Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXI | *AUGUST, 1942* | NUMBER 8 CONSECUTIVE NO. 15

The Assay of Digitalis

III. The Potency of U. S. P. XII Digitalis*†

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The question of the relative potencies of U. S. P. X and U. S. P. XI Digitalis has been reviewed by Edmunds (2), who has also set forth the events leading up to the adoption of the International Standard Digitalis (1926) and the U. S. P. Reference Digitalis Powder (3). When the latter reference standard became available, it was generally expected that its use would result in fixing the U. S. P. XI standard of potency at a level about 25% above the U. S. P. X potency. Considerable literature developed which raised doubt as to whether this expectation was actually realized. Some observers have reported that the increase was of the order of 50% to 70% (4, 5, 6, 7, 8). The exhaustion of the original (1926) supply of international standard as well as the acknowledged unsuitability of ouabain as a reference standard for digitalis are but two factors of many which preclude the satisfactory resolution of the controversy.

Difficulties of a similar nature complicate the problem of estimating the expected decrease in potency which will result in changing from U. S. P. XI to U. S. P. XII standards of strength. The object of this report is to make available certain data bearing on this question. These data were obtained in comparisons of the various reference digitalis standards among themselves and in assays of samples of commercial digitalis preparations which have entered interstate commerce. The data justify the conclusion that the potency of U. S. P. XII Digitalis will be substantially less than that of U. S. P. XI Digitalis and indicate the extent of the decrease attributable to the change in reference standard and, in general, the decrease arising from the change in test animal.

Most of the material used in this study represents official samples submitted to this laboratory in the course of regulatory activities connected with the enforcement of the Federal Food, Drug, and Cosmetic Act. The series does not represent a cross section of the digitalis in interstate commerce, since an inordinate proportion of the samples reported have formed the basis for regulatory action which has terminated. Such samples are, as a matter of course, much

* Received July 3, 1942, from the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.

† The second paper of this series, entitled "The Assay of Digitalis. II. Absorption as Influenced by the Site of Injection" (1), was presented before the American Society for Pharmacology and Experimental Therapeutics at its annual meeting in New Orleans, March 13–16, 1940.

more exhaustively examined than those which give evidence of meeting the official requirements. The additional examination has included assays on cats, the results of which permit a calculation of the potency in terms of the new U. S. P. XII requirements. In view of the great interest in the changing standards of strength, it seems desirable to publish the information so obtained and to support it by similar data on other samples whose potencies are within the allowed tolerances. The data are further supplemented by extensive comparisons of the U. S. P. Reference Digitalis Powder with both of the International Standard Digitalis powders (1926 and 1936) and the "study powder."[1]

EXPERIMENTAL

Preparatory to assay of those samples in solid form (powders and tablets), tinctures were prepared by adding to a weighed amount of the preparation in a glass stoppered centrifuge tube (9), a volume corresponding to 10 cc./Gm. of U. S. P. digitalis menstruum. In the case of powdered extracts, a liquid preparation was obtained similarly by using a proportion of 10 cc. of menstruum per 0.25 Gm. as directed in N. F. VI. The mixtures were shaken mechanically and continuously for 24 ± 2 hrs. Following this maceration period the liquid phase was clarified by centrifuging and decanted into a hard glass bottle in which it was stored under refrigeration. Suitable precautions were taken to prevent evaporation during storage. The tinctures were usually assayed promptly and in no case longer than thirty days after their preparation.

In carrying out the U. S. P. XI method, all the specified directions were followed closely. Test dilutions of the standard and of the two or three preparations to be assayed were prepared fresh each day and were so adjusted that the dosage per Gm. of frog was contained in 0.02 cc. Each test dilution contained approximately 23% of alcohol regardless of the actual concentration of digitalis which varied, of course, with the dosage. In routine assays, three dosage levels were used which stood in relation to each other as $1 : \sqrt{2} : 2$ so that the logarithm of the dosage interval was 0.15. When the low dose of such a series produced positive results (systolic standstill of the ventricle) in 25% of the frogs injected, such a dosage range usually assured approximately 50% and 75% of positive results with the intermediate and high doses, respectively. Every effort was made to insure homogeneity within the various groups of frogs receiving the different dosage levels. This precaution not only complies with the principles of good practice, but also makes possible the use of recently developed statistical methods (10) in calculating the most probable potency and its standard error, which is a measure of the reliability associated with the estimate of potency.

In using the 18-hr. (overnight) method, the test dilutions were prepared as for the U. S. P. XI method except that the three doses were usually related as $1 : \sqrt[4]{2} : \sqrt{2}$ so that the dosage interval corresponded to log-dose 0.075. As with the U. S. P. XI assay, precautions were observed for insuring homogeneity among the frogs so that the same means of calculating the potency and its standard error were available.

In employing the U. S. P. XII method in which the cat is the test animal, the directions for selecting and preparing the cats and for preparing and injecting the test dilutions were followed.[2] Ordinarily six cats were injected simultaneously, two being injected with the diluted Standard Preparation of Digitalis, and two each with dilutions of two different preparations to be assayed. In this way, day-to-day (secular) variations in the responses of the cats were minimized. It was observed that such variations could assume considerable magnitude over and above what may prove to be a seasonal variation in the average lethal dose of the standard preparation. In this study the most probable potency and its standard error were determined by a method of calculation[3] essentially that suggested by

[1] Grateful acknowledgment is hereby recorded for adequate supplies of International Standard Digitalis (1936) and of the study powder obtained through the kindness of Professor E. Fullerton Cook, General Chairman of the U. S. P. Committee of Revision.

[2] The U. S. P. XII assay directions substantially in their final form were made available through Dr. E. E. Nelson, Chairman of Subcommittee No. 3 on Biological Assays of the U. S. P. Revision Committee.

[3] The calculation is a slight modification, with certain corrections, of that developed by Dr. Bliss for consideration by the subgroup on digitalis assay of the U. S. P. Subcommittee No. 3 on Biological Assays. It is carried out as follows:

Express in logarithms the number of doses of test dilution required by each cat. To the logarithms of the observations on those cats receiving the standard, add the logarithm of the number of cc. of Standard Preparation of Digitalis contained in each 100 cc. of the test dilution of the standard. Average (arithmetically) the sums so obtained to get $\bar{y}_S$, the average log-dose of the standard. Similarly, add to each of the logarithms of the observations on the cats receiving the preparation being assayed (the unknown) the logarithm of the number of cc. of the unknown in each 100 cc. of the test dilution of the latter. Average these sums to obtain $\bar{y}_U$, the average log-dose of the unknown.

Obtain M, the logarithm of the number of U. S. P. Digitalis Units per cc. of the preparation being assayed, by subtracting $\bar{y}_U$ from $\bar{y}_S$.

