A STUDY OF THE STANDARDIZATION OF DIGITALIS. I. A METHOD FOR CLINICAL STANDARDIZATION

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(Received for publication April 18, 1935)

It was at least partly because clinical observations led to the conclusion that different specimens of digitalis varied in their potency that the need for assay of the drug was realized. It would seem natural, therefore, to suppose that much attention would have been given to the clinical standardization of digitalis. In an exact sense, however, this is not the case. The problem is, indeed, a difficult one. It is necessary to know the rate of absorption of digitalis, and desirable to know its fate and its rate of elimination. The rates of absorption and elimination of some preparations have been worked out, though not in any large series or with great accuracy; the results show considerable variation from person to person, so that the averages reached stand for a wide range in a relatively small group of cases. When the drug is given by mouth, the speed and amount of absorption varies with different preparations and perhaps with the degree of congestive failure, if that condition is present. The tincture, however, is apparently fairly promptly and evenly absorbed in most cases (1). Intravenous or subcutaneous administration eliminates certain variables, but is restricted to certain preparations and is not commonly necessary in clinical practice. Very little, indeed, is known of what becomes of the digitalis that is absorbed. Some effect of a full dose may last as long as three weeks (2). The Eggleston method (3) of giving the drug involves the calculation of the amount required for an individual on the basis of his weight. This plan coupled with the average daily excretion found by Pardee (4) has been widely used; it assumes the possibility of clinical standardization.

Clinical experience with the body-weight method of calculating the dose of digitalis gives variable results, even with the same sample of the drug. This is not astounding, since two patients in exactly the same condition can hardly be found. The weight of the body is but one of its variable functions. The number of its functions which influence the effect of digitalis must be great. It has been shown that age is important, for young children require up to two or two and a half times the body-weight dose for adults (5). The Eggleston method (3) of giving the drug has been found very useful, and has done much to clarify the problems of the clinical use of digitalis.

It is remarkable that we have not been able to find any report of the clinical standardization of digitalis in which effort was made to study the limits of accuracy of the methods employed, by giving the same preparation in different strengths to the same individual. The most interesting attempts at exact clinical comparison of different preparations are those of Gilchrist and Lyon (6, 7) and Martin (8). Both of these reports are based on the study of three samples of leaves supplied by the Hygiene Committee of the League of Nations. The samples were extensively "standardized" by both frog and cat methods in many laboratories (9). Gilchrist and Lyon finally restricted their considerations to observations made on patients with auricular fibrillation and adopted as their criterion for the effect of the drug the drop in ventricular rate in terms of the initial ventricular rate. Martin employed patients with cardiovascular disease, both with and without auricular fibrillation, and took into consideration clinical evidence of improvement, minor toxic symptoms and pulse rate, blood pressure, and A–V conduction time. In both of these groups there was a variable degree of heart failure. In neither was special effort made to give all samples to the same individuals. Briefly summarized, the potencies of leaves "A" and "B" are given in terms of leaf "C." It should be pointed out that with improved understanding of the problem and with better standardization of technique, more consistent results can readily be attained. The two clinical studies reversed the position of leaf "B" with respect to "C." That the results are

so divergent is, of course, due in large part to the fact that various methods and techniques were used. The divergence illustrates the difficulties involved.

TABLE I

Comparison of assays from literature

Assay \ Leaf	A	B	C
Frog methods, averages (9)	50.1	94.3	100
Cat methods, averages (9)	60.5	116.7	100
Clinical (6)	64.3	88.9	100
Clinical (8)	75.8	141.5	100

In consideration of the desirability of accuracy, purely clinical criteria seem out of the question for these tests. The degree of congestive failure changes spontaneously and with time. The clinical effects of digitalis seem to vary with the degree of failure. The occurrence of congestive failure is, furthermore, a general indication for digitalis treatment, so that it is difficult to withhold the drug in its presence. The long interval which must elapse to secure freedom from previous doses renders it impossible to reproduce the same conditions in successive tests. It seems best to eliminate, therefore, the factor of clinical congestive failure, as far as possible.

