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 8Mμ 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).

This work was made possible by a generous grant from the Cancer Research Campaign.

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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, Roberton, Holloway & Knight, 1967).

We have presented evidence that a significant proportion of the radioactivity of [^{14}C]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

using the methods described by Cattell *et al.* (1970) a crude proteolipid preparation was obtained from mitochondrial membranes that had previously been incubated with 1 nmol of [^{14}C]dicyclohexylcarbodiimide/mg of protein. This was chromatographed on a column of Sephadex LH-20 (Soto, Pasquini, Placido & La Torre, 1969). Two major protein peaks were eluted that contained 81% of the radioactivity recovered from the column. These peaks contained 38.1 and 45.1 nmol of dicyclohexylcarbodiimide/mg of protein. The radioactivity associated with the fractions obtained during this procedure was shown to be neither [^{14}C]dicyclohexylcarbodiimide nor [^{14}C]dicyclohexylurea. Since the radioactivity did not migrate with phospholipid during t.l.c. it is assumed to be associated with the protein component of the extracts. In agreement with the findings of Eichberg (1969), who worked with ox heart-tissue proteolipid, we found cardiolipin to be the major phospholipid in the proteolipid fractions from the Sephadex LH-20 column.

Electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate (Lenard, 1970) showed that a protein of approx. 10000 molecular weight was present in the membranes and was the major component of protein-containing fractions eluted from the Sephadex LH-20 column. The distribution of radioactivity in the polyacrylamide gels was determined. It was found that 90–100% of the radioactivity recovered from the gels was associated with this protein.

These results suggest that dicyclohexylcarbodiimide is reacting with a single membrane proteolipid, thus emphasizing the specificity of dicyclohexylcarbodi-imide as an inhibitor of oxidative phosphorylation. The function of this proteolipid could be either as an intermediate in ATP synthesis or as a factor involved in the maintenance of the mitochondrial membrane in a conformation necessary for energy conservation.

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