of lysophosphatidylcholine the remaining particulate material was rapidly fixed in glutaraldehyde. Electron microscopy indicates that the two phases of the solubilization process involve a structural transition of the myelin membrane.

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Amino Acid Sequence of the Basic Protein of Human Brain Myelin

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The central nervous system contains an unusual basic protein that comprises 30% of the total protein in myelin (Eng, Chao, Gerstl, Pratt & Tavaststjerna, 1968). The sequence of the 170 amino acids in the protein has been determined (Carnegie, 1971). Micro-heterogeneity occurs in the protein as a result of methylation of a single arginine residue. A cytoplasmic enzyme from brain transfers methyl groups to arginine-107 with the formation of ω -N-monomethyl- and ω -NN'-dimethyl-arginine (Baldwin & Carnegie 1971). This methylated arginine occurs adjacent to a non-polar region in the molecule that has been isolated and shown to induce an autoimmune attack by lymphocytes on the central nervous system (Lennon, Wilks & Carnegie, 1970).

The basic protein has been shown to be present in the intraperiod line in the lamellar structure of myelin (Dickinson, Jones, Aparicio & Lumsden, 1970). As the protein has numerous aspartic acid, histidine and serine residues interspersed along the molecule it is speculated that in the non-polar environment within myelin they may be participating in a 'charge-relay system' (cf. Blow, Birktoft & Hartley, 1969). Fernandez-Moran (1959) has suggested that membranes with a lamellar structure may be functioning as semi-conductors.

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Trypan Blue: Reaction with Myelin

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A simpler and more sensitive method for estimating the basic encephalitogenic protein of myelin in myelin fractions than is afforded by conventional extraction and ion-exchange purification techniques was required. It had been claimed (Adams & Bayliss, 1968) that the tetra-azo-tetrasulphonic acid dye, Trypan Blue, in certain conditions combines specifically with this protein and with the nucleohistones in frozen sections of central-nervous-system tissue. Reaction of central-nervous-system myelin, purified in the ultracentrifuge and dissolved in dry chloroform-methanol (2:1, v/v), with the dye dissolved in methanol (to give chloroform-methanol, 1:1, v/v, final composition) leads to a precipitate, which, after the washing away of excess of dye, is dissolved in 0.1 M-sodium dodecyl sulphate solution, and the precipitated dye is determined from the extinction at 585 nm. The method satisfies criteria for quantitative use.

Different myelin preparations have different capacities for reacting with the dye: correlation plots with all lipid components show, as expected, weak negative regressions. Only plots against total nitrogen and total protein show strong positive regressions, and only that against protein gives a regression line that has an intercept not significantly different from zero ($0.7 > P > 0.6$) as well as a strong correlation ($P < 0.001$). [Total protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) on solutions of myelin in 0.1 M-sodium dodecyl sulphate; the values obtained are always equal to, or greater than, those obtained after alkali digestion with or without prior lipid extraction, presumably owing to reduced handling losses and degradation.]

The weight fraction of myelin precipitated with dye is very similar to the total protein content, and the amino acid compositions of the precipitate and the parent myelin are closely similar, even though these may differ considerably for different myelins. The sum of the uncorrected amino acid residue weights accounts for more than 90% of the non-dye part of the precipitates.

Thus, in these restricted conditions, Trypan Blue combines with all proteins and not specifically with basic protein, and the dye-combining power in dry organic solvents may be used as a measure of protein content in proteolipids (that is protein and lipid complexes soluble in chloroform-methanol, 2:1, v/v). The method is reproducible, readily standardized and has a slightly greater sensitivity (*E* units/mg of protein per test) than the standard method of Lowry *et al.* (1951).