men of tincture of digitalis for examination because of the failure to secure any toxic effect with total amounts of as much as 149 Cc. administered to a patient, weighing 140 pounds, during a single course of treatment. This amount merely caused slowing. I found the tincture to be active by the biologic test.

The chloroform-soluble fraction varies in the percentage of the total active constituent, but the specimen just described showed an unusually low percentage, this fraction constituting only one-fourth of the total activity of the leaf. At the other extreme in the series thus far tested, is a specimen placed in alcohol by Professor Newcomb immediately after gathering a leaf of the first year. In this the chloroform-soluble constituent constituted about 65 percent of the active constituents.

It is my purpose to examine specimens of the leaf obtained from various sources, and variously treated, both from the first and second year, in order to determine whether one can be found of constant composition, or that which yields constantly the highest percentage content of the more absorbable constituent.

I have been fortunate in securing the coöperation of growers and dealers and hope to be able to report much progress during the coming year.

One specimen of fluidextract at least 38 years old was fractioned, and while the amount available was too small for satisfactory study (especially as it involved a probable accident) it seems that the content of the chloroform-soluble constituent in this specimen is very high.

The work raises numerous problems which cannot be discussed now, but it seems clear that the higher the digitoxin content (if digitoxin is present in the leaf) the lower should be the biologic activity measured by the official assay, but the more active by oral administration. It seems probable from previous work done in our laboratory that the water-soluble constituent is relatively more actively emetic than the chloroform-soluble. Not only is the chloroform-soluble fraction absorbed more readily than the water-soluble, but it is almost certainly more lasting in its effects, in other words, its action probably persists for as many weeks as that of the other in days.

I believe that we must assay digitalis with reference to the content of this more readily absorbable fraction, and that a specimen containing a high percentage of this fraction will be more active clinically than one showing a greater total activity but with a low percentage of this fraction.

Incidentally, I believe that this chloroform-soluble fraction may be made available for intravenous use, since it mixes perfectly with water. The permanence of such mixtures has not been sufficiently studied.

DETERIORATION OF HIGH TEST AMERICAN-GROWN DIGITALIS.*

BY J. F. O'BRIEN AND J. P. SNYDER.

In the Fall of 1916 we received a shipment of American-grown Digitalis from the state of Washington, which upon assay gave such a high test by the U. S. P.

*Read before Scientific Section, A. Ph. A., New York meeting, 1919.

IX method that we decided to prepare a tincture and a fluidextract and determine the deterioration after standing for a considerable length of time. Accordingly we prepared, following out the methods as outlined in the Pharmacopoeia, a tincture and a fluidextract, and after making tests by the U. S. P. frog method, the 24-hour guinea pig method and the Hatcher & Brody cat method these two preparations were allowed to stand in the laboratory under conditions which closely paralleled that in which tinctures and fluidextracts of Digitalis are kept in the average drug store. After a period of two and one-half years the tincture and the fluid were tested under similar conditions by the same methods as when first prepared.

TINCTURE OF DIGITALIS BY GUINEA PIG METHOD.

Animal.	No.	Wt.	Dose per 250 Gm.	Dilution.	Dose of dilution.	Results after 24 hours.
Guinea pig	1	255	0.4	1–1	0.4	Died
Guinea pig	2	315	0.6	1–1	0.75	Died
Guinea pig	3	280	0.8	1–1	0.9	Died
Guinea pig	4	310	1.0	1–1	1.24	Died
Guinea pig	5	255	1.1	1–1	1.12	Died
Guinea pig	6	215	1.2	1–1	1.03	Died
Guinea pig	2	315	0.4	1–1	0.5	Died
Guinea pig	4	305	0.3	1–1	0.36	Died
Guinea pig	5	370	0.2	1–1	0.3	Recovered
Guinea pig	6	290	0.4	1–1	0.46	Died
Guinea pig	7	320	0.3	1–1	0.38	Died
Guinea pig	1	250	0.2	1–1	0.2	Recovered
Guinea pig	2	360	0.1	1–1	0.14	Recovered

Accepting the standard for a 250 Gm. guinea pig as 1 Cc., we find this Tincture Digitalis to test 330 percent, we finding the M. L. D. for a 250 Gm. pig to be 0.3 Cc.

