17243. Acetylcholine-Like Action of a Product Formed by an Acetylating Enzyme System Derived from Brain.*

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In 1943 Nachmansohn and Machado extracted from brain an enzyme, choline acetylase, which forms acetylcholine in cell-free solution using the energy of adenosine triphosphate.[1] The acetylation of choline is a complex reaction requiring for full activity in addition to the enzyme system and ATP, the substrates choline and acetate, a co-enzyme, K^+, Mg^{++}, Ca^{++} ions and cysteine.[2,3] The choline ester formed enzymatically, assumed to be exclusively acetylcholine, was determined by bioassay with the rectus muscle of the frog. In spite of the intense interest of physiologists in acetylcholine, no adequate chemical methods were available and investigators studying the occurrence or formation of the ester used by necessity bioassays, which are of questionable specificity.

Recently high yields of choline ester were obtained by a purified and concentrated enzyme solution which was prepared from acetone dried powder of rabbit brain by fractional ammonium sulphate precipitation. These high yields made possible the determination of choline ester by a chemical method introduced by Hestrin.[4] The method is based on the reaction of O-acyl groups with hydroxylamine in alkaline medium and may therefore be used for the determination of acetylcholine in presence of choline and acetate.

When tested by bioassay the values of acetylcholine formed showed a striking discrepancy with the figures obtained by chemical determination. Less than half of the total biological activity of the enzymatically formed product could be accounted for by the chemical method. Consequently, the greater part of the effect obtained in the bioassay must be attributed to a product which appears to have the same biological action as acetylcholine but may be distinguished from the latter chemically.[3] The formation of a biologically active product is observed in absence of added choline, whereas under these conditions no acetylcholine formation is observed.

It appeared necessary to ascertain whether the product has acetylcholine-like action in other biological systems besides that observed on the frog rectus. Experiments will be described in this paper in which the action of the enzymatically formed product, henceforth referred to as e.f. product, has been tested on the frog heart and on the blood pressure of cats.

The tests of the effect of the product on blood pressure were performed on cats. The animals were anesthetized with Dial (0.07 - 0.08 g/kilo), injected intraperitoneally. Arterial blood pressure was measured in the carotid artery with a mercury manometer and recorded on smoked paper. The compounds tested were injected into the external jugular vein in a volume of 1 ml.

For the experiments on the frog's heart, bullfrogs (*Rana catesbeiana*) were used. The hearts were removed and perfused with frog's Ringer solution according to the method of Straub, as modified by Krayer.[5] Ventricular contractions were recorded with an isotonic lever attached to the apex of the heart.

The enzymatically formed product used in

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1 Nachmansohn, D., and Machado, A. L., *Neurophysiol.*, 1943, **6**, 397.

2 Nachmansohn, D., and Weiss, M. S., *J. Biol. Chem.*, 1948, **172**, 677.

3 Nachmansohn, D., Hestrin, S., and Voripaieff, H., *J. Biol. Chem.*, 1949, **180**, 249.

4 Hestrin, S., *J. Biol. Chem.*, in press.

5 Krayer, O., Linstead, R. P., and Todd, D., *J. Pharm. and Exp. Therap.*, 1943, **77**, 113.

TABLE I.

Depressor Effect of the Enzymatically Formed Product on Arterial Blood Pressure of Cat. Bioassay with frog's rectus indicated a content of 30-40 μg ACh equivalents per ml of sample. a and b indicate assays done at different periods of the same experiment.

	Acetylcholine		e. f. product		
Exp. No.	μg ACh	Decrease of blood pressure, %	Dilution of product	Decrease of blood pressure, %	μg ACh equivalents/ml
1	0.25	28	400	26	100
2	0.2	22	400	23	80
	0.5	35	200	35	100
3 a.	0.25	25	400	23	100
b.	0.5	30	200	28	100
4	0.25	13	400	12	100
	0.5	35	200	29	<100
5 a.	0.2	17	400	14	80
b.	0.5	26	200	23	100
6 a.	0.2	20	400	24	80
	0.25	36			
b.	0.5	43	200	35	<100
7	0.2	45	400	42	80

all the experiments was obtained by incubation of the choline acetylating system in a reaction mixture as described recently.[3] No choline was added to the mixture. The blank solutions used as controls were aliquot parts of the reaction mixture which formed on incubation the product. The controls were used at O time (without incubation), after the enzyme had been inactivated by short boiling.

