seven- to ten-fold that of neurons in myelinating brain, and two- to three-fold that of neurons in mature brain.

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An Enzyme Catalysing the Hydrolysis of Phosphoric Acid Diesters in Rat Liver

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One of the primary mammalian metabolites of insecticidal phosphoric acid triesters such as 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate is the corresponding diester, in this case 2-chloro-1-(2,4,5-trichlorophenyl)vinyl methyl hydrogen phosphate (I) (Akintonwa & Hutson, 1967; Donninger, Hutson & Pickering, 1967; Hutson, Pickering & Donninger, 1967). In a study of the further metabolism of phosphate diesters in mammalian liver an enzyme catalysing the hydrolysis of compound (I) to 2,4,5-trichlorophenacyl chloride was found in the soluble fraction of rat and pig liver homogenates.

The enzyme was purified 210-fold from rat liver homogenate by centrifuging at 105 000g for 60 min, precipitation of the enzyme from this supernatant at between 60% and 90% saturation with ammonium sulphate followed by chromatography of the precipitate after dialysis on a DEAE-cellulose column eluted with a gradient of tris-HCl buffer between 0.1M, pH 7.2, and 0.6M, pH 8.0. Assay of activity was by incubation with [1,2-¹⁴C]vinyl-labelled substrate followed by a benzene/water partition in which unchanged substrate remained in the aqueous phase and the labelled product entered the organic phase. The ¹⁴C content of both phases was measured by liquid-scintillation spectrometry.

High concentrations (1mM) of Cu²⁺, Zn²⁺ and Fe³⁺ were inhibitory. Phosphoric acid monoesters and inorganic phosphate also inhibited, whereas EDTA (10mM) activated the enzyme. The enzyme had optimum pH 6.9 at 37°C. Over 30% of the activity was retained after 20 min at 60°C.

The enzyme showed no activity towards RNA 'core', unlike snake venom (Privat de Garilhe & Laskowski, 1955) and spleen (Hilmoe, 1960) phosphodiesterases (EC 3.1.4.1). The latter did not hydrolyse the substrate used in this investigation. The enzyme also differed from these and similar phosphodiesterases (Razzell, 1961; van Venrooij & Poort, 1969) in other properties.

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Solubilization Procedures Cause Changes in the Response of Brain 'Neurotoxic Esterase' to Inhibitors

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Homogenates of hen brain contain cholinesterases and numerous other partially characterized esterases associated with microsomal membranes. Organophosphorus inhibitors have been used to show the presence of at least four distinct hen brain esterases that hydrolyse phenyl phenylacetate. Two are highly sensitive to paraoxon (Poulsen & Aldridge, 1964). One of the paraoxon-resistant enzymes is sensitive to Mipafox (NN'-di-isopropyl-phosphoryldiamidic fluoride) and phosphorylation of its active site is associated with the delayed neurotoxic effects of some organophosphorus compounds (Johnson, 1969, 1970).

A partially purified preparation of 'neurotoxic esterase' was obtained by centrifuging a homogenate of hen brain in sucrose (1.0M) at 20 000g_{av} for 30 min; the floating and sedimented materials were discarded and the suspension was diluted with an equal volume of tris buffer (50mM, adjusted to pH 8.0 with HCl) and centrifuged at 105 000g_{av} for 90 min; the sedimented material was resuspended in tris buffer. Paraoxon-sensitive enzymes were inhibited by incubating the suspension for 20 min at 37°C with 40 μM-paraoxon. After preincubation the suspension was either cooled to 0°C and immediately treated with detergent or was diluted 20-fold before analysis of the Mipafox-sensitive 'neurotoxic esterase'.

Plots of the logarithm of the percentage activity remaining after incubation of particulate enzyme with Mipafox (20 min at 37°C) versus Mipafox concentration gave a first-order plot, indicating a single enzyme with I_{50} about 5 μ M. This activity also behaved as a single enzyme when analysed similarly with 2,2-dichlorovinyl di-isopropyl phosphate (I_{50} 23 nM).

Treatment of the particulate suspension with either sodium deoxycholate (0.8% final concn.) or Triton X-100 (1% final concn.) gave opalescent suspensions, and the solubilized proteins were transferred to lower detergent concentrations by passage through Sephadex G-25 loaded with deoxycholate (0.04%) or Triton X-100 (0.03%) in tris buffer. The eluents were found to behave as mixtures of two phenyl phenylacetate esterases with distinctly different I_{50} . The nature of the detergent did not alter the observed I_{50} but did alter the proportion of the two components. The majority of the activity (55–80%) had unchanged I_{50} , but the remainder was highly sensitive to inhibition, with I_{50} of less than 0.2 μ M for Mipafox and less than 4 nM for 2,2-dichlorovinyl di-isopropyl phosphate.

Centrifugation of the solubilized preparation at high speeds yielded particles containing esterase activity of normal sensitivity while the supernatants were enriched in the highly sensitive activity.

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Antigen–Antibody Interaction in the Presence of Sodium Deoxycholate

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Detergents have been used with considerable success to solubilize cell membranes (e.g. Dulaney & Touster, 1970; Emmelot & Vaz Dias, 1970; Miller, 1970; Allan & Crumpton, 1971). Although some of the biological activities of the membrane may survive solubilization, the subsequent separation of the biologically active molecules has, in general, been unrewarding. However, if fractionation methods based on specific molecular interaction were applicable in the presence of detergent, then it should be possible to readily purify certain selected membrane components. The utility of this approach was examined initially by investigating antigen–antibody interaction in the presence of detergent. Sodium deoxycholate was chosen for investigation because it effectively solubilized the plasma membrane of lymphocytes (Allan & Crumpton, 1971)

and because it appeared that detergents such as sodium dodecyl sulphate destroy protein–protein interaction (Weber & Osborn, 1969).

Amounts of precipitate formed by increasing amounts of sperm-whale metmyoglobin with a constant volume of an antiserum to the native protein were determined in the absence and presence of 1% (w/v) sodium deoxycholate in 75 mM-tris-HCl–75 mM-NaCl, pH 8.1. In each case the same amount of metmyoglobin was required for maximal precipitation, but slightly less precipitate was formed in deoxycholate. A comparison of the absorption spectra of solutions of the precipitates indicated that the deoxycholate caused no detectable dissociation of the haem from metmyoglobin. Determination of the amounts of precipitate formed at the equivalence point in the presence of increasing concentrations of sodium deoxycholate revealed slightly more precipitate (11%) in 0.5% deoxycholate, whereas 1%, 2% and 4% deoxycholate gave decreases of 14, 32 and 72% respectively. Similar results were obtained by using pig immunoglobulin G and DNP-bovine serum albumin with rabbit antisera to pig immunoglobulin G and the DNP group respectively. In the former case 1%, 2% and 4% deoxycholate gave decreases of 6, 23 and 32% respectively in the amount of precipitate formed at equivalence.

The results indicate that antigen–antibody interaction is not grossly affected by concentrations of sodium deoxycholate (1%) in which cell membranes are soluble. Consequently immunoadsorbents and affinity chromatography can probably be used in 1% deoxycholate to selectively purify solubilized membrane components. Further, since the immunological reactivity of globular proteins is determined by the conformation of the surface of the molecules (Crumpton, 1968), it seems likely that deoxycholate (1%) does not bind to the surface or cause gross conformational changes.

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