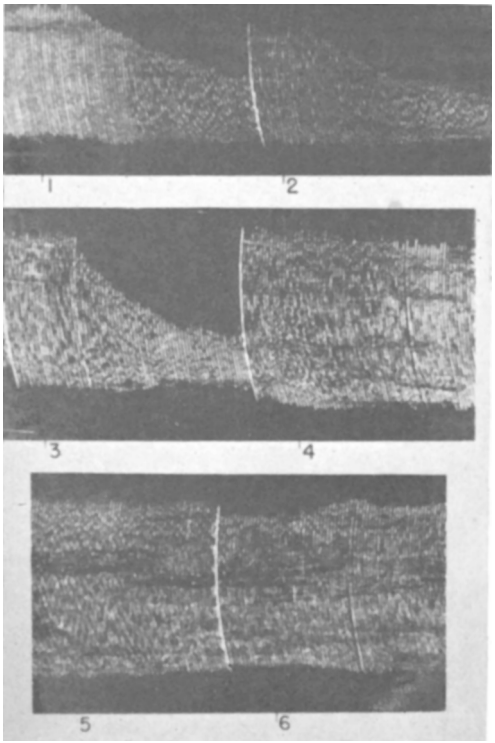

FIG. 2.
Effect of e.f. Product upon the Ventricular Contractions of the Frog Heart.

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.

We wish to thank Dr. David Nachmansohn for suggesting this research and for his interest and encouragement, and to Dr. S. R. Korey for providing the e.f. product.

Received July 1, 1949. P.S.E.B.M., 1949, **71**.

17244. New Substrates for Cholinesterases.

E. ALBERT ZELLER,† GERARD A. FLEISHER, ROBERT A. MCNAUGHTON AND JOHN S. SCHWEPPE. (Introduced by Charles F. Code.)

From the Mayo Foundation, Rochester, Minn.

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-

† On leave from the University of Basel, Switzerland.

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.

TABLE I.
Enzymatic Hydrolysis of a Mixture of Acetylcholine and Ethoxyethanol Acetate.*

Substrates	Conc. of substrate	Q	$Q_{ACh} + Q_{EtA}$	$Q_{ACh + EtA}$
ACh	.005 M	5,730	7,490	5,600
EtA	.05 M	1,760		
ACh	.005 M	5,730	8,630	5,080
EtA	.20 M	2,900		
ACh	.01 M	4,700	6,460	4,190
EtA	.05 M	1,760		
ACh	.01 M	4,700	7,600	4,840
EtA	.20 M	2,900		

* 0.1 ml of purified erythrocyte ChE, 1.0 ml total volume.

strates and without enzymes, and all results recorded in this paper were obtained by subtracting the corresponding blanks. They are expressed in terms of Q, which gives the number of microliters of carbon dioxide evolved from bicarbonate-Ringer per hour per milligram of venom or per milliliter of the enzyme solution.

Sources of e-ChE were dry snake venoms (from the family of the Colubridae) and human erythrocytes. The latter were washed 4 times with saline solution and then hemolyzed with 5 volumes of distilled water. The hemolyzed cells were centrifuged at 8,800 g until a reddish precipitate separated. The supernatant was discarded and the precipitate was resuspended in water; this procedure was repeated 6 to 8 times until the supernatant was clear. The orange-red precipitate contained 50 to 60% of the original ChE activity of the erythrocytes. This preparation will be referred to as "purified erythrocyte ChE."

In the venoms[5] and in the purified erythrocyte ChE preparation no indication of the presence of an "ali"-esterase appeared. The activity toward methyl butyrate, which is found in hemolyzed erythrocytes, completely disappeared after purification. Thus, when both preparations attack an ester, it is highly probable that the e-type of ChE is responsible for this reaction. The sensitivity toward eserine and caffeine[6] and, when possible competition experiments with acetylcholine were used to check this conclusion.

Results. A. Ethoxyethanol Acetate (EtA). This glycol derivative is easily attacked by the venoms of *Naia melanoleuca* (Q_{EtA} = 1,720) and *Elaps frontalis* (Q_{EtA} = 170), using a substrate concentration of 0.1 M. Eserine (0.2mM) gives complete inhibition. Purified erythrocyte ChE also catalyzes the hydrolysis of ethoxyethanol acetate. In all cases acetylcholine competed with the noncholine ester, as is seen from the results listed in Table I. The same substance is also hydrolyzed by human serum, a rich source of s-ChE ("pseudo" or "unspecific" ChE). Using a purified erythrocyte ChE and diluted human serum of a similar activity toward acetylcholine (Q_{ACh}), the first enzyme preparation catalyzes the ethoxyethanol acetate much more rapidly than the s-type, in spite of the fact that the affinity between the substrate and the two enzymes is of the same order (compare with Michaelis constant, Fig. 1). Since human serum contains some "ali"-esterase activity[7] which probably is partly responsible for the hydrolysis of the noncholine ester, the ratio might be even more in favor of the e-type.

