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

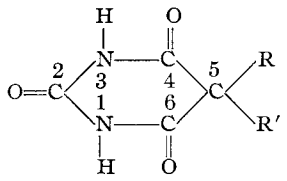
By Henry M. Lee and Edward E. Swanson

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.



wherein R denotes an alkyl radical (normal or secondary with 2 to 8 C-atoms) and R′ an ethyl radical. A number of thiobarbituric acid derivatives were also studied, wherein R′ indicates crotyl (3-methyl allyl) and R an alkyl radical (normal or secondary with 2 to 5 C-atoms).

The apparatus used in testing the hemolytic action was a modification of that described by Ponder (6). Small test-tubes containing the hemolytic system were suspended in a glass water-bath maintained at a constant temperature (37.7° C.). Each test-tube contained 0.4 cc. of a suspension of thrice-washed sheep blood cells, representing 0.05 cc. of whole sheep blood plus 1.6 cc. of a 0.05*M* barbituric acid sodium salt solution and 0.154*M* sodium chloride. The hydrogen-ion concentration of each suspension was adjusted to p_H 10.

As shown in Chart 1, certain barbituric acid derivatives caused the blood to hemolyze. The hemolytic time was dependent on the number of C-atoms in the substituted alkyl radical. In the primary alkyl substituted compounds, diethyl barbituric acid (2 C-atoms in the alkyl radical) produced hemolysis in 288 minutes. As the alkyl radical increased in length from 2 to 7 C-atoms, there was a rapid decrease in the hemolytic time from 288 minutes to less than 1 minute. In the secondary alkyl-substituted barbituric acid derivatives, with the exception of the secondary propyl (3 C-atoms), the increase in the number of C-atoms on the alkyl radical from 4 to 8 lowered the hemolytic time from 260 minutes to 1 minute. The primary and secondary alkyl-substituted derivatives of the alkyl crotyl thiobarbituric acid derivatives showed an even greater decrease in the hemolytic time. As the number of C-atoms increased in the alkyl radical from 2 to 5, the hemolytic time decreased from 348 minutes to 6 minutes. Thus, there appears to be a definite relationship between the hemolytic action on the red blood cells and the chemical structure of certain barbituric acid derivatives.

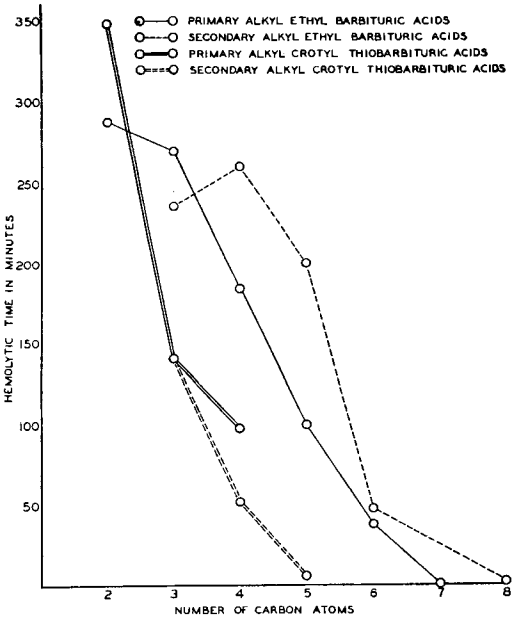


Chart 1.—Hemolytic Time of Barbituric Acid Derivatives.

The authors are indebted to Dr. K. K. Chen, Director of Pharmacologic Research, for his valuable suggestions and criticisms and to Dr. W. W. Davis for his technical assistance.

SUMMARY

1. A series of primary and secondary alkyl-substituted barbituric acid and thiobarbituric acid derivatives were studied for their hemolytic action on the sheep's blood.

2. The hemolytic time decreases as the number of C-atoms increases in the substituted alkyl radical.

3. There is obviously a relationship between the hemolytic action and the chemical structure of certain barbituric acid derivatives.

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The following scientists have been honored by France by portrayal on postage stamps: Marcelin Berthelot, pioneer in organic synthesis; Claude Bernard, physiologist; Louis Pasteur, Pierre Curie and Marie Curie, discoverers of polonium and radium; Leon Calmette, research worker in tuberculosis.