed only a slight difference in the susceptibility of frogs to cardiac stimulants through the various seasons.

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STUDIES ON THE BIOASSAY OF DIGITALIS.*,[1]

III. A NEW DIURETIC OLIGURIC CAT METHOD.

BY JAMES H. DEFANDORF.

Digitalis in toxic amounts has a peripheral constrictor effect on the blood vessels of the kidney in animals (2, 8, 13, 17) which, together with its weakening effect on the circulation, results in a decreased output of urine (oliguria). Since digitalis has a cumulative action, oliguria should follow the repeated administration of small doses and the effect observed by measuring the urine output at short

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* Scientific Section, A. PH. A., continued from Madison meeting, A. PH. A., 1933.

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intervals. The increase in toxic activity of the drug, as shown by a constantly decreasing output of urine, should thus serve as a guide to the rate and amount of administration in intravenous methods of assay, and, by indicating the approach of death, make possible a more accurate determination of the minimum lethal dose than can be obtained by the continuous intravenous injection method of Hatcher and Brody (10, 11) and its modifications (1, 12, 19).

The cat was selected as the experimental animal because it can readily be made to exhibit diuresis. Moreover, it is the animal most used in this country for the assay of digitalis by an unofficial method.

Three tinctures of digitalis were tested, and their effects on urine output were observed following repeated intravenous administration of sub-lethal doses. The change from diuresis to oliguria was studied in its relation to the approach of death. The minimum lethal doses were determined and compared with those of the subcutaneous (15), new leg-vein (5) and new intramuscular (5) minimum lethal dose guinea-pig methods, and with systolic standstill doses of the U. S. P. X (18), modified four-hour U. S. P. X (4), Smith-McClosky intravenous (16) and Dooley-Higley intramuscular (6) frog heart methods.

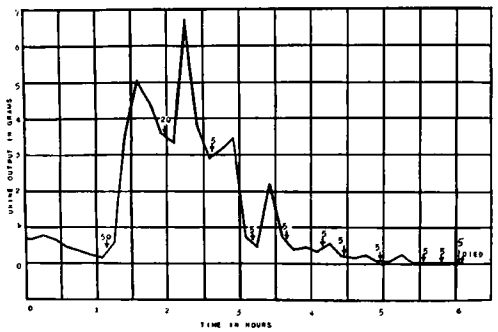

Fig. 1.—Graphic representation of urine output in the cat following intravenous administration of repeated doses of digitalis. (Cat No. 13, 10/17/33; also see protocol.)

EXPERIMENTAL PROCEDURE.

Male and female cats, ranging in weight from 1.5 to 4.3 Kg. were used. They were placed on a milk diet for about a week previous to the experiment, and in a few cases raw liver was added to the diet, but was later omitted when found to be unnecessary. The animals were weighed immediately before the experiment, and the dosages of both the anesthetic and digitalis were calculated on this basis. In some of the first experiments water was given by stomach tube immediately before administration of the anesthetic in order to insure an adequate output of urine, but this also was found to be unnecessary and was omitted in the later experiments. Sodium barbital, 0.25 to 0.3 of a gram per Kg., was given by stomach tube, and usually excellent anesthesia was obtained by the end of the hour; occasionally a few inhalations of ether were necessary in the induction of anesthesia, and at times during the course of the experiment, which usually lasted several hours.

When anesthesia was complete, the femoral vein was exposed for use in intravenous injection. A small median incision was made in the lower abdomen, exposing the bladder, and the urine removed by gentle compression or by a hypodermic syringe and needle. A small thistle tube cannula was then inserted in the bladder through a small incision, and carefully tied in so as to avoid obstructing the ureters. By means of rubber tubing this was connected with a long glass tube, and the system filled with warm saline. As fluid began to drop from the distal end of the tube it was collected in tared flasks at stated intervals, usually of ten minutes, throughout the course of the experiment, and weighed, the amount of urine excretion being plotted on graph paper.

The digitalis solution was prepared for injection by evaporating all alcohol from the tincture and diluting the residue with physiological saline to a 5 per cent solution, so that 1 cc. represented 50 mg. of the leaf. When the urine secretion reached a normal level, which on the average was about thirty minutes after cannulation of the bladder, the first injection of digitalis was made with

an accurately graded Luer tuberculin syringe into the exposed femoral vein, at the rate of 1 cc. per minute. The first dose of 50 mg. per Kg., which is about 50 per cent of the minimum lethal dose of the standard leaf for cats, has been found to be entirely satisfactory with the tinctures examined. Marked diuresis usually appeared in ten to fifteen minutes, lasting for upward of an hour (Fig. 1). When the increments of urine began to decrease, another and smaller injection of 10 to 20 mg. of digitalis per Kg. was made, and the same observations made as described above. The same procedure was repeated with successive doses of 5 mg. per Kg., until the urine excretion began to approach or fall below the normal obtained at the beginning of the experiment. This condition, which may be the result of a failing circulation or of peripheral constriction of the kidney blood vessels, or both, has proved to be a sign of approaching death. The 5-mg. doses were then injected at 10- to 20-minute intervals, and the time of death noted, cardiac stoppage being observed by mediate auscultation.

