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On the Biological Assay of Digitalis by the Over-Night Frog Method

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In biological assays which depend on a quantal response the use of dosage-response curves is becoming widely adopted. This is particularly true in the assay of digitalis when frogs are used as the test animals. Dosage-response curves offer a means of overcoming difficulties in interpretation of results which are due to the variability of animals (1), and also give the necessary data for computing the errors of an assay (2, 3). The slope and position of these curves give requisite information for the design of a test, for theoretically, if it can be shown that the slope does not change significantly, only one dose each of the standard and the unknown are necessary (2, 4), and if the position is constant, the standard may be dispensed with after a preliminary determination of the "characteristic" curve.

Simplicity is considered of great importance in the design of any method of assay intended for general routine use. The ease with which the results of a one-dose assay may be calculated is in itself a strong recommendation for its adoption, provided, of course, that it is as accurate as other methods.

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The results from assays of samples by both one-dose and multiple-dose procedures as reported in this paper indicate that a method using a single dose each of the standard and sample, and based on a "characteristic" curve, may be used for the assay of digitalis by the over-night frog method. The results obtained from the assay of samples by this method check very closely with those of the more laborious three-dose procedure.

EXPERIMENTAL

In previous papers (5, 6) the care of the frogs, the type of apparatus used and the preparation of dilutions were described. For the construction of regression lines in Tables I, II and III, the Canadian Standard Digitalis, Lot No. 1135, and Canadian Standard Ouabain, Lot No. 330, were used. For the experiments in Table I exactly 0.6 Gm. of powder was extracted in each Soxhlet for two hours, using absolute alcohol as the menstruum. Various methods of extracting the Canadian Standard powder were employed for the experiments in Table II. The dilutions for injection were made up so that the dose per Gm. of frog was contained in 0.02 cc. of a liquid having 20 to 25 per cent by volume of ethyl alcohol. All frogs used for this investigation had been kept in concrete storage tanks with running water at approximately 10 ± 5° C. from two weeks to three months previous to use.

Table I.—Data Used in Computing Regression Lines for Digitalis

Powders extracted by Soxhlet method only

Date	Dose in Gm. per Gm. of Frog × 10^{-6}	% Mortality	Sex	b Slope of Regression Line
1938				
Oct. 24	41	40.0	M	17.6
	49	86.7		
Oct. 28	41	13.3	M	20.0
	49	66.7		
Nov. 2	41	16.7	M	23.4
	49	80.0		
Nov. 4	41	30.0	M	16.2
	49	76.7		
Nov. 8	41	23.3	M	20.3
	49	80.0		
Nov. 10	35	3.3	M	15.5
	40	3.3		
	46	23.3		
	53	63.3		
	61	83.3		
	70	100.0		
Nov. 14	35	3.3	M	13.0
	40	13.3		
	46	33.3		
	53	60.0		
	61	86.7		
	70	100.0		
Nov. 16	40	6.7	M	11.4
	46	20.0		
	53	53.3		
	61	70.0		
Nov. 16	40	13.3	M	11.9
	53	63.3		
Nov. 18	41	20.0	M	12.0
	49	53.3		
Nov. 18	40	6.7	M	19.9
	46	10.0		
	53	63.3		
	61	93.3		
	70	100.0		

Note: 30 frogs were used on each dose.

Table II.—Data Used in Computing Regression Lines for Digitalis

Powders extracted by various methods

Date	Dose in Gm. per Gm. of Frog × 10^{-6}	% Mortality	Sex	b Slope of Regression Line
1938				
Oct. 11	26	6.7	F	10.0
	31	20.0		
	35	6.7		
	40	23.3		
	46	53.3		
	53	76.7		
Oct. 13	26	0.0	F	13.3
	31	3.3		
	35	13.3		
	40	53.3		
	46	63.3		
	53	90.0		
	61	100.0		

Table II.—(*Continued*)

