SCIENTIFIC SECTION

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THE EFFECT OF AGING UPON THE POTENCY OF DIGITALIS TINCTURES.*

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There has been considerable disagreement among different workers as to the effect of aging upon the potency of digitalis tinctures. Wokes, in two papers (1, 2), discussed the assay of aged digitalis tinctures of which the potency as estimated by assay in the frog was considerably reduced; assay of the same tinctures in the cat, however, indicated that there was no loss of potency. Woke's work, therefore, suggests that the glucosides which kill the frog are different from those which kill the cat. It is clearly important also to determine which method of assay is more suitable for the clinic. The experiments reported in this paper were undertaken not only to confirm Woke's observations by different methods but also to attempt to ascertain why the deterioration of aged tinctures could be shown only by assay in the frog. It is hoped later to report the results of clinical assays of aged and fresh tinctures.

METHODS.

Assays.—Assay in the cat was performed by the common method of continuous intravenous infusion, at a constant rate of about 1 cc. per minute, till death occurred. The cats were not fed on the day of assay. Artificial respiration was routinely administered by means of a Starling pump used with the anesthetizing apparatus designed by one of us (3). The injection was stopped and the final burette reading was taken when the blood-pressure fell to zero. Most of the tinctures used were sufficiently concentrated (25 per cent) so that the fluid injected contained only two per cent of alcohol by volume.

The technique used for assay in the frog depended upon the intravenous injection, by the method of Smith and McClosky (4), of a diluted tincture (always containing 10 per cent of alcohol by volume), or of an aqueous extract of the residue of a tincture after removal of the alcohol in a vacuum desiccator at room temperature. Only male frogs were used. The brain was pithed and a suitably diluted extract was injected in a volume equivalent to 0.01 cc. per Gm. body weight; the same dose of the same preparation was ordinarily administered to 20 frogs. The hearts were examined in the usual way two hours after injection and the per cent mortality recorded.

Temperature control, which is important in any frog assay method, was effected by means of a specially constructed water-bath (Fig. 1).

The apparatus was made of rustless metal ("Allegheny"). The water of the large reservoir D, was maintained at 20° C. by means of a coil, through which cold water circulated, and a knife-blade heater controlled thermostatically. Water from D flowed through the outlet E into the individual frog compartments and thence through F, G, H and I_1. I_1 was connected with I_2 in Fig. 2 which illustrates the water pump operated by compressed air. When bottle R became filled, the rubber dam attached to cork float S closed the tubes O and T. Air from N raised the pressure in R and the water was forced up tube M and emptied into the main reservoir D of Fig. 1. When the bottle was emptied, the air escaped through the outlet tube M and as the pressure fell the valve S opened and the cycle was repeated. Tubes J, J, J and K served as outlet drains for emptying and cleaning the compartments. The 40 compartments were separated by partitions (A) and closed by hinged doors (B). X indicates small wire screen inserted in the

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overflows from each tier to prevent the frogs from closing the tube openings. The apparatus itself was enclosed in a larger asbestos board cabinet mounted on a galvanized steel framework with casters.

One hour before an experiment, the frogs were placed in the water-bath, usually a pair in each compartment. Before pithing, weighing and injecting, each was dried and the urine was expressed from its bladder. All injections were made into the musculocutaneous vein. If only two preparations were to be assayed, the standard and unknown solutions were injected alternately into pairs of similar frogs; each solution was given in the same dose per Gm. body weight to a group of 20 frogs.

The Accuracy of Assay in the Frog.—By the use of the intravenous technique described above a curve relating percentage mortality to *relative* dose was constructed by a method similar to that of Trevan (5). This was accomplished by twelve experiments each of which involved the use of two groups of twenty frogs. Inasmuch as all doses are computed as median lethal doses ("LD 50" (5)) Table I was calculated from the curve to facilitate the calculation of the LD 50, the median lethal dose having been given an arbitrary value of unity. If a standard and an unknown solution were to be compared in a given group of frogs, the median lethal dose of each was found by multiplying the dose administered by the factor appropriate for the mortality observed.