In these studies cardiac failure occurred before or simultaneously with respiratory failure, never after; artificial respiration applied in some of the earlier experiments had no beneficial effect, and was abandoned. Control injections of physiological saline, equal in liquid content to the largest amount of fluid injected with the digitalis preparations, had no effect on urinary output.

The following protocol is typical of the procedure and results obtained from the diuretic oliguric cat method (see Fig. 1).

10/17/33.	Cat, female, 2.86 Kg., on milk diet.
9:35 A.M.	Administered sodium barbital by stomach tube, 0.3 Gm. per Kg. (0.86 Gm.).
10:30	Anesthesia complete, respiration slow, deep and regular.
10:33	Began operation. Tracheotomy performed, femoral vein exposed, bladder cannulated.
10:52	Operation completed.
10:55–12:05 P.M.	Collected and weighed urine at ten-minute intervals until satisfactory control was obtained.
12:08–12:14	Injected tincture "C," 5 per cent, alcohol-free, 50 mg. per Kg., into femoral vein of right leg.
12:14–12:35	Marked diuresis.
12:30	Animal swallows, breathes rapidly and violently, alternating for several half-minute periods (nausea); heart rate markedly slowed.
12:57½–12:59½	Injected tincture "C," 20 mg. per Kg.
1:02	Marked diuresis, symptoms as at 12:30.
1:37–1:37½	Injected tincture "C," 5 mg. per Kg.; slight diuresis.
2:11–2:11½	" " " " " " " marked "
2:38–2:38½	" " " " " " " slight "
3:09½–3:10	" " " " " " " " "
3:28½–3:29	" " " " " " " " "
3:57–3:58	" " " " " " " " "
4:25	Anuria
4:30–4:31	Injected tincture "C," 5 mg. per Kg.
4:47–4:48	Injected tincture "C," 5 mg. per Kg.
4:53	Labored respiration; heart rate alternately fast and slow.
5:06–5:07	Injected tincture "C," 5 mg. per Kg.
5:07–5:11	Heart irregular; stops for a few seconds, then beats rapidly; respiration also irregular.
5:11	Heart stopped.
5:11½	Respiration ceases; no urine since 3:58.

Summary: Total mg. per Kg. of tincture "C" to produce anuria:* 100.
Total mg. per Kg. of tincture "C" to produce death: 115.

* Often anuria does not occur until death.

The average minimum lethal doses in Table I indicate that tincture "D" is 92 per cent and tincture "B" 78 per cent of the strength of tincture "C."

The minimum lethal doses for tincture "B" are exceptionally uniform, but this is not true for tinctures "C" and "D." The greatest variations from the average minimum lethal doses for "C" and "D" occurred with animals below (3, 15, 24) 2 Kg. or above 3 Kg. (4, 30).

TABLE I.—COMPARISON OF MINIMUM LETHAL DOSES OF TINCTURE "B," "C" AND "D" BY THE DIURETIC OLIGURIC CAT METHOD.

Tincture.	Cat Number.	Sex.	Weight in Kg.	Minimum Lethal Dose, Mg. per Kg.	Average Minimum Lethal Dose, Mg. per Kg.	Average Deviation.
B	17	F	2.29	80		
B	18	M	2.62	75		
B	19	F	1.54	75		
B	20	F	2.35	70	73.6	± 3.1
B	21	F	2.55	70		
B	22	M	2.20	75		
B	26	F	2.04	70		
C	3	F	1.87	62.5		
C	4	M	3.13	125		
C	6	M	2.42	85		
C	8	F	2.27	100	94.6	±15.3
C	13	F	2.86	115		
C	14	M	3.27	100		
C	15	F	1.44	80		
C	16	F	1.64	90		
D	24	F	1.84	105		
D	25	M	2.80	80		
D	27	F	2.61	80		
D	28	F	2.22	70	87.1	±15.3
D	29	F	2.76	65		
D	30	M	4.30	120		
D	31	F	2.60	90		

TABLE II.—A COMPARISON OF TINCTURES "B," "C" AND "D" BY CAT, FROG AND GUINEA-PIG METHODS.

