

TOXICITY OF DIGITALIS TO WHITE RATS.*

BY WALTER E. WENTZ, JR.

Many investigations have been made to determine the effects of digitalis and related drugs upon various animals with several purposes in mind. First, to discover the pharmacological action of the drug upon the animal employed. Second, to demonstrate the variations in the action of the drug dependent upon the animal used. Third, to show the influence of season, food, weight, sex and ventilation upon the susceptibility of the test-animal to the drug. Fourth, to devise the most suitable method for the biological assay of the drug.

The present accepted "One-Hour Frog Method" (1) for the biological assay of digitalis and its related drugs is, in the minds of many, far from ideal. For this reason, other animals have been subjected to the action of digitalis in the hope that some better method might be discovered.

Vanderkleed (2) and Haskell (3) have experimented independently with guinea-pigs; both agree that the guinea-pig is superior to the frog in that the pharmacological action on the guinea-pig more closely approximates that on man; also, that the guinea-pig under standard conditions shows less variation in minimum fatal dose than does the frog. Haskell claims that age, sex, and weight of a guinea-pig do not affect its susceptibility to digitalis, but that there is a seasonal variation in the resistance of the animals to this drug, which must be considered in the standardization of digitalis. Vanderkleed finds that age, sex, weight, food and season influence the susceptibility of the guinea-pig to digitalis intoxication. Haskell demonstrates (4) that the alcohol-content of preparations of digitalis will markedly influence the results obtained from their subcutaneous injection into guinea-pigs and rabbits.

The cat has been employed by Hatcher and Brody (5) as the laboratory test-animal in the biological assay of digitalis and its preparations. Macht and Colson (6) report some very interesting experiments in which they found that fatal doses of digitalis in vagatomized cats gave more uniform results than animals with vagi intact, also that the toxicity of digitalis compounds is greater in vagatomized cats. Colson (7) made extensive studies of the "One-Hour Frog Method" and the M. L. D. cat method. He concludes from his experimentation that the cat method is capable of greater absolute, as well as relative, accuracy in the assay of the Digitalis series.

Pittenger and Vanderkleed (8) have proposed the use of gold-fish (*Carassius auratus*) as test-animals. Their experiments show that the fish die in direct proportion to the strength of the solution (1:75-1:3800) in which they are placed. Alcohol to the extent of that contained in the U. S. P. tincture did not appear to affect results. They state that goldfish show much less individual variation than do frogs, that the weight of the fish may be disregarded in these experiments, and that goldfish are less expensive than frogs.

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EDITOR'S NOTE: It will be evident to the readers that the purpose of publishing the results recorded in this paper and the succeeding one (on *Strophanthus*) is not to present a better method, but for the information of others, who have contemplated investigations along the same lines, *i. e.*, using white rats.

Inasmuch as the white rat is now extensively used as a test-animal and has many advantages (9), this animal has been used.

Large numbers of well-cared-for rats of approximately the same age and weight are used in testing arsphenamine and similar products. This offered a test-animal which might be obtained very cheaply after being used for these tests. The rats used in the following observations were furnished by the Powers-Weightman-Rosengarten Company after they had been used in making tests for therapeutic efficiency.

The chief purpose of these observations was to ascertain the toxicity of an official digitalis preparation to the white rat. *Tinctura Digitalis*, U. S. P., was selected as the preparation. One hundred mils of U. S. P. tincture represents ten grams of digitalis and contains about seventy-one per cent of alcohol. The tincture used was assayed biologically by Grant Favorite as follows:

The tincture was diluted with equal parts of physiological salt solution.

One mil of this dilution was equivalent to 0.5 mil of the original tincture.

TABLE I.—BIOLOGICAL ASSAY OF TINCTURE OF DIGITALIS, U. S. P.