If a dose of digitalis producing a given mortality is to be considered significantly different from another dose producing the same mortality, providing that the probability of such a difference in random sampling is less than 0.01, the deviation in the median lethal dose is found graphically to be ± 14.4 per cent (-14.2 and $+14.6$ per cent); by calculation, in which K is estimated, the deviation of the median lethal dose is estimated at ± 15.3 per cent (see Trevan (5)). Therefore, if one assumes that the deviation of the median lethal dose is ± 14 per cent, the deviation in the ratio of potency of one preparation in terms of another will amount approximately to ± 28 per cent. In Table II are given the data of a group of experiments in which the LD 50 and ratio of potency were calculated for each pair of experiments in which the same dose of digitalis was administered to two similar groups of frogs. From the data on the possible ratios of potency (theoretical = 1.00), the standard deviation of the ratio of potency has been calculated as ± 0.086. For $p = 0.01$, the deviation must be increased to ± 0.289. It might, therefore, be concluded that a ratio of potency should be altered by more than ± 0.29 if such an alteration is to be regarded as clearly significant. However, if one computes the deviation of the ratio of potency from the experiments in which the observed mortalities fell on the "straight line" portion of the curve (excluding Experiments 1 and 8) the standard deviation of the ratio becomes ± 0.060 or, for $p = 0.01$, $3.7 \times 0.060 = \pm 0.222$.

The Effect of Alcohol upon Assay in the Frog.—In most of the experiments the solutions injected into the frog contained 10 per cent alcohol by volume. The effects of alcohol alone were first studied by injecting into seven groups of frogs 0.01 cc. of 20 per cent alcohol at assay-temperatures of 20° C. and 30° C. At the lower temperature no effect was observed in frogs recently obtained; however, two of 19 frogs, stored for several weeks, were killed by such a dose of alcohol. At 30° C. about 50 per cent of frogs stored for several weeks were killed by the alcohol

solution; from 14 to 35 per cent of fresh frogs were killed. It therefore appears that the effect of alcohol, especially at higher assay-temperatures, must not be disregarded.

TABLE I.—FACTORS FOR THE CONVERSION OF DOSES INTO THOSE KILLING FIFTY PER CENT OF A GROUP OF FROGS.

Observed Mortality.	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.
0						1.47	1.44	1.41	1.39	1.37
10	1.34	1.32	1.30	1.29	1.27	1.25	1.24	1.23	1.22	1.21
20	1.20	1.19	1.18	1.17	1.16	1.15	1.14	1.13	1.13	1.12
30	1.11	1.10	1.10	1.09	1.08	1.08	1.07	1.07	1.06	1.05
40	1.05	1.04	1.04	1.03	1.03	1.02	1.02	1.01	1.01	1.00
50	1.00	1.00	0.99	0.99	0.98	0.98	0.97	0.97	0.97	0.96
60	0.96	0.95	0.95	0.94	0.94	0.93	0.93	0.92	0.92	0.91
70	0.91	0.90	0.90	0.89	0.89	0.88	0.88	0.87	0.87	0.86
80	0.85	0.85	0.84	0.83	0.83	0.82	0.81	0.81	0.80	0.79
90	0.78	0.77	0.76	0.75	0.74	0.74				

TABLE II.—CONTROL ASSAYS IN THE FROG.

Experiment No.	Tr. Digit.	Dose, Mg./Kg.	No. of Frogs.	Observed Mortality. Per Cent.	Calculated LD 50, Mg./Kg.	Possible Ratios of Potency.
1	A 13	208	20	15	260	1.16
		208	20	35	225	0.86
2	A 13	208	21	43	214	1.03
		208	20	50	208	0.97
3	A 13	208	20	40	218	1.05
		208	20	50	208	0.95
4	B 11	169	21	33	184	1.05
		169	22	41	176	0.95
5	C 5	159	20	25	183	1.13
		159	20	45	162	0.89
6	C 5	170	21	48	172	1.03
		170	20	55	167	0.97
7	C 5	191	20	70	174	1.00
		191	20	70	174	1.00
8	C 5	192	20	65	179	1.13
		192	20	85	157	0.88
9	C 12	189	20	60	181	0.98
		189	20	55	185	1.02