Oct. 18	26	0.0	M	8.8
	31	3.3		
	35	26.7		
	40	50.0		
	46	60.0		
	53	80.0		
	61	86.7		
	70	100.0		
Oct. 19	26	3.3	M	13.0
	31	3.3		
	35	10.0		
	40	26.7		
	46	46.7		
	53	86.7		
	61	96.7		
	70	100.0		
Oct. 20	31	0.0	M	13.9
	35	3.3		
	40	23.3		
	46	60.0		
	53	73.3		
	61	96.7		
	70	96.7		
Oct. 24	41	33.3	M	11.1
	49	66.7		
Oct. 24	41	43.3	M	10.2
	49	73.3		
Oct. 24	41	26.7	M	14.8
	49	70.0		
Oct. 28	41	23.3	M	17.5
	49	73.3		
Oct. 28	41	16.7	M	12.5
	49	50.0		
Oct. 28	41	26.7	M	10.3
	49	56.7		
Nov. 2	41	30.0	M	21.1
	49	86.7		
Nov. 2	41	43.3	M	18.7
	49	90.0		
Nov. 2	41	40.0	M	15.8
	49	83.3		
Nov. 4	41	16.7	M	12.5
	49	50.0		
Nov. 4	41	26.7	M	16.0
	49	73.3		
Nov. 4	41	30.0	M	13.5
	49	70.0		
Nov. 22	40	23.3	M	16.7
	46	46.7		
	53	93.3		
	61	96.7		
	70	100.0		
Nov. 22	43	50.0	M	10.7
	53	83.3		
Nov. 24	41	36.7	M	10.0
	49	66.7		
Dec. 20	41	20.0	M	22.1
	45	40.0		
	49	80.0		

Table II.—(*Continued*)

1939				
Jan. 26	28	16.7	F	8.7
	31	46.7		
	34	36.7		
	38	66.7		
Jan. 27	28	13.3	F	9.6
	31	40.0		
	34	36.7		
	38	63.3		
Jan. 30	28	15.0	F	15.7
	31	23.3		
	35	61.6		
	39	86.6		
Feb. 1	28	46.7	F	15.0
	31	80.0		
	35	86.7		
	39	100.0		

Note: Thirty frogs were used on each dose, except on December 20, 1938, and January 30, 1939, when 10 and 60 frogs were used, respectively.

Table III.—Data Used in Computing Regression Lines for Ouabain

Date	Dose in Gm. per Gm. of Frog $\times 10^{-8}$	% Mortality	Sex	b Slope of Regression Line
1939				
Mar. 10	25	36.7	M	22.8
	27	50.0		
	29	90.0		
Mar. 16	20.5	0.0	M	17.2
	23	20.0		
	26	70.0		
	29	73.3		
Mar. 17	23	10.0	M	32.7
	25	36.7		
	27	83.3		
Mar. 22	23	3.3	M	22.5
	25	40.0		
	27	70.0		
	29	73.3		
Oct. 5	26	10.0	M	16.6
	29	43.3		
	32	60.0		
Oct. 6	26	60.0	M	19.4
	29	93.3		
	34.5	100.0		
Oct. 27	26	4.0	M	29.1
	30	60.0		
	34.5	96.0		
Oct. 30	26	16.7	M	24.0
	30	76.0		
	34.5	96.7		
Nov. 7	26	63.3	M	13.5
	30	83.3		
	34.5	98.3		

Table III.—(*Continued*)

Nov. 10	20	13.3	F	21.9
	23	53.3		
	26	93.3		
	30	96.7		
Nov. 13	20	3.3	F	21.6
	23	20.0		
	26	56.7		
	30	96.7		
Nov. 14	20	0.0	F	24.1
	23	20.0		
	26	63.3		
	30	96.7		

Note: Thirty frogs were used on each dose except as follows: Oct. 27, 1939, 25 frogs were used on each dose; Oct. 30, 1939, 25 frogs were used on the middle dose; Nov. 7, 1939, 60 frogs were used on the high dose.

Regression Lines.—Tables I, II and III present the data from which the individual regression lines were obtained. The methods used in computing the parameters for the curves were those described by Bliss (3).