Animal.	Method Used.	Tincture "B." Minimum Systolic* or Lethal[1] Dose in Mg. per Gm.	Tincture "C." Minimum Systolic* or Lethal[1] Dose in Mg. per Gm.	Tincture "D." Minimum Systolic* or Lethal[1] Dose in Mg. per Gm.
Frog	U. S. P. X one-hour lymph-sac	0.8	...	0.7
	Modified U. S. P. X four-hour lymph-sac	0.8	...	0.8
	Intramuscular (Dooley-Higley)	0.8	...	0.8
	Intravenous (Smith-McClosky)	0.4	...	0.4
Guinea Pig	Subcutaneous (Reed and Vanderkleed)	0.25	0.3	0.3
	New Intramuscular	...	...	0.225
	New leg-vein	0.175	0.2	0.2
Cat	New diuretic oliguric	0.074	0.095	0.087

* Frog methods.

[1] Guinea-pig and cat methods.

Table II summarizes the results of assays of tinctures "A," "B" and "C" on frogs, guinea pigs and cats. Tincture "C" was exhausted before frog assays could be made. Analysis of this table permits the following deductions:

Tinctures "B" and "D" are practically identical in activity as tested by four frog heart methods.

Tincture "B" is slightly stronger than "C" and "D" by the guinea-pig methods, "C" and "D" being of equal strength by these tests.

Tincture "B" is strongest by the diuretic oliguric cat method, whereas "C" is weaker than "D."

The results of the frog and guinea-pig methods are in closer agreement with each other than they are with those of the cat method.

When measured by the results of the intravenous frog, new intravenous guinea-pig and new diuretic oliguric cat methods, digitalis is about twice as toxic to the cat as to the guinea pig and about twice as toxic to the guinea pig as to the frog:

Cat = 1. Guinea pig = 2. Frog = 4.

DISCUSSION.

The assays reported above corroborate many observations made in the literature concerning the variations in susceptibility of cats to digitalis (7, 9, 14). The assays on tincture "B" are remarkably uniform, in contrast to the large positive and negative variations in the results obtained with tinctures "C" and "D." Large variations in susceptibility in frogs and guinea pigs are of less importance because large numbers of these animals can be used in contrast to cats, where the experimental procedure is much more prolonged, and the animals more difficult to obtain in large numbers. When small numbers of animals are used for an assay it seems logical to agree with Burn (3) that it is indefensible to set aside the results obtained from one or two cats which show a great difference from the others, such as Cats No. 3 and No. 4 in tincture "C" (Table I). Burn points out that owing to the large range in sensitivity of cats, "the average of three cats, for example, can never be assumed to be nearer the true value than that of four," and states that the average of results on a small number of cats is more nearly the true value as the number of cats is increased, the true results being obtained from the average of a very large number, such as one hundred.

In the new diuretic oliguric cat method just described the drug is not injected continuously, and this would appear to be an advantage not possessed by the intravenous methods usually employed. As digitalis is a drug which acts slowly, it would appear impossible to get an accurate determination of its true activity when employing a comparatively rapid continuous injection method.

Following the early diuretic effect, the repeated administration of small doses produces a constantly decreasing output of urine (oliguria), a condition due to toxic action, and an excellent indicator of the approach of death, more easily discernible than the heart changes ordinarily observed by auscultation in the usual cat method. Anuria occurs sometimes, before death.

It should also be observed that the best results were obtained with cats ranging in weight from 2 to 3 Kg., in the relatively small number of animals used. Cats above or below these weight limits showed a wider variation in susceptibility. These weight extremes may be identical with extremes in age which might account for the variations in resistance.

SUMMARY AND CONCLUSIONS.

A new intravenous cat method for the bioassay of digitalis is described, which utilizes the changes produced in urinary output to determine the amount and frequency of administration of the drug.

The progress from diuresis to oliguria following intravenous injection of successive doses of digitalis is a useful index of the development of toxic activity of the drug, and thus serves as a guide to the frequency and amount of its administration.

Cats showed wide individual variations in their susceptibility to digitalis, and their value in the assay of this drug appears questionable since in practice only a few animals are used.

Results of frog and guinea-pig assays agreed more closely with each other than with those of the cat method.

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THE RECTAL ABSORPTION OF DIGITALIS IN CATS.*

BY W. ARTHUR PURDUM.

INTRODUCTION.

Digitalis and its preparations, when administered by mouth, frequently cause nausea and vomiting. To overcome this untoward effect, Eichhorst, first (3), and later, other investigators have suggested administration by rectum. Therefore, digitalis preparations intended for rectal administration in suppository form and in solution have been offered to the medical profession within the last few years.

The available clinical reports on the administration of digitalis by rectum are favorable. It, therefore, seemed desirable to determine the preparation best suited for rectal use. With this objective in view, two preliminary experiments were conducted to determine whether or not digitalis is absorbed from the cat rectum. Large

* From the laboratories of A. G. DuMez, Professor of Pharmacy, and Marvin R. Thompson, Professor of Pharmacology, School of Pharmacy of the University of Maryland. Compiled from a thesis submitted to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Master of Science.