TINCTURE DIGITALIS BY GUINEA PIG METHOD AFTER STANDING TWO AND ONE-HALF YEARS.

Animal.	No.	Wt.	Dose per 250 Gm.	Dilution.	Dose of dilution.	Result.
Guinea pig	1	600	0.4 mil	1–1	0.96	Died
Guinea pig	2	540	0.5 mil	1–1	1.08	Recovered
Guinea pig	3	415	0.6 mil	1–1	0.99	Died
Guinea pig	4	480	0.7 mil	1–1	1.34	Died
Guinea pig	5	585	0.8 mil	1–1	1.87	Died
Guinea pig	6	575	0.9 mil	1–1	2.07	Died
Guinea pig	7	660	0.3 mil	1–1	0.79	Recovered
Guinea pig	8	405	0.4 mil	1–1	0.65	Recovered
Guinea pig	9	480	0.4 mil	1–1	0.77	Recovered
Guinea pig	10	435	0.43 mil	1–1	0.75	Died
Guinea pig	11	455	0.45 mil	1–1	0.82	Recovered
Guinea pig	12	380	0.47 mil	1–1	0.71	Recovered
Guinea pig	13	495	0.5 mil	1–1	1.0	Recovered
Guinea pig	14	595	0.55 mil	1–1	1.3	Recovered
Guinea pig	15	360	0.57 mil	1–1	0.82	Died
Guinea pig	16	480	0.55 mil	1–1	1.05	Recovered
Guinea pig	17	455	0.57 mil	1–1	1.03	Died

M. L. D. is 0.57 per 250 Gm. Pig percent of activity, 175.

TINCTURE DIGITALIS BY ONE-HOUR FROG METHOD.

No.	Dose per Gm.	Weight.	Dose.	No. Cc.	Result after one hour.	Dilution
127	0.006	28.9	0.1734	0.17	Stopped	20–100
128	0.005	31.9	0.15	0.16	Stopped	20–100
129	0.004	24.9	0.099	0.19	Stopped	20–100
130	0.003	27.9	0.083	0.08 = 0.4	Stopped	20–100
131	0.002	24.8	0.049	0.05 = 0.25	Recovered	20–100
132	0.003	26.2	0.078	0.08 = 0.4	Stopped	20–100
127	0.001	27.7	0.0277	0.0277 = 0.13	Beats	20–100
128	0.002	23.0	0.046	0.046 = 0.23	Stopped	20–100
129	0.003	27.0	0.081	0.081 = 0.40	Stopped	20–100

From the above we selected the M. L. D. as 0.0025 with ouabain at 0.000,00055, which in terms of percentage would be 264.

TINCTURE DIGITALIS BY ONE-HOUR FROG METHOD AFTER STANDING TWO AND ONE-HALF YEARS.

After standing we find that the tincture digitalis tests by the one-hour frog method 120%, we selected the M. L. D. as 0.006 with ouabain at 0.000,0006, which shows preparation to test 120%.

No.	Wt.	Dose per Gm.	Dose.	Dilution.	Dose of dilution.	Result.
1	20.2	0.003	0.06	1–1	0.06	B
2	18.0	0.004	0.07	1–1	0.07	B
3	17.3	0.005	0.085	1–1	0.085	B
4	12.5	0.006	0.075	1–1	0.075	S
5	15.2	0.007	0.1	1–1	0.1	S
2	18.4	0.008	0.15	1–1	0.15	S
1	18	0.0055	0.1	1–1	0.1	B
2	17.6	0.0057	0.1	1–1	0.1	B
3	15	0.006	0.09	1–1	0.09	S
4	18.4	0.0057	0.1	1–1	0.1	B
5	20	0.006	0.12	1–1	0.12	S

M. L. D.—0.006 with ouabain at 0.000,0006. Percent of activity, 120.

TINCTURE DIGITALIS BY CAT METHOD.