Application of Bliss' equation 20*a* (7) demonstrated that the apparent differences in the slopes of the lines for digitalis, Tables I and II, and for ouabain, Table III, were not significant, and could have occurred as a result of sampling variation.

The position of the lines for digitalis and ouabain, however, were shown by a modification (8) of Bliss' equation 19 (7) to differ to a significant degree. The use of a standard with each test is, therefore, necessary. Data from Table II were not used in computing chi-square for position because several methods of extracting the Canadian Standard powder were used in these experiments.

Since the slopes of the separately determined lines did not differ significantly from their means, composite lines for both digitalis and ouabain were computed by methods described elsewhere (3). The composite regression coefficient for digitalis was found to be 13.1, for ouabain 21.2 and the disagreement between these two values was found to be significant.

Comparison of Methods.—In Table IV the results of 50 assays are shown. Three doses each of the standard and samples have been used and potencies were computed by the procedure recommended by Gaddum (2) and Bliss (3, 7), and by curve numbers (5, 6, 9). In the former case the slope and position of each regression line were computed separately, while in the latter the slope of the line already determined for the standard was employed, and each experiment treated as three separate assays; the low, middle and high doses of the unknown being compared with the corresponding doses of the standard. The figures given in Columns 7 and 8 of the table are in each instance the arithmetic mean of the three results calculated in this way when a standard curve for digitalis (Curve *D*) and for ouabain (Curve *O*) were used. The slopes for Curve *D* and Curve *O* were obtained from the data shown in Tables I, II and III.

Table IV.—Comparison of Methods

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose Method — One-Dose Curve *D*	One-Dose Curve *O*
1939									
Oct. 27	Std.	2.5	25	52.0					
		2.9	25	52.0					
		3.4	25	88.0					
Oct. 27	U	5.3	25	12.0	42	43	45	2.4	7.1
		6.2	25	40.0					
		7.2	25	88.0					
Dec. 7	Std.	2.7	30	23.3					
		3.2	30	40.0					
		3.9	30	93.3					
Dec. 7	V	2.5	31	48.4	122	117	111	4.1	9.0
		3.1	30	76.7					
		3.9	30	96.7					
Dec. 8	Std.	2.7	20	20.0					
		3.2	20	40.0					
		3.9	25	76.0					
Dec. 8	V	2.5	25	40.0	122	118	112	3.3	8.2
		3.1	20	60.0					
		3.9	25	96.0					
1940									
Jan. 6	Std.	2.51	10	20.0					
		3.16	10	70.0					
		3.98	10	100.0					
Jan. 6	A	1.78	10	0.0	129	135	137	4.7	6.2
		2.24	10	60.0					
		2.82	10	90.0					
Jan. 8	Std.	2.51	15	46.7					
		2.82	15	33.3					
		3.16	15	80.0					
Jan. 8	A	2.00	15	40.0	137	135	131	1.5	4.4
		2.24	15	66.7					
		2.51	15	100.0					
Jan. 10	Std.	2.51	15	40.0					
		2.82	15	60.0					
		3.16	15	93.3					
Jan. 10	A	2.00	15	40.0	130	130	128	0.0	1.5
		2.24	15	73.3					
		2.51	15	100.0					
Feb. 8	Std.	2.5	15	33.3					
		3.1	15	40.0					
		3.9	15	80.0					
Feb. 8	B	1.9	15	6.7	114	119	122	4.4	7.0
		2.4	15	26.7					
		3.0	15	73.3					
Feb. 8	C	1.0	15	33.3	269	266	263	1.1	2.2
		1.2	15	46.7					
		1.45	15	86.7					
Feb. 9	Std.	2.5	15	20.0					
		3.1	15	60.0					
		3.9	15	100.0					
Feb. 9	B	1.9	15	26.7	118	127	128	7.6	8.5
		2.4	15	40.0					
		3.0	15	78.6					
Feb. 9	C	1.0	15	13.3	253	257	255	1.6	0.8
		1.2	15	73.3					
		1.45	15	86.7					
Apr. 4	Std.	2.24	15	13.3					
		2.51	15	13.3					
		2.82	15	33.3					