The results of Gilchrist and Lyon suggest that the drop in ventricular rate, in the presence of auricular fibrillation, expressed in terms of the initial ventricular rate, may be a good criterion for the effect of digitalis. This method apparently cannot be used for the trial of different samples in the same individuals, however, because the effect is demonstrably different at different times. (Is this because of variation in the degree of congestive failure (16)?) The method must be confined to statistical studies in which large numbers of individuals preferably without failure, not available to us, are employed. (It is well known that the effect of digitalis on the rate of the normal mechanism is too variable to be applied for the purpose in hand.)

METHODS

In planning our investigation, it was decided to rely principally and finally on objective observations, and to give to the same subjects all the samples of digitalis to be compared. The subjects selected were normal individuals or patients with cardiovascular disease, but without clinically demonstrable congestive failure. They were studied by the usual clinical methods and were under observation for considerable periods before the tests began. Except in one instance (19 days), subjects were without digitalis for three weeks, and in many cases for much longer periods before each trial. No patient had significant fever, and no other important drug was given at the time of the tests.

The samples of digitalis were supplied by the Department of Pharmacology, in the form of tinctures, the strengths of which were unknown to us until the tests were completed. Because it was not our intention to compare the effects in different patients, their weights were not taken into consideration, although they were recorded; in no case was there important change in an individual's weight between trials. In each series each subject received the same dose of the preparations given him, except in one case. In the morning, after preliminary observations, 8 cc. (in the second series 10 cc.) were given. In the first and second series this was followed by three doses of 2 cc. each at 4-hour intervals; in the third series by a single dose of 2 cc. after 4 hours. This plan was adopted in the hope of detecting early differences among the effects of the samples. (The results show that a single dose is preferable.) The order in which the samples were given to patients was varied, but this variation does not seem necessary.

The special observations made included weight, height, blood pressure, and electrocardiograms. Symptoms of minor intoxication were carefully noted. In a few cases teleroentgenograms were made; although in some cases interesting changes occurred, the number of observations is not sufficient to justify their consideration at present. The present observations were made the day before the tests, immediately before the first dose, at 4-hour intervals during the day of the trial, and on the morning after (24 hours after the first dose). The usual leads of the electrocardiograms were taken with the subjects lying down at rest. The resistance was always satisfactorily low. The string deflection was standardized in the usual manner, and the standard deflection was recorded with each lead. The study of the electrocardio-

grams comprised measurement of the cycle length, the " A–V " conduction time (" P–R " interval), the duration of " electrical systole " (" Q–T "), the amplitude and direction of the T wave, and observation of the form of other waves. Measurements were made with calipers under a magnifying glass, in most cases in Lead II, but always in the same lead for different samples of digitalis in the same individual. Some of the measurements are difficult and perhaps unreliable in the presence of auricular fibrillation. The few results from cases of fibrillation which we present are averages of ten consecutive cycles.

RESULTS

A survey of the features observed convinced us that it seemed impossible to use most of them in any exact way for the comparison of the different samples of digitalis. Most of the usual effects of moderate doses occurred. Symptoms were encountered only in a very few instances. The blood pressure, as may be expected, showed no significant changes. Slowing of the rate, though slight in many cases, occurred in 38 out of 44 observations, but was irregular. The " A–V " conduction time was usually lengthened, often very slightly. The T wave was often decreased in amplitude but sometimes not. No other important changes in the form of the deflections were seen.

It has long been known that digitalis shortens the duration of systole in experimental animals. Cheer (13) and Berliner (14) first called attention directly to the effect on the " Q–T " interval in human electrocardiograms. This interval is apparently a function of the cycle length (which stretches from end of one " T " to end of the next " T," and includes the " Q–T " interval to

TABLE II

Electrocardiographic measurements and constant "K" immediately before and 24 hours after giving samples of digitalis of unknown potencies

