tially normal levels by androgen therapy. The seminal vesicle is more sensitive than the prostate gland in this respect.

Androgen therapy caused hypertrophy of the glands beyond normal size, but under the conditions of these experiments, the acid and alkaline phosphatase activity was not increased beyond normal levels.

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17190. Investigations on the Use of Eserine for the Differentiation of Mammalian Esterases.*

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In most mammalian tissues there exist 3 types of esterase[1] capable of facilitating the hydrolysis of noncholine aliphatic esters, *i.e.*, ali-esterase,[2] pseudo-cholinesterase[1] (s-type)[3] and true cholinesterase[1] (e-type).[3] Most esters of aliphatic acids can be hydrolysed by enzymes from two of these 3 groups, certain non-choline esters (*e.g.*, triacetin) seem to be hydrolysed by enzymes of all 3 types[4-6] and, further, esterases of all 3 types are present in most crude tissue preparations. To distinguish between that portion of the hydrolysis of aliphatic esters which is due to the cholinesterases and that portion which is due to the ali-esterases in tissue preparations, Easson and Stedman[7] employed low concentrations of eserine or prostigmine to inhibit selectively any activity due to the cholinesterases (ChE's). Later investigators, *e.g.*, Richter and Croft,[2] Mendel and co-workers,[1] and others, also found low concentrations of eserine to be a very satisfactory tool for differentiating cholinesterase and ali-esterase activity. Thus it has been found that a concentration of 10^{-6}M eserine inhibits completely, or almost completely, the activities of both the true and the pseudo-ChE's of most mammalian tissues while the activity of the ali-esterases remains relatively unaffected by as much as 100 times this concentration.

Recently, however, McNaughton and Zeller[8] have concluded from their observations that "the use of eserine to decide whether a hydrolysis is catalysed by a cholinesterase or another esterase is of limited value because the s-type (pseudo-cholinesterase) is inhibited by this substance only in the presence of acetylcholine but not in that of ethyl chloroacetate." The evidence obtained in the present investigation has indicated, however, that eserine in low concentrations (10^{-6}M) can, in fact, be used to inhibit completely the activity of pseudo-ChE toward ethyl chloroacetate (ECIA) and that differentiation of cholinesterase activity from ali-esterase activity is therefore still possible by this means. Moreover, even though this concentration of eserine may not be adequate to effect complete inhibition of the ChE's from certain non-mammalian organisms,[9] in no case tested has the sensitivity of any cholinesterase to eserine been found to be altered significantly when one

* Supported by a grant from the National Research Council of Canada.

1 Mendel, B., and Rudney, H., *Biochem. J.*, 1943, **37**, 59.

2 Richter, D., and Croft, P. G., *Biochem. J.*, 1942, **36**, 746.

3 Zeller, E. A., and Bissegger, A., *Helv. chim. acta*, 1943, **26**, 1619.

4 Bodansky, O., *Ann. N. Y. Acad. Sci.*, 1946, **47**, 521.

5 Hawkins, R., Ph.D. Thesis, Toronto, 1947.

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

7 Easson, E. H., and Stedman, E., *Biochem. J.*, 1937, **31**, 1723.

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

9 Hawkins, R. D., and Mendel, B., *J. Cell. Comp. Physiol.*, 1946, **27**, 69.

TABLE I.
Inhibition of the Activity of Human Plasma towards ECIA Brought about by Various Inhibitors.

Inhibitor	Conc. of inhibitor (M)	Inhibition of Bch hydrolysis (%)	Inhibition of ECIA hydrolysis (%)
Eserine	2×10^{-6}	100	43*
	2×10^{-5}	100	45
DFP	4×10^{-8}	100	45
	1×10^{-6}	100	44
	1×10^{-4}	100	42

* With 12 different samples of human plasma the eserine-sensitive portion of the ECIA hydrolysis varied from 7-45% (average ca 15%) of the total activity.

substrate is substituted for another, whether this substrate be a choline or a non-choline ester.