Calculate the standard error of M, s_M, by one of the following formulas:

(*a*) Where an equal number of cats has been used on both standard and unknown,

$$s_M = \sqrt{\frac{S(y^2) - \overline{N(\bar{y}_S{}^2 - \bar{y}_U{}^2)}}{N(N-1)}}$$

in which $S(y^2)$ = sum of the squares of the log-doses for all the cats used, N = number of cats on standard, $\bar{y}_S$ = average log-dose of standard and $\bar{y}_U$ = average log-dose of unknown. (*Continued, p. 227*).

TABLE I.—COMPARISON OF *USP* REFERENCE DIGITALIS POWDER WITH INTERNATIONAL STANDARD DIGITALIS POWDERS (1926) AND (1936)

Date	Method	Standard	No. of Animals	Amount of Standard Equivalent to 1 Gm. of *USP RDP* Grams	Log ± *S. E.*
May, 1936[a]	*USP* XI	*ISD* (1926)	179	2.06	0.3136 ± 0.033
June, 1936[a]			80	1.68	0.2253 ± 0.050
Dec., 1937[a]			130	1.93	0.2867 ± 0.039
Apr., 1939			180	2.24	0.3512 ± 0.0248
Weighted average, all assays				2.07 ± 0.08	0.3156 ± 0.0166
Mar., 1938	*USP* XI	*ISD* (1936)	330	1.37	0.1358 ± 0.0306
Apr., 1939			435	1.25	0.0982 ± 0.0237
Aug., 1939			540	1.31	0.1174 ± 0.0231
Sept., 1939			129	1.20	0.0809 ± 0.0646
Oct., 1939			380	1.25	0.0982 ± 0.0296
June, 1940			144	1.12	0.0504 ± 0.0345
Weighted average, all assays				1.27 ± 0.04	0.1028 ± 0.0121
Apr., 1939	4-Hr.	*ISD* (1936)	240	1.38 ± 0.10	0.1391 ± 0.0319
Mar., 1938	18-Hr.	*ISD* (1936)	380	1.29	0.1057 ± 0.0080
Aug., 1939			540	1.21	0.0811 ± 0.0166
Feb., 1940			160	1.37	0.1375 ± 0.0250
July, 1940			80	1.26	0.0991 ± 0.0258
Weighted average, 18-Hr. assays				1.27 ± 0.02	0.1036 ± 0.0067
June, 1938[b]	Cat	*ISD* (1936)	18	1.18	0.0711 ± 0.0351
Sept., 1941			12	1.11	0.0433 ± 0.0308
Nov., 1941			11	1.29	0.1100 ± 0.0336
Weighted average, 1941 assays				1.19 ± 0.06	0.0738 ± 0.0227

[a] These assays were carried out using the single-dose standard curve method which does not provide an inherent check on the accuracy and therefore the data do not yield an estimate of the standard error. The standard error values given are approximations obtained by taking into account the number of frogs used in these assays and the observed standard errors in three-dose assays.

[b] This assay on the cat involved a faster rate of injection than specified in the *USP* XII assay so that the mean death time was 39 min. It was not included in the weighted average.

Bliss and Hanson (11), in which the individual lethal doses are transformed to logarithms. In contrast to the method of calculation supplied in U. S. P. XII, this calculation facilitates combining the results of two or more assays in a weighted mean to which each individual result contributes in proportion to the information it affords. Assigning weights in this way involves the assumption that an assay yielding a low calculated standard error provides, in general, a more reliable estimate of the true potency and, therefore, more information relative to it than does a similar assay having a high standard error.

An effort was made to carry out sufficient assays by each method so that the calculated standard error of the weighted mean was not greater than 10% of the mean. Ordinarily to accomplish this, it was necessary to conduct at least three U. S. P. XI assays, each of which involved the use of 40 frogs on the standard and on each preparation being assayed, or a total of 160 for the assay of three samples. Two 18-hr. assays on the same number of frogs were quite sufficient because of the greater accuracy characteristic of this method, an observation first substantiated by objective evidence in the initial paper of this series (10). With the U. S. P. XII assay, a comparable accuracy usually results from employing six to nine cats on both the standard and the preparation to be assayed.

A Comparison of the U. S. P. Reference Digitalis Powder with the 1926 and 1936 International Standard Digitalis Powders.—During the period the U. S. P. Reference Digitalis Powder[4] has been available, numerous comparisons have been made in this laboratory to determine its relative potency in terms of the 1926 and the 1936 international standards. The results of all these assays are compiled in Table I, in which the individual assay values have been combined arbitrarily by months as a matter of simplifying the table. The data are expressed in terms of the amount of the respective international standard which is equivalent to 1 Gm. of *USP RDP* by the method of assay specified.

The exceptionally high potency of the *USP RDP*

[3] (*Continued*). (*b*) Where an unequal number of cats has been used on standard and unknown,

$$s_M = \sqrt{\frac{[S(y^2) - N_S\bar{y}_S^2 - N_U\bar{y}_U^2](N_S + N_U)}{(N_S + N_U - 2)N_SN_U}}$$

in which $S(y^2)$ = sum of the squares of the log-doses for all the cats used, N_S = number of cats on standard, $\bar{y}_S$ = average log-dose of standard, N_U = number of cats on unknown and $\bar{y}_U$ = average log-dose of unknown.

Where more than one assay has been made, combine the individual results by the procedure suggested by Miller, Bliss and Braun (10).

[4] Toward economy of space, the following abbreviations will hereafter be used: *USP* in referring to the U. S. Pharmacopœia, *USP RDP* in referring to the U. S. P. Reference Digitalis Powder; *ISD* (1926) and *ISD* (1936) in referring to the International Standard Digitalis (1926) and (1936), respectively, and *USP DRS* in referring to the U. S. P. Digitalis Reference Standard (1942).

is apparent at once from these data. By the *USP* XI method it appears to be more than twice as potent as the *ISD* (1926), the weighted average of all the assays indicating that 1 Gm. of *USP RDP* is equivalent to 2.07 Gm. of *ISD* (1926) or 207% of this international standard. The latter value is to be compared with the observation of Edmunds, Moyer and Shaw (3) that, in their hands, the *USP RDP* was 134.22% of *ISD* (1926). It was their value, of course, on which the "factor" for the *USP RDP* was based. The present observation of 207% is somewhat higher than any heretofore reported as may be seen in Table V. Because the supply of *ISD* (1926) was so severely limited, assays by methods other than the official were not attempted.

The comparisons of *USP RDP* and *ISD* (1936) bring out several points. With respect to methods, it is clear that there is no difference in the relative potencies of these two powders as indicated in results of frog assays involving three different periods of observation. While only two assays were carried out with the 4-hr. method, the results of these are not significantly different from the weighted mean results of either the *USP* XI or the 18-hr. assays. The latter represent a total of 14 and 9 assays respectively. The observation that the *USP* XI and the 18-hr. assays agree very well is in confirmation of the results obtained in the first comparison of the *USP* digitalis study using frogs (12). It is in conflict with the conclusion drawn by Edmunds, *et al.* (3), with respect to these two powders. As indicated below (Table V), their conclusion was that the *USP RDP* appeared stronger with the longer period of observation. It seems unlikely, in view of the limited number of frogs used in their assays, that this conclusion was justified. Differences of the magnitude they observed cannot be established with a satisfactory degree of certainty without the use of several hundred frogs.

