12084

Effect in Man of Curare and Metrazol Administration on Choline Esterase Activity of Blood Serum. II.*†

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In a previous publication[1] we reported that small amounts of curare *in vitro* inhibit the choline esterase activity of human serum, the extent of the inhibition varying with the amount of curare added. In this paper we wish to report upon the effect of the intravenous injection of curare‡ in man upon the choline esterase activity of the serum and the duration of this effect.§ It was hoped that such determinations would yield information as to the relation of the induced muscular paralysis to the choline esterase activity and also afford a means of approximating the amount of the drug present in the blood stream at various periods after its administration. Information regarding the latter might be of value in studies on the therapeutic use of the drug in various neuromuscular conditions.

A review of the literature revealed no studies of this nature carried out in man. Felger and Kowarzyk[2] reported that the intravenous injection of curare in the rabbit had, contrary to their

* We wish to acknowledge the assistance of Dr. Bernard L. Pacella and Dr. William A. Horwitz in the administration of the medication.

† Squibb & Sons supplied their curare preparation known under the trade name of "Intocostrin" for these studies. Each milliliter of this curare solution was equivalent to the physiological activity of 10 mg of the crude drug.

1 Harris, M. M., and Harris, R. S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 619.

‡ The blood was obtained from a group of mental patients who were receiving curare as a preventive measure against fractures during metrazol convulsant therapy and also from a neurological patient, W.M., who was suffering from coarse tremors of the upper and lower extremities.

§ One must keep in mind the fact that curare is not a pure chemical substance but a mixture and it is possible that some of the substances present may be physiologically active without affecting the enzymatic activity. As indicated in the previous publication it is planned to investigate this phase of the subject. Unpublished experiments carried out by us indicate that the incubation of either serum or defibrinated blood at 37.5°C for 1 hr with curare does not alter the inhibiting power of the curare on the choline esterase activity of the serum. The red blood cells of the defibrinated blood do not appear to take up any measurable amount of the material in the curare which inhibits choline esterase activity.

2 Felger, J., and Kowarzyk, H., *Compt. Rend. Soc. de Biol.*, 1938, **127**, 1149.

expectations, an inhibiting effect upon the choline esterase activity of the blood. They stated, however, that the curare first produced blocking of the neuromuscular junction without affecting the choline esterase activity of the blood and only subsequently was the latter affected. No detailed experimental data were given in their paper.

Procedure. Samples of blood were withdrawn from the cubital vein of the arm both before and at various intervals after medication. Compression was applied only for a short interval to facilitate venipuncture. Care was taken to avoid hemolysis. The blood was allowed to clot and the serum was removed in about one-half to one hour after the venipuncture. The curare solution was injected intravenously at the rate of one milliliter per 30 to 45 seconds until the desired degree of muscular paralysis was produced. This usually required from 7 to 10 ml of the curare solution. In 2 cases no other medication was given and blood samples were taken at several intervals up to 30 minutes after the injection as shown in Table I. In 4 cases metrazol was injected intravenously following the injection of curare in order to produce convulsions. Blood samples were collected at various intervals up to 6 hours from the time of completion of the injection of curare as indicated in Table II.

The choline esterase activity of each sample of serum was determined as described in a previous publication[1] by the continuous titration method as adapted by Hall and Lucas.[3] The number of milliliters of N/100 NaOH required to neutralize the acetic acid

TABLE I.
Effect of Intravenous Injection of Curare on Choline Esterase Activity of Serum.

Patient	Wt lbs	Time after curare injection*	Choline esterase activity of 0.5 ml of serum, units	Decreased choline esterase activity, %	Remarks
(S.S.)	124	Before	3.18	0	8 ml curare sol. inj. in
(F)		6 min.	1.13	64.5	6 min.
		18 "	1.44	54.7	
		30 "	1.55	51.3	
(W.M.)		Before	2.90	0	10 ml curare sol. inj.
(M)		Immed. after	1.25	57.0	in 7 min.
		15 min.	1.68	42.7	
		30 "	2.11	27.3	

*Time indicated is that which elapsed between the completion of the injection of curare and the obtaining of the particular blood specimen.
M—Male. F—Female.

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

TABLE II.
Effect of Intravenous Injection of Curare and Metrazol on Choline Esterase Activity of the Serum.

Patient	Wt lbs	Time after curare injection*	Choline esterase activity of 0.5 ml of serum, units	Decreased choline esterase activity, %	Remarks
(A.F.) (M)	135	Before 11 min. 5 hr. 8 min.	3.66 1.68 3.01	0 54.1 17.8	9.6 ml curare sol. inj. in 7 min. followed immediately by 7 ml of metrazol. Convulsion produced.
(V.T.) (M)	156	Before 24 min. 5 hr. 25 min.	4.63 2.22 3.35	0 52.0 27.6	9.5 ml curare inj. in 7 min. followed immediately by 6 ml of metrazol and 8 ml 5 min. later. Convulsion produced by second dose of metrazol.
(V.D.) (F)	108	Before Immediately after curare 6hr	3.23 0.95† 2.84	0 71.0† 12.1	7 ml curare inj. in 4½ min. followed by 6 ml of metrazol. Convulsion produced.
(B.S.) (F)	120	Before 6 min. 6 hr.	4.40 1.28† 2.71	0 68.3† 39.4	8 ml curare in 7 min. followed by 5 ml of metrazol. Convulsion produced.

