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'.