Since the potency estimates between the three frog methods do not differ significantly, it is probably safe to combine them into a weighted mean. Doing this yields the observation that 1.0 Gm. of *USP RDP* is equivalent in the frog to 1.27 ± 0.02 Gm. of *ISD* (1936). By definition, 0.080 Gm. of the latter is established as currently representing the potency of 1.0 International Digitalis Unit; thus, 0.0630 Gm. of *USP RDP* exhibits in the frog an activity of 1 International Digitalis Unit. The difference between 0.0630 and 0.0745 Gm., the latter being the amount of *RDP* fixing the *USP* Digitalis Unit, is 15.4% and is a measure of the discrepancy between the *USP* Digitalis Unit and the International Digitalis Unit as both are currently defined under the provisions of *USP* XI. Actually, it is known that this must be an underestimate of the discrepancy since the factor of 0.8 for *ISD* (1936) is an average of results by three methods of assay. Actual assay on frogs (presumably by the overnight method) showed that *ISD* (1936) was 138% of *ISD* (1926) (13), so that, strictly speaking, a factor of 0.725 is more appropriate for relating the potency of the two standards by the frog method. On this basis the real difference between the *USP* and the international unit is closer to 23%. In any event, the discrepancy would scarcely seem to be of any great moment to the practicing physician and suggests a search elsewhere for the causes of untoward clinical reactions which have been reported (14).

On the cat, 1.19 ± 0.06 Gm. of *ISD* (1936) is equivalent to 1 Gm. of *USP RDP* or slightly less than the equivalence by any of the frog methods. Calculated as above, it is seen that in the cat, 0.067 Gm. of *USP RDP* exhibits the activity of 1 International Digitalis Unit and when determined on this species the discrepancy between the *USP* and international units is only 10%. Making allowance for the relationship actually observed between the two international standards, which indicates that 0.0862 Gm. of the *ISD* (1936) represents 1 International Digitalis Unit in the cat (13), the discrepancy between the latter unit and the *USP* unit is only 3% and entirely negligible.

Potency of the Study Powder.[5]—The study powder has been compared against the *USP* Standard Preparation of Digitalis (containing 0.0745 Gm. of *USP RDP* per cc.) in assays by each of three methods. Eighty frogs were used in each of the *USP* XI assays while 77, 90, 80 and 90 frogs were used, respectively, in the 18-hr. assays listed in that order in Table II. The three cat assays represent the use of 12, 17 and 20 cats, respectively. The results, listed in Table II, have all been expressed in terms of the volume of the standard preparation which was equivalent to 0.1 Gm. of the original study powder. It will be noted that the agreement between the results of individual assays by the same method is quite satisfactory. Although there is a slight indication of higher potency in the assay conducted in 1940 by the *USP* XI method, this value is not significantly higher than those obtained later. The agreement in general between the various assays by the same method at different times assumes added significance when it is considered that the data represent determinations made independently by each writer using each of the three methods. With one exception, the *USP* XI assays of November 26, 1941, and December 1, 1941, no two assays were made on the same pair of standard tinctures.

The data in Table II reveal that the results of the 1-hr. and 18-hr. methods on frogs agree very well,

[5] As used throughout this paper the term "study powder" refers exclusively to the material distributed as samples 3 and 5, respectively, for the second and third comparisons (using frogs) of the *USP* (1939–1941) digitalis study. Actually only the 1940 assays reported in Table II were conducted on the study powder in its original concentration. Developments discussed elsewhere (15) resulted in a stepwise dilution of the study powder in the proportion of 1 : 1.351 to yield what is now the *USP* Digitalis Reference Standard (1942). Information on this dilution has been obtained through the courtesy of Professor E. Fullerton Cook making it possible to adjust the results of the 1941 and 1942 assays to the basis of the original study powder.

TABLE II.—SUMMARY OF ASSAYS OF THE STUDY POWDER AGAINST *USP* STANDARD PREPARATION OF DIGITALIS

USP XI Method			18-Hr. Frog Method			*USP* XII Method		
	Potency[a]			Potency[a]			Potency[a]	
Date	Cc.	Log ± *S. E.*	Date	Cc.	Log ± *S. E.*	Date	Cc.	Log ± *S. E.*
May 8, 1940	1.20	0.0779 ± 0.0539	July 2, 1940	1.09	0.0374 ± 0.0226	Sept. 29, 1941	1.13	0.0544 ± 0.0283
Nov. 26, 1941	1.06	0.0241 ± 0.0548	July 15, 1940	0.90	−.0461 ± 0.0276	Oct. 31, 1941	1.16	0.0637 ± 0.0256
Dec. 1, 1941	1.01	0.0053 ± 0.0563	July 30, 1940	1.02	0.0075 ± 0.0409	May 29, 1942	1.13	0.0528 ± 0.0507
Dec. 4, 1941	1.00	−.0019 ± 0.0556	June 8, 1942	1.01	0.0044 ± 0.0410			
Weighted mean	1.06	0.0273 ± 0.0276		1.01	0.0044 ± 0.0150		1.14	0.0585 ±0.0178

[a] The potency value given in columns 2, 5 and 8 is the volume of *USP* XI Standard Preparation of Digitalis (containing the activity of 0.0745 Gm. of *USP* Reference Digitalis powder per cc.) equivalent to 0.1 Gm. of the original study powder; this value, multiplied by 100, gives the potency of the study powder expressed as a percentage of the *USP* XI requirements. The values given in columns 3, 6 and 9 are the logarithms of the volume and its standard error, respectively, which are conventionally known as $M \pm s_M$ (10).