The observation of McNaughton and Zeller that ECIA is split with great rapidity in the presence of human serum or suspensions of guinea pig parotid gland has been confirmed and this rapid hydrolysis has similarly been found to be only slightly inhibited by 10^{-5}M eserine. However, while these preparations have been shown[10,11] to contain relatively large amounts of pseudo-ChE, no evidence was presented by these authors[8] to substantiate their assumption that the high activity towards ECIA is not due, in part at least, to the presence of other esterases. To investigate the nature of the enzyme or enzymes responsible for the hydrolysis of ECIA by crude tissue preparations, the following experiments were carried out:

(1) To exclude the possibility that the low percentage inhibition of total ECIA hydrolysis by 10^{-5}M eserine (a concentration which inhibits completely pseudo-ChE activity towards acetylcholine, benzoylcholine, tributyrin, etc.) might in some way be associated with the fact that the inhibition produced by this compound is 'competitive' and reversible[12] the crude preparation was incubated with diisopropyl fluorophosphonate (DFP), an irreversible inhibitor with very high affinity for pseudo-ChE.[13] In the presence of 5×10^{-8}M DFP, pseudo-ChE was completely inactivated, as indicated by the total absence of activity towards benzoylcholine (Bch), a substrate specific for the pseudo-ChE's of most mammalian tissues.[10] Despite the complete and irreversible inactivation of its pseudo-ChE, however, human plasma tested in the presence of this concentration of the inhibitor still displays a high activity towards ECIA. Moreover, the degree of inhibition of total ECIA hydrolysis produced by this concentration of DFP was almost identical with that observed with eserine in concentrations which also completely abolish the activity of human plasma towards benzoylcholine. Since evidently the portion of the hydrolysis of ECIA which is insensitive to eserine is also insensitive to DFP, the eserine-insensitive portion of the ECIA hydrolysis is most likely due to a type of esterase distinct from the enzyme responsible for the eserine-sensitive portion of the activity. This supposition is further confirmed by the fact that considerable increases in the concentration of either inhibitor fail to augment the inhibition appreciably (Table I), in contrast to the results which would be expected if only one enzyme, the pseudo-ChE, were responsible for the total activity displayed towards ECIA.

(2) To prevent any ali-esterases from participating in the activity displayed towards ECIA, preferential inactivation of these enzymes was effected by heating undiluted oxalated human plasma at 52-53°C for 60-90 minutes. Plasma treated in this manner still displayed 65-75% of the activity of the untreated plasma towards Bch and all of its residual activity towards ECIA was inhibited by eserine. These results demonstrate that the eserine-insensitive hydrolysis of ECIA is

[10] Mendel, B., Mundell, D. B., and Rudney, H., *Biochem. J.*, 1943, **37**, 473.

[11] Mendel, B., and Rudney, H., *Science*, 1943, **98**, 201.

[12] Straus, O. H., and Goldstein, A., *J. Gen. Physiol.*, 1943, **26**, 559.

[13] Hawkins, R. D., and Mendel, B., *Brit. J. Pharmacol.*, 1947, **2**, 173.

TABLE II.
Effect of Various Concentrations of Eserine and DFP on the Activities Displayed towards ECIA and Bch by Heated* Human Plasma.

Inhibitor	Conc. of inhibitor (M)	Inhibition of Bch hydrolysis (%)	Inhibition of ECIA hydrolysis (%)
Eserine	1×10^{-6}	97	99
	1×10^{-7}	87	87
	1×10^{-8}	51	52
	1×10^{-9}	11	8
DFP	8×10^{-9}	87	90
	5×10^{-9}	43	43

* Heated at 53°C for 90 minutes.

due to an enzyme or combination of enzymes distinct from the pseudo-ChE. That the eserine-sensitive residual ECIA hydrolysis by the heated plasma preparation was due to pseudo-ChE could be concluded from the following evidence: (a) various concentrations of eserine and DFP inhibited the activities towards ECIA and Bch to the same degree (Table II); (b) when hydrolysis was measured in the presence of a mixture of both substrates (ECIA and Bch) no addition of the separate activities was observed—rather, only an activity similar to that exhibited towards Bch alone was displayed; (c) exposure of the treated plasma to a temperature of 56-57°C for 30 minutes depressed the activities towards Bch and towards ECIA to the same degree (76% and 73% respectively).

(3) To demonstrate conclusively that the pseudo-ChE does not contribute to the eserine-insensitive portion of the hydrolysis of ECIA by crude tissue preparations, the activity of highly purified pseudo-ChE preparations towards ECIA was also investigated. Using a 9000-fold purified preparation of the pseudo-ChE obtained from horse serum,[14] the ratio activity towards ECIA (0.04M) / activity towards Bch (0.006M) was found to be about 1.5, as compared with a ratio of 130 with a sample of untreated horse serum. Moreover, although a low concentration (10^{-5}M) of eserine inhibits only a minute fraction of the activity of untreated horse serum towards ECIA, the activity towards ECIA of the pseudo-ChE purified from the same source is completely abolished by the same concentration of eserine. It is evident, therefore, that the major portion of the hydrolysis of ECIA by horse serum is due to the presence of eserine-insensitive ali-esterases which are removed from the preparation upon extensive purification of the pseudo-ChE contained therein.