Table IV.—(*Continued*)

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose Method One-Dose Curve *D*	One-Dose Curve *O*
Apr. 4	D	2.24	15	6.7	100	100	99	0.0	1.0
		2.51	15	13.3					
		2.82	15	46.7					
Apr. 4	S	2.24	15	26.7	125	122	112	2.4	10.4
		2.51	15	66.7					
		2.82	15	80.0					
Apr. 5	Std.	2.51	15	26.7					
		2.82	15	26.7					
		3.16	15	66.7					
Apr. 5	D	2.51	15	33.3	109	106	105	2.8	3.7
		2.82	15	53.3					
		3.16	15	73.3					
Apr. 5	S	2.24	15	26.7	125	124	120	0.8	4.0
		2.51	15	66.7					
		2.82	15	100.0					
Apr. 8	Std.	2.51	15	20.0					
		2.82	15	53.3					
		3.16	15	93.3					
Apr. 8	D	2.51	15	26.7	100	99	99	1.0	1.0
		2.82	15	66.7					
		3.16	15	73.3					
Apr. 8	T	2.24	15	26.7	112	111	111	0.9	0.9
		2.51	15	73.3					
		2.82	15	66.7					
Apr. 11	Std.	2.51	15	26.7					
		2.82	15	46.7					
		3.16	15	80.0					
Apr. 11	D	2.51	15	46.7	98	102	102	4.1	4.1
		2.82	15	46.7					
		3.16	15	73.3					
Apr. 11	T	2.24	15	13.3	109	110	111	0.9	1.8
		2.51	15	53.3					
		2.82	15	80.0					
Apr. 19	Std.	3.16	15	33.3					
		3.55	15	53.3					
		3.98	15	86.7					
Apr. 19	E	2.82	15	33.3	103	105	108	1.9	4.9
		3.16	15	53.3					
		3.55	15	46.7					
Apr. 19	F	1.41	15	40.0	233	233	230	0.0	1.3
		1.59	15	73.3					
		1.78	15	86.7					
Apr. 19	G	2.24	15	13.3	122	121	125	0.8	2.5
		2.82	15	46.7					
		3.16	15	73.3					
Apr. 19	H	1.26	15	33.3	253	254	253	0.4	0.0
		1.41	15	60.0					
		1.59	15	86.7					
May 8	Std.	3.16	15	46.7					
		3.55	15	60.0					
		3.98	15	66.7					
May 8	M	1.78	15	20.0	167	164	162	1.8	3.0
		2.24	15	80.0					
		2.82	15	86.7					
May 9	Std.	3.16	15	60.0					
		3.55	15	53.3					
		3.98	15	86.7					
May 9	M	1.78	15	26.7	153	161	167	5.2	9.2
		2.00	15	53.3					
		2.24	15	60.0					

Table IV.—(*Continued*)

