12083

Effect *in Vitro* of Curare and Beta-Erythroidin Hydrochloride on Choline Esterase of Human Blood Serum. I.*

MEYER M. HARRIS AND RUTH S. HARRIS.

From the Department of Internal Medicine, New York State Psychiatric Institute and Hospital, New York City.

Because of the similarity of some preparations of curare and beta-erythroidin in producing paralysis of the skeletal musculature both have been used in man for the relief of some non-voluntary muscular rigidities and spasms due to involvement of the nervous system[1, 2] and more recently for the prevention of fractures resulting from metrazol convulsant therapy in mental patients.[3, 4, 5]

Some investigators[4] have found that it required about 10 to 20 times the dose of beta-erythroidin hydrochloride to that of some curare preparations to produce similar states of paralysis in man for the purpose of preventing fractures in metrazol convulsant therapy.

Owing to these differences in potency in producing paralysis, and the rôle of acetylcholine[6, 7] in the mechanism of muscular contraction, it was planned to investigate the effect of these substances on the choline esterase activity of human serum.[8]

Procedure. The choline esterase activity of the serum was determined by the continuous titration method as described by Hall and Lucas,[9] using acetylcholine bromide or acetylcholine iodide as the substrate. The amount of acetic acid liberated in 20 minutes by the choline esterase activity of 0.5 ml of serum, expressed in terms of

* A preparation of curare under the trade mark of "Intocostrin" was supplied for experimental purposes by E. R. Squibb & Sons, New York City. A sample of beta-erythroidin hydrochloride used in this study was obtained from Dr. D. Ewen Cameron. This drug is a purified isomer of erythroidin-hydrochloride prepared by Merck & Company of Rahway, N.J., and made available by them for clinical investigation.

1 West, R., *Proc. Roy. Soc. Med.*, 1935, **28**, 565.

2 Burman, M. S., *Arch. Neur. and Psychiat.*, 1939, **41**, 307.

3 Bennet, A. E., *J. Am. Med. Assn.*, 1940, **114**, 322.

4 Rosen, G. R., Cameron, D. E., and Ziegler, J. B., *Psychiat. Quart.*, 1940, **14**, 3.

5 Gray, R. W., Spradling, F. L., and Fechner, A. H., *Psychiat. Quart.*, 1941, **15**, 159.

6 Brown, G. L., *Physiol. Rev.*, 1937, **17**, 485.

7 Eccles, J. C., *Physiol. Rev.*, 1937, **17**, 538.

8 Ammon, R., and Voss, G., *Pflüg. Arch. f. d. ges. Physiol.*, 1934-5, **235**, 393.

9 Hall, G. E., and Lucas, C. C., *J. Pharm. and Exp. Therap.*, 1937, **59**, 34.

the milliliters of N/100 sodium hydroxide, required for its neutralization, was used as the measure of the enzymatic activity.

Corrections were made as recommended by Hall and Lucas. Burette readings taken every 5 minutes over the 20-minute period of enzymatic hydrolysis and plotted against time gave a straight line as indicated by the above authors. The stock solution of curare ("Intocostrin") was equivalent in physiological activity to 10 mg of crude curare per ml. The milligrams of curare indicated in the tables were calculated on this basis. Various dilutions of the curare were made with boiled distilled water. The beta-erythroidin was obtained in dry crystalline form and was dissolved in boiled, distilled water in the various amounts used in the experiments. The total volume containing all the constituents was always kept equal to that described in the method of Hall and Lucas. Blanks containing all the constituents, except the serum were always run at the same time and were used to correct for spontaneous decomposition of the acetylcholine.

In one experiment the effect of the simultaneous addition of curare and beta-erythroidin was investigated.

Observations. The results of typical experiments are recorded in Tables I, II, and III.

Discussion. It will be seen from the tables that very minute amounts of curare produced a marked inhibition of choline esterase activity. The degree of inhibition, up to about 60%, was approxi-

TABLE I.
Effect of Curare on Choline Esterase Activity of Human Serum.

Curare added mg	Choline esterase activity, of 0.5 ml serum, units*	Decreased choline esterase activity, %
0.0	3.11	0
0.016	0.46	85.2
0.004	1.19	61.7
0.002	2.01	35.4
0.001	2.56	17.7

TABLE II.
Effect of Beta-Erythroidin Hydrochloride upon Choline Esterase Activity of Serum.

