When water is introduced to the dye-myelin system the situation becomes more complex. The amount of dye remaining insoluble when the previously prepared precipitates are washed with chloroform-methanol-water (1:4:5, by vol.) is decreased to about half. Preliminary experiments indicate that in these conditions, and when freshly prepared myelin is treated with Trypan Blue in purely aqueous buffer (0.1M-acetate, pH 5.0, the conditions used for histological staining), a fraction of the protein is released into the aqueous medium. It is likely that this protein is a particular molecular species.

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Proteins of the Plasma Membrane Lining the Lumen of the Rat Bladder

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Rat transitional epithelium acts as a barrier to the flow of water between the underlying tissue fluids and the hyperosmotic urine of the bladder. The maintenance of this permeability barrier appears to depend on the luminal plasma membrane (Hicks, 1966). This membrane, when fixed and sectioned for electron microscopy, appears to be asymmetrically thickened in transverse section (Hicks, 1965) and to have a subunit structure in tangential section. Negative-contrast staining of this membrane again indicates that it is composed of subunits and that these subunits are arranged in a hexagonal lattice (Hicks & Ketterer, 1969). When suitable images obtained from negative-contrast stained material are subjected to optical diffractometry it is found that the hexagonal subunits can be resolved into dodecamers composed of subunits of equal diameter, suggesting that they represent spheres approx. 30 Å in diameter (Warren & Hicks, 1970).

The bladder luminal membrane has been separated from homogenates of bladder epithelium by centrifugation through sucrose density gradients (Hicks & Ketterer, 1970). Owing to the fact that it can occur either as a membrane limiting the cell surface or as intracellular fusiform vesicles, thickened membrane occurs in several fractions. The various fractions have been analysed for lipids and amino acid content. The fraction of greatest purity is

unusual in having a higher-than-average proline content (Hicks, Ketterer & Beale, 1969).

The protein content of this fraction has now been analysed by polyacrylamide-gel electrophoresis in an 8M urea-sodium dodecyl sulphate medium containing a mercaptan. At least 12 polypeptide components have been observed, ranging in molecular weight from 10000 to 100000, with most of the material greater than 20000 in molecular weight.

If the particles making up the dodecameric subunits were spherical proteins they might be expected to have a molecular weight of approx. 10000; however, the protein analysis does not suggest that sufficient protein of this molecular weight is present to account for these spheres. If the interaction of protein molecules with sodium dodecyl sulphate can be taken as a model then it is more likely that the proteins in membranes are extended polypeptides rather than globular in shape (Reynolds & Tanford, 1970).

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The Identification of the Site of Action of Dicyclohexylcarbodi-imide as a Proteolipid in Mitochondrial Membranes

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The mode of action of dicyclohexylcarbodi-imide as an inhibitor of mitochondrial oxidative phosphorylation suggests that it reacts specifically and covalently with a membrane component that is essential for energy-conservation reactions (Beechey, Robertson, Holloway & Knight, 1967).

We have presented evidence that a significant proportion of the radioactivity of [¹⁴C]dicyclohexylcarbodi-imide-treated mitochondrial membranes is bound to a chloroform-methanol-soluble protein fraction, i.e. proteolipid (Cattell, Knight, Lindop & Beechey, 1970). Here we show that the radioactivity is associated with a single proteolipid. By