SUSCEPTIBILITY OF THE GUINEA PIG TO POISONING BY DIGITALIS.

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A method of drug assay to be suitable for an official assay process should be accurate. This point outweighs all others, and many objectionable features can be overlooked if the method in question enables us to measure accurately the therapeutic properties of the drugs examined. Nevertheless, should two methods show no appreciable difference regarding the degree of accuracy attainable, the cheapness, simplicity, and rapidity of performance come to have considerable influence in the choice between them.

The advocates of the frog methods believe that careful investigation, extending over many years in different laboratories has shown that these methods carried out upon "standardized" animals enable the pharmacologist to estimate the strength of digitalis preparations with an error not greater than 10 per cent. So far as we have been able to learn, the valid objections to the use of frogs are removed when a reliable standard, such as ouabain or Houghton's crystalline strophanthin, is used. Compared with the guinea-pig method, proposed by Dr. Reed, the frog methods are much cheaper. In the assay of digitalis preparation by the one-hour frog heart method, we practically never use more than 18 frogs, the cost of which varies from forty to seventy-five cents. Our ignorance of the routine use of the guinea-pig method prevents us from stating definitely the cost of an assay. It scarcely seems probable, however, that, on an average, less than six or eight animals would suffice. The guinea pigs we have been able to obtain have cost us from 40 to 75 cents apiece, making the probable cost of an assay from two to six dollars, about 800 per cent. greater than the frog method.

Closely connected with the cost, are the rapidity and simplicity of execution. By the one-hour frog heart method, an assay can be completed by one man in three hours at the utmost. By the guinea pig method, at least twelve hours must be consumed, and probably twenty-four. Moreover, an assistant is required in injecting the solution into the guinea pig and accuracy of dosage is more difficult to obtain. It would seem, from the investigations that we have carried out, that an alcoholic strength of 25 per cent. has no appreciable influence on the reaction of frogs to ouabain, so that removal of the alcohol from official digitalis preparations is not necessary for their assay upon frogs, since the necessary dilution accomplishes the desired reduction.

But if it be granted that the guinea-pig method is deficient in these points, is the accuracy attainable by it sufficient to counterbalance the less important considerations? Dr. Reed and Dr. Githens are convinced that this is so, and the opinions of such careful investigators is worthy of much consideration.

The question of the lethal action of digitalis upon mammals is of some interest. Dr. Githens believes that guinea pigs die as a result of the action of the drug upon the heart, all the phenomena observed being due to circulatory embarrass-

ment. It would seem advisable to adduce experimental evidence in support of a view which is denied by such men as Edmunds, Hale, Cushny, and, apparently, has been disproven for rabbits by Nestor.

This point, however, is relatively unimportant if it can be proven that the lethal power of digitalis for guinea pigs runs parallel with the theapeutic efficiency of the drug. That such parallelism exists, however, is a dangerous assumption if our knowledge concerning the chemistry of digitalis is correct. It is held that the glucosides tend to break up under certain poorly understood conditions, giving rise, probably, to numerous decomposition products, among which toxiresin and digitalresin are prominent. According to Sollman, these bodies appear to be more poisonous than the oroginal glucosides, so it is conceivable that an old, deteriorated preparation, one that is not only therapeutically weak but capable of doing serious damage if used clinically, would seem of good strength when tested by a lethal dose method. If we are right in the assumption that the therapeutically active glucosides are the only substances present in digitalis preparations which are capable of bringing about the cardiac changes upon which the one-hour frog heart method is based, it is evident that this method, being a qualitative as well as quantitative test of digitalis, possess a great advantage over the lethal dose methods.

Aside from these theoretical considerations, what evidence is there for or against the guinea pig as a test animal? So far as we can discover, there have been no reports of experiments carried out to show that the susceptibility of guinea pigs to digitalis poisoning is constant regardless of age, weight, sex, diet, season, or locality. On the other hand, Dr. Houghton has found them less useful than frogs, and, as the result of his earlier work, he discarded guinea pigs for the latter animal in the assay of strophanthus.