TABLE III.—*USP* XI AND *USP* XII POTENCIES OF SEVERAL SAMPLES OF DIGITALIS

Sample No.	Type of Preparation	Potency[a] *USP* XI Method, Cc.	Potency[a] *USP* XII Method, Cc.	Ratio of *USP* XII to XI	*USP* XII Potency[b]	Excess of *USP* XI over *USP* XII *USP* Digitalis Units[c]
7707	Tincture	1.49 ± 0.08	1.77 ± 0.20	1.19 ± 0.16	2.11	0.42 ± 0.19
7935		1.72 ± 0.08	2.15 ± 0.14	1.25 ± 0.10	2.56	0.49 ± 0.12
8088		0.57 ± 0.04	1.01 ± 0.06	1.77 ± 0.16	1.20	1.11 ± 0.19
8228		0.52 ± 0.06	0.75 ± 0.03	1.42 ± 0.17	0.89	0.69 ± 0.21
8429		1.58 ± 0.12	2.32 ± 0.10	1.46 ± 0.12	2.76	0.74 ± 0.15
8435		1.30 ± 0.14	1.42 ± 0.08	1.09 ± 0.14	1.69	0.30 ± 0.16
8474		0.88 ± 0.08	1.02 ± 0.05	1.16 ± 0.12	1.21	0.38 ± 0.14
8502		1.58 ± 0.10	2.35 ± 0.16	1.48 ± 0.13	2.80	0.77 ± 0.16
Study	Powder	1.06 ± 0.07	1.14 ± 0.05	1.07 ± 0.08	1.35	0.28 ± 0.10
ISD (1936)		1.06 ± 0.03	1.13 ± 0.04	1.07 ± 0.06	1.35	0.27 ± 0.07
7556		0.57 ± 0.05	0.82 ± 0.06	1.45 ± 0.16	0.92	0.73 ± 0.19
7608		1.07 ± 0.11	0.97 ± 0.06	0.91 ± 0.11	1.20	0.09 ± 0.16
8229		0.53 ± 0.07	0.70 ± 0.07	1.33 ± 0.21	0.83	0.59 ± 0.26
8000	Leaves	1.28 ± 0.11	1.10 ± 0.07	0.86 ± 0.09	1.31	0.02 ± 0.15
7171	Tablet	0.44 ± 0.03	0.62 ± 0.04	1.40 ± 0.14	0.74	0.66 ± 0.17
7967		0.32 ± 0.02	0.51 ± 0.04	1.59 ± 0.16	0.61	0.89 ± 0.19
7873	Powdered	1.15 ± 0.09	1.66 ± 0.07	1.44 ± 0.13	1.98	0.72 ± 0.15
7893	extract	1.55 ± 0.12	2.32 ± 0.18	1.49 ± 0.16	2.76	0.78 ± 0.19

[a] The potency values in columns 3 and 4 are expressed in terms of the volume of *USP* Standard Preparation (containing 0.0745 Gm. of *USP RDP* per cc.) to which 1 cc. of tincture (or 0.1 Gm. of powder, whole leaf or powdered extract) is equivalent by the method of assay indicated.
[b] Calculated by multiplying data in column 4 by 1.19.
[c] The potency values in columns 6 and 7 are expressed in *USP* Digitalis Units, each of which represents 0.1 Gm. of *USP* Digitalis Reference Standard (1942).

the difference between them being clearly insignificant. A weighted average of all the data obtained on frogs indicates that 0.1 Gm. of the study powder was equivalent to 1.02 ± 0.03 cc. of the *USP* Standard Preparation of Digitalis, each cc. of which represented 0.0745 Gm. of *USP RDP*. It is thus seen that, as originally blended, the study powder met exactly the potency requirements of *USP* XI.

On cats, the study powder exhibited a slightly greater relative effect than the *USP* Standard Preparation, the weighted average being 114% as compared with 102% on frogs. From this determination on cats it is possible to calculate the relation between the *USP RDP*, which was available from the time of its release early in 1936 until recently, and the *USP DRS* which is the new digitalis standard for the United States. The latter should exhibit 74% (1/1.351) of its original potency.[6] Thus, it should establish the potency of *USP* XII digitalis at about 84% (0.74 × 114) of the standard of strength of *USP* XI, a reduction of about 16%. This reduction may be looked upon as the decrement attributable to the change in reference standards and is, of course, a fixed amount. That it is not the total reduction is indicated in Table III, from which it is clear that an additional decrement results from changing the test animal.

Relative Potency of USP XI and USP XII Digitalis.—In view of statements in the literature (16, 17) tending to discredit the use of frogs in assaying digitalis and, finally, the action of the *USP* Committee of Revision in adopting a method based on cats for *USP* XII, it was of great interest to compare the results of assays of the same samples by the *USP* XI and *USP* XII methods. Since the useful-

[6] It is a curious coincidence that had the study powder been adopted in its original potency as a new reference standard to be used with the *USP* XII method, the necessary factor would have been almost exactly 0.745, that currently in force for *USP RDP*. The desirability of dispensing with the somewhat confusing factor is manifest.

ness of such a comparison depends solely upon the reliability of the respective determinations involved, the basic data in Table III were extended to the limits of practicality. In column 2 is indicated the type of the preparations identified by numbers in column 1. Column 3 indicates the *USP* XI potency of each preparation and the average standard error of the estimate. As pointed out in the introduction, the *USP* XI potencies of many of these preparations were such as to indicate regulatory action. The series of tinctures includes both subpotent and overstrength samples, the potency values of which range from 52% to 172% *USP* XI. The variation in potency of the six samples of powdered digitalis or whole leaf is less extreme; three of the powders meet the *USP* XI requirements exactly although only one of the three (7608) is a standardized product. Sample 7556 is the much-studied (16, 17, 18) New York Heart Association Powder No. 7.[7] Sample 8229, listed as a powder, was submitted in capsule form. The two samples of tablet preparations reported show a very low potency by the official method. Assays were made of these on cats since each of the manufacturers involved claimed that his product exhibited full potency upon cats. The degree to which these claims are substantiated is seen in the data. It is of interest to note that sample 7967 was purported to consist of *Digitalis lanata*.

Column 4 lists the potency indicated by each preparation when compared by the *USP* XII technique against the same standard used in the *USP* XI method. A comparison of the data in columns 3 and 4 reveals, as indicated by the ratios listed in column 5, that the activity exhibited by the *USP* XII method (on cats) is generally greater than that shown by the *USP* XI method (on frogs). This species difference in response to the same preparation is such that nine of the eighteen samples examined appear at least 40% stronger on the cat than on the frog. Thus, if the new reference standard were fully as active (on cats) as the reference standard heretofore available and if these preparations met the *USP* XI requirements exactly, their greater relative activity on the cat would make it necessary to dilute them at least 30% to bring them into compliance with the *USP* XII requirements. As a matter of fact, few of them are of *USP* XI strength so that in comparison with the weaker reference standard the overstrength preparations greatly exceed the *USP* XII potency, while those markedly deficient by *USP* XI standards come close to meeting the *USP* XII standards. This is clearly indicated in column 6 which gives the potency of the samples in terms of the *USP* XII standard of strength as calculated from the values in column 4 coupled with the information presented in Table II that 1 cc. of *USP* Standard Preparation (of *USP RDP*) represents the activity of 1.19 (1/0.84) cc. of *USP* XII Tincture of Digitalis.

[7] A quantity of New York Heart Association Digitalis Powder No. 7 was obtained through the kindness of Dr. Harry Gold, Cornell Medical College.