*The time indicated is that which elapsed between the completion of the injection of curare and the obtaining of the blood specimen.

†These second blood specimens were obtained just before the metrazol was injected in cases (V.D.) and (B.S.). The second specimen was taken after metrazol had also been injected in cases (A.F.) and (V.T.).

liberated from acetylcholine in 20 minutes at 37.5°C at pH 8.0 by the choline esterase present in 0.5 ml of serum and corrected according to the recommendations of Hall and Lucas,[3] is reported as so many units of enzyme activity.

Observations. It will be seen from Table I and patients (V.D.) and (B.S.) in Table II, that immediately or within a few minutes after the injection of curare there is a marked decrease in choline esterase activity ranging from 57% to 71% of the initial values. In patients (A.F.) and (V.T.) (Table II) the second specimens of blood were taken after metrazol also had been injected and the patients had had a convulsion. The intervals which had elapsed between the completion of the injection of curare and the taking of the second blood samples were 11 minutes and 24 minutes and the decrease in choline esterase 54.1% and 52.0% respectively. In the subsequent intervals the choline esterase activity in each case tended to rise toward its initial value at variable rates. These rates of re-

covery were independent of the size of the dose of curare or the weight of the patient. (Tables I and II.) In the cases which received metrazol in addition to the curare (Table II) the rate of recovery from inhibition of choline esterase activity appeared to bear no relation to the dose of metrazol which was administered.

It is of interest to note that, even after 5 to 6 hours the choline esterase activity was still from 12.1% to 39.4% below the respective initial values.

It may be of interest to point out at this time that 30 minutes after the injection of curare the patients showed no evidence of paresis although some patients stated that their muscles felt tired.

Discussion. It would be beyond the scope and purpose of this paper to enter upon any extensive discussion of the various phases of physiology and pharmacology pertaining to the rôle of acetylcholine and choline esterase and the action of various antidotes to curare. Some references to this phase of the subject have been made in the previous paper and also have been considered to some extent in a number of reviews.[1, 3, 4] It may be of interest to mention, however, in view of the marked inhibition of choline esterase activity that Pusitz[5] and his coworkers indicate that the injection of mecholyl tended to aggravate rather than relieve curare intoxication.

Our findings in the human are in agreement with the findings of Felger and Kowarzyk[2] in the rabbit that the injection of curare produces an inhibition of the choline esterase activity of the blood. However, our finding that marked inhibition of choline esterase activity is still present when muscular paralysis has disappeared would appear to differ from their findings in the rabbit.

The degree of inhibition of choline esterase activity produced by the intravenous injection of curare is of the same order of magnitude as that obtained in the *in vitro* experiments reported in our previous publication.[1] It, therefore, should be possible from the amount of inhibition of choline esterase activity to estimate the amount of the inhibiting portion of the curare present in the blood stream at any period after injection by carrying out preliminary *in vitro* experiments with serum or blood from the experimental subject as described in our previous publication.[1] It also should afford a tool for the investigation of clinical, physiological and pharmacological problems pertaining to curare.

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

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

Summary and Conclusions. 1. The intravenous injection of curare produces a marked inhibition of the choline esterase of human serum. 2. The inhibition of this esterase activity gradually diminishes at a variable rate in different patients, but is still present 5 to 6 hours after the injection of curare. 3. The degree of inhibition of the esterase activity was similar to that obtained in *in vitro* experiments previously reported. 4. A method is indicated for estimating the amount of the choline esterase inhibiting constituent of curare in the blood by the use of its inhibiting effect on esterase activity. Some of the possible applications of the method are pointed out. 5. The relation between the effect of curare on choline esterase and the production of muscular paralysis is discussed.

12085 P

Strychnine Tetanus and the Mechanism of Neuronal Synchronization.*

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Oscillographic study indicates that spinal strychnine tetanus is the most perfect expression of the synergy of elementary electrical activities in a mass of nerve cells. Strychnine tetanus was studied in the curarized cat under light dial anesthesia (10 to 20 mg per kg) with the special purpose of analyzing the mechanism of neuronal synchronization. The following facts were demonstrated:

1. Tetanic waves, recorded from any segmental level of the spinal cord by means of an active electrode thrust into the grey matter of the anterior horn, have regularity of form, amplitude and rhythm of an alternating current. Their frequency varies from 30 to 10 per second, their voltage from 500 to 100 microvolts. Usually there is an inverse relationship between voltage and frequency. This relationship becomes most apparent when the frequency of the tetanic waves has been increased as the result of an intravenous injection of nicotine.

2. With each tetanic wave there is a corresponding strong discharge of centrifugal action potentials in the anterior roots. This

* This work was completed in May, 1940, but because of war conditions its publication has been delayed until now.