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 moeity, 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 128μM-Mipafox but not by 16μM-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 128μM, 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; 10–50mg. of each), triglycerides (tributyrin and tristearin, 5–10mg. of each) and a phospholipid preparation (40–200mg. of bovine lecithin; Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.) on Sephadex LH-20 columns (50cm. × 2·4cm. diam. approx.; flow rate 1ml./min.) equilibrated with either chloroform or 20% methanol in chloroform