The ratios listed in column 5 vary considerably with no apparent relation to either the absolute level of potency or the type of preparation. However, the highest ratios are observed in the tinctures and generally lower ratios prevail among the powders. Of the latter, samples 7556 and 8229 are grayish brown in color, the chlorophyll having faded noticeably. The ratios of these samples are slightly higher than those of the study powder and *ISD* (1936) which powders, of course, have been carefully preserved so that the chlorophyll is still bright green. Samples 7608 and 8000, which are English digitalis, are also bright green and are unique in showing less activity on the cat than on the frog. The observations on powders are obviously too scanty to support any speculation, but it may prove significant that the highest cat-to-frog potency ratios are seen in the two powders which are definitely "off-color" and subpotent by *USP* XI standards. The two samples of powdered extract examined were also definitely subpotent since 0.1 Gm. should be equivalent to at least 2.75 cc. of *USP* Standard Preparation of Digitalis (19).[8] It is obvious, of course, that these findings are in harmony with the view generally held (20) with respect to deterioration in these products, *i. e.*, that their activity on the frog decreases while that on the cat remains practically unchanged.

All of the tinctures in the series are known to be several months old. They have been stored under refrigeration since their receipt in the laboratory and some of them have been reassayed by the *USP* XI method on several occasions. Sample 7707, for example, was assayed at 12 and 18 months after the initial series of assays. In no case has any evidence of a loss in potency been observed under these conditions. No correlation between the known age of the tinctures and the cat-to-frog ratio of potencies is evident in this limited series. It is regrettable that circumstances preclude detailed studies of the histories of the samples which might lead to helpful generalizations. Unfortunately, also, the variation in the cat-to-frog ratio is so great from one tincture to another that it precludes the possibility of striking an average that might satisfactorily represent the ratio of *USP* XII to *USP* XI standards of potency for even this dosage form of digitalis. Statistical analysis indicates that there is less than one chance in a hundred that the difference in the ratios for these specimens of tincture is due simply to sampling error. This is forcibly shown in the data in column 7 of Table III. These values indicate the extent to which these various specimens of digitalis would exceed the *USP* XII requirements if all were adjusted to 100% *USP* XI. The excess varies from over 1 *USP* Digitalis Unit (as represented by the new reference standard) to practically zero in those cases where the calculated excess is less than twice the standard error.

[8] In order to simplify the discussion of the powdered extracts, which are official National Formulary items, their potencies are expressed throughout in terms of *USP* requirements.

With respect to appraising the change in potency of digitalis preparations resulting from the revision of the standards, the leeway or allowed tolerance in standardizing to *USP* XI requirements must be taken into consideration. Thus, if a *USP* XI Tincture of Digitalis having a potency of 0.8 *USP* Digitalis Unit per cc. were considered acceptable, it is seen that its potency might very well be practically that required for *USP* XII Tincture of Digitalis. If, however, the *USP* XI product exhibited in the frog a potency of 1.32 *USP* Digitalis Units per cc., it would be expected to show, per cc., at least 1.5 and possibly 2.6 Digitalis Units as these are defined under *USP* XII. In order to adjust such a preparation to the *USP* XII standard of strength, a very substantial reduction is necessary.

It will be noted that the range of values in both columns 3 and 5 is such that the strongest is three times as potent as the weakest. A similar spread between the weakest and strongest of a series of commercial digitalis tinctures has been reported by Gold and his co-workers (16, 17, 21) as evidence of variation in the potency manifested in the cat in samples presumed to meet the *USP* XI requirements. While this presumption may be correct, it is hardly likely, in view of the data of Table III, that all of them were of the *same* potency by any method of assay. It is not widely realized that two samples differing as much as 65% in strength might both be considered as meeting the *USP* XI requirements under a liberal interpretation of the footnote on page 398, *USP* XI, which reads "Owing to many variable factors in the standardization of digitalis, evidence of potency in all digitalis assays to within 20 per cent above or 20 per cent below the standard, is acceptable."

In this connection, it is worthy of note that the *USP* XII assay provides for a ±20% tolerance and at the same time defines what is acceptable in the way of evidence that the tolerance has not been exceeded. The potency of the preparation being assayed is expressed in the customary manner as the ratio of the respective average lethal doses of standard and unknown. To insure satisfactory accuracy in this ratio, an upper limit has been set upon the allowable variation in the determination of each average lethal dose, the extent of the variation being calculated as the standard error. If this limit on the individual standard errors of ±5.7% is not exceeded, the standard error of the ratio will not exceed ±10% of the ratio. By virtue of the conventional definition of the standard error (22), it follows then that the chances are only one in twenty that the observed ratio differs from the true ratio by as much as ±20%. As applied to Tincture of Digitalis, *USP* XII, this provision affords a basis for confidence that the actual potency will not differ by more than ±0.2 *USP* Digitalis Unit per cc. from the prescribed potency of 1.0 unit per cc. It should be noted that if the accuracy is just sufficient to meet the *USP* XI requirements, *i. e.*, that the calculated standard errors are each just 5.7%, subsequent adjustment of the potency to either more or less than 1.0 unit per cc. of tincture involves the risk of having the finished product vary more than ±0.2 unit from the requirement (23) that "1 cc. shall be equivalent to 1.0 *USP* Digitalis Unit." A tincture adjusted to a potency of 0.85 unit per cc. on the basis of such an assay would scarcely be an acceptable *USP* XII product since it can be shown that the likelihood that its true potency is 1.0 unit per cc. is very much less than that of its being less than 0.8 unit per cc. or 20% below the requirement. Under *USP* XI, however, such a tincture presumably would have been satisfactory since there is "evidence of potency . . . . to within . . . . 20 per cent below the standard." It is clear, of course, that by achieving greater accuracy in the assay (by using more cats or by adopting means of reducing the variability in their responses) the amount of likely variation may be reduced to materially less than ±0.2 unit. The new requirements will demand more careful standardization of this class of drugs in which the need for accurate dosage is second only to that for insulin.

Comparison of the Potencies of Digitalis Preparations Indicated by the USP XI and 18-Hr. Methods.— For more than three decades the question of the length of time to be allowed for injected digitalis to react in assays on frogs has been a live issue (3, 5, 24). Until recently, attention was focused upon the completeness of absorption attained in the assay rather than upon the influence of variations in the length of the assay upon the relative potency of the preparations being assayed. In view of the lack of conclusive evidence on the latter point, two comparisons of the *USP* (1939–1941) digitalis study (15) were devoted to concurrent assays on frogs of the same material by the two methods most widely used which depend, respectively, upon observations for 1 hour and 18 hours. The conclusion was reached (12) that the two methods gave substantially the same result. In arriving at this conclusion little consideration was paid to the fact that the materials studied were good quality powdered digitalis. Importance of the type of preparation in this respect is brought out in Table IV. This table is similar to Table III in arrangement and content except that a comparison is drawn between the ratios of assays by both the *USP* XI and 18-hr. methods. The data designated as the "first series" were obtained in 1938–1939 before the details of the 18-hr. method had been standardized to the extent accomplished later in connection with the *USP* (1939–1941) digitalis study (15). With few exceptions the assays in this series were carried out by both methods on the same day using frogs from the same batch. Hence, there was no question of a change in either the sample or in the frogs between assays by the two methods. Column 5 of Table IV lists the ratios of the potencies observed by the 18-hr. and the *USP* XI method, respectively. It will be noted that in general this ratio is higher for the samples of leaf than for the samples of tincture; the one sample of fluidextract examined behaved like the tinctures. This pointed