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose Method — One-Dose Curve *D*	One-Dose Curve *O*
May 9	N	1.78	15	73.3	212	193	187	9.0	11.8
		2.00	15	86.7					
		2.24	15	86.7					
May 9	O	1.78	15	66.7	187	181	180	3.2	3.7
		2.00	15	66.7					
		2.24	15	80.0					
May 17	Std.	3.16	15	53.3					
		3.55	15	73.3					
		3.98	15	80.0					
May 17	M	1.78	15	33.3	147	160	167	8.8	13.6
		2.00	15	46.7					
		2.24	15	60.0					
May 17	N	1.59	15	26.7	166	177	185	6.6	11.4
		1.78	15	46.7					
		2.00	15	60.0					
May 20	Std.	3.16	15	46.7					
		3.55	10	80.0					
		3.98	15	86.7					
May 20	M	1.78	15	33.3	147	153	163	4.1	10.9
		2.00	10	30.0					
		2.24	15	60.0					
May 20	N	1.59	15	13.3	181	179	187	1.1	3.3
		1.78	10	60.0					
		2.00	15	80.0					
May 20	O	1.59	15	60.0	186	188	193	1.1	3.8
		1.78	10	50.0					
		2.00	15	73.3					
May 20	Std.	2.00	15	26.7					
		2.24	15	60.0					
		2.51	14	42.9					
May 20	P	2.00	14	35.7	101	102	101	1.0	0.0
		2.24	12	33.3					
		2.51	12	66.7					
May 20	Q	2.82	14	35.7	68	70	71	2.9	4.4
		3.16	15	33.3					
		3.55	15	53.3					
May 21	Std.	2.00	15	6.7					
		2.24	15	53.3					
		2.51	15	53.3					
May 21	P	2.00	15	13.3	98	99	100	1.0	2.0
		2.24	15	33.3					
		2.51	15	53.3					
May 21	Q	2.82	15	20.0	68	70	71	2.9	4.4
		3.16	15	33.3					
		3.55	15	40.0					
May 22	Std.	2.24	15	40.0					
		2.51	14	57.1					
		2.82	15	60.0					
May 22	P	2.24	15	40.0	107	107	105	0.0	1.9
		2.51	14	71.4					
		2.82	14	85.7					
May 22	Q	3.16	15	0.0	66	71	69	7.6	4.5
		3.55	14	35.7					
		3.98	14	78.6					
July 9	Std.	5.62	15	33.3					
		7.03	15	66.7					
		8.78	15	100.0					
July 9	I	4.47	15	13.3	107	110	115	2.8	7.5
		5.62	15	33.3					
		7.03	15	86.7					

Table IV.—(*Continued*)

Date	Sample	Dose, Cc./Kg.	No. of Frogs	% Mortality	Potency in % of Standard — Method — Three-Dose	One-Dose Curve *D*	One-Dose Curve *O*	% Variation from Three-Dose One-Dose Curve *D*	% Variation from Method One-Dose Curve *O*
July 9	J	1.78	15	13.3	259	270	285	4.2	10.0
		2.24	15	26.7					
		2.82	15	80.0					
July 9	K	4.47	15	26.7	127	125	125	1.6	1.6
		5.62	15	73.3					
		7.03	15	100.0					
July 9	L	1.78	15	33.3	339	346	331	2.1	2.4
		2.24	15	93.3					
		2.82	15	86.7					
July 10	Std.	5.62	15	20.0					
		7.03	15	73.3					
		8.78	15	100.0					
July 10	I	4.47	15	6.7	112	113	117	0.9	4.5
		5.62	15	53.3					
		7.03	15	86.7					
July 10	J	1.78	15	13.3	261	285	297	9.2	13.8
		2.24	15	40.0					
		2.82	15	66.7					
July 10	K	4.47	15	46.7	138	140	134	1.4	2.9
		5.62	15	86.7					
		7.03	15	100.0					
July 10	L	1.78	15	33.3	302	322	320	6.6	6.0
		2.24	15	66.7					
		2.82	15	80.0					
Aug. 16	Std.	2.51	15	6.7					
		3.16	15	53.3					
		3.98	15	80.0					
Aug. 16	R	2.51	15	13.3	81	91	95	12.3	17.3
		3.16	15	20.0					
		3.98	15	40.0					
Aug. 19	Std.	3.16	15	20.0					
		3.98	15	33.3					
		5.01	15	86.7					
Aug. 19	R	3.16	15	26.7	98	99	99	1.0	1.0
		3.98	15	33.3					
		5.01	15	73.3					
Aug. 21	Std.	3.16	15	13.3					
		3.98	15	53.3					
		5.01	15	80.0					
Aug. 21	R	3.16	15	13.3	95	97	98	2.1	3.2
		3.98	15	46.7					
		5.01	15	66.7					
Aug. 23	Std.	2.82	15	20.0					
		3.55	15	26.7					
		4.47	15	60.0					
Aug. 23	W	2.82	15	6.7	74	89	93	20.3	25.7
		3.55	15	13.3					
		4.47	15	26.7					
Aug. 27	Std.	3.16	10	40.0					
		3.98	7	57.1					
		5.01	9	100.0					
Aug. 27	R	3.16	10	20.0	92	98	98	6.5	6.5
		3.98	9	66.7					
		5.01	10	80.0					

