than either the acyl groups of phosphatidylethanolamine or free palmitic acid. Sphingosine is not significantly labelled by either EGP-alkenyl or -acyl groups. The relatively poor labelling of lecithin (phosphatidylcholine) in the [^3H]EGP incubations indicates that *N*-methylation of EGP does not occur in myelinating brain. The labelling of sphingolipid acyl groups by EGP-alkenyl groups suggests that the aldehydes derived from EGP become involved in fatty acid chain-elongation processes.

The results below are in accord with this suggestion. (i) Brain EGP-alkenyl groups (almost exclusively $\text{C}_{16:0}$, $\text{C}_{18:0}$ and $\text{C}_{18:1}$) are turning over more rapidly in myelinating rat brain than EGP-acyl groups of corresponding chain length (Debuch, 1964). (ii) Brain contains a mitochondrial aldehyde dehydrogenase of very broad specificity. The K_m for palmitaldehyde is $1.3 \times 10^{-6} \text{ M}$ (Erwin & Deitrich, 1966). (iii) Brain plasmalogenase is associated with the mitochondria, the site for aldehyde dehydrogenase activity and fatty acid chain-elongation processes. Further, these are specific for the C_{16} and C_{18} acids (Aeberhard & Menkes, 1968). (iv) Brain cerebroside acyl groups are formed by chain-elongation processes involving palmitic acid derived from two pools, namely *de novo* from acetate and from 'complex lipids' (Kishimoto & Radin, 1966).

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Separation at Neutral pH of a ^{32}P -Labelled Membrane Protein Associated with the Sodium-plus-Magnesium Ion-Activated Adenosine Triphosphatase from Ox Brain

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The Na^+ -dependent acyl-bound phosphate formed during the hydrolysis of ATP by cerebral microsomes has only been clearly demonstrated after acid-denaturation of the labelled membrane proteins. At neutral pH the nature of the bond has

not been described, leaving open the possibility of an acid-catalysed migration of phosphoryl groups during denaturation at low pH. Attempts to characterize the phosphorylated membrane-component, now widely considered to be an intermediate in the $\text{Na}^+ + \text{K}^+ + \text{Mg}^{2+}$ -activated adenosine triphosphatase (Kanazawa, Saito & Tonomura, 1967; Fahn, Koval & Albers, 1968; Schoner, Beusch & Kramer, 1968), at physiological pH values have been hampered by the lability of the bond.

Sodium dodecyl sulphate was used by Nagano *et al.* (1967) to stabilize and solubilize membrane ^{32}P -labelled protein with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ as precursor. Using 0.1% sodium dodecyl sulphate to terminate the reaction we have confirmed that a Na^+ -dependent increment of 50 pmol of bound P/mg of protein can be isolated from ox brain microsomes, labelled as described by Rodnight, Hems & Lavin, (1966), after separation on Sephadex G-25 at pH 7. Precipitation of labelled protein with acetone and treatment with 0.8M-hydroxylamine at 0°C for 10 min at pH 7 resulted in rapid hydrolysis of the label. Hydrolysis rates over the range pH 2-9 gave a profile characteristic of an acyl phosphate.

A fivefold purification of acyl-labelled protein was achieved with a column of Sepharose 6B; by markers the molecular weight of the component at this stage was estimated at 570 000. After concentration of the labelled protein by rapid ultrafiltration further fractionation was attempted by electrophoresis on polyacrylamide gel (10%, w/v) containing 0.1% sodium dodecyl sulphate, 5M-urea and 0.01% mercaptoethanol at pH 8.7. Twelve protein bands were observed and despite the increased lability of the acyl bond at this pH enough hydroxylamine-sensitive label was present after electrophoresis (40 min) to demonstrate its presence in a single band. From mobilities of markers (Shapiro, Vinuela & Maizel, 1967) the molecular weight of the labelled band was estimated as 102 000.

Incubation of microsomes with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ also results in a Mg^{2+} -dependent transfer of phosphate to protein serine residues (Rodnight & Lavin, 1966). Membrane proteins labelled for 2 min with 1mM- MgATP were solubilized with sodium dodecyl sulphate and fractionated on Sepharose 6B. Three peaks of phosphorylserine labelling were obtained; incorporation of label into the first two peaks was stimulated by $100 \mu\text{M}$ -3':5'-cyclic-AMP (Weller & Rodnight, 1969). The second peak was eluted in the same position as the acyl-labelled protein. On polyacrylamide gels the phosphorylserine labelling was confined to two major protein bands of lower molecular weight than the acyl-labelled band.

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The Use of Zonal Centrifugation in the Preparation of Subcellular Fractions from Brain Tissue

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The first attempted separation of a brain homogenate into its subcellular components in a zonal centrifuge was by Barber, Rankin & Anderson (1966), who loaded whole brain on to a continuous density gradient of sucrose. Later, Cotman, Mahler & Anderson (1968) used a similar gradient to subfractionate material comparable with the crude mitochondrial fraction (P_2) of Gray & Whittaker (1962). A discontinuous gradient was used by Rodnight, Weller & Goldfarb (1969), who were able to prepare relatively large quantities of components of the crude mitochondrial fraction from ox brain. Suitably adapted discontinuous gradients in a zonal head offer a method of obtaining certain fractions more quickly and in larger amounts than is possible with the conventional bucket rotor and we have been investigating the possibilities.

The crude mitochondrial fraction obtained from whole rat brain or up to 15 g of cat cerebral hemispheres and prepared by the method described by Gray & Whittaker (1962) was loaded on to a discontinuous gradient of sucrose (0.4–1.7M) in a B-XIV aluminium rotor (capacity 650 ml) which was run in a modified MSE 50 ultracentrifuge. The separation was allowed to proceed for 2 h at 67 000g, after which the rotor was unloaded and fractions (20 ml) were collected. After the distribution of protein had been determined, from the extinction at 280 nm, the nature of the subcellular fractions in the different peaks was examined by a number of methods, particularly enzyme assay.

A fraction moving through a band of 1.4 M-

sucrose and clearly separated from mitochondria gave the orange fluorescence with Acridine Orange characteristic of lysosomes and, in several preparations examined, green fluorescence caused by mitochondria was not observed (Koenig, Gaines, McDonald, Gray & Scott, 1967). The succinate dehydrogenase concentration in this fraction was less than 0.025% of that of the mitochondria on a protein basis, a value that is comparable with that of Koenig *et al.* (1967). The method can be used to prepare a lysosomal fraction rapidly from 12–15 g of brain tissue. The synaptosomal fraction was also clearly defined and exhibited the high occluded free lactate dehydrogenase ratio characteristic of this material (Marchbanks, 1967). The distribution of 5'-nucleotidase and acetylcholinesterase in all fractions was also studied.

The methods employed and their probable value will be discussed.

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Biochemical Studies of Taurine in the Developing Rat Brain

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Taurine is found in high concentration in immature neural tissue although, unlike glutamate and γ -aminobutyrate, there is a striking decrease in its concentration during development of the brain (Agrawal, Davis & Himwich, 1968). Since taurine is probably formed through decarboxylation of cysteinesulphinic acid, we have examined the activity of cysteinesulphinic acid decarboxylase during development of the rat brain in relation to taurine concentration. Surprisingly, there is a steady increase of decarboxylase activity from after birth to 20 days *post partum*, closely resembling the increase in glutamate decarboxylase activity seen during the same period.