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

containing 1.25% of water. Sand (1 cm.) was placed on top of the column to prevent the gel floating during elution with solvent of specific gravity higher than that of the Sephadex. This also facilitated application of the lipid samples (dissolved in 2 ml. of solvent) without disturbing the column.

When chloroform only was used as eluent, tristearin, tributyrin, stearate, *n*-decanoate, *n*-butyrate and acetate were separated into well defined peaks [elution volumes (V_e) 65, 85, 225, 320, 450 and 575 ml. respectively]. Linolenate was not separated from stearate. The recovery of both fatty acids and triglycerides was approx. 85%. Two peaks, each containing less than 10% of the phospholipid applied, were eluted; one near the void volume of the column (V_e , 60 ml.) and the other (V_e , 200 ml.) before the stearate peak. After elution of the fatty acids by chloroform, a third peak containing 50% of the phospholipid applied was eluted when the column was washed with methanol. Thin-layer chromatography (Vaskovsky & Kostetsky, 1968) indicated marked differences in the major phospholipid components of each peak.

When these fractionations were carried out on a column equilibrated with 20% methanol in chloroform containing 1.25% of water, very poor resolution of the polar lipids was obtained. The phospholipids were eluted as a single peak (V_e , 55 ml.) near the void volume, closely followed by the fatty acids. Stearate, *n*-decanoate and *n*-butyrate were eluted as three ill-defined peaks (V_e , 70, 85 and 96 ml. respectively).

The separation of the individual fatty acids and phospholipids is apparently effected mainly by the adsorptive characteristics of Sephadex LH-20, whereas the triglycerides are fractionated by molecular sieving. This preliminary investigation indicates that the combined molecular-sieving and adsorptive properties of Sephadex LH-20 render it a versatile material for the chromatography of both polar and non-polar lipids.

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Amino Acids Involved in Chromophore-Protein Linkages in Phycoerythrins

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The phycoerythrins contain the covalently-bound bile pigment, phycoerythrobilin, as their chief

prosthetic group. Recent evidence indicates that this tetrapyrrolic chromophore is doubly linked to the apoprotein; one bond, probably ester-type (Ó Carra, Ó hEocha & Carroll, 1964), involves a propionic acid side-chain of the chromophore and the other involves the lactam grouping of its ring A (Rüdiger & Ó Carra, 1968).

To investigate the amino acids bound to the pigment, R-phycoerythrin was digested with proteolytic enzymes, the chromopeptide material was purified, and the attached amino acids were identified.

The best results were obtained by digestion of the reduced aminoethylated phycoerythrin (Raftery & Cole, 1963) first with pancreatin (Tong & Chaikoff, 1958) in the presence of mercaptoethanol (0.14 M), then with hog intestinal peptidases (Sigma Chemical Co.) and finally with carboxypeptidase A, leucine aminopeptidase and prolidase. The chromopeptide fraction was isolated and purified by chromatography on talc (Dus, Bartsch & Kamen, 1962) followed by gel filtration on Sephadex G-50 in phenol-acetic acid-water (1:1:1, by vol.) (Synge & Youngson, 1961). The chromophore was then removed by preliminary hydrolysis (11N-HCl for 6 hr. at 15°) followed by adsorption on talc, and the released peptide material was completely hydrolysed (6N-HCl for 20 hr. at 110°). The resulting amino acids, after dinitrophenylation, were identified by thin-layer chromatography (Brenner, Niederwieser & Pataki, 1961).

The following amino acids were found: glutamic acid, serine, aspartic acid, threonine, glycine and alanine. It seems unlikely that glycine or alanine could be involved in the linkage, but repeated efforts to eliminate them from the chromopeptide preparations by further proteolytic digestion failed. Glutamic acid and serine were always present in higher concentration, and in some experiments in much higher concentration, than aspartic acid and threonine. Preliminary results with B- and C-phycoerythrins indicate a similar pattern. It seems likely, therefore, that at least the majority of the phycoerythrobilin units are linked through glutamic acid and serine. This is consistent with the results of Rüdiger & Ó Carra (1968) mentioned above, since serine could form an ester bond with a propionic acid side-chain of phycoerythrobilin, and the γ -carboxyl group of glutamic acid could be bound to the lactam grouping.

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