Recent studies have shown that the chromaffin granules of the adrenal medulla, which are concerned with the storage and release of catecholamines, are capable of polyphosphoinositide synthesis. The significance of this biosynthesis in relation to the functions of the granules is being investigated.

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The Molecular Organization of Lipids in the Membrane of *Escherichia coli*

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Available evidence shows that the fatty acid composition of membrane lipids of unsaturated fatty acid auxotrophs of *Escherichia coli* depends on the physicochemical properties of the supplemental unsaturated fatty acid (Esfahani *et al.*, 1969, 1971). X-ray-diffraction techniques (Engelman, 1970) have been used to investigate the physical state of the lipids in membranes of an *E. coli* mutant grown on various fatty acids (M. Esfahani, A. R. Limbrick, S. Knutton, T. Oka & S. J. Wakil, unpublished work). Wide-angle X-ray-diffraction measurements show that the lipids in these membranes undergo reversible thermotropic phase transitions. Below the transition temperature the X-ray-diffraction pattern features a sharp ring at 0.42 nm (4.2 Å) due to the close hexagonal packing of the apolar groups of the lipids; above the transition temperature the lipids are in a less organized liquid-crystalline state that gives rise to a diffuse band centred at a Bragg spacing of 0.46 nm (4.6 Å). Transition from the condensed to the liquid state occurs at temperature ranges of 19–29°C for membranes derived from cells grown on oleate or 12-bromostearate, 30–40°C for elaidate-enriched membranes and 36–46°C for membranes containing myristoleate or linolenate. These temperatures are below or about the temperature at which the cells were grown.

The effect of temperature on the proline-uptake and succinate dehydrogenase activities of these membranes has also been studied. Arrhenius plots for these two activities are found to be biphasic.

The temperatures at which discontinuities in the Arrhenius plots for proline uptake were observed are in decreasing order from elaidate (26°C) to oleate (19°C) to linolenate (14°C), indicating a sensitivity of the proline-transport process to the degree of unsaturation in the supplemental fatty acids. Similar sensitivities were reported by Wilson & Fox (1971) for both the glucoside- and galactoside-transport systems. The succinate dehydrogenase activity of the membranes shows discontinuities in the Arrhenius plots at 11°, 23° and 22°C for oleate-, linolenate- and elaidate-enriched membranes respectively. The response of the dehydrogenase to temperature and lipid composition is therefore different from that of proline uptake, and suggests an association