While the variations in the susceptibility of guiena pigs to other poisons cannot be accepted as positive proof that similar variations will occur when digitalis is the toxic agent, yet such evidence is very suggestive.

Dr. Arms has called attention to the fact that very striking individual variations are encountered when tests are made upon guinea pigs with the virus of rabies or with material containing infectious organisms. Hunt has shown that guinea pigs vary in their susceptibility to acetonitrile according to season and diet. In January, the m. l. d. of acetonitrile per gram guinea pig was 0.20; in July, 0.45, a difference of 125 per cent. One series of pigs fed on green food survived a dose of 0.53 gm., while a second series, kept on an oats diet under similar conditions, succumbed to a m. l. d. of 0.23, a difference of 130 per cent.

Sudmersen and Gleny found that guinea pigs varied in their susceptibility to diphtheria toxin according to season and inversely as to age. In January, the approximate m. l. d was 0.006; in July, 0.009; a variation of 50 per cent. It is worth noting that these investigators found that the animals' resisting power to diphtheria toxin was greater in summer and fall, agreeing with Hunt's results when acetonitrile was used.

If similar variations in the susceptibility of guinea pigs to digitalis poisoning should be found present, it is obvious that these animals would be suitable for

the assay of the members of the digitalis group only when a standard preparation is used on a control series of pigs, and the method would possess absolutely no advantage over the frog methods, while being much more cumbersome. In view of these facts, we have taken up the study of the susceptibility of guinea pigs to poisoning by members of the digitalis series in the attempt to learn whether this susceptibility is the same in a series of animals kept under similar conditions is uninfluenced by artificial respiration; by the weight of the animals; by the diet of the animals or by the season of the year. Further, we wish to investigate the action of old preparations of digitalis upon frogs and upon guinea pigs to see whether the rate of deterioration is the same as determined by the two methods.

In this paper we present only a preliminary report. Two factors, the lack of time and the great difficulty of securing guinea pigs, have forced us to postpone much of that we had contemplated doing, but we have secured some rather incomplete data upon a few of the points mentioned.

We found it impossible to obtain a sufficient number of pigs, although we tried dealers in Indianapolis, Terre Haute, Lexington, Chicago, and Boston. The pigs we secured were, except when stated otherwise, fed liberally on oats, hay and cabbage, and had access to water constantly.

In these earlier experiments, we have used the crystalline gratus strophanthin or ouabain of Merck. A stock solution of 1 to 1000 was made up with 70 per cent alcohol, and this was diluted 1-10 with normal salt solution before injection. We realize that there are points of difference in the action of the various members of the digitalis group, but we do not believe that these differences are great enough to vitiate the results we have secured. The solution was injected under the skin of the abdomen by means of the Hitchens syringe, by which accuracy of dosage was secured.

Dr. Reed at first considered that any animal receiving a lethal dose would succumb within three hours. The time was subsequently extended to twelve hours, but the impossibility of working a twelve hour limit in the ordinary day led us to adopt a twenty-four hour limit. One pig succumbed after thirty hours; another after fifty hours had elapsed.

In every instance that we observed the lethal action of tincture of digitalis or of ouabain upon guinea pigs, we noted that the animals made violent ineffectual inspiratory efforts, the phenomena suggesting obstruction of the air passages. In a few minutes the struggles ceased, the animals lost consciousness and were apparently dead, but in every case the heart could be distinctly felt beating, and continued to beat several minutes. On opening the chests of such animals after the heart had ceased beating, the organ was always in diastole. We were convinced from this that the cause of death from poisoning by ouabain was due to respiratory failure, and to prove this we instituted artificial respiration on six animals, commencing to operate only when voluntary respiration had entirely ceased and the pigs were apparently dead. In all but two instances, marked temporary improvement occurred in the condition of the animals, but we were not able to save any of the series. From this we were led to conclude that death

of guinea pigs from poisoning by ouabain is due primarily to respiratory failure, but that cardiac poisoning is concerned in this and would ultimately, in itself, cause death. Possibly, by the earlier institution of artificial respiration, death could be averted, but we did not consider the point of sufficient importance to justify the necessary infliction of pain.