In a second set of experiments (Table III), the same dose of the same preparation with and without alcohol was administered to similar groups of frogs. The alcohol was removed from the digitalis tincture by the evaporation at room temperature of a measured volume in an agate mortar over P_2O_5 in a vacuum desiccator. The residue was triturated with saline solution and quantitatively transferred to a pyrex centrifuge tube, placed in a bath of boiling water for 5 minutes, cooled, made up to known weight and centrifuged. The results in Table III indicate, without exception, that a greater dose of glucoside(s) must be used in the absence of alcohol. Ten per cent of alcohol by volume appears to be equivalent, roughly, to an increase of 17 per cent in the dose of glucoside(s) (excluding the experiments with digitoxin).

Finally it may be asked whether or not the estimation of the ratio of potency of two preparations is affected by the presence of alcohol. Data in answer to this

question may be found in Table IV. Aged and fresh digitalis tinctures were compared in these experiments. The ratios of potency do not differ from each other significantly. The conclusion that a concentration of 10 per cent alcohol by volume does not seriously affect the estimation of the potency-ratio is justified, we believe, if the assay is carried out at 20° C.

TABLE III.—THE EFFECT OF ALCOHOL UPON ASSAYS IN THE FROG.

Experiment No.	Drug Used.	Alcohol Concentration by Volume, Per Cent.	No. of Frogs.	Observed Mortality, Per Cent.	Calculated LD 50, Mg./Kg.	Ratio of Dose without Alcohol to Dose with Alcohol.
212	Digitoxin	5.2	20	10	1.35	1.21
	"	15.2	20	30	1.12	
215	"	5	20	30	1.33	1.11
	"	15	21	48	1.20	
180	Ouabain	0	20	55	0.203	1.15
	"	10	21	81	0.176	
183	"	0	20	50	0.207	1.14
	"	10	21	76	0.182	
208	"	0	20	60	0.203	1.23
	"	10	20	90	0.165	
211	"	0	20	15	0.231	1.30
	"	10	20	60	0.177	
287	"	0	20	80	0.179	1.08
	"	10	21	62	0.165	
282	Tr. Digitalis A-15	0	20	75	227.0	1.15
	" " "	10	20	70	197.0	
286	" " "	0	20	55	253.0	1.17
	" " "	10	20	45	217.0	

TABLE IV.—THE LACK OF EFFECT OF ALCOHOL UPON THE RATIO OF POTENCY OF TINCTURES OF DIGITALIS AS DETERMINED BY ASSAY IN THE FROG.

Experiment No.	A Potency-Ratio (Alcohol, 10 Per Cent by Volume).	B Potency-Ratio (No Alcohol).	$\frac{A}{B}$
238, 240	1.35	1.22	1.11
254, 244	1.29	1.23	1.05
250, 252	1.40	1.40	1.00
262, 276	1.44	1.53	0.94

METHODS OF EXTRACTION.

The method of Joachimoglu (6), slightly modified, was employed to insure the complete extraction of the different powdered digitalis samples. By the use of absolute alcohol in a Landsiedl extraction-apparatus, the leaves were continuously extracted for eight hours by alcohol near its boiling point. This time was chosen as a result of some control experiments which are shown graphically in Fig. 3. However, the potency of the extracts after only four hours' extraction is not significantly less than that after eight hours' extraction.

The adequacy of the method of extraction has also been studied by the assay of a number of fresh extracts of three different lots of digitalis leaves. Twenty-five such assays have been done in the cat, the average number of cats used for each assay being about seven. Only in three assays did the question of a significant difference in potency arise. In these three cases the probabilities that one would

obtain as great differences in random sampling were 0.017, 0.015 and 0.017 (calculated by the method of Fisher (7)). We have therefore, in subsequent assays, assumed that a significant change in potency as estimated by assay in the cat, has not occurred unless the probability of the occurrence of such a value, in random sampling is less than 0.01.

