is concluded (a) that activation by NaCl can almost match the activation by tris and (b) that the effect is not on k_3 but on k_2 .

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Shay Chloroma Alkaline Phosphatase

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The characterization of normal human and animal tissue alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) has been described (Morton, 1955) and studied in great detail (Moss, Eaton, Smith & Whitby, 1967; Sussman, Small & Cotlove, 1968). Since such studies have not to date been reported for a tumour enzyme we studied the Shay Chloroma, a myeloblastic tumour in the rat (Moloney, Dorr, Dowd & Boschetti, 1962). This tumour contains an average of 8.0 i.u. of alkaline phosphatase/g. wet wt. of tumour. The enzyme was purified by butanol extraction, acetone fractionation, ammonium sulphate fractionation, gel filtration on Sephadex G-200 and chromatography on DEAE-cellulose DE52.

Enzyme activity was eluted in 3 peaks from DEAE-cellulose DE52 at 50, 100 and 250 mM-NaCl, designated respectively peaks A, B and C. The purification achieved was 200-fold for peak B and 90-fold and 120-fold for peaks A and C. Enzyme B was eluted in two peaks of activity from Sephadex G-200, an included and excluded peak, and enzymes A and C were both totally excluded from the gel.

The K_m values for the three enzymes with *p*-nitrophenyl phosphate as substrate were 0.46, 0.37 and 0.44 mM and the pH optimum was pH 10.2 in each case. Enzyme A was the most resistant to urea inactivation, 55% of the activity remaining in 3.0 M-urea compared with 20% with enzymes B and C. The urea inhibition was non-competitive. On polyacrylamide-gel electrophoresis, enzyme B moved as a single band and had the highest mobility, enzyme A separated into three bands and enzyme C into two bands of activity. One component of enzyme A and of enzyme B was retarded after neuraminidase treatment. Extensively dialysed samples of the three enzymes were activated by Mg^{2+} , all to different degrees. EDTA inactivated enzymes A, B and C, the inactivation being a function of time and concentration; the activity was only partially restored by addition of Mg^{2+} .

The three enzymes varied in their heat stability but were all inhibited to the same extent (20%) by L-phenylalanine. The ratio of alkaline phosphatase activity to inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) activity was not constant through all the steps of purification (Moss *et al.* 1967); the ratios for enzymes A, B and C were 13, 20 and 12 respectively.

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The Human Pseudocholinesterase System

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Multiple forms of human pseudocholinesterase have been demonstrated on starch-gel (La Motta, McComb, Noll, Wetstone & Reinfrank, 1968) and polyacrylamide-gel (Juul, 1968) electrophoresis. It has been shown (Svensmark, 1961) that there are several molecules of *N*-acetylneuraminic acid (NANA) associated with each molecule of pseudocholinesterase, but that these play no part in the enzyme function (Augustinsson & Ekedahl, 1962).

A 460-times enriched preparation of the enzyme was obtained from pooled outdated plasma by ammonium sulphate precipitation and DEAE-cellulose fractionation at pH 4.2. Polyacrylamide-gel electrophoresis at pH 8.9 followed by enzyme detection with butyrylthiocholine iodide showed that some enzyme components of whole serum were lost during this purification process. The consistency of the distribution of the enzyme components of whole serum on polyacrylamide gels under various conditions of gel-pore size and buffering indicated that the heterogeneity may not be completely explained by assuming polymer forms for the enzyme (La Motta *et al.* 1968).

The enriched enzyme preparation, after treatment with neuraminidase (EC 3.2.1.18) for 7 days at 30° was quite homogeneous on polyacrylamide-gel electrophoresis and its mobility was less than that of the major component of the heterogeneous (6–10 components) control. Both the neuraminidase-treated preparation and its control yielded three fractions on elution from Sephadex G-75 with dilute ammonia solution at pH 8.2. The two major protein fractions were poorly resolved and eluted at about the void volume and the third minor fraction was eluted at about twice the void volume. Whereas enzyme activity was associated

only with the first two protein fractions by a spectrometric assay with benzoylcholine chloride (Kalow & Lindsay, 1955), all three fractions were active with butyrylcholine iodide in conjunction with 5,5'-dithiobis-2-nitrobenzoate (Ellman, Courtney, Andres & Featherstone, 1961). Further, each fraction gave the same number and distribution of enzyme components on polyacrylamide gels as did the unfractionated preparations.