Weight of cat.	Sex.	Mils of digitalis injected.	Mils of ouabain injected.	Dilution.	1 mil ouabain.	Results.
2 kilos	female	4	5	1–10	0.00002	40 mg. = C.U.

Here we find that 50 mg. of digitalis is equal to one cat unit, as Hatcher and Brody consider the cat unit for digitalis to be about 100 mg. We find this in terms of percentage to be about 250.

TINCTURE DIGITALIS BY CAT METHOD AFTER STANDING TWO AND ONE-HALF YEARS.

Weight of cat.	Sex.	Mils of digitalis injected.	Mils of ouabain injected.	Dilution.	1 mil ouabain.	Results.
2.7 kilos	female	4	10	1–10	0.0002	57.7 mg. = C.U.

Here we find that 1 C. U. = 57.7 mg. or 173%. The check analysis gave 174%.

F. E. DIGITALIS BY THE GUINEA PIG METHOD.

Animal.	No.	Wt.	Dose per 250 Gm.	Dilution.	Dose of dilution.	Result after 24 hours.
Guinea pig	1	250	0.01	1–20	0.2	Recovered
Guinea pig	2	255	0.02	1–20	0.4	Recovered
Guinea pig	3	275	0.03	1–20	0.66	Recovered
Guinea pig	4	300	0.04	1–20	0.96	Died

Animal.	No.	Wt.	Dose per 250 Gm.	Dilution.	Dose of dilution.	Results after 24 hours.
Guinea pig	1	320	0.02	1–20	0.51	Recovered
Guinea pig	2	2 0	0.03	1–20	0.67	Died
Guinea pig	3	260	0.03	1–20	0.62	Died
Guinea pig	4	245	0.04	1–20	0.78	Died
Guinea pig	5	250	0.03	1–20	0.6	Died
Guinea pig	6	265	0.04	1–20	0.84	Died
Guinea pig	1	245	0.02	1–20	0.4	Recovered

We selected from the above 0.03 Cc. as the M. L. D. for a 250 Gm. guinea pig, which, in terms of percentage, would be 330.

F. E. Digitalis by Guinea Pig Method after Standing Two and One-Half Years.

Animal.	No.	Weight.	Dose per 250 Gms.	Dose.	Dilution.	Dose of dilution.	Result.
Guinea pig	1	545	0.04	0.087	1–10	0.87	Recovered
Guinea pig	2	475	0.05	0.095	1–10	0.95	Recovered
Guinea pig	3	640	0.055	0.14	1–10	1.4	Recovered
Guinea pig	4	520	0.06	0.12	1–10	1.2	Died
Guinea pig	5	515	0.055	0.11	1–10	1.1	Recovered
Guinea pig	6	410	0.057	0.093	1–10	0.93	Died
Guinea pig	7	435	0.06	0.104	1–10	1.04	Died
Guinea pig	8	425	0.055	0.093	1–10	0.93	Recovered
Guinea pig	9	465	0.057	0.106	1–10	1.06	Died

M. L. D. 0.057. Percent of activity, 175%.

F. E. Digitalis by One-Hour Frog Method.

No.	Dose per Gm.	Weight.	Dose.	No. Cc.	Results after one hour.	Dilution.
127	0.0006	26.4	0.0158	0.016 = 0.8	Stopped	20–1000
128	0.0005	23.1	0.01155	0.011 = 0.55	Stopped	20–1000
129	0.0004	24.8	0.0099	0.0099 = 0.49	Stopped	20–1000
130	0.0003	29.9	0.0089	0.0089 = 0.49	Stopped	20–1000
131	0.0002	23.8	0.0047	0.0047 = 0.23	Beats	20–1000
132	0.0001	30.6	0.00306	0.00306 = 0.15	Beats	20–1000
127	0.0002	31.9	0.00638	0.00638 = 0.32	Stopped	20–1000
128	0.00025	27.8	0.00695	0.00695 = 0.34	Stopped	20–1000
129	0.0003	26.9	0.00807	0.00807 = 0.4	Stopped	20–1000
129	0.0003	25.0	0.0075	0.0075 = 0.37	Stopped	20–1000

Here we figure the M. L. D. to be 0.00025, which gives us in terms of percentage 264 percent, with ouabain at 0.000,00055.