Subject*	Digitalis** sample	P–R interval		T wave		Cycle length		Q–T interval		Constant "K"†	
		Before	After	Before	After	Before	After	Before	After	Before	After
		seconds	*seconds*	*mm.*	*mm.*	*seconds*	*seconds*	*seconds*	*seconds*		
1 Normal heart	A (3)	0.150	0.160	3.0	2.5	0.720	0.860	0.320	0.325	0.377	0.350
	B (2)	0.140	0.160	3.0	2.0	0.920	0.960	0.365	0.310	0.381	0.316‡
	C (1)	0.140	0.160	3.4	2.5	0.915	0.800	0.360	0.315	0.376	0.352
2 Luetic CVD	A (1)	0.170	0.180	−3.5	−3.0	1.280	1.010	0.480	0.410	0.424	0.408
	B (2)	0.170	0.180	−1.5	−1.7	1.140	1.390	0.440	0.420	0.412	0.356
	C (3)	0.170	0.180	−1.0	−1.5	1.070	1.245	0.410	0.400	0.397	0.358
3 AS CVD AF	A (1)	?	?	3.5	2.0	0.650	0.910	0.320	0.370	0.397	0.388
	B (2)	?	?	4.0	1.5	0.690	1.360	0.350	0.400	0.421	0.343‡
	C (3)	?	?	3.0	2.0	0.600	1.030	0.320	0.365	0.413	0.360
4 Normal heart	A (1)	0.140	0.150	3.0	2.0	0.745	0.645	0.325	0.300	0.377	0.374
	B (2)	0.130	0.170	3.5	2.0	0.705	0.750	0.335	0.318	0.399	0.367
	C (3)	0.145	0.160	4.0	2.0	0.740	0.730	0.335	0.310	0.389	0.363
5 Hy CVD	B (2)	0.160	0.180	1.6	1.4	0.805	0.860	0.355	0.340	0.396	0.367
	C (1)	0.150	0.160	1.0	1.0	0.780	0.820	0.350	0.350	0.396	0.387
6 Rh CVD	A	0.165	0.170	1.3	1.5	0.800	0.850	0.330	0.325	0.369	0.353
7 Rh CVD	B	0.195	0.210	1.4	1.3	0.625	1.200	0.320	0.365	0.405	0.333
8 Hy CVD AF	C	?	?	1.8	1.3	0.560	0.900	0.310	0.330	0.414	0.348

* "CVD" is cardiovascular disease; "AS" is arteriosclerotic; "Hy" is hypertensive; "Rh" is rheumatic; "AF" is auricular fibrillation.

** "A," "B" and "C" are dilutions of a standard tincture of digitalis of unknown relative strengths. The numbers indicate the order of their administration.

† "K" is "Q–T" interval divided by square root of cycle length; see text.

‡ Marked nausea.

be measured). The cycle length must, therefore, be taken into account. Although there is difference of opinion as to the formula best expressing the relationship, that used by Bazett (15) seems to fit well for usual heart rates, say 50 to 120, and we have employed it in this clinic. According to it the "Q–T" interval should equal the square root of the cycle multiplied by a constant ($Q\text{–}T = K\sqrt{C}$). Variations in "$Q\text{–}T$" can then be expressed as variation in "K." [1]

A study of the values of "K" led us to believe we could do best by relying principally upon it, as the results seem fairly consistent. The number of observations is very small, but it was not our intention to apply the statistical method in this study. Only the results immediately before the first dose and after a standard interval are presented. The intermediate values were neither more discordant nor helpful.

In the first series (Tables II and III) three strengths of the same tincture of digitalis, "A," "B," and "C," were given, known to differ by as much as 25 per cent. In three of the four cases in which all three tinctures were given sample "B" clearly had the greatest effect, and similarly in three of the four cases "C" had a greater effect than "A," as shown by the values of "K." The incomplete cases (5 to 8) happen to fit in with this conclusion. On the basis of the values of "K" these samples were put in the order B > C > A. This conclusion was confirmed by data supplied by the Department of Pharmacology, according to which the relative strengths of

[1] In this investigation we are not concerned with the question of the normal value of "K" which is in the neighborhood of 0.38. On the whole matter, see the reports of Cheer and his co-workers (16, 17).

