of the determination of b greatly simplifies the calculations. If a standard or characteristic curve, such as that employed in this work, can be used in the assay of digitalis (4) it is to be preferred because of the simplicity in performing an assay and calculating results.

CONCLUSION

1. Seven samples of digitalis, powder and leaf, are compared by the one-hour and eighteen-hour methods. Good agreement was observed.

2. The U. S. P. Reference powder possesses 152.5% of the potency of the 1936 International Standard when the latter is considered to possess an activity of one International Unit per 0.08 Gm.

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Progress Report on the U. S. P. (1939-1940) Digitalis Assay Study*

By Lloyd C. Miller

As a result of a conference on the Assay of Digitalis held in connection with the Atlanta Meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION, a collaborative study of the assay of digitalis using frogs has been in progress during the past year. The program is a U. S. P. project made possible by the coöperation of the participating laboratories. As a consequence of my offer to calculate and compile the results of the study, it now falls to my lot to serve as a narrator in presenting a summary of the progress to date.

* Report prepared at the request of Dr. C. W. Chapman, Chairman, A. PH. A. Committee on Physiological Testing, with the approval of Dr. E. E. Nelson, Chairman, Committee on U. S. P. (1939–1940) Digitalis Assay Study.

EXPERIMENTAL

The conference decided that the U. S. P. reference digitalis powder was unsuitable for use in this collaborative program and requested Professor Cook to collect immediately samples of both domestic and imported digitalis leaves of good quality to be composited as study material for this project. As a result 110 lb. were collected from various sources and upon the very prudent suggestion of Dr. C. W. Chapman, who had had considerable experience in compositing the present Canadian digitalis standard powder, each of these samples of leaves was subjected to bioassay. The findings in these assays have just been reported by Dr. Chapman.

It was the consensus of the Atlanta conference that probably the most important issue was the question of the alleged superiority of the so-called "over-night" frog method over the official U. S. P. one-hour method. Of interest also was the most suitable means of preparing an extract of a standard powder prior to assay, *i. e.*, a hot exhaustive extraction as compared with a cold maceration procedure. Then and in subsequent correspondence the cat method was considered, but it was decided finally to seek to perfect one method at a time.

It was felt that the details of preparing a macerate could be specified rigidly enough to insure a preparation sufficiently uniform to enable a valid comparison between the one-hour and eighteen-hour methods. It is to be noted that the term "eighteen-hour" was applied to designate the so-called "over-night" method, a decision which was indicative of the resolve of the conference to make a fresh start. It was freely expressed at the conference that great care should be exercised toward devising a workable and well-defined plan for the assays. It may now be said that this desire was thoroughly justified.

THE FIRST COMPARISON

It was decided that the program should be made up of a series of what may be termed major comparisons, each of which would bear directly on a well-defined and vital phase of the problem. Thus the first comparison was designed to determine (1) the relative merits of the one- and eighteen-hour methods when conducted under as nearly identical conditions as practicable and (2) the practicability of diluting a standard powder with exhausted marc. Toward this end two samples of digitalis powder, Samples 1 and 2, were submitted to nearly 20 laboratories. One of these powders was a dilution of the other, the diluent being thoroughly exhausted marc (the inactivity of which was proved biologically) in a proportion known to none of the collaborators. Each collaborator was requested to determine the relative potency of these two samples by both the one- and eighteen-hour methods under conditions quite rigidly specified.

The data for the one-hour and eighteen-hour methods were compared on the basis of four criteria which were proposed in a paper presented to the

Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION at Atlanta and published in the October 1939 number of the JOURNAL. As a result of this objective comparison, certain unmistakable advantages appeared to be attached to the eighteen-hour method. One of these is indicated in the following table which gives the results submitted on the first comparison.

Results of First Comparison—U. S. P. Digitalis Study

Values indicate potency of Sample 2 in terms of Sample 1 and are weighted means ± the standard errors obtained by each aboratory. Figures in parentheses indicate number of assays if other than two.

Laboratory Number	One-Hour Method, Per Cent Potency	Eighteen-Hour Method, Per Cent Potency
1221	122 ± 8 (3)	126 ± 6 (3)
1223	130 ± 13	148 ± 9 (1)
1226	134 ± 10	152 ± 12
1227	130 ± 18	126 ± 12 (1)
1228	148 ± 11	130 ± 4
1229	127 ± 8 (4)	144 ± 6 (4)
1235	144 ± 12 (1)	142 ± 6 (1)
1237	172 ± 61 (1)	
1238	120 ± 7	143 ± 6
1241	165 ± 25 (1)	163 ± 17 (1)
Weighted mean—all laboratories	130.9 ± 3.4	138.4 ± 2.3

Actual relationship, 140.0 per cent

THE SECOND COMPARISON

It was decided that the second comparison should be planned to supply data on the reproducibility of a hot extraction procedure as compared with cold extraction (maceration). It became clear at once that if assays were to be made upon extracts prepared by two different methods from a single powder and only these two variables were studied, there would be no way of associating any variability observed with one method or the other. That is, while we assumed in the first comparison that a perfectly uniform liquid preparation could be prepared by all collaboratores by the maceration technique, this assumption was not valid in the present case. Consequently, it was decided to provide a 4-oz. sample of tincture as a standard against which the macerate and the hot extract could be compared. Thus a tincture and a sample of the powder from which it was prepared were then submitted to each collaborator.

Wherever comparable, the results submitted thus far on the Second Comparison bear out the data submitted earlier. The principal features of the data as a whole are (1) the remarkable uniformity in the potencies of macerates prepared from the same digitalis powder in different laboratories and (2) the very low error of the eighteen-hour method.

Russia has issued a postage stamp in honor of Dimitri Ivanovich Mendeléjeff for his outstanding work as a scientist.

Barbituric Acid Derivatives*

Relationship between Hemolytic Action and Chemical Structure

By Henry M. Lee and Edward E. Swanson

In a previous communication (1), it was observed that there is an obvious relationship between the pharmacological action and the chemical structure of certain barbituric acid derivatives. In the primary or secondary alkyl-substituted compounds, with an increase in the number of C-atoms in the alkyl group, both the minimal anesthetic dose (M. A. D.) and the minimal lethal dose (M. L. D.) grow relatively smaller, but when the alkyl radical is longer than 5 C-atoms, the amount required to produce anesthesia or death in rats again increases. As the alkyl chain lengthens, the therapeutic index or ratio between M. L. D. and M. A. D., appears to be gradually greater. In general, the duration of action shows similar features; that is, it is shorter when the alkyl group becomes lengthened. In normal alkyl-substituted derivatives, the critical compound is the one that possesses 6 C-atoms in one of the 5–5 positions; in the secondary alkyl-substituted derivatives, the critical compound is the one having 7 C-atoms in one of the 5–5 positions, beyond which the duration of action begins to increase. More recently, it has been reported that the substitution of a methyl, ethyl or allyl radical on the nitrogen (nitrogen alkyl-substituted barbituric acid derivatives) (2), or the substitution of an alkyl, methallyl (2-methyl allyl) (3), or crotyl (3-methyl allyl) (4) on one of the 5–5 positions, or a sulfur atom (5) in place of the oxygen on the 2 C-atom obviously reduces the duration of action. This shorter duration of action is independent of the amount of drug administered.

EXPERIMENTAL

The present investigation deals with the study of the relationship between the hemolytic action on the red blood cells and the chemical structure of certain barbituric acid derivatives synthesized by Shonle and his associates of this laboratory, with the general formula:

* From the Lilly Research Laboratories, Indianapolis, Indiana.