F. E. Digitalis by One-Hour Frog Method after Standing Two and One-Half Years.

No.	Weight.	Dose per Gm.	Dose.	Dilution.	Dose of dilution.	Result.
1	18.1	0.0003	0.0054	1–10	0.054	B
2	19.0	0.0004	0.0072	1–10	0.07	B
3	15.8	0.0005	0.008	1–10	0.08	B
4	17.1	0.0006	0.01	1–10	0.1	S
5	18.0	0.00057	0.01	1–10	0.1	B
6	16.0	0.0006	0.009	1–10	0.09	S
7	15.4	0.0057	0.0087	1–10	0.09	B
8	10.2	0.0006	0.011	1–10	0.11	S

M. L. D. 0.0006 with ouabain at 0.000,0006 or 120%.

F. E. DIGITALIS BY CAT METHOD.

Weight of cat.	Sex.	Mils of digitalis injected.	Mils of ouabain injected.	Dilution.	1 mil ouabain.	Results.
2.6 Kilos	female	4	8.5	1–100	0.00002	44.4 mg. = C. U.
2.7 Kilos	male	4	8.2	1–100	0.00002	33.7 mg. = C. U.

The average of 44.4 and 37.7, the number of mgs. of digitalis form the fluidextract which we find by this method to be equal to a cat unit, is 41; and, based upon 100 mg. as the standard cat unit, we find the percentage strength of this to be about 244 percent.

F. E. DIGITALIS BY CAT METHOD AFTER STANDING TWO AND ONE-HALF YEARS.

Weight of cat.	Sex.	Mils of digitalis injected.	Mils of ouabain injected.	Dilution.	1 mil ouabain.	Results.
2 kilos	male	4	7.5	1–100	0.00002	57.6 mg. = C. U.

Test is 173%. Check 175%.

DETERMINATION OF LETHAL DOSE OF OUABAIN ON CATS.

Female Cat

Weight 2.5 kilos

Time	4.05	4.10	4.15	4.20	4.30	4.40	4.50	5.00	5.10	5.20	5.30	5.40	5.45	5.50	6.00	6.10
No. of Cc..	1	1	1	1	1	1	1	1	1	1	1	0.5	0.5	0.2	0.3	dead

Male Cat

Weight 2 kilos

Time	10.00	10.10	10.20	10.30	10.40	10.50	11.00	11.10	11.20	11.30	11.40	11.50	12.00	12.15	12.30	
No. of Cc..	1	1	1	1	1	1	1	1	1	0.5	0.5	0.5	0.3	0.2	dead	

From the above we find that in cat No. 1 the cat unit is 0.1 mg. per kilo, and in cat No. 2, 011 per kilo, and that these both closely check the standard selected by Hatcher and Brody which is 0.1 mg. per kilo weight of cat.

CONCLUSION.

We find from the results of the above experiments that tincture and fluidextract of digitalis prepared from this particular lot of drug does deteriorate and that the deterioration in the tincture and fluidextract is practically the same. In the case of the guinea pig method, we first find it to test 330 percent, and after standing, 175 percent, or a loss in activity by the guinea pig method of 47 percent. In the case of the tincture and fluidextract by the one-hour frog method we formerly found it to test 264 percent and after standing, 120 percent, or a loss in activity of 55 percent. In the case of the cat method we at first found it to test approximately 250 percent and after standing, 175 percent, or a loss in activity of 30 percent. We therefore find that the frog method shows the greatest deterioration and that the cat method shows the least, and that the guinea pig method more closely checks with the frog method than it does with the cat method as far as deterioration is concerned, although in the case of the guinea pig method no attempt was made to standardize these animals against seasonable variation, which has been shown to exist. Furthermore, that in the case of this high-test digitalis, after being made into a tincture and fluidextract and standing two and one-half years neither the frog method, guinea pig method, nor the cat method shows the preparations to have deteriorated below the U. S. P. minimum standard for the tincture and fluidextract, and the test would indicate that this fluidextract