In nearly every test 15 frogs were injected with each dose. The procedure outlined in the United States Pharmacopœia XI, page 397, was followed in preparation of extracts, and the alcohol content of the material to be injected was adjusted to between 20 and 25 per cent by volume. The doses were contained in 0.02 cc., except on April 4th and 5th when the volume was 0.01 cc. Several standards were used for these comparisons.

Table V presents an analysis of the results given

in Table IV. Differences in potencies computed by the different methods from the same experimental data are shown. Figures obtained by Bliss' method of calculation (3, 7) are used as a basis of the comparison. The true potency of the samples was not known except in one case, and it is therefore impossible to judge which method gave the more accurate results. Differences between the results obtained from the methods are all that can be shown here.

Table V.—Analysis of Results in Table IV

Per Cent Difference from the Three-Dose Method	Percentage of Assays by One-Dose Method	
	Curve *D*	Curve *O*
0–5	78.0	62.0
5–10	18.0	22.0
Over 10	4.0	16.0

The results are classified according to the difference in the potencies when calculated by curve numbers and when computed by the longer procedure of the three-dose method. There was 10 per cent or less variation from the results of the three-dose method in 96 per cent of the assays using Curve *D*, and in 84 per cent when Curve *O* was used. When the variation was greater than 10 per cent the mortalities for some of the doses were low or high. If all of the mortalities fall between 20 and 80 per cent for both standard and sample the agreement between the methods will be very close.

The actual potency of Sample *W*, August 23, 1940, Table IV, was known to be 96 per cent of the standard, and the result by the one-dose method was much closer to this value than by the three-dose procedure.

It should be pointed out that if these experiments had been designed as routine assays by the one-dose method, all of the frogs would have been injected with the middle doses shown in the table. The greater weights obtained by having the mortalities for all groups of animals close to 50 per cent would reduce the errors to a minimum.

If the slope of the composite curve for digitalis be taken as 13.1, and for ouabain 21.2, Tables VI and VII show the effect of the number of animals, the per cent response and the slope of the regression line on the limits of error when assaying digitalis

Table VI.—Limits of Error Computed for One-Dose Method, Digitalis Curve

No. of Frogs Used on Standard and Sample	Sex	Limits of Error from True Potency 95 Times in a 100 When Per Cent Responses are 20 and 80	When Per Cent Response is 50
5	M or F	74–136	76–131
10	M or F	81–124	83–121
15	M or F	84–119	86–117
20	M or F	86–117	88–114
25	M or F	87–115	89–113
30	M or F	89–113	89–112
35	M or F	89–112	90–111
40	M or F	89–112	90–110

The equation for the composite regression line for digitalis used in calculations of the above table is $Y = 4.98 + 13.1 (X - 1.63)$.

Note: In computing this equation all doses were multiplied by 10^6.

Table VII.—Limits of Error Computed for One-Dose Method, Ouabain Curve

No. of Frogs Used on Standard and Sample	Sex	Limits of Error from True Potency 95 times in a 100 When Per Cent Responses are 20 and 80	When Per Cent Response is 50
5	M or F	83–121	85–118
10	M or F	87–114	89–113
15	M or F	90–112	91–110
20	M or F	91–110	92–109
25	M or F	92–109	93–108
30	M or F	93–108	93–107
35	M or F	93–108	94–107
40	M or F	94–107	94–106

The equation for the composite regression line for ouabain used in calculations of the above table is $Y = 5.12 + 21.2 (X - 1.42)$.