B. Desoxycorticosterone Acetate (DOCA). The extremely low solubility of this substance in water prevented any rapid reaction. The substance was added to the reaction vessels in dry form. Venom from *Naia melanoleuca* (Q_{ACh} = 30,800)[5] produced an easily readable hydrolysis (Q_{DOCA} = 8.7). Eserine (0.2 mM) inhibited this reaction 90%. Even purified erythrocyte ChE caused a considerable hy-

[6] Zeller, E. A., and Bissegger, Alfred, *Helvet. chim. acta*, 1943, **26**, 1619.

[7] Adams, D. H., and Whittaker, V. P., *Biochem. J.*, 1949, **44**, 62.

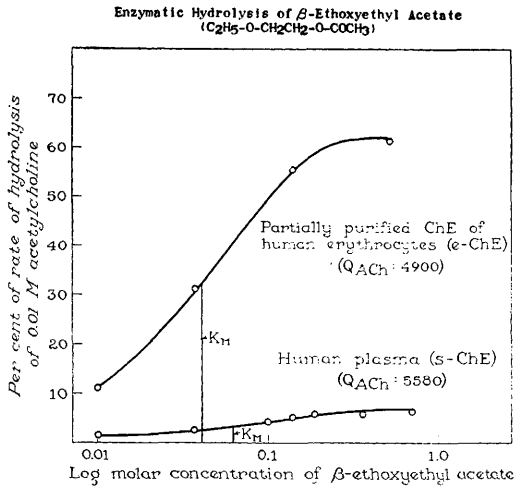

FIG. 1.

Hydrolysis of ethoxyethyl acetate in the presence of human serum and purified erythrocyte cholinesterase; 0.1 ml serum, 0.1 ml purified erythrocyte ChE, total volume 1.0 ml. Reaction velocity expressed in percentage of the reaction velocity caused by 0.01 M acetylcholine. K_M represents the substrate concentration which produced half of the maximal reaction velocity.

drolysis of this ester ($Q_{DOCA} = 48$). On account of the enormous difference between Q_{ACh} and Q_{DOCA} no competitive experiments with a mixture of acetylcholine and DOCA were attempted.

C. Ketopropanol Acetate (KPA). This substance, which, like DOCA, is a keto-alcohol ester, was attacked by the ester of the venom of *Bungarus fasciatus* ($Q_{ACh} = 25{,}000$).[5] At 0.2 M concentration the reaction velocity was $Q_{KPA} = 705$. Eserine (0.2 mM) and caffeine (0.1 M) depressed the rate 100 and 64 per cent respectively.

D. Phenyl Acetates. Phenyl acetate (PA) and various derivatives are hydrolyzed rapidly by preparations of e-ChE and s-ChE (Table II). Like another noncholine ester, ethyl chloroacetate,[4] the hydrolysis of phenyl acetate is inhibited by eserine only in the presence of e-ChE, but not in the presence of human serum. The very rapid hydrolysis of p-nitrophenyl acetate by various snake venoms, by purified erythrocytes ChE and by human serum, and the inhibition of this reaction by the addition of eserine and acetylcholine can easily be followed by the development of the yellow color, due to the liberation of free p-nitrophenol.‡

Comment. Besides acetylcholine the acetates of very different hydroxyl derivatives are hydrolyzed by e-cholinesterase ("true" or "specific" ChE). Acetyl derivatives of unsubstituted aliphatic alcohols and halogenated alcohols have been mentioned previously,[1,3,4] while in the present paper representatives of keto-alcohols, poly-alcohols, steroid alcohols and phenols have been added to the list of the noncholine substrates of e-ChE. On the other hand, much less variation is possible on the part of the acyl group, as has been pointed out by the preliminary results obtained by Bovet Nitti,[8] by Adams and Whittaker[9] and by our laboratory. Of the unsubstituted aliphatic acids acetic acid gives the best results, while acids with more than 3 carbon atoms are not as well hydrolyzed. This behavior separates the e-ChE from the s-ChE ("pseudo"-ChE) and from the "ali"-esterase, because these latter enzymes are active toward esters of acids with more than 3 carbon atoms. At the present time the e-ChE can be considered to be an *acetyl*esterase (e-acetylesterase) rather than a *cholin*esterase.