β-Erythroidin HCl added mg	Choline esterase activity of 0.5 cc serum, units*	Decreased choline esterase activity, %
0	3.9	0
0.16	3.9	0
0.84	3.9	0
50.0	3.9	0

TABLE III.
Effect of Adding Curare and Beta-Erythroidin to Same Mixture upon Choline Esterase Activity of the Serum.

Curare added mg	β-Erythroidin HCl added, mg	Choline esterase activity of 0.5 cc serum, units*	Decreased Choline esterase activity, %
0.0	0.0	3.75	0
0.004	0.0	1.45	61.3
0.0	0.16	3.80	0
0.004	0.16	1.54	58.9

*Units are equivalent to the numbers of ml of N/100 NaOH required to neutralize the acetic acid liberated from the acetylcholine. The figures have been corrected as indicated in the text.

mately proportional to the amount of curare added. The maximum inhibition of esterase activity obtained was 85%. (Table I.)

In contrast, the addition of beta-erythroidin hydrochloride, in amount up to 3000 times that of the largest amount of curare, had no effect upon the choline esterase activity. (Table II.) The combined addition of curare and beta-erythroidin hydrochloride produced the same inhibition as that of an equal amount of curare alone. (Table III.)

The important rôle attributed to acetylcholine and choline esterase in the physiology of muscle and the nervous system[6, 7] should lead one to expect that 2 substances which differ so markedly in their effect upon this enzyme system would also differ markedly in physiological action. The production of paralysis of the muscular system by both substances is probably only a gross similarity of action. This view finds substantiation in a number of physiological experiments with curare and curare-like substances and their antidotes in warm and cold blooded animals. The fact that eserin has been used as an antidote for curare and the explanation offered by some that eserin produces its effect by the inhibition of the choline esterase would appear to be rather questionable in view of the marked inhibition produced by curare alone. That other mechanisms of action may be involved seems likely from the findings of other investigators.[10-14] Further knowledge regarding the differences in the mode of action of these substances may throw additional light upon neurophysiological

[10] Ing, H. R., *Physiol. Rev.*, 1936, **16**, 527.

[11] Jacobsohn, D., and Kahlson, G., *Skand. Arch. f. Physiol.*, 1938, **79**, 26.

[12] Roepke, M. H., *J. Pharm. and Exp. Therap.*, 1937, **59**, 264.

[13] Pusitz, M. E., Lattimore, J. L., Gold, A., and Ebendorf, H., *J. Kansas Med. Soc.*, 1940, **41**, 374.

[14] Rosenbluth, A., Lindsley, D. B., and Morison, R. S., *Am. J. Physiol.*, 1936, **115**, 53.

mechanisms and also help to explain certain differences in the therapeutic action of these substances. Such information should be of value in the clinical use of these substances.

Since the curare used is not a pure chemical substance but represents a mixture of various alkaloids,[15] it will be important to study some of the pure alkaloids such as curarine and curine and other curare constituents regarding their effect upon choline esterase in order to determine which substance or substances in the curare produce the marked inhibition of the choline esterase activity and whether these parallel the paralytic action or toxicity of the curare. Plans are under way for an investigation of this phase of the problem.

The finding, as previously indicated, that within a certain range the degree of inhibition of choline esterase activity is proportional to the amount of curare added to the experimental mixture, may afford an *in vitro* method for assaying curare preparations as far as the constituent inhibiting choline esterase is concerned. Such determinations may help to explain some of the differences obtained clinically with different curare preparations. This requires further study.

Summary and Conclusions. 1. *In vitro* experiments have been carried out regarding the effect of curare and beta-erythroidin hydrochloride upon the activity of choline esterase of human serum. 2. Very small amounts of curare had a marked inhibiting effect upon the choline esterase activity of human serum. 3. The degree of inhibition of enzyme activity up to 60% was approximately proportional to the amount of curare added. The possible application of this finding for assaying curare preparations is indicated. 4. Beta-erythroidin hydrochloride in amounts 3000 times as large as that of the largest amount of curare had no effect upon the choline esterase activity of the serum. 5. The possible importance of the marked difference in effect between curare and beta-erythroidin hydrochloride upon choline esterase activity is discussed. 6. Plans for further investigation are indicated.

[15] Henry, T. A., *The Plant Alkaloids*, P. Blakiston's Sons, Philadelphia, Pa., 1939.