Note: In computing this equation all doses were multiplied by 10^8.

and ouabain by a one-dose method. The figures given in these tables were computed from Irwin's equation 39 (4) in which the error of the slope of the standard curve is omitted. The error of the slopes for both composite curves is very small and may be neglected in comparison with the errors from other sources. The importance of the slope of the curve, the number of animals and the per cent response may be readily estimated from the tables.

DISCUSSION

The agreement between the slopes of the individual regression lines estimated by equation 20*a* (7), when considered in conjunction with the small differences in results of the two methods, Tables IV and V, is good evidence that one dose each of the standard and sample are sufficient for a test, and that the results may be calculated by curve numbers obtained from the standard Curve *D*.

The ouabain curve (Curve *O*) may be used in a similar way for the assay of strophanthus preparations, but, since Curve *D* and Curve *O* have different slopes, it would appear that the latter should not be used in calculating the results of digitalis assays. However, it is an interesting fact that, in 84 per cent of the assays, the differences from the three-dose method due to the use of this curve in calculation of results were 10 per cent or less. In those assays in which the variation was greater some of the moralities fell outside of the range 20 to 80 per cent.

An important reason advanced for the use of a three-dose method is the determination of significant changes in the slope of the dosage-response curve which might occur from time to time (2, 7), and it is recommended that the method be used in

routine assays for this purpose. Routine assays are necessarily carried out on limited numbers of animals, and even under ideal conditions, where the responses obtained are most effective for the calculations, the differences in slope which can be detected are relatively large. This may be shown by reference to the figures given in Table III, in which twelve separate determinations of the slope of the regression line for ouabain are reported. From seventy-five to one hundred and twenty frogs were used in each of the experiments and the slopes varied from 13.5 to 32.7, yet no *significant* difference could be shown in these twelve determinations. Chi-square for b was determined by Bliss' equation 20*a* (7) and also for each individual test in comparison with the composite line by his equation 20 (7).

In most cases it would appear, therefore, that relatively large differences in slope cannot be shown to have significance when the determinations are carried out in routine assays using the restricted numbers of animals which can be economically employed for this purpose. The evidence in Table III indicates that changes which have occurred in the case of ouabain were not large enough to be detected. When the slopes of the digitalis curve shown in Tables I and II were compared, no significant differences could be proven by equation 20*a* (7) and only three lacked agreement with the mean when equation 20 (7) was used. What appears to be of greater importance for the purpose of routine tests is the small variation in the results of assays when computed by the two methods, Tables IV and V. It appears, therefore, that a one-dose assay based on a standard curve will give results that are quite satisfactory in testing market samples of digitalis, and the method is to be preferred from the standpoint of simplicity.

Although the extent of the variability in the sensitivity of frogs to ouabain and digitalis is not the same, from a practical standpoint either the digitalis or ouabain curve may be used, providing the mortalities fall between 25 and 75 per cent. This observation has been emphasized in a recent paper by Chapman (10).

SUMMARY

1. Data for the construction of composite dosage-response curves for digitalis (*Digitalis purpureum*) and ouabain (*Strophanthus gratus*) are presented.

2. The composite curve for digitalis (*Digitalis purpureum*) has been found to differ significantly from the composite curve for ouabain (*Strophanthus gratus*).

3. A comparison of methods shows good agreement between a three-dose method and a one-dose method of assay for digitalis.

4. The maximum errors of the one-dose methods of assay for digitalis and also for ouabain are given.

5. A one-dose method is recommended for routine assays of digitalis and strophanthus preparations.

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Book Review

Handbook of Chemical Microscopy. Volume II. Chemical Methods and Inorganic Qualitative Analysis. EMILE MONNIN CHAMOT and CLYDE WALTER MASON. 2nd Edition. 438 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York, N. Y., 1940. Price, $5.00.

The second edition of Volume II of this handbook describes the essential manipulative methods employed in chemical microscopy and presents a compilation of the most dependable tests for the inorganic cations and anions. The references to original sources of information are unusually complete. This is believed to be the best and most complete authority in the field of microscopical qualitative analysis.—A. G. D.