TABLE IV.—COMPARISON OF THE POTENCIES OF DIGITALIS PREPARATIONS INDICATED BY THE *USP* XI AND THE 18-HR. METHODS

Sample No.	Type of Preparation	Potency[a] *USP* XI Method, Cc.	Potency[a] 18-Hr. Method, Cc.	18-Hr./*USP* XI Ratio ± *S. E.*
		FIRST SERIES		
6817	Tincture	0.78 ± 0.14[b]	0.58 ± 0.06	0.74 ± 0.15
6837		0.58 ± 0.04	0.47 ± 0.02[c]	0.81 ± 0.07
6869		0.66 ± 0.08	0.63 ± 0.05	0.95 ± 0.14
6881		1.23 ± 0.24	1.00 ± 0.07[b]	0.81 ± 0.17
6883		1.13 ± 0.18	0.68 ± 0.05	0.60 ± 0.11
6816	Fluidextract	0.49 ± 0.05[c]	0.38 ± 0.02	0.78 ± 0.10
6885	Leaves	0.88 ± 0.18	0.83 ± 0.08	0.94 ± 0.21
6886		1.30 ± 0.30	1.04 ± 0.06	0.80 ± 0.19
6898		1.00 ± 0.20	1.05 ± 0.05	1.05 ± 0.22
6927		0.96 ± 0.15	0.94 ± 0.06	0.98 ± 0.17
6857	Capsules	0.91 ± 0.13	0.73 ± 0.03[c]	0.80 ± 0.12
6855	Tablets	0.61 ± 0.10	0.42 ± 0.06	0.69 ± 0.15
		SECOND SERIES		
7707	Tincture	1.49 ± 0.08	0.99 ± 0.06	0.67 ± 0.05
7935		1.72 ± 0.08	1.30 ± 0.07	0.76 ± 0.06
8088		0.57 ± 0.04	0.45 ± 0.03	0.80 ± 0.07
8228		0.52 ± 0.06	0.48 ± 0.02	0.90 ± 0.11
8429		1.58 ± 0.12	1.50 ± 0.07	0.95 ± 0.08
8435		1.30 ± 0.14	0.90 ± 0.07	0.69 ± 0.09
8474		0.88 ± 0.08	0.54 ± 0.03	0.62 ± 0.06
8502		1.58 ± 0.10	1.17 ± 0.08	0.74 ± 0.07
Study *ISD* (1936)	Powder	1.06 ± 0.07	1.01 ± 0.04	0.95 ± 0.07
		1.06 ± 0.03	1.06 ± 0.02	1.00 ± 0.03
7556		0.57 ± 0.05	0.60 ± 0.03	1.07 ± 0.10
7608		1.07 ± 0.11	1.03 ± 0.05	0.97 ± 0.11
8229		0.53 ± 0.07	0.53 ± 0.03	1.00 ± 0.14
8000	Leaves	1.28 ± 0.11	1.28 ± 0.06	1.00 ± 0.10
7171	Tablet	0.44 ± 0.03	0.55 ± 0.05	1.24 ± 0.14
7967		0.32 ± 0.02	0.31 ± 0.03	0.96 ± 0.10
7873	Powdered	1.15 ± 0.09	0.88 ± 0.08	0.77 ± 0.09
7893	extract	1.55 ± 0.12	1.34 ± 0.06	0.87 ± 0.08

[a] The potency values in columns 3 and 4 are expressed in terms of the volume of *USP* Standard Preparation (containing 0.0745 Gm. of *USP RDP* per cc.) to which 1 cc. of tincture (or 0.1 Gm. of powder, whole leaf or powdered extract) is equivalent by the method of assay indicated. Unless otherwise indicated, the values in the first series represent single assays. In the second series, each value represents two or more assays.

[b] Weighted average of three assays.

[c] Weighted average of two assays.

to a definite trend in favor of the conclusion that the two methods give the same potency for samples of leaves, but not for liquid preparations. Such a conclusion is borne out by a critical statistical examination which indicates that the differences observed in this series would occur by chance only 3 times in 200 if no difference actually existed between leaves and tincture in this respect. A similar discrepancy between the potency of a tincture indicated by the *USP* XI and overnight methods was noted in the A. PH. A. cooperative assays (4).

The second series of assays completed recently on the same samples listed in Table III adds sufficient further evidence to demonstrate conclusively the difference between the results of assays by the two methods on the two popular dosage forms of digitalis. In this series the assays were not carried out strictly simultaneously, but sufficient checks were made to insure that the differences observed could not be due to a change in potency between the time of the USP XI and 18-hr. assays. Further, in this series no less than two assays of each sample were made by each method so that the individual data are much more reliable for establishing the existence of a discrepancy between the two methods than are the data of the first series.

The powders studied in the second series are not representative of the powdered digitalis generally available since two of the six specimens were reference standards. The data, therefore, are obviously too scanty to support generalization, but it is interesting to note that the two powders which show the highest cat-to-frog potency ratios have identical potencies by the *USP* XI and 18-hr. methods. The two samples of powdered extract give ratios characteristic of tinctures, an observation which probably reflects their kinship to tinctures in mode of preparation.

