tration; in the case of lard, this is between 0.05 and 0.10% of α-tocopherol.[15] Very recently, in similar experiments,[16] there is a suggestion that 0.5 mg α-tocopherol is an optimum dosage for the storage of vitamin A in the liver; the effect on vitamin A storage in the kidney was irregular. The liver stores of vitamin A from administered β-carotene were diminished by the larger doses of α-tocopherol and kidney storage was again irregular.

Unfortunately, no indication was secured as to the vitamin A stores of our animals on α-tocopherol acetate, following the assay period. The larger of the two dosages, 0.6 mg per day, even though it was within the optimum range and extended over both depletion and assay periods, may have been insufficient; it can hardly have been excessive. In any case, there was no response. The stabilization which the acetate can provide for vitamin A in the alimentary tract is limited to the amount of α-tocopherol available from it in the interval between hydrolysis and absorption. Perhaps, as with triglycerides, complete hydrolysis of the ester is not necessary for absorption, in which case any protective action in the tissues would depend upon the amount absorbed and the rate of subsequent hydrolysis. Information is needed on the rate of hydrolysis and absorption of tocopherol esters. The possible antioxygenic role of tocopherols in the tissues remains to be clarified.

Summary. 1. In bio-assays of vitamin A, α-tocopherol, but not α-tocopherol acetate, increased the rate of gain in the assay period. In a post-assay depletion period, animals that received α-tocopherol during the assay period survived 30-80% longer than controls.

2. As judged by gain in weight in the assay period, there is an optimum dosage of α-tocopherol; animals receiving 1.5 mg per day gained less than those receiving 0.5 mg. This conforms to the antioxygenic behavior of α-tocopherol.

3. Some of the possible causes for the failure of α-tocopherol acetate to conserve vitamin A are briefly discussed.

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16860 P

On the Specificity and Differentiation of Cholinesterases.

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Cholinesterases (ChE) of different sources may be separated into two types, the "s" and the "e."[1-3] The behavior of the two enzymes toward noncholine esters has been supposed to be the major difference between them. It has been assumed that the e-type could catalyze only the hydrolysis of certain choline esters;[4] therefore it has been also called "specific" or "true" cholinesterase.

The venoms of the colubrid snakes can split acetylcholine[5] and some noncholine esters.[6] In a recent investigation, it was shown that

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several noncholine esters—for example, ethyl chloroacetate (ECIA)—are hydrolyzed at a high rate by many of the colubrid venoms, and that this hydrolysis is due to cholinesterase.[7] Since the colubrid cholinesterase belongs to the e-type,[8] the question arose as to whether other e-cholinesterases also have the ability to split EC1A.

In order to solve the problem of whether other cholinesterases display a pattern of specificity similar to that of the snake venom enzyme, experiments were carried out with the e-cholinesterase of the erythrocytes of human beings. The hemolyzed erythrocytes and a purified preparation derived therefrom[2] catalyzed the hydrolysis of EC1A at an easily measurable rate. With the use of mixtures of acetylcholine and EC1A, it could be shown that with the concentration of acetylcholine varying from 0.004 M to 0.025 M, and with the concentration of EC1A varying from 0.035 M to 0.075 M, the resulting hydrolysis rate ($Q_{ACh} + Q_{EC1A}$). One typical example is given in Fig. 1.

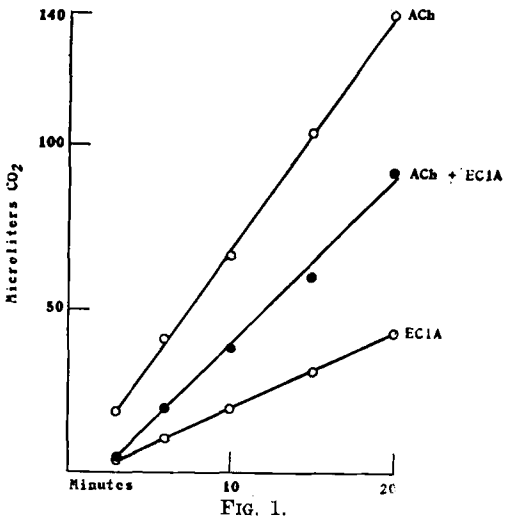

FIG. 1.