Nine pairs of different extractions were assayed in the frog by the method used in Table II. The only difference lay in the use of different extracts of the same leaf in the same dose for the comparison instead of the same extract in the same dose. On the assumption that in this set of experiments the theoretical ratio of potency is 1.000, the standard deviation is found to be ± 0.087. The results (including four experiments in which one or both mortalities lay outside the "straight line" portion of the curve relating relative dose and mortality) are there-

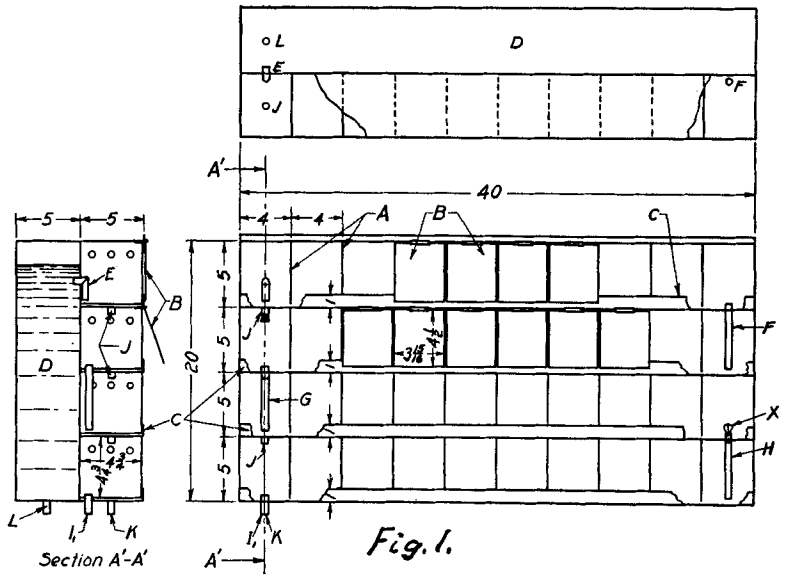

Fig. 1.—Constant temperature apparatus for assay in the frog. (Dimensions are given in inches.)

fore the same as those of the experiment in which pairs of extracts known to be identical were compared. Assay of different extracts in the frog, therefore, demonstrates that the extraction-procedure yields comparable extracts of uniform potency.

Extracts were also made by means of cold absolute alcohol. For this purpose extraction was carried out in a glass tube one and one-half by thirty-six inches; by the removal of small amounts of alcohol each day, the extraction was continued for two to three weeks. We are aware that extraction at room temperature can be performed adequately only with more dilute alcohol; however, it seemed best for purposes of comparison to employ absolute alcohol.

DIGITALIS SAMPLES.

Three different samples of digitalis leaves were employed: "A," Canadian, 1927, "B," Canadian, 1928 and "C," Minnesota, 1928.[1] Each sample was thor-

[1] Kindly donated by Mr. F. A. Upsher Smith.

oughly mixed and stored in brown glass bottles at room temperature. Dried to constant weight at 105° C., A was found to contain 7.74 per cent water, B and C, 8.30 and 7.36 per cent, respectively. The data on the relative potency of the three samples are given in Table V. The mean lethal doses for the cat were obtained by the use of 50 cats each for Samples A and B, and 60 cats for Sample C. One experiment each was performed in comparing potency in the frog by direct comparison (the three samples being assayed simultaneously) and by comparison in pairs. Finally two sets of determinations were carried out in the frog by the use of ouabain as a standard. In the first set of experiments, comparisons with ouabain were made twice with each sample; similar frogs were used for all comparisons. The second set of experiments represents the mean ratios of potency observed at different times in six experiments each with Samples A and B and seven experiments with Sample C. In the frog, one Gm. of A was found to be equivalent to approximately 0.95 mg. of U. S. P. ouabain.[1]