It must be remembered that the different members of the digitalis group differ among themselves as regards the relative intensity of their action upon the central nervous system and upon the heart. Possibly, ouabain has more of the direct cardiac action than have the bodies that Nestor used, which would account for our contradictory results.

The following are records of observations typical of the series, save in the two animals where death occurred before commencement of artificial respiration:

No. 1. 1:15 P. M.—Pig weighing 485 grams received 0.00035gm. ouabain per gram body weight, subcutaneously.
2:50 P. M.—Complete cessation of respiration. Animal limp and apparently dead, except for strong, rapid heart-beat.
2:52 P. M.—Artificial respiration begun.
2:58 P. M.—Animal struggles feebly; heart beat strong and regular.
3:27 P. M.—Thorax opened. Heart contracting feebly; slow and regular rate.
3:45 P. M.—Heart ceases in systole.

No. 2. 1:45 P. M.—Weight 280 gm. received 0.00000025 gm. ouabain per gm. body weight, subcutaneously.
2:30 P. M.—Violent convulsions.
2:35 P. M.—Respiration ceased. Limp and unconscious.
2:38 P. M.—Artificial respiration begun.
2:40 P. M.—Heart beating strongly.
4:00 P. M.—Heart-beat faintly felt.
4:05 P. M.—Chest opened: feeble auricular contractions. Ventricles contracted.

An attempt was made to learn with what degree of accuracy the minimum lethal dose of ouabain could be determined upon a series of pigs kept under the same conditions. The following were the results secured:

TABLE No. I.

Dose per gm.	Survived	Died
0.00020 mgm.	1	0
0.00023 mgm.	1	0
0.00024 mgm.	1	0
0.00025 mgm.	4	0
0.00026 mgm.	1	1
0.00027 mgm.	4	2
0.00028 mgm.	4	3
0.00029 mgm.	4	5
0.00030 mgm.	2	6
0.00031 mgm.	0	1
0.00032 mgm.	1*	0
0.00033 mgm.	0	1
0.00035 mgm.	0	2
0.000375 mgm.	0	1
0.00040 mgm.	0	1

* Died during night over thirty hours after injection.

From this table it appears that 0.00026 mgm. killed one pig, while 0.00032 mgm. failed to kill another within the twenty-four hour limit, a difference of 23 per cent. It must be understood, however, that the pigs in this series differed

in sex, weight, and the locality from which they came. The individual variation was not nearly as large as we had expected to find, and we are inclined to think that even less variation would be encountered when the pigs are of the same age and weight and are grown in the same locality. We regret that the impossibility of getting a sufficient number of animals prevented us from making this series as complete as it should be.

The next point of practical importance was a study of the influence of diet. We were struck by the rapid increase in the weight of those pigs fed liberally, and it occurred to us that this rapid increase in weight might cause increase in susceptibility.

Two lots of six pigs each were selected and placed under the same general conditions. One lot was fed liberally on a mixed oat, clover hay, cabbage, and carrot diet for seven days. The other lot was fed a restricted amount of the same diet for the same length of time. The well-fed pigs gained an average of 21 grams per pig, while those on the restricted diet lost on the average 14 grams per pig. The minimum lethal dose per gram weight for the pigs on a liberal diet was found to be 0.00026 mg., while that for those on the restricted diet was 0.00025 mg.

TABLE No. II.