TABLE III

Constant "K" before and after giving digitalis. Data from Table I

Subject	Sample A			Sample B			Sample C		
	Before	After	Difference	Before	After	Difference	Before	After	Difference
1	0.38	0.35	0.03	0.38	0.32	0.06	0.38	0.35	0.03
2	0.42	0.41	0.01	0.41	0.36	0.05	0.40	0.46	0.04
3	0.40	0.39	0.01	0.42	0.34	0.08	0.41	0.36	0.05
4	0.38	0.37	0.01	0.40	0.37	0.03	0.39	0.36	0.03
Sum			0.06			0.22			0.15
5				0.40	0.37	0.03	0.40	0.39	0.01
6	0.37	0.35	0.02						
7				0.41	0.33	0.08			
8							0.41	0.35	0.06

TABLE IV

Electrocardiographic measurements and constant "K" immediately before and 16 hours after giving samples of digitalis of unknown potencies *

Subject	Digitalis tincture †	P–R interval		T wave		Cycle length		Q–T interval		Constant "K"	
		Before	After	Before	After	Before	After	Before	After	Before	After
		seconds	*seconds*	*mm.*	*mm.*	*seconds*	*seconds*	*seconds*	*seconds*		
3	A (2)	?	?	2.2	1.2	0.855	0.890	0.360	0.340	0.389	0.376
AF	B (1)	?	?	4.0	3.5	0.790	0.970	0.355	0.330	0.383	0.335
9	A (1)	0.155	0.160	−1.0	+0.7	0.905	1.130	0.430	0.390	0.452	0.367
Rh CVD	B (2)	0.155	0.160	−1.0	+1.0	1.060	1.090	0.445	0.385	0.432	0.369
10	A (1)	0.150	0.145	3.5	4.0	0.730	1.070	0.350	0.400	0.410	0.387
Rh CVD	B (2)	0.140	0.150	3.7	5.5	0.660	0.990	0.320	0.350	0.394	0.352
11	A (2)	0.150	0.145	3.2	3.7	0.760	0.910	0.355	0.380	0.407	0.398
Normal heart	B (1)	0.155	0.160	1.5	1.6	0.620	0.855	0.320	0.358	0.406	0.387
12	A (1)	0.165	0.190	2.5	3.0	0.725	0.920	0.358	0.390	0.420	0.407
Normal heart	B (2)	0.160	0.175	3.5	3.6	0.840	1.030	0.360	0.390	0.393	0.384
13	A (1)	0.160	0.160	3.5	4.0	0.850	1.000	0.360	0.390	0.390	0.390
Rh CVD	B (2)	0.150	0.160	3.0	4.5	0.760	1.000	0.345	0.380	0.396	0.380

* See notes to Table I.

† "A" and "B" are samples of standardized aged and fresh tinctures of digitalis (not known apart before the conclusions were reached). Numbers denote the order of administration.

the samples were B = 125 per cent, C = 100 per cent, and A = 75 per cent.

The second series (Tables IV and V) included two tinctures, A and B, one known to be old and the other fresh. By misunderstanding, they were both given to us in weak form (4.5 per cent), instead of the usual concentration of 10 per cent.

TABLE V

Constant "K" before and after giving digitalis. Data from Table III

Subject	Tincture A			Tincture B		
	Before	After	Difference	Before	After	Difference
3	0.39	0.38	0.01	0.38	0.34	0.04
9	0.45	0.37	0.08	0.43	0.37	0.06
10	0.41	0.39	0.02	0.39	0.35	0.04
11	0.41	0.40	0.01	0.41	0.39	0.02
12	0.42	0.41	0.01	0.39	0.38	0.01
13	0.39	0.39	0.00	0.40	0.38	0.02
Sum			0.13			0.19

The effects were, therefore, not marked. Nevertheless, we concluded that "B" was somewhat stronger than "A," for in four of the six cases the changes in the value of "K" were in that direction, though the difference did not seem to be great. Data supplied after this decision was reached showed that standardization of the tinctures just before the first tests gave these results (the differences are not statistically significant):

Sample	Potency		
	Dog	Cat	Frog
A (Aged)	0.90	0.95	0.84
B (Fresh)	1.00	1.00	1.00

The same method was applied to the study of a third pair of unknown samples, the nature of which together with the results of the work is described elsewhere (18).