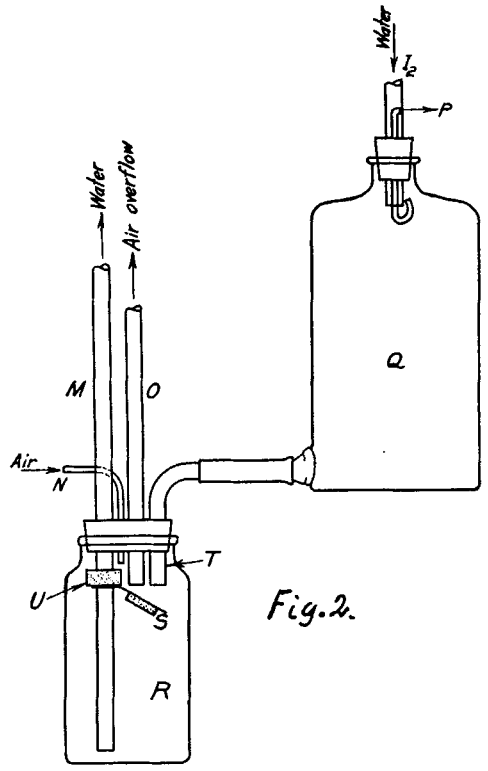

Fig. 2.—Water pump operated by compressed air.

In all the determinations, A is found to be the least potent. B and C appear to be of the same potency except when their potency is compared directly in the frog. However, more experiments should be done to establish this apparent difference.

TABLE V.—A COMPARISON OF THE POTENCY OF THE THREE DIGITALIS SAMPLES.

Digitalis Leaf.	Cat Assay. Mean LD. mg./Kg.	Cat Assay. Relative potency.	Frog Assay. Relative Potency as Determined by Direct comparison.	Comparison in pairs.	Use of ouabain (1) as standard.	(2)
A	76.8	90	76	73	86	82
B	68.5	99	89	88	97	99
C	68.9	100	100	100	100	100

THE LOSS OF POTENCY THROUGH AGING.

If tinctures of digitalis are permitted to age in glass-stoppered, paraffin-sealed bottles in the cupboard at room temperature, the potency as tested in the frog unquestionably falls. The potency as tested in the cat falls less and not so convincingly (Table VI). Assays in both the cat and frog were done at intervals of 5 to 29 weeks; an effort was made to perform both assays in the same week although

[1] Kindly supplied by Dr. W. T. McClosky of the Bureau of Food and Drug Administration, Department of Agriculture.

this was not always possible. The values given for potency in the cat are based on a comparison with the assay performed when the tincture was first made. In the cases of three of the tinctures (A9, C1 and C4) the potency was significantly reduced

TABLE VI.—THE LOSS OF POTENCY OF AGING TINCTURES OF DIGITALIS.

	Assay in Cat.				Assay in Frog.			
Sample.	Age of Tr. weeks.	Number of cats.	A potency.	Standard error of mean, per cent of mean LD.	Age of Tr. weeks.	B potency.	Approx. Age of Tr. Weeks.	B — A
A-6	7	5	1.00	10.9	23	0.82	24	0.93
	24	5	0.88	7.8	29	0.80		
	36	10	0.93	3.8	37	0.92	37	0.99
	53	10	0.95	7.0	38	0.82		
	73	10	0.88	5.3	48	0.67[1]	51	0.71
					73	0.58[1]	73	0.66
A-8	4	10	1.00	5.5				
	30	10	0.93	3.4	30	0.68[1]	30	0.73
	53	10	0.86	4.4	52	0.67[1]	52	0.78
A-9	0	5	1.00	7.7				
	17	10	0.98	4.0	19	0.81	18	0.83
	34	10	0.74[1]	2.7	35	0.82	35	1.11
B-1	0	5	1.00	3.4	14	0.93		
	16	5	0.89	7.1	19	0.84		
	29	9	0.95	3.9	29	0.77[2]	29	0.81
	45	10	0.91	5.3	42	0.74[2]	44	0.81
	65	10	0.91	2.7	65	0.62[1]	65	0.68
B-4	3	8	1.00	4.0				
	32	10	0.92	6.4	32	0.78[2]	32	0.85
	52	10	0.93	4.3	52	0.67[1]	52	0.72
B-9	0	5	1.00	6.2				
	18	10	0.94	3.9	19	0.86	19	0.91
	38	9	0.90	2.5	36	0.86	37	0.96
C-1	1	5	1.00	4.4	14	0.81	15	1.01
	8	4	0.85	4.9	25	0.76[2]		
	16	5	0.80	5.4	30	0.77[2]	30	0.93
	29	10	0.83	5.2	44	0.72[1]		
	46	10	0.79[1]	2.7	45	0.69[1]	46	0.87
	65	11	0.64[1]	5.8	65	0.60[1]	65	0.94
C-4	1	4	1.00	6.0				
	29	10	0.87	4.1	30	0.73[2]	30	0.84
	51	10	0.77[1]	4.3	51	0.68[1]	51	0.88
C-5	0	5	1.00	6.8				
	16	10	0.93	4.6	19	0.80	18	0.86
	37	9	0.82	6.2	41	0.76[2]	39	0.93
Digitoxin	0	7	1.00	4.4	0	1.00* (1.00**)		
	11	4	0.50[1]	2.8	5	0.88 (0.79)		
					20	0.49[1] (0.59)		
	41	9	0.49[1]	5.8	41	0.46[1] (0.60)	41	0.94* (1.22**)
	58	10	0.53[1]	6.4	59	0.52[1] (0.73)	59	0.98 (1.38)