Results. A. Action of product on arterial blood pressure of cats. Table I summarizes the results obtained in this series of experiments. In all the experiments performed, the e.f. product exerted on arterial blood pressure a depressor action that closely resembles the fall in blood pressure induced by acetylcholine.

The samples used in Experiments 1 to 3

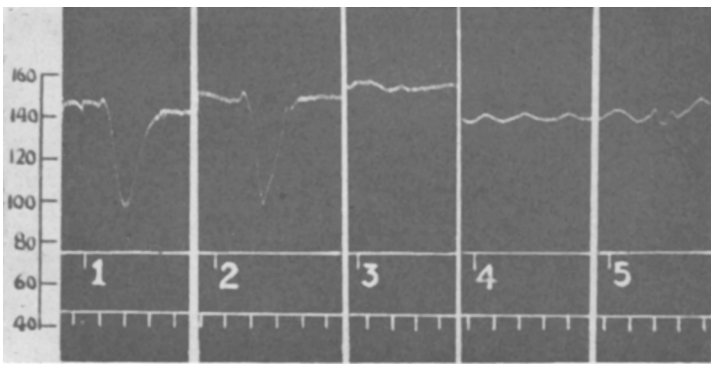

FIG. 1.

Effect of e.f. Product upon Arterial Blood Pressure. Cat. Upper line, arterial blood pressure; second line, injection marks; third line, time in 10 sec. Scale on the left: mm of Hg. 1. ACh 0.5 μg. 2. e.f. product diluted 1:200. 3. Control diluted 1:50. Between 3 and 4, atropine sulfate (0.4 mg). 4 and 5, as in 1 and 2.

TABLE II.
Effect of the Enzymatically Formed Product on the Amplitude of the Frog Heart Contraction. Bioassay with frog rectus indicated a content of 30-40 μg ACh equivalents per ml of sample.

	Acetylcholine		e. f. product		
Exp. No.	μg ACh	Decrease of amplitude, %	Dilution of product	Decrease of amplitude, %	μg ACh equivalents/ml
1	0.01	60	4000	66	50–60
	0.02	78			
2	0.025	58	1600	48	>40
3	0.05	22			
	0.1	40	1600	20	80
4	0.05	32	2000	32	
			1600	40	100
5	0.05	35	1600	37	80
		40	800	50	
6	0.1	50	800	43	
	0.3	64	400	50	40–60
			200	68	
7	0.025	60	1600	68	50–60
	0.05	82			

were derived from the product of the same experiment, 4 to 7 from another experiment. Compared with bioassay on frog rectus the values obtained were higher, although in the same order of magnitude. The figures in all experiments indicate strikingly good constancy. It may be noted that experiments 6 and 7 were carried out about 2 weeks later. During this interval, however, no loss of activity seems to have occurred.

It was also observed that the depressor action of the e.f. product is regularly suppressed by atropine in doses sufficient to abolish the depressor action of acetylcholine.

Fig. 1 shows a typical experiment. It can be observed that a dose of e.f. product (at 1) and of acetylcholine (at 2) which elicit a marked fall of blood pressure before atropine, becomes completely ineffective once the animal has been atropinized (5 and 6). The injection of a control solution at 3 does not modify the level of blood pressure.

B. Action of product on the frog's heart. The results obtained are summarized in Table II. In 7 experiments carried out the e.f. product given in adequate concentrations produced depression of the activity of the heart, similar to that elicited by the administration of acetylcholine. The depressor action affected primarily the amplitude of the cardiac contractions, the frequency being only slightly decreased if at all.