Frog.	Weight in grams.	Mils of tincture per gram body weight.	Dose of dilution.	Effect on heart.	
1	36	0.0055	0.396 mil	Beating slowly	
2	30	0.006	0.36 mil	Stopped	M.L.D.
3	31	0.0065	0.403 mil	Stopped	
4	29	0.007	0.406 mil	Stopped	
5	29	0.0075	0.435 mil	Stopped	

The drug was tested on twenty frogs. The "One-Hour Frog Method" was used. The minimum lethal dose was 0.006 mil per gram of body weight.

To insure accuracy, the technic employed in these tests was modeled after that standardized by the Hygienic Laboratory, Washington, D. C. (10). Healthy, non-pregnant rats weighing between one hundred and fifty and two hundred grams were weighed before injection and the dose of the drug was administered in proportion to body weight. The animals were fed twelve to fifteen hours before the time of injection to render the dosage more accurate. After the animal was bound to the operating board, the skin on the anterior surface of the thigh was cleansed with alcohol and the hair shaved off. A small incision was made over the saphenous vein and all the subcutaneous tissue was cleared away from the vein for a distance of about one centimeter. Injection into this vein was made at a given rate of flow, one mil per sixty seconds.

Since Haskell found that the alcohol content of digitalis preparations markedly influences the drug's effect upon guinea-pigs, a series of experiments was performed to determine the toxicity of alcohol to the white rat. Preliminary tests were made to determine the most satisfactory dilution of alcohol. The solution adopted contained sixty parts of ninety-five per cent alcohol to forty parts of physiological saline solution by volume. The smallest fatal dose was found to be 0.003450 mil per gram of body weight. The fatal dose produced death within ten minutes of the time of injection.

Having determined the toxicity of alcohol, an effort was made to keep the alcohol content of the doses of the tincture below this toxic dose. A series of preliminary tests was performed using various dilutions of the tincture of digitalis in

physiological salt solution. A dilution containing three parts by volume of the tincture to one part by volume of physiological salt solution was found to be most satisfactory for white rats of the weight employed in this experiment.

TABLE II.—EFFECT OF ALCOHOL.

Rat.	Weight in grams.	Mil of 95% alcohol per gram of body weight.	Dilution of alcohol injected.	No. of mils injected.	Results.
1	153	0.00333	60%	0.85	Rat lived
2	145	0.00339	60%	0.82	Rat lived
3	160	0.003412	60%	0.91	Rat lived
4	153	0.003412	60%	0.87	Rat lived
5	167	0.003413	60%	0.95	Rat lived
6	165	0.003418	60%	0.94	Rat lived
7	167	0.003418	60%	0.95	Rat lived
8	161	0.003428	60%	0.92	Rat lived
9	140	0.003428	60%	0.80	Rat lived
10	175	0.003428	60%	1.00	Rat lived
11	175	0.003428	60%	1.00	Rat lived
12	150	0.003440	60%	0.86	Rat lived
13	150	0.003440	60%	0.86	Rat lived
14	150	0.003440	60%	0.86	Rat lived
15	160	0.003450	60%	0.92	Rat died
16	160	0.003450	60%	0.92	Rat died
17	160	0.003450	60%	0.92	Rat died
18	165	0.003454	60%	0.95	Rat died
19	158	0.003455	60%	0.91	Rat died
20	142	0.00346	60%	0.82	Rat died
21	173	0.00346	60%	1.00	Rat died
22	150	0.003480	60%	0.87	Rat died
23	155	0.003484	60%	0.90	Rat died
24	160	0.003488	60%	0.93	Rat died
25	140	0.00351	60%	0.82	Rat died
26	145	0.00352	60%	0.85	Rat died
27	170	0.00353	60%	1.00	Rat died
28	135	0.00355	60%	0.80	Rat died
29	150	0.00360	60%	0.90	Rat died
30	140	0.00428	60%	1.00	Rat died
31	115	0.00522	60%	1.00	Rat died

The smallest fatal dose was found to be 0.003950 mil of tincture of digitalis, U. S. P. per gram of body weight. All animals, which died as a result of the intravenous injection of the tincture, died within fifteen minutes after injection.

TABLE III.—ACTION OF TINCTURE OF DIGITALIS, U. S. P.