[1] Potency significantly reduced ($p = < 0.01$).

[2] Potency perhaps significantly reduced.

* In terms of absolute doses.

** In terms of ouabain.

(in the case of A9 after only 34 weeks' aging). Although without exception all other tinctures appear weaker, in no single case is this convincingly shown.

To estimate potency in the frog, each aged solution was compared with one freshly made. The potency was significantly reduced in six of the nine tinctures and probably was reduced in a seventh (C5). In Tinctures A9 and B9, aged 35 and 36 weeks, respectively, the potency was not significantly lower. That the frog assays indicate a greater degree of deterioration than do those in the cat is shown by the ratios given in the last column of Table VI.

A limited amount of a single sample of Merck's digitoxin was the only purified digitalis principle available to us for aging experiments. The initial mean lethal dose of this preparation for the cat was 0.344 mg. per Kg. (7 cats, S. E. of mean = ±0.015 mg.); the LD 50 for the frog was about 0.73 mg. per Kg. or one-fourth the potency of U. S. P. ouabain. As is shown in Table VI, this sample of digitoxin dissolved in absolute alcohol rapidly deteriorated so that after 20 weeks it had lost half of its potency; during the succeeding 39 weeks there was probably no additional deterioration. Inasmuch as the same reduction in potency was observed by assays in both the cat and the frog, the experiments with the digitoxin sample do not aid in interpreting the other data of Table VI.

THE EFFECT OF TEMPERATURE UPON ASSAY IN THE FROG.

Assays in the frog were undertaken at 30° C. not only to determine the alteration in absolute LD 50 but also to learn whether or not the potency of aged solutions was found to be as low as when assays were done at 20° C. A characteristic curve determined for 30° C. was not available; the "LD 50 doses" were calculated from the curve as determined at 20° C. The experiments could not be performed at the two temperatures simultaneously; it is believed, however, that by the use of similar frogs, the change in absolute dose has been determined fairly accurately. The "LD 50 dose" at 30° C. is 43 per cent (about half) of the LD 50 dose at 20° C. This statement is based upon 14 determinations at 30° C. and 15 determinations at 20° C. Similar results were obtained with both alcohol-free ouabain solutions and digitalis tinctures.

Assays carried out at 30° C. with aged and fresh tinctures were in no case significantly different from those done at 20° C. In general, however, the apparent loss of potency appeared to be less at the higher temperature (six comparisons at 20° C. and eight comparisons at 30° C.). Before any further conclusion can be drawn, however, more experiments must be performed.

EXPERIMENTS WITH TINCTURES MADE BY EXTRACTION AT ROOM TEMPERATURE.

Extraction of the digitalis samples was carried out with absolute alcohol at room temperature by the method described in the first part of this paper. Although such extraction may be carried out slowly over several weeks, the glucosides are still incompletely removed. A portion of each of these extracts was heated, by immersing in a bath of boiling water, in a reflux condenser for eight hours and then compared with a corresponding unheated portion to determine whether or not there was a significant change in the potency in the frog compared with that in the cat. The results are shown in Table VII and suggest that such heating reduces the potency as estimated in the frog. In individual experiments none of the differences are

TABLE VII.—A COMPARISON OF THE POTENCY OF FRESH HEATED AND UNHEATED TINCTURES MADE BY EXTRACTION WITH COLD ABSOLUTE ALCOHOL.

