14662

Effect *in vitro* of Curare Alkaloids and Crude Curare Preparations on "True" and Pseudo-Cholinesterase Activity.

MEYER M. HARRIS AND RUTH S. HARRIS.
From the Department of Internal Medicine, N. Y. State Psychiatric Institute and Hospital, New York City.

In previous publications,[1] we reported that intocostrin, which is prepared from an Indian trade curare, strongly inhibited the choline esterase activity of human serum. It was pointed out that this preparation of curare was not a pure chemical substance and that it would be desirable to investigate some of the pure curare alkaloids. Since it has been found that crude curare preparations vary in their lissive action and also in toxicity further information regarding the constituents of the crude preparations should be of value in clinical and physiological studies.

Recently McIntyre and King[2] reported that d-tubocurarine chloride had no inhibiting effect on the choline esterase activity of serum although it produced muscular paralysis. We were fortunate in having placed at our disposal for this study the following pure curare alkaloids; d-chondocurine, d-isochondodendrine, d-isochondodendrine dimethyl-ether, alkaloid 4, and d-tubocurarine chloride which had been isolated from chondodendron tomentosum.*

It had been shown by a number of investigators[4, 5] that the red blood cells of human blood contain a cholinesterase (called "True cholinesterase") which differs from that present in serum (called pseudo-cholinesterase). The effect of the alkaloids upon both enzymes therefore was investigated using acetylcholine bromide and mecholyl (acetyl-beta-methyl-choline) as substrates. (Note: The "pseudo-esterase of serum will not hydrolyze mecholyl whereas the "true" esterase hydrolyzes both acetylcholine bromide and mecholyl; the latter substrate is therefore specific for the "true" esterase.[4] Benzoylcholine, which is specific for the pseudo-esterase, was not available for study).

Procedure. The alkaloid solutions were prepared as follows: d-tubocurarine chloride was dissolved in distilled water; d-isochondodendrine and alkaloid 4 were dissolved in a few milliliters of N/100 HCl and d-chondocurine in N/100 H_2SO_4 and made up to volume with distilled water so that one milliliter was equivalent to approximately one milligram of alkaloid.

The enzymatic hydrolysis was determined by the continuous electrometric titration method[5] at pH 7.35 and 37°C; 0.2 ml of serum or 0.2 ml of washed hemolyzed red blood cells of human blood prepared according to Mendel,[4] was used in a total volume of 20 ml. The solution contained approximately 0.7% of sodium chloride in order to minimize the error due to varying concentrations of sodium. A 0.2% solution of acetylcholine iodide or bromide or mecholyl was used as substrate. N/100 NaOH was used to neutralize the acetic acid liberated by the enzymatic hydrolyses. Burette readings taken every 5

* Dr. O. Wintersteiner and Dr. J. D. Dutcher of the Squibb Institute for Medical Research recently isolated these compounds and kindly supplied them for this study. Chemical and physiological data regarding these compounds are given in their paper.[3] They have also supplied us with various other crude curare preparations which have been included in this study as indicated in the table. According to a communication from Dr. Wintersteiner, only *d*-tubocurarine, which is a quaternary base, possesses the typical curare (lissive) action. The other alkaloids mentioned are tertiary bases and were found ineffective in the test for lissive action.

1 Harris, M. M., and Harris, R. S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 619; *Ibid.*, **46**, 623.

2 McIntyre, A. R., and King, R. E., *Science*, 1943, **97**, 69.

3 Wintersteiner, O., and Dutcher, J. D., *Science*, 1943, **97**, 467.

4 Mendel, B., and Rudney H., *Biochem. J.*, 1943, **37**, 59; *Ibid.*, **37**, 473.

5 Alles, G. A., and Hawes, R. C., *J. Biol. Chem.*, 1940, **133**, 375.

TABLE I.*

Effect of Various Curare Alkaloids on the Cholinesterase Activity of Serum and Hemolyzed Red Blood Cells at pH 7.35 and 37°C.

Alkaloid		Cholinesterase activity					
		Acetylcholine bromide substrate				Mecholyl substrate	
		Serum		Hemolyzed R.B.C.		Hemolyzed R.B.C.	
Type	Amount mg	Units	% inhibition	Units	% inhibition	Units	% inhibition
No. 1	0	1.18	0	1.86	0	1.30	0
d-chondocurine	0.5	.88	25	1.25	33	.94	27
	1.0	.76	36	.99	47	.81	37
No. 2	0	1.27	0	1.95	0	1.34	0
d-isochondodendrine	.25	1.13	11	1.93	1	1.29	4
	.50	1.05	17	—	—	1.23	8
No. 3	0	1.11	0	2.17	0	1.61	0
Alkaloid 4	.53	.45	59	1.13	48	.77	52
No. 4	0	1.03	0	2.16	0	1.45	0
d-Tubocurarine chloride	1.04	.89	14	—	—	—	—
	2.0	.69	33	1.82	16	1.11	24
No. 5	0	1.15	0	2.23	0	1.57	0
d-isochondodendrine dimethylether	1.0	.66	43	1.93	13	1.24	20
No. 6	0	1.11	0	2.17	0	1.67	0
Intocostrin (Lot No. 69904) (unauthenticated curare)	0.016	.13	88	2.11	3	1.49	11
No. 7	0	.99	0	2.09	0	1.36	0
Curare 4901-1″ (unauthenticated)	0.01	.06	94	2.02	3.5	1.40	--1.7
No. 8	0	1.30	0	2.27	0	1.56	0
Curare (Gill) (Lot No. 25064)	.25	.91	30	2.10	4	1.47	6
No. 9	0	1.26	0	2.34	0	1.58	0
Serpa A	.25	.83	34	2.19	6	1.53	3
No. 10	0	1.19	0	2.76	0	1.79	0
Curare (Gill), ether extract†	.05	.66	44	2.59	6	1.75	2
No. 11	0	1.27	0	2.76	0	1.91	0
Curare (Gill), chloroform extract†	.1	.87	31	2.59	6	1.76	8

* This table contains only representative data of many experiments carried out with the various alkaloids.