A. Non-Fatal Doses.

Rat.	Weight in grams.	Mil of Tincture of Digitalis per gram of body weight.	Mils injected of dilution of: Tincture of Digitalis 3 parts P. Salt Solution 1 part.
1	246	0.003049	1.00
2	177	0.003135	0.74
3	143	0.003147	0.60
4	237	0.003164	1.00
5	230	0.003219	0.99
6	231	0.00324	1.00
7	207	0.00326	0.90
8	155	0.00348	0.72
9	194	0.003706	0.96

10	190	0.003789	0.96
11	175	0.00385	0.90
12	151	0.003926	0.79
13	187	0.003930	0.98
14	158	0.003936	0.83
15	177	0.003940	0.93
16	152	0.003945	0.80

B. Fatal Doses.

1	155	0.00435	0.90
2	144	0.00416	0.80
3	171	0.00412	0.94
4	144	0.004062	0.78
5	163	0.004049	0.88
6	165	0.004000	0.88
7	188	0.00399	1.00
8	143	0.00398	0.76
9	164	0.003979	0.87
10	172	0.003957	0.91
11	180	0.003955	0.95
12	150	0.003950	0.79
13	150	0.003950	0.79

CONCLUSION.

Tincture of digitalis is more toxic to the white rat than it is to the frog.

The toxic dose of tincture of digitalis for the guinea-pig as determined by Vanderkleed (11) was quite near to that for the white rat.

Tincture of strophanthus, another digitalis substance, which was determined by a colleague, D. W. Beddow, was more than twice as toxic as tincture of digitalis.

These facts are shown by this table:

Tincture of Digitalis U. S. P.	Mil per gram of body weight.
White Rat.....	0.00395
Guinea-pig (Vanderkleed).....	0.0025
	0.003125
	0.004166
Frog (U. S. P. Biological Assay).....	0.006
Tincture of Strophanthus U. S. P.	
White Rat (Beddow).....	0.001719

The variations in the toxic doses of the tincture of digitalis on the guinea-pig were shown by Vanderkleed to be due to variations in the digitoxin content of the tinctures.

The tincture of digitalis in toxic doses on white rats produces death in a much shorter time than on the frog or guinea-pig. The toxic doses tabulated above produced death in one hour in the case of the frog, and in two hours in the case of the guinea-pig. In this experiment, no rat which lived more than fifteen minutes died as a result of the drug, but recovered and was active as the normal rats in six to twelve hours.

The relation of the contained alcohol to the toxicity of the tincture of digitalis is shown by the following:

The toxicity of alcohol was shown to be 0.003450 mil per gram of body weight.

The alcohol in the toxic dose of the tincture was about 0.00280 mil per gram of body weight.

Since the amounts of alcohol injected in these experiments are so near the minimum fatal dose of alcohol, further experiments must be made to determine the relation of the alcohol present to the minimum fatal dose of the tincture of digitalis.

My thanks are due the Powers-Weightman-Rosengarten Company for the presentation of the rats used in these experiments.

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TOXICITY OF STROPHANTHUS TO WHITE RATS.*

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An attempt was made, in the following investigation, to ascertain the toxicity of *Strophanthus* to white rats. The preparation used in this experimentation was the official preparation, *Tinctura Strophanthi*, U. S. P., each one hundred mils of the tincture representing ten grams *strophanthus* in approximately ninety-two per cent of alcohol.

Before beginning the experimental work, it was deemed advisable to standardize the particular tincture which was to be used. A biological assay of the tincture was made by Grant Favorite, according to the one-hour frog method, as described in the United States Pharmacopœia, IX (2). As a result of his determinations, it was found that the minimum lethal dose of this tincture was 0.00006 cc. for each gram of body weight of the frog.

White rats, ranging in weight from one hundred and fifty to two hundred grams, were obtained and prepared for injection in a manner similar to the method adopted by the Hygienic Laboratory at Washington, D. C. (1). The animals were fed twelve to fifteen hours before injection of the drug in order to insure accuracy. Just prior to injection each animal was weighed, marked, and the amount of the drug

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