Sample.	Number of cats.	Assay in Cat. Mean LD and standard error of mean, Mg./Kg.	U/H potency.	Assay in Frog. LD 50, mg./Kg.	U/H potency.
AP-1H	5	180.3 ± 6.6	1.08	375	0.86
AP-1U	5	194.6 ± 9.4		322	
AP-5H	5	180.8 ± 14.6	0.99	437	0.88
AP-5U	5	178.7 ± 10.6		385	
BP-1H	5	178.9 ± 11.4	0.91	341	0.91
BP-1U	5	162.5 ± 9.0		309	
BP-2H	5	150.1 ± 6.8	0.84	394	0.76
BP-2U	5	126.2 ± 4.8		301	
CP-1H	7	143.2 ± 4.9	1.02	245	0.90
CP-1U	5	146.2 ± 12.1		220	
CP-2H	5	140.4 ± 10.2	0.86	300	0.84
CP-2U	5	120.1 ± 5.9		252	

H indicates a heated extract.
U indicates an unheated extract.

significant. On the other hand, the mean potency (six experiments) of the heated sample is 95 per cent of the unheated sample by assay in the cat, and 86 per cent of the unheated sample by assay in the frog.

Samples BP-1H and BP-1U were permitted to age for 39 weeks; two comparison assays were then made in the frog. The absolute doses were increased in both from about 325 mg. per Kg. to about 500 mg. per Kg. However, the heated sample was then found to be the stronger (109 and 105 per cent)—an observation suggesting that the unheated sample underwent more rapid deterioration.

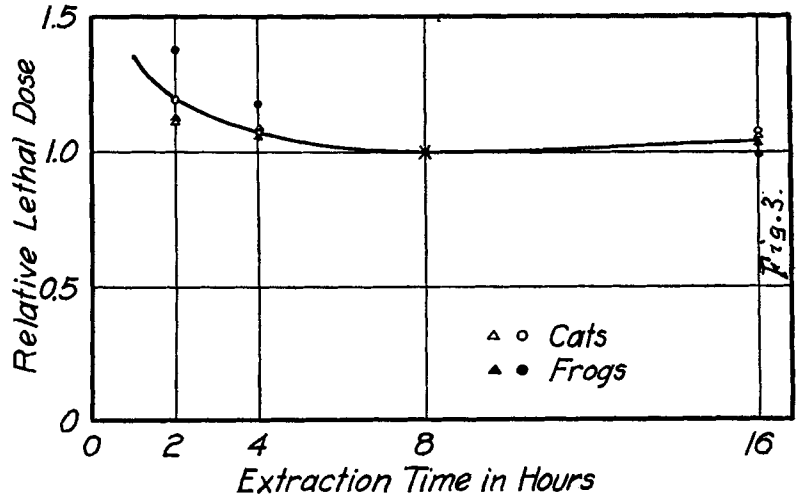

Fig. 3.—Efficiency of continuous extraction by hot absolute alcohol as determined by assay in the cat and in the frog.

DISCUSSION.

Our results confirm Woke's finding that tinctures of digitalis, if assayed in the frog, rather rapidly become weaker. On the other hand we found that although the

loss of potency as determined in the cat is usually much less, it may be nearly as great. Clinical assays of tinctures, which appear weaker only in the frog, are being undertaken in order to ascertain which method of assay is more accurate for the clinic. In so far as a conclusion may be drawn from one set of observations, it appears that a digitoxin solution, in aging a few months, loses about half of its potency as estimated in both the frog and the cat and therefore does not resemble an ordinary tincture of digitalis. Our data from experiments with tinctures made by extraction at room temperature suggest that at least part of the toxic action in the frog, rather than in the cat, is due to heat-labile substances.