No.	Sex			Dose in mg.	*Result of liberal mixed diet.*
1.....	M	328	356	0.00024	Survived.
2.....	M	241	280	0.00025	Survived.
3.....	M	678	684	0.00025	Survived.
4.....	M	173	194	0.00026	Died 1 hr. 10 min.
5.....	M	233	253	0.00027	Died 1 hr. 20 min.
6.....	M	305	319	0.00029	Died 1 hr. 30 min.
					Result of restriced mixed diet.
1.....	M	162	159	0.00024	Survived.
2.....	M	291	280	0.00025	Died 1 hr. 50 min.
3.....	M	579	531	0.00025	Died 1 hr. 30 min.
4.....	F	229	239	0.00026	Died 1 hr. 15 min.
5.....	F	261	250	0.00027	Died 1 hr. 15 min.
6.....	M	343	321	0.00029	Died 1 hr. 7 min.

Hunt found variations in diet to influence markedly the susceptibility of guinea pigs to acetonitrile poisoning and suggests that oats diet has some specific action upon the thyroid. It seemed to us that the susceptibility of these animals to digitalis might be similarly influenced, but the impossibility of securing sufficient numbers of pigs and the limited time at our disposal, prevented us from securing really conclusive evidence on this point.

Sixteen sound, healthy pigs, raised by the same breeder, under similar conditions, were divided into two groups of eight. The members of the first group were fed as much oats as they would take and were given a small amount of cabbage once. The members of the second group were fed as much cabbage and carrots as they would take. As may be seen from the table, the whole time of the experiment was fourteen days, and if such results were observed after this limited time on these different diets, more striking differences would be expected when the experiment extends over considerable time. One pig of Group I became sick and was not used; one pig of Group II was discarded because a part of the ouabain solution escaped from the syringe when the injection was being made.

TABLE No. III.

Series I. Oats Diet.

No.	7-25-11 Weight	8-7-11 Weight	8-8-11 Weight	Dose in gms.		
1	140	167	—	0.00000022	Survived.	
2	200	—	200	0.00000022	Survived.	
3	117	—	116	0.00000023	Survived.	(Died 50 hrs. after injection.)
4	174	—	230	0.00000024	Died.	
5	293.5	—	318	0.00000024	Died.	
6	189	179	—	0.00000024	Died.	
7	174	175	—	0.00000026	Died.	

Series II. Cabbage and Carrots.

1	147	152	—	0.00000026	Survived.
2	315	—	295	0.00000026	Survived.
3	175	178	—	0.00000026	Survived.
4	186	177	—	0.00000026	Survived.
5	221	—	213	0.00000026	Survived.
6	216	—	215	0.00000027	Survived.
7	226.5	—	220	0.00000028	Survived.

The results secured by such a limited number of animals are, of course, not absolutely conclusive. None of the pigs of Group II seemed seriously sick, although a dose of 0.00000028 gm. was given one, while pig No. 3 of Group II succumbed to 0.00000023 after 50 hours and doses greater than this were invariably fatal within 24 hours. It is unfortunate that the scarcity of guinea pigs and the lack of time prevented us from going more fully into this question. We hope to do this later.

In conclusion, we may say that the individual variations in the susceptibility of guinea pigs to poisoning by ouabain, as determined in a small series of animals, varies as much as 23 per cent. This may be due to age, weight, or source of the animals.

2. That the life of a guinea pig that has received a lethal dose of ouabain can be prolonged by the employment of artificial respiration, but in no instance were we able to save the animal.

3. That a diet of oats with a minimum amount of food seems to cause a decided increase in the susceptibility of guinea pigs; while a diet exclusively of green food seems to diminish susceptibility.

The first and third points are of great practical importance, and, if they be confirmed, would seem to render the guinea pig method as now employed unsuited for standardization of the members of the digitalis group.

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THE CRIMES OF RESPECTABILITY.

"Theft and murder are awful crimes, yet in any single year the aggregate sorrow, pain and suffering they cause in a nation is microscopic when compared with the sorrows that come from the crimes of the tongue. Place in one of the scale-pans of Justice the evils resulting from the acts of criminals, and in the other the grief and tears and suffering resulting from the crimes of respectability, and you will start back in amazement as you see the scale you thought the heavier shoot high in air."—*William George Jordan.*