and tincture after standing this length of time still retains sufficient activity for them both to be considered standard preparations.

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ABSTRACT OF DISCUSSION ON DIGITALIS PAPERS BEFORE SCIENTIFIC SECTION, A. PH. A.

ROBERT A. HATCHER: I have a number of questions. I hope I won't seem too inquisitive. I have been very much put to it to secure my favorite animal, the cat, in testing, and I have been casting about to see if I could substitute frogs for cats. That might come as a shock to a good many members of this assembly, but it has been impossible at times to secure an adequate supply of cats. I have been experimenting on frogs, and while I had from time to time standardized various preparations on frogs, I recently purchased a batch of five hundred frogs with which I was utterly unable to standardize a single preparation of digitalis and could not even standardize the frogs against ouabain. On three hundred and seventeen out of five hundred frogs I could not standardize the frogs against ouabain. Now there are some here who will throw up their hands in horror at my inability to do this. I have, however, consulted with others, who informed me that the uniformity of absorption is the bane of the work in the hygienic laboratory; that they occasionally have similar experiences. Now if gold fish eliminate the uncertainty due to variation in susceptibility I am certainly glad because of the saving in time consumed. I want to ask if the same ratio exists between the toxicity of tincture of strophanthus and tincture of digitalis when tested on gold fish or when tested on cats or frogs?

PAUL S. PITTENGER: I had better answer the questions as they come up. We have not gone far enough with the gold fish method to find the exact ratio between digitalis and the other drugs. We intend to take up strophanthus and the other drugs later on. We have, however, started with ouabain and found that it took a very much greater amount of ouabain in comparison with the amount of digitalis required to produce toxic effects than on either frogs or guinea pigs.

ROBERT A. HATCHER: More ouabain?

PAUL S. PITTENGER: No, not more ouabain than tincture of digitalis, but many times more ouabain *in comparison with the amount of digitalis* than by either the guinea pig or frog method.

ROBERT A. HATCHER: Where it is not necessary to be absolutely accurate what are the ranges of accuracy with slight variations in temperature? It is rather difficult to maintain a temperature that does not vary over a degree.

PAUL S. PITTENGER: All these tests were carried out with a variation in the temperature bath from one to one and a half degrees, with the accuracy of the test well within two and a half percent.

HENRY KRAEMER: I was very much interested in the paper of Dr. Pittenger because I have long realized that gold fish were exceedingly sensitive to chemical substances. A few years ago a client came to me to investigate a problem. He had some trees which had been destroyed by a Public Service Corporation tearing up the streets, affecting the gas mains. So I was called in about eighteen months after the damage had been done, to act in the capacity of an expert. I went out and saw the trees were all dead. The question was, what had killed them? It occurred to me that there ought to be some definite, scientific way of showing the cause. The soil was clay, so I had a man dig down about six feet and obtained some samples. It occurred to me that probably a very simple test could be performed by comparing my samples with ordinary soil from some other locality. I bought a couple dozen gold fish and it was surprising as to how immediate the reaction was. After putting a sample of the soil, which I had obtained from the roots which were injured, in the water the gold fish immediately succumbed. One thing you have to watch in the city, where you have flowing water, is the effect of the chemicals used in treating the water. One time, for instance, I was making starch water, and was surprised to see how much the water tested of chlorine. Such things are very apt to affect some work, when you are expecting definite reactions you may have those from the chemically treated water. I merely interject this so you will appreciate that there are some things apart from the