From the evidence obtained it can be concluded that the pseudo-ChE does hydrolyse ECIA but only at about 1½ times the rate at which it hydrolyses Bch and, further, its activity towards ECIA is inhibited just as effectively by eserine as is its activity towards Bch. While various crude enzyme preparations may exhibit extremely high activity towards ECIA—the ratio activity towards ECIA / activity towards Bch varying from 4 in some samples of human plasma to 130 in horse serum and over 1000 in rat plasma—only that small portion of the total hydrolysis which is inhibited by low concentrations of eserine can be attributed to a cholinesterase. The large eserine-insensitive portion of this activity would, on the other hand, seem to be due to a distinctly different enzyme, an ali-esterase. This ali-esterase, it might be noted, is probably not identical with the ali-esterases responsible for the eserine-insensitive hydrolysis of tributyrin and/or triacetin by tissue preparations, as is evidenced by differences in sensitivity to certain inhibitors (*e.g.*, DFP) and by the fact that human plasma displays little or no eserine-insensitive activity towards these substrates although exhibiting a large eserine-insensitive activity towards ethyl chloroacetate[8] and certain substituted acetates of nitrophenol.[15]

[14] Strelitz, F., *Biochem. J.*, 1944, **38**, 86.

[15] Huggins, C., and Smith, D. R., *J. Biol. Chem.*, 1947, **170**, 391.

Investigations with two 'irreversible' inhibitors—DFP as a preferential inhibitor of pseudo-ChE and tri-o-cresyl phosphate (TOCP) as a preferential inhibitor of ali-esterases[16]—showed that these compounds, unlike eserine, cannot be used as a general means of distinguishing pseudo-ChE and ali-esterase activities: certain ali-esterases, *e.g.*, the ali-esterase of rat plasma hydrolysing ECIA, exhibit abnormally high sensitivity to DFP, while others, *e.g.*, the ali-esterase of rat brain hydrolysing tributyrin, proved to be quite insensitive to TOCP. In other words, the portion of the total activity inhibited by low concentrations of DFP may not always be a reliable index of pseudo-ChE activity and similarly the portion of the total activity inhibited by TOCP may not always be a reliable criterion of ali-esterase activity. However, in all cases tested as yet the ali-esterases have proved to be much less sensitive to eserine than the cholinesterases of the same tissue. In fact, so far as is known, a concentration of 2×10^{-6}M eserine can be used to inhibit completely or almost completely the cholinesterase activities in mammalian tissue preparations while leaving those of the ali-esterases unaffected.

Summary. Recent observations by McNaughton and Zeller on the high 'eserine-insensitive' activity displayed towards ethyl chloroacetate by various crude preparations of pseudo-ChE have been confirmed but the experimental results reported here fail to substantiate their conclusion that the activity of pseudo-ChE towards ECIA is insensitive to eserine. The evidence presented here has, in fact, indicated that the activity of any of the cholinesterases towards ethyl chloroacetate is inhibited by low concentrations of eserine, the remaining eserine-insensitive activity being due to ali-esterases. The value of eserine for distinguishing that portion of the hydrolysis of aliphatic esters which is due to the cholinesterases from that due to ali-esterases in crude tissue preparations has been re-affirmed. It would seem that of the three inhibitors tested (eserine, diisopropyl fluorophosphonate, tri-o-cresyl phosphate), only eserine can be relied upon to give a clear cut distinction between cholinesterase and ali-esterase activities in all cases.

[16] Mendel, B., and Mortimer, E. M., Report to National Research Council of Canada, October, 1944.

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17191. Histamine and Other Imidazole Compounds as Bacterial Growth Stimulators.*

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In the course of a study it was observed that small amounts of histamine markedly stimulated the growth of certain bacteria. This report represents typical results of a comparative study of this effect with histamine and other derivatives of the imidazole nucleus.

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Materials and methods. The culture medium was composed of NaCl (0.5%), $MgSO_4 .7H_2O$ (0.02%), $(NH_4)H_2PO_4$ 0.1%, K_2HPO_4 (2.4%), KH_2PO_4 (0.6%), glucose (0.2%) and distilled water. The reaction of the medium was pH 7.0. It was passed through a sintered glass filter to sterilize. Solutions of the test substances in the salt-glucose medium were also sterilized by filtration. No significant drop in pH occurred during growth with this high phosphate buffer concentration. A stock strain of *Klebsiella pneumoniae* was