recombined materials with ether and ether-ethanol (1:3, v/v) appeared to be comparable with those of the erythrocyte 'ghosts', even among different mammalian species. These observations, together with p.m.r. (proton-magnetic-resonance) (Kamat, Chapman, Zwaal & van Deenen, 1970) and spin-label studies (Berger, Barratt & Kamat, 1970) on these recombined preparations, suggest that the association of lipids and proteins in these experiments *in vitro* at pH 4 first involves electrostatic attractions that are followed by apolar interactions. Comparative electron-microscopic studies demonstrated that the recombined materials show similar trilamellar images to those observed in erythrocyte membranes.

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Separation of Lipid-Protein Complexes of Rat Brain Myelin by Isopycnic Centrifugation

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The lipid-protein complexes released by solubilization of rat central-nervous-system myelin with lysophosphatidylcholine $1.4 \times W$ min (Gent, Gregson, Gammack & Raper, 1964) were separated by density-gradient centrifugation on continuous sucrose gradients (5–20% sucrose in tris-HCl buffer, pH 8 and I 0.05) at 10°C for 16 h at 40000g. Two regions of light-scattering were seen on observing the resultant gradients by an axial beam of white light. One region of lower density showed a very small amount of scattering, whereas the other, a much wider band at a denser region of the gradients, showed high scatter in which three distinct bands were clearly visible.

The gradients were fractionated into approx. 1 ml samples and E_{280} values were read. The samples were analysed for protein (Lowry, Rosebrough, Farr & Randall, 1951), phosphorus (Bartlett, 1959) and radioactive [^3H]lysophosphatidylcholine, used as a marker for lysophosphatidylcholine. The enzyme 2':3'-cyclic AMP 3'-phos-

phohydrolase was assayed (Kurihara & Tsukada, 1967) and the samples were tested for complement-fixing antigen (Gregson, Kennedy & Leibowitz, 1971). The refractive index of the fractions was measured in a refractometer to test the gradients for linearity and to determine the flotation densities of the components.

The various analyses showed two main fractions corresponding to the light-scattering components. The less-dense fraction, whose flotation density was 1.023, contained 60% of the recovered protein, almost 100% of [^3H]lysophosphatidylcholine and showed a denser shoulder with almost 100% of the enzyme activity. The peak for the dense components was at density 1.043. They contained the remaining protein.

The fractions from both the light and the heavy regions were pooled, dialysed and freeze-dried. The residues were taken up in phenol-acetic acid-water (Cotman & Mahler, 1967) and subjected to electrophoresis on disc polyacrylamide gels. The heavy component was found to contain the major part of the basic protein, whereas the lighter components contained very little of this protein and mainly less cationic proteins.

The resulting lipid-protein complexes from myelin treated with various amounts of lysophosphatidylcholine ($0.3 \times W$ min– $3.0 \times W$ min) were separated by isopycnic centrifugation in the way described. The results indicate that the heavy component is released initially by small quantities of lysophosphatidylcholine followed by the lighter components on further treatment. A modification of the lighter components occurs on treatment with amounts of lysophosphatidylcholine greater than $1 \times W$ min, but the heavy component is unaffected.

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