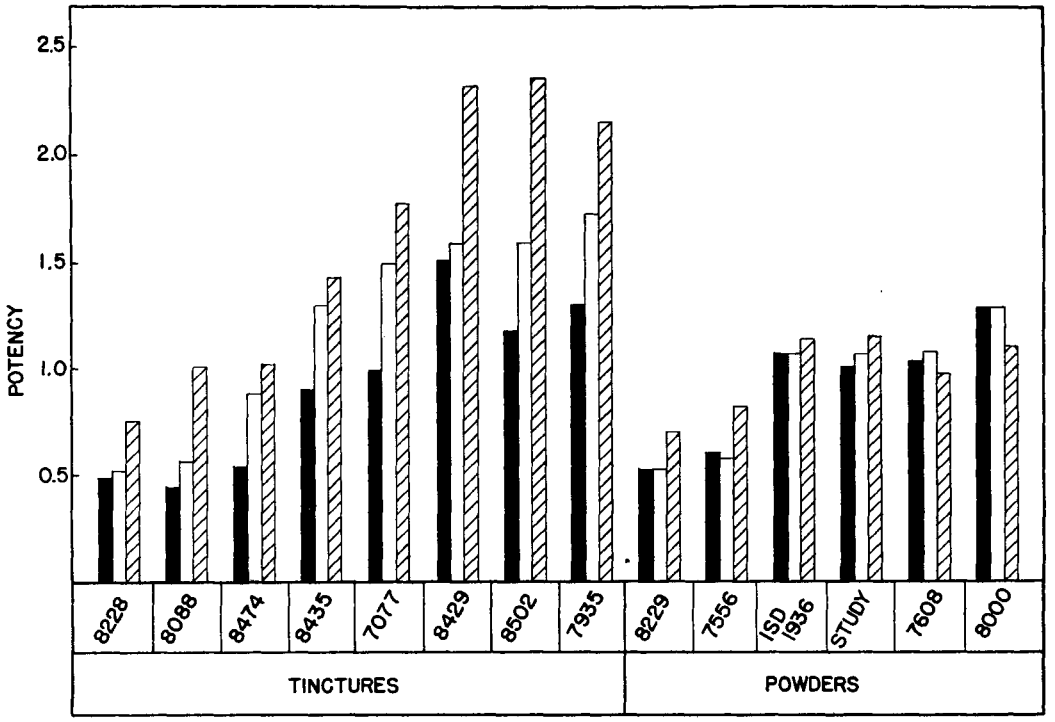

Figure 1.

The vertical bars represent the potency, determined as indicated and expressed on the ordinate as cc. of *USP* Standard Preparation of Digitalis (each cc. representing 0.0745 Gm. of *USP* Reference Digitalis Powder), of the samples identified by number along the bottom of the graph. Solid bars, 18-hr. method; open bars, *USP* XI method; hatched bars, *USP* XII method. Data taken from Tables III and IV.

The USP Digitalis Unit.—Figure 1 brings together the essential data of Tables III and IV in order to provide a graphic comparison of the results of the three methods of assay as applied to tinctures and powders. If it is true that assays on cats of the galenical preparations are more indicative of the true therapeutic efficacy (17), it is obvious that adoption of the 18-hr. method would have materially increased the discrepancy between biological assay and human response in the case of many preparations. Furthermore, it is clear that there is sufficient difference between the results of official assays and those on cats to afford ample basis, quite apart from variations in the *USP* XI potency, for the reports (14) of digitalis overdosage. The physician's difficulties cannot be attributed wholly to the discrepancy between the species of animals, however, since a substantial proportion of the variation in potency from one brand of digitalis to another might be traced to the original standardization.

It may be true that refinements in assay technique and slight variations in potency are of more concern to the biological assayist than to the physician, but it is important to the latter to be able universally to procure digitalis of uniform potency. A complaint was voiced recently (25) against the practice of expressing digitalis potency in units on the grounds that "the dose for each (patient) is best considered in grains or in minims, regardless of the method used for standardization." This statement neglects entirely two facts: (*a*) The native activity of digitalis varies widely from specimen to specimen, and (*b*), as illustrated in Fig. 1, the method of standardization is of paramount importance in determining the activity. It is unquestionably desirable to eliminate the possibility of confusion by limiting the variety of expressions for denoting digitalis activity. However, this depends primarily upon general acceptance by the medical profession of some expression of activity such as the *USP* Digitalis Unit.

As has been pointed out to the medical profession by Nelson (26) the absolute potency of the unit of digitalis activity that will prevail under the *USP* XII standards of strength is less than that fixed by the *USP* XI standards. The reduction is the result of changing not only the reference standard but also the test animal. *USP* XII Digitalis will be adjusted, on the basis of the new assay procedure, to the potency established by the International Standard Digitalis (1936). As emphasized by Nelson, and as may be calculated from Table V, the USP XII preparations will be about 25% stronger than those of *USP* X. This holds true for products which exhibit the same relative activity in both cats and frogs. The data of this paper show that for such preparations the *USP* XII potency will be about 16% weaker than that of *USP* XI. In the case of those preparations which exhibit a substantially greater activity on the cat than on the frog the data

TABLE V.—COMPILED DATA ON THE RELATIVE POTENCIES OF *USP* X DIGITALIS, *USP* XI DIGITALIS AND INTERNATIONAL DIGITALIS STANDARDS (1926) AND (1936)

Comparison	Method	Potency Ratio	Observer
USP X Digitalis/*ISD* (1926)	1-Hr. frog	0.77	Edmunds, Lovell and Braden (30)
		0.81	Rowe (31)
		0.83	Rowe and Pfeifle (5)
USP XI Digitalis/*USP* X Digitalis	1-Hr. frog	1.55	A. PH. A. cooperative study (4)
		1.52	Rowe and Pfeifle (5)
		1.40	Rowe (6)
		1.53	Ichniowski and Thompson (7)
		1.60	Swoap and Pabst (8)
USP RDP/ISD (1926)	1-Hr. frog	1.34	Edmunds, Moyer and Shaw (3)
		1.35	Chen (reported by Edmunds, *et al.* (3))
		1.81	Rowe and Pfeifle (5)
		2.07 ± 0.08	Braun and Miller (this paper)
	Overnight frog	1.91	Edmunds, Moyer and Shaw (3)
		1.91	Rowe and Pfeifle (5)
USP RDP/ISD (1936)	1-Hr. frog	1.13	Edmunds, Moyer and Shaw (3)
		1.18	Rowe and Pfeifle (5)
		1.41	Rowe (6)
		1.27 ± 0.04	Braun and Miller (this paper)
	4-Hr. frog	1.19	Edmunds, Moyer and Shaw (3)
		1.38 ± 0.10	Braun and Miller (this paper)
	Overnight frog	1.36	Edmunds, Moyer and Shaw (3)
		1.18	Rowe and Pfeifle (5)
		1.48	Rowe (6)
		1.22	Chapman (32)
		1.27 ± 0.02	Braun and Miller (this paper)
	Cat	1.16	Edmunds, Moyer and Shaw (3)
		1.19 ± 0.06	Braun and Miller (this paper)

show that in meeting the *USP* XII requirements the potency may not be much more than half that required under *USP* XI. This is not to be interpreted as evidence of fluctation in the *USP* Digitalis Unit, which was fixed under *USP* XI as the activity on frogs of 0.0745 Gm. of *USP RDP* and under *USP* XII (as a result of a coincidence) as the activity on cats of 0.1 Gm. of *USP DRS*. The variability is resident in the somewhat unpredictable characteristics of the digitalis preparations being standardized.

The question will arise in the minds of many as to why the time-honored expression "cat unit" should be abandoned in favor of the *USP* Digitalis Unit. The reasons have been set forth quite adequately by Burn (27) who has marshaled the arguments against the use of units defined in terms of physiological effects. While there will be limitations on even the *USP* unit in determining the human dosage of the purified glycosides (28), the way is open for avoiding potency differences between laboratories due to variations in determinations of the fatal dose of digitalis to cats as well as similar differences due to variations within the confines of a single laboratory. As demonstrated recently in carefully controlled experiments (29) the latter can assume quite significant proportions. Until a chemical method of assay is developed for digitalis, it will be the responsibility of the biological assayist to insure uniformity in potency from one lot to another of each digitalis preparation. The correct dosage in *USP* Digitalis Units of each type of preparation can be determined only by clinical investigation and experience.