† The chloroform and ether extracts from curare (Gill) contain, in varying proportions, the tertiary alkaloids listed in the table and perhaps others which have not been isolated.

minutes for 20 minutes and plotted against time gave a straight line. The amount of acetic acid liberated in 20 minutes by the choline esterase activity of 0.2 ml of serum, or hemolyzed red cell solution expressed in terms of the milliliters of N/100 sodium hydroxide required for its neutralization, was used as the measure of the enzymatic activity and one milliliter is called one unit of esterase activity.

The effect on the choline esterase activity of the addition of varying amounts of the different alkaloids was determined. Corrections were made for the spontaneous hydrolysis of the acetylcholine derivatives. Preliminary tests showed that all of the alkaloids remained in solution at pH 7.35. However, at a more alkaline pH some of the pure alkaloids tended to precipitate.

Observations. (a) *Effect of Alkaloids on "Pseudo-cholinesterase" of Serum.* It will be seen from the table that d-tubocurarine

chloride had the least inhibiting effect on the enzymatic hydrolysis by pseudo-cholinesterase of any of the pure or crude alkaloid preparations tested. In comparison d-isochondodendrine was approximately twice as effective, d-chondocurine three times, and alkaloid 4 about 8 to 10 times as effective as d-tubocurarine chloride. Intocostrin (No. 6) one of the preparations used in the studies previously reported[1] which was prepared from unauthenticated curare was about 1500 times as effective while intocostrin prepared from curare (Gill) had an inhibiting effect slightly less than that of d-isochondodendrine. (1 mg gave 21% inhibition, data not given in the table).

Preparation (No. 7), some of the unauthenticated curare which originally had been used to prepare intocostrin, supplied to us by Dr. Wintersteiner, also had a similar marked inhibiting effect. All of the other curare preparations (No. 8, 9, 10, 11) had an exceedingly weaker inhibiting effect.

(b) *Effect of Alkaloids on "True Cholinesterase."* The unauthenticated curare (No. 6 and 7) in the amounts which almost completely inhibited the pseudo-esterase had little or no effect upon the true esterase. The other curare preparations (No. 8, 9, 10, 11) also had only a slight inhibiting effect on the latter enzyme in contrast to the effect upon the pseudo-esterase. This is in contrast to the effect produced by the pure alkaloids (especially No. 1 and No. 3) where both esterases are equally affected.

Discussion. It will be noted that, contrary to the report of McIntyre and King,[2] d-tubocurarine chloride has some inhibiting effect on choline esterase activity of both the true and "pseudo-cholinesterase" *in vitro*. However, this is not demonstrable in the low concentration of the alkaloid. It is possible that a difference in methods from those used by the above investigators may account for the difference between their findings and ours.

While the tertiary alkaloids which were tested do not possess the lissive action which is manifested by the quaternary alkaloid, d-tubocurarine chloride, they have a more pronounced inhibiting effect upon the cholinesterases. However, it would not be safe at present to conclude that the lissive action is therefore totally unrelated to the effect on the cholinesterases.[6,7]

The findings with the different curare preparations is of considerable interest. The marked inhibition of the pseudo esterase by the unauthenticated curare with little or no effect upon the true esterase is further evidence for the existence of 2 enzymes. It will be of interest to determine the nature of the substance producing this specific effect upon the pseudo-esterase. It would offer a means of studying the effects of inhibiting this esterase while the true esterase remains active. Information regarding the physiological role of the pseudo esterase may thus be obtained.

The inhibiting effect of the curare specimens (No. 8, 9, 10, and 11) upon the pseudo esterase, although considerably less than that of the unauthenticated curare, cannot be accounted for by the pure alkaloids investigated and points to the presence of some other inhibiting substance. Whether this is due to a small amount of the same substance as that in the unauthenticated curare cannot be stated at present. The marked inhibition reported in our previous studies is now shown to have been due to the effect upon the pseudo-esterase of some substance in the crude curare preparations.

Summary. (1) Five pure curare alkaloids and several curare preparations were investigated for the effect on the activity of "true" and pseudo-cholinesterase. (2) A very active substance which specifically inhibits the pseudo-esterase has been found in some curare preparations. (3) The value of this substance for special studies regarding the role of the pseudo-esterase is discussed. (4) The difference in the inhibiting action of the pure alkaloids and the crude curare preparation is demonstrated.

[6] Roepke, M. H., *J. Pharm. and Exp. Therap.*, 1936, **59**, 264.

[7] Mendel, B., and Hawkins, R. D., *J. Neurophysiol.*, 1943, **6**, 431.