Hydrolysis of ethyl chloroacetate and acetylcholine by hemolyzed erythrocytes. Manometric measurement of the CO_2 produced from bicarbonate by the liberation of acids.[9] Erythrocytes were washed three times with saline, hemolyzed with an equal volume of distilled water and diluted with two volumes of bicarbonate-*Ringer* ("Ringer-30"). Three tenths milliliter of this preparation was used in a total volume of 3 ml. Final concentration of acetylcholine: 0.0067 M; final concentration of EC1A: 0.075 M. Blanks resulting from the enzyme solutions and the substrates were subtracted.

TABLE I.
Inhibition of the Enzymatic Hydrolysis of EC1A by Eserine.

Source of cholinesterase	Type	Inhibition, %
Erythrocytes (human) purified*	e	84-94
Snake venom (Naia melanoleuca)†	e	100
Plasma (human)‡	s	4-11
Parotid (guinea pig)§	s	13-

Same method as described in Fig. 1.

* 0.2 ml of erythrocyte-ChE, prepared according to the method of Mendel and co-worker.

† 40 μg of dried venom of Naia melanoleuca.[10]

‡ 0.083 ml of heparinized plasma of human beings.

§ 0.5 cc of extract from homogenized parotids from guinea pigs, prepared with 10 volumes of saline solution. Final concentration of eserine: 0.2-0.3 10^{-3}; final concentration of EC1A 0.016-0.05 M.

The enzymatic hydrolysis of EC1A in the presence of snake venoms[10] and of purified ChE from human erythrocytes[2] is inhibited by caffeine and other hydroxylpurines and by eserine (Table I) and prostigmine in the same way, and to the same extent, as has been shown to be true of acetylcholine.[3,11]

Serum from human beings and extracts from guinea pig parotid glands, typical sources of the s-ChE ("unspecific," "pseudo" ChE), split EC1A very rapidly. This hydrolysis is only slightly inhibited by eserine (Table I).

Another striking difference between the two esterases was found when β-chloroethyl acetate was used. This noncholine ester is easily attacked by purified erythrocytes and snake venom. Experiments carried out with mixed substrates and with eserine gave the same results as with EC1A, indicating that this substance is also hydrolyzed by e-ChE. Human serum is comparatively less able to

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TABLE II.

Enzymatic Hydrolysis of β-Chloroethyl Acetate and Acetylcholine.

Same method as described in Fig. 1; same enzyme preparations as in Table I. Final concentration of acetylcholine: 0.0067 M; final concentration of β-chloroethyl acetate; 0.033 M for the first three and 0.016 M for the last preparation. Q_{ACh} resp. $Q\beta_{C1A}$ give the number of microliter of CO_2 produced in 1 hour, calculated from reaction of zero order.

Source of cholinesterase	Type	Q_{ACh}	$Q\beta_{C1A}$
Erythrocytes (human) purified	e	669	249
Snake venom (Naia melanoleuca)	e	540	129
Plasma (human)	s	405	39
Parotid (guinea pig)	s	711	72

split this ester (Table II).

Detailed information about methods and enzyme and substrate preparations will be given in an extensive publication.

Conclusions. The ability to hydrolyze noncholine esters can no longer be used as a criterion to distinguish the two cholinesterase types, since both groups of enzymes are able to split noncholine esters; for example, ethyl chloroacetate. Another noncholine ester, β-chloroethyl acetate, is even preferentially attacked by the e-type ("true" or "specific" ChE).

The use of eserine to decide whether a hydrolysis is catalyzed by a cholinesterase or another esterase is of limited value, because the s-type ("pseudo" or "unspecific" ChE) is inhibited by this substance only in the presence of acetylcholine, but not in that of ethyl chloroacetate. The e-type is inhibited in both cases.

Ethyl chloroacetate plus eserine and β-chloroethyl acetate can be used as new means of distinction of the two groups of cholinesterases.

16861 P

Variations in the Vitamin B_{12} Content of Selected Samples of Pork and Beef Muscle.*

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In a recent paper[1] a fairly quantitative assay procedure for a growth factor in liver preparations was described. This assay procedure involves placing rats on the basal ration for a 2 week depletion period and following the growth response during another 2 week period when the material to be tested is given. Further work has shown that crystalline vitamin B_{12} will give a quantitative response in this assay (Fig. 1).

Henderson *et al.*[2] have reported wide variations in the ability of pork muscle, when fed to female rats as the source of protein, to produce normal lactation. Less than one-third of the young were reared in certain experiments; however, in other experiments the lactation was comparable to that obtained with rations containing beef. Beef-fed rats

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