The ability of the ChE of human erythrocytes to split acetylsalicylic acid and desoxycorticosterone acetate may play a role in the liberation of the corresponding hydroxyl derivatives after the therapeutic application of these and similar substances.

The fact that under certain physiologic and pathologic conditions the ChE activity of a given tissue changed has previously led to far-reaching hypotheses concerning the presence of acetylcholine. Later, similar assumptions were restricted to the e-ChE. In the light of our present results even this latter conclusion can no longer he held. Unless new evidence is produced, no enzymologic clues are available at present to show that acetyl-

‡ The use of phenyl acetate and derivatives thereof for the colorimetric determination of ChE will be discussed elsewhere.

8 Bovet Nitti, F., *Experientia*, 1947, **3**, 283.

9 Adams, D. H., and Whittaker, V. P., *Biochem. J.*, 1948, **43**, xiv.

TABLE II.
Hydrolysis of Phenyl Acetates in the Presence of Various Cholinesterase Preparations.

Source of ChE	Type	Q_{ACh}	Substrate	Q	Q substrate + 0.2 mM eserine
Venom of *Bungarus fasciatus*†	e	25,000	Phenyl acetate, .002 M	16,200	0
Hemolyzed erythrocytes‡	e	11,750	" " .002 M	7,250	0
Plasma*	s	6,500	" " .002 M	117,000	102,000
Purified erythrocyte ChE§	e	12,000	Acetylsalicylic acid, .06 M	160	0
Venom of *Naia melanoleuca*‖	e	30,800	" " .08 M	650	0
Purified erythrocyte ChE¶	e	5,550	*p*-Nitrophenyl acetate, .003 M	2,680	

Total volume in all cases = 1 ml.
* 0.002 ml of heparinized human plasma.
† 0.02 mg of dry venom.
‡ 0.1 ml of washed and hemolyzed erythrocytes; washed 3 times with saline solution and hemolyzed with 3 volumes of distilled water. This suspension was diluted with 9 volumes of "Ringer-30."
§ 0.3 ml (*cf.* "Methods").
‖ 1 mg of dry venom.
¶ 0.025 ml.

choline is the physiologic substrate of the e-ChE.

Summary. e-Cholinesterase ("true" cholinesterase) from human erythrocytes and snake venoms is capable of catalyzing the hydrolysis of ethoxyethanol acetate, desoxycorticosterone acetate, ketopropanol acetate, phenyl acetate, p-nitrophenol acetate and acetylsalicylic acid. The first mentioned ester is attacked more effectively by e-cholinesterase than by s-cholinesterase ("pseudo"-cholinesterase).

Received June 10, 1949. P.S.E.B.M., 1949, **71**.

17245. Mobilization of Radioactive Sodium from the Gastrocnemius Muscle of the Dog.*

PETER W. STONE† AND WILLIAM B. MILLER, JR.‡ (Introduced by J. V. Warren.)
From the Whitehead Department of Surgery, Emory University, Atlanta, Ga.

The experimental study reported here was based on the clinical application of intramuscular injection of radioactive sodium 24 in studies of peripheral vascular disease by Elkin *et al.*[1] This technic and the inferences drawn from the results of those studies are predicated upon the disappearance of the radioactive sodium by way of the blood stream. However, the possibility existed that a portion of the sodium was being removed by the lymphatic system. A further consideration was that the isotope might diffuse along intramuscular planes, thereby removing itself from the range of the Geiger-Mueller counter, in which event it would erroneously appear that the sodium had been removed by the circulating blood. The study reported here was undertaken, therefore, to determine:

(*1*) The role of the lymphatics in the mobilization and removal of intramuscularly injected radioactive sodium.

(*2*) A means of quantitative estimation of

* This investigation was supported in part by research grants from the U. S. Public Health Service, and the Medical Department of the U. S. Army.

† Research Fellow, Whitehead Department of Surgery.

‡ Electronics Consultant, Whitehead Department of Surgery.

1 Elkin, D. C., Cooper, F. W., Jr., Rohrer, R. H., Miller, W. B., Jr., Shea, P. C., Jr., and Dennis, E. W., *Surg., Gynec. and Obst.*, 1948, **87**, 1; Cooper, F. W., Jr., Elkin, D. C., Shea, P. C., Jr., and Dennis, E. W., *ibid.*, 1949, **88**, 711.