DISCUSSION

In the first series, the method employed enabled us to distinguish correctly between three samples of the same preparation of digitalis, which differed in strength by 25 per cent. The results do not lead us to believe we can confidently and reliably distinguish between preparations differing by significantly smaller strengths. It is recognized that they need confirmation. The statistical method applied to a large series of cases might clarify the situation.

It should be noted that we are not prepared to say that the strengths of the samples are in direct proportion to the changes produced in the value of "K." There are some indications that the formula used may not apply to very slow or very fast rates. In slow rates a constant factor may be needed to express a natural limit of relative increase in "Q–T." Such a constant factor would then be taken into consideration in comparing proportionately changes in the value of "K."

On the basis of these results the common practice of giving adult patients of usual size a total dose of digitalis of 1.0 to 1.2 gram in terms of potent powdered leaves, seems justified. In other words, calculation of the dose is not in general necessary. In very small or in very large individuals, factors with which this investigation did not deal may modify the dose. There are suggestions that in advanced age susceptibility to digitalis is increased, but the question has apparently not been seriously studied. The maintenance dose does not seem to depend upon weight in any case (19), but rather on the amount in the body (20).

Standardization of digitalis for clinical use is necessary in order to establish the fact that a preparation is not inert, unduly feeble, or too strong.

As to the second series, the differences found seem to be slight. They were in the same direction as those shown by animal assays, even though these were not statistically significant. It is possible that the fresh tincture was a little more potent than the aged preparation. Our chief interest lies in the fact that there was no discrepancy between the animal and clinical standardizations.

It is evident that the greatest care is necessary in attempting to carry out the clinical standardization of digitalis. The only methods so far suggested which seem to offer the promise of reliable results are those of Gilchrist and Lyon, in which the effect of a sample is judged in patients the subject of auricular fibrillation by the drop in

ventricular rate in terms of the initial ventricular rate, with statistical control; and the method here reported of using the decrease in the "Q–T" interval in relation to the cycle length ("T–T" interval, preferably). For the latter method the use of a comparator, which has not been available to us, seems desirable. Careful study of the "P–R" interval and "T" wave should be made, as it is still uncertain to what extent these phenomena may be important aids in arriving at final conclusions. Those portions of the cardiac cycle which are used for correlation (such as cycle length and "Q–T" interval) should be measured in the same cycles. In both cases it is necessary to test the capacity of the method by giving different strengths of the same preparation to the same subject. It is clearly desirable likewise to give the various samples under trial to the same patients. For the second method, at present it seems desirable to use both normal subjects and patients with cardiovascular disease, but it is important to know to which group the subjects belong. The drug is best given in a single dose of a size determined from assay by a standard animal method, but well below the amount which might be expected to produce effects so marked as to mask differences between the samples being compared, at a definite time in relation to meals. The same dose of each of the samples to be compared should be given. It is believed that the following conditions should be met in making comparisons of the strengths of digitalis preparations:

Age limits, 20 to 50 years; careful study as to whether cardiovascular disease is present; no clinically demonstrable congestive failure; no fever for two weeks; no recent serious illness; no diarrhea or vomiting; no digitalis for 30 days; no important drug therapy (especially no opiate) during the tests; hospitalization and a constant diet throughout, or certainly during, the tests; the observations to be compared should, if possible, be made at the same time of day, and under conditions as nearly comparable as they can be made.

SUMMARY

Past efforts to standardize digitalis by clinical study are briefly reviewed.

Attempt was made to ascertain the feasibility of separating by clinical assay different strengths of the same tincture of digitalis. In the conditions of the experiments, among the phenomena observed (which included symptoms, weight, blood pressure, cardiac rate, and various aspects of the electrocardiogram), only one seemed clearly helpful. The effect, under standard conditions, of three such samples, on the "Q–T" interval of the electrocardiogram, stated in relation to the cardiac cycle length, made it possible to distinguish potencies related as 75 : 100 : 125. Aged and fresh samples of the same tincture, were similarly tested.

The conditions for satisfactory clinical assay of digitalis are discussed, and a standard set outlined.

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