The figures of Table II show that the concentration of the e.f. product in the samples used is fairly constant, as estimated by their action on the frog's heart. It fluctuates between 40 and 80 μg of acetylcholine equivalents per ml in one sample (1 and 2), between 40 and 100 μg per ml in the second sample used (3 to 7).

The cardiodepressor action of the e.f. product was regularly blocked by atropine in concentrations sufficiently high to abolish the cardiodepressor effects of acetylcholine.

The record in Fig. 2 is typical of this series. At 1 and 2, e.f. product and at 3 acetylcholine, produce a marked decrease in the amplitude of cardiac contractions. At 4, a control solution is used; no effect on cardiac activity is obtained. The heart is then atropinized and at 5 and 6, e.f. product and acetylcholine are applied again as in 2 and 3; the

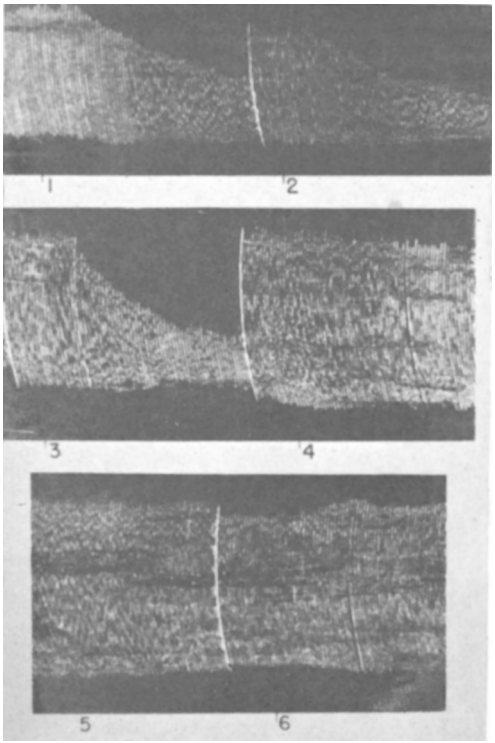

FIG. 2.
Effect of e.f. Product upon the Ventricular Contractions of the Frog Heart.

1. e.f. product diluted 1:100. 2. e.f. product diluted 1:50. 3. ACh 0.3 μg. 4. Control diluted 1:50. 4 and 5. Atropine sulfate 1 mg/liter. 5. ACh as in 3. 6. e.f. product as in 2.

effect on the heart is now abolished.

Summary. The pharmacological properties of an enzymatically formed product described recently by Nachmansohn, Hestrin and Voripaieff have been tested. This product, obtained in the choline acetylating system derived from brain extracts, is distinctly different from acetylcholine but has an acetylcholine-like action in the bioassay with frog rectus.

In the present paper the acetylcholine-like action of the product has been confirmed and extended. The product decreases the arterial blood pressure of cats and the amplitude of the isolated frog heart in the same way as acetylcholine. Atropine regularly suppresses both actions.

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17244. New Substrates for Cholinesterases.

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In previous reports it has been shown that the cholinesterases (ChE) of snake venoms[1-3] and of human erythrocytes[3,4] catalyze the hydrolysis of noncholine esters such as ethyl chloroacetate and β-chloroethyl acetate. Since both enzymes are typical members of the e-group of cholinesterases,[5] the former assumption that the e-ChE was unable to attack noncholine ester, therefore, should be discarded. Thus, a wide field for the search for new substrates of the e-ChE has been opened.

Methods. Esterase activity was measured by the usual manometric procedure.* In each case tests were also run without sub-

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1 Zeller, E. A., *Helvet. physiol. pharmacol. acta*, 1948, **6**, C36.

2 Zeller, E. A., and Utz, D. C., *Helvet. chim. acta*, 1949, **32**, 338.

3 Zeller, E. A., Fleisher, G. A., and McNaughton, R. A., *Fed. Proc.*, 1949, **8**, 268.

4 McNaughton, R. A., and Zeller, E. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 165.

5 Zeller, E. A., *Helvet. chim. acta*, 1949, **32**, 94.

* Twelfth communication: Zeller, E. A., *Helvet. chim. acta*, 1949, **32**, 484.