Compiled Data on the Relative Potencies of USP X Digitalis, USP XI Digitalis and International Digitalis Standards (1926) and (1936).—Table V lists a number of values compiled from the literature indicating the relationship between the *USP* X and *USP* XI standards of strength for digitalis and certain comparisons of these with the two international standards (1926) and (1936). The investigative effort represented by the data compiled in Table V is truly monumental and yet by no means does it indicate the full amount of research conducted on the assay of digitalis in the past decade. Unfortunately, there is a great deal of variation in the amount of experimental work supporting the various values and no attempt has been made to determine their inherent reliability. This might be done by calculating the respective standard errors as has been done for the data in this paper. In the light of present information on the assay of digitalis, however, it is known that many of the ratios in Table V represent so few experimental observations that they can be little more than rough approximations of the true relationships. In spite of this, there is a striking agreement throughout the series with one notable exception, namely, the comparison of the *USP RDP* against *ISD* (1926). It is a regrettable circumstance that this comparison of such critical importance should have proved to be the only controversial one. Had almost any of the other comparisons listed in Table V been made the basis of establishing the potency of *USP* XI Digitalis, a great deal of subsequent research and journal space could have been directed into more profitable channels. No explanation of the discrepancy in

these determinations is apparent. Of the possible explanations which have been explored in this laboratory, such as the influence of alcoholic content of the test dilutions and possible heterogeneity of the ampuls of *ISD* (1926), none has given any basis for the variation observed. In view of recent developments the question becomes one solely of academic importance with little promise of a satisfactory solution.

SUMMARY

1. By the *USP* XI assay, the *USP* Reference Digitalis Powder which was released in 1936 was found to be 2.07 ± 0.08 times as potent as International Standard Digitalis (1926).

2. By the *USP* XI assay and assays on frogs involving longer periods of observation than one hour, the *USP* Reference Digitalis Powder was found to be 1.27 ± 0.02 times as potent as International Standard Digitalis (1936) and in assays on cats by the *USP* XII procedure, it was found to be 1.19 ± 0.06 times as potent as International Standard Digitalis (1936).

3. In assays on frogs by the *USP* XI and 18-hr. methods, the *USP* study powder was found to meet almost exactly the potency requirements for *USP* XI Powdered Digitalis, while in assays on cats the potency observed was 114% of that required for powdered digitalis of *USP* XI strength. Since the *USP* Digitalis Reference Standard (1942) is a dilution of the *USP* study powder, these data indicate that insofar as can be determined by the *USP* XII method of assay, the *USP* XII standard of potency will equal that established by International Standard Digitalis (1936) and will be about 16% weaker than the *USP* XI standard of potency.

4. The *USP* XI and *USP* XII potencies of several samples of the various pharmaceutical forms of digitalis were determined. The data show that complying with the revised standards of strength will result in a substantial reduction in the potency to which the American physician has become accustomed during the past six years. The indication is that the change may be greater in tinctures than in powdered digitalis.

5. Assays on frogs by the *USP* XI and 18-hr. methods gave substantially identical results with digitalis leaves and powdered digitalis; with tinctures, the potency indicated by the 18-hr. method is significantly lower than that shown by the *USP* XI assay.

6. A review is presented of the published data on the relative potencies of *USP* X Digitalis, *USP* XI Digitalis and International Digitalis Standards (1926) and (1936).

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Comparison of Digoxin, Digilanids *A*, *B* and *C*, and Deacetyldigilanids *A* and *B**†

By K. K. Chen, Chester C. Hargreaves and E. Brown Robbins

After *Digitalis lanata* was shown to have more physiologic units quantitatively than *Digitalis purpurea* (1), chemical investigations were carried out in a few laboratories. The most pertinent question to settle is whether or not the active constituents of one species are identical with those of the other. Smith (2, 3) succeeded in isolating digoxin and gitoxin, and studied the constitution of the aglycone of digoxin, digoxigenin (4). Mannich and his associates (5) announced the separation of lanata-glycosides I, II, III and IV, and later made several revisions (6, 7, 8) in the light of other workers' findings. Much systematic information has been furnished by Stoll, Kreis and Hofmann (9, 10, 11, 12, 13, 14, 15, 16). According to their results, the leaves of *Digitalis lanata* contain three polysides, digilanids *A*, *B* and *C*. The latter under suitable conditions of hydrolysis give rise to glycosides of smaller molecular sizes as follows:

Digilanid *A* → Deacetyldigilanid *A* + CH_3COOH
↓
Digitoxin + $C_6H_{12}O_6$ (glucose)
↓
Digitoxigenin + 3 $C_6H_{12}O_4$ (digitoxose)

Digilanid *B* → Deacetyldigilanid *B* + CH_3COOH
↓
Gitoxin + $C_6H_{12}O_6$
↓
Gitoxigenin + 3 $C_6H_{12}O_4$

Digilanid *C* → Deacetyldigilanid *C* + CH_3COOH
↓
Digoxin + $C_6H_{12}O_6$
↓
Digoxigenin + 3 $C_6H_{12}O_4$

* Received June 8, 1942, from the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.

†The authors are indebted to Dr. Henry M. Lee, and Messrs. Robert C. Anderson, Frank A. Steldt, William T. Winchester and Harold Worth.

Interestingly, Stoll and Kreis (17) have shown that deacetyldigilanids *A* and *B* also occur in the leaves of *Digitalis purpurea*, although, heretofore, digitoxin and gitoxin have been long considered as the main crystalline principles of this well-known plant (18). The simpler glycosides of digilanid *C*, namely, deacetyldigilanid *C* and digoxin, are apparently absent in the leaves of *D. purpurea*.

The potencies of the above compounds have been repeatedly determined. The results as published by different workers are compiled in Table I. The discrepancies are undoubtedly due to many factors such as the strain of animals, the atmospheric temperature and variations in experimental procedures. The anesthetic in cats exerts a great influence on the outcome of tests, as is well illustrated by Kaplan and Visscher (23). Digoxin has been assayed on guinea pigs, rabbits, pigeons and dogs (23, 26, 28, 29). Quantitative evaluation of digoxin and digilanid *C* can also be made on the embryonic chick heart (30). Moe and Visscher (22), by means of the heart-lung preparation, presented evidence that digilanid *C* was especially effective in increasing the cardiac efficiency in relatively small doses, as compared with digilanids *A* and *B*. This has been questioned by Cattell and Gold (31).

The present study deals with the evaluation of digoxin, digilanids *A*, *B* and *C*, and deacetyldigilanids *A* and *B*, in cats and frogs under as nearly comparable conditions as possible. The results so obtained furnish secure grounds for correct assignment of the order of activity. Besides, they reliably demonstrate the significance of the