$3.8 \pm 0.2\%$ (five determinations) together with rat myelin lipid composition (Cuzner, Davison & Gregson, 1965) for which there is $12.9 \mu\text{g}$ of N/mg of myelin lipid, and an assumed protein nitrogen content of 16%, then the protein content of rat myelin is 17%. Similar treatment of ox and human myelin nitrogen contents (Wolfgram & Kotorii, 1968) leads to values of 19% protein.

The difference between extinction values at 215 and 225 nm is proportional to protein concentration in solution (Waddell, 1956) and depends on peptide-bond absorptions. Because of light-scattering by particulate myelin suspensions anomalous protein contents are to be expected (Winder & Gent, 1970b), differing from instrument to instrument because of the variable inclusion of forward-scatter components in the transmitted beam. After appropriate corrections rat myelin protein averages at 44%. Part of the myelin absorption in the short-u.v. region is shown to be due to cerebroside, and allowance for this effect lowers the protein content to 28%. Solubilization of myelin by lysophosphatidylcholine by the procedure of Gent, Gregson, Gammack & Raper, (1964) eliminates turbidity, and with correction for cerebroside absorption a protein content of 20% is indicated. Lipid-protein interaction may modify the chromophore function of peptide linkages. Results are presented that indicate considerable modification by lipid interactions of the chromophores responsible for 280 nm absorption.

Estimates by the tyrosine-dependent method of Lowry, Rosebrough, Farr & Randall (1951) suggest for rat myelin a protein content of 20.0%, S.D. 1.3% (17 determinations). Results are also reported for other species and different brain regions by this method.

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Improved Separation of Viable Neurons and Glia from Rat Brain

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The preparation of greatly enriched fractions of neurons and glial cells in workable amounts is requisite for a detailed study of glia-neuron synergic relationship at the biochemical level. Information derived from the application of the method of Rose (1967) is subject to some doubt on the grounds of reliability of the fractionation technique and integrity of the separated cells (Cremer, Johnston, Roots & Trevor, 1968). Norton & Poduslo (1970) introduced considerable technical improvement by using trypsin before tissue disruption. With a modified disruption procedure and zonal centrifugation their method has now been extended to yield large numbers of viable cells.

Seven brains of 14–40-day-old rats were cut into small pieces ($0.5\text{--}1.0 \text{ mm}^3$) and incubated for 60 min at 37°C with 10 vol. of trypsin medium (5% fructose–5% glucose–1% serum albumin–1% Difco 1:250 trypsin–100 mM-sodium-potassium phosphate buffer, pH 6.2). After three rinses in cold trypsin-free medium the pieces of tissue were disrupted on 110-mesh bolting cloth stretched over a glass funnel by gentle abrasion with a large paint brush, while the medium was continuously dropped on the preparation. The suspension was filtered three times through stainless-steel sieves (150 and 200 mesh). In an attempt to separate cells from processes, the sample (15–20 ml) was loaded on to a discontinuous sucrose gradient comprising a lower layer of 60% sucrose (10 ml) with superjacent 22% sucrose prepared in the medium (10 ml). After 5 min centrifugation at 3000 rev./min in an MSE Mistral 6L centrifuge, cells were collected from the 22%/60% sucrose interface and loaded on to a continuous sucrose gradient (40–60% in medium) in an MSE H.S. zonal rotor. After centrifugation for 30 min at 4500 rev./min 7.5 ml or 15 ml samples were collected.

The cells were identified under phase-contrast illumination and estimated as percentage of total brain DNA by the diphenylamine method (Burton, 1956). They comprised six different cell fractions located as follows: glia A (40% sucrose, 3% DNA); glia B (42% sucrose, 2% DNA); capillaries (46% sucrose, 2% DNA); neurons and capillaries (49% sucrose, 5% DNA); neurons A (53% sucrose, 8% DNA); neurons B (58% sucrose, 4% DNA). The characters of the cell types will be described.

Viability was tested by measuring the incorporation rate of DL-[^{14}C]valine *in vitro*. When referred to DNA, the incorporation rate of glial cells was

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'.