We have not been able to correlate the degree of deterioration of a tincture (assay in cat or frog) with increased or increasing "p_H." On the contrary, Tinctures B1, B4 and B9 (Table VI), the potency of which in the cat was least affected by aging, were found to yield the highest glass-electrode potentials (+0.1937, 0.1909 and 0.1919 v. in comparison with 0.1858, 0.1811 and 0.1798 v. for the A group and 0.1752, 0.1709 and 0.1714 v. for the C group; 0.1 N HCl = −0.0494 v.). The estimates of the p_H of fresh two per cent aqueous extracts (infusions) were 5.78, 5.83 and 5.90 for Samples A, B and C.

The large number of assays performed enabled us to gather additional data bearing upon a few of the general questions relating to assay. In all seasons of the year, for example, U. S. P. ouabain was assayed in frogs. Our data consistently show that the frog (*R. pipiens*) is least susceptible in August (about 0.22 mg. per Kg.) and most susceptible in January (about 0.18 mg. per Kg.). Others (8) consider that frogs are more sensitive in summer. We observed no seasonal variation in cats nor could we correlate susceptibility in the cat more satisfactorily with heart weight than with body weight. Like McFarlane and Masson (9) we have noticed that cats exhibiting a pronounced bradycardia in the course of the injection commonly required somewhat larger doses than others receiving the same preparation.

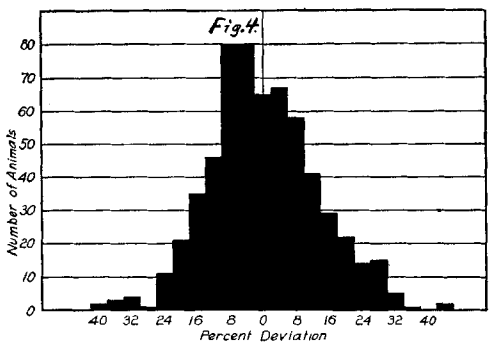

Fig. 4.—Results of assays of different tinctures of digitalis in 601 cats. Five to ten cats were used in each assay. Ordinate: number of animals killed. Abscissa: per cent deviation from mean.

The histogram shown in Fig. 4 probably is not as accurate as that of de Lind van Wijngaarden (10) inasmuch as it is based upon assays of different solutions in different sized groups (five to ten cats). Assays in 601 cats are represented in the histogram. The distribution probably is normal; there is no highly resistant group, such as McFarlane and Masson believe that they encountered.

SUMMARY.

1. The effect of aging upon the potency of digitalis tinctures was determined in the cat and frog (*R. pipiens*). In all cases the tinctures were made by extraction with absolute alcohol, usually with hot alcohol in a Landsiedl extractor. Assay in

the frog was performed by an intravenous technique. Various types of control experiments are described.

2. Aged tinctures were always found to be less potent by assay in both species; however, the greater reduction in potency was usually observed in the frog.

3. Deterioration of tinctures could not be correlated with increased "p_H;" on the contrary the tinctures most stable by cat-assay were shown to yield the highest glass-electrode potentials.

4. A sample of digitoxin in absolute alcohol lost about half of its toxicity toward both the frog and the cat after a few months. There was no further deterioration during the period of observation (59 weeks).

5. Frogs were found to be least susceptible to ouabain in the late summer (August).

6. The results of assays in 601 cats indicated that the distribution of susceptibility is probably normal.

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AMINO-ALCOHOLS. IX. BIOLOGIC ASSAY OF PROPADRIN AND EPHEDRINE.*[1]

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The physiologic assay of phenylpropanolamine (propadrin) and phenylpropanolmethylamine (ephedrine) is a problem which still awaits completely satisfactory solution. Although the qualitative action of ephedrine has been elucidated fairly thoroughly, and it is known to act on most, if not all, of the structures innervated by the autonomic system, none of these actions lend themselves to accurate quantitative analysis. Its actions on each of these structures differ so widely, quantitatively at least, from one animal to another and all are so markedly influenced by slight experimental differences which are not readily analyzed, that it is not possible to obtain quantitative determinations by comparing the action on one animal directly with that on another.

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* Presented under title "Bioassay of Propadrin Solutions," by T. S. Githens, James C. Munch and W. H. Hartung, before Scientific Section, A. PH. A., Toronto meeting, 1932.

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