It would seem that the more anionic components of this heterogeneous enzyme system may be due to various amounts of *N*-acetylneuraminic acid being associated with, possibly, a common protein moiety, whereas the less anionic components, poorly resolved on gel electrophoresis, may be explained by the polymerization phenomenon.

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An Enzyme in Hen Brain Hydrolysing Phenyl Phenylacetate: a Possible Connection with the Delayed Neurotoxic Effect of some Organophosphorus Compounds

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The phenyl esters of 1-phenylacetic acid (PPA) and 2-phenylpropionic acid (PPP) are structurally analogous to the neurotoxic esterase inhibitor phenylsaligenin phosphate. Poulsen & Aldridge (1964) showed that there are two enzymes in hen brain that hydrolyse both PPA and PPP, although the ratio of activity towards the two substrates differed. Aldridge & Barnes (1966) investigated whether inhibition of these enzymes was involved in the genesis of the delayed neurotoxic effect produced by dosing hens with some organophosphorus compounds: they showed that there was no clear correlation between neurotoxicity and high inhibition of the two enzymes. Moreover, Poulsen & Aldridge (1964) showed *in vitro* that the pI_{50} values for total PPA hydrolysis and PPP hydrolysis were lower for the non-neurotoxic inhibitors tetraethyl pyrophosphate (TEPP) and Paraoxon (*O*-*p*-nitrophenyl di-*O*-ethyl phosphate) than for the neurotoxic di-isopropyl phosphorofluoridate (DFP).

Recently a technique has been developed that identifies a protein in chicken brain that is phosphorylated by neurotoxic organophosphorus compounds administered *in vivo* (Johnson, 1967, 1968; Aldridge, Barnes & Johnson, 1968). The protein is phosphorylated *in vitro* by 128 μ M-Mipafox but not by 16 μ M-TEPP. Labelling of this protein by ^{32} P[DFP] *in vitro* is blocked in the presence of PPA: few other esters out of a large range investigated are effective. The hydrolysis of PPA by chicken brain homogenate was therefore reinvestigated. It has now been shown that about 10% of the total hydrolysis is much less sensitive to TEPP and Paraoxon than is the remainder. Inhibition experiments with Mipafox, which is neurotoxic, show that this 10% of the activity is due to more than one enzyme: activity is increasingly inhibited as Mipafox concentration is increased to about 128 μ M, but there is no greater inhibition at higher concentrations.

It is therefore possible that the TEPP-insensitive Mipafox-sensitive enzyme that hydrolyses PPA is the same protein as that identified with radioactive DFP. This view is supported by the fact that all compounds that have been tested so far and are known to be neurotoxic inhibit this enzyme *in vivo*, whereas structurally analogous but non-neurotoxic esterase inhibitors do not.

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Lipid Separation on Sephadex LH-20

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The development of Sephadex LH-20 has extended the application of gel filtration to the fractionation of lipids (Maxwell & Williams, 1967; Joustra, Söderqvist & Fischer, 1967). We investigated the separation of artificial mixtures containing fatty acids (acetate, *n*-butyrate, *n*-decanoate, stearate and linolenate; 10–50 mg. of each), triglycerides (tributyrin and tristearin, 5–10 mg. of each) and a phospholipid preparation (40–200 mg. of bovine lecithin; Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.) on Sephadex LH-20 columns (50 cm. $\times$ 2.4 cm. diam. approx.; flow rate 1 ml./min.) equilibrated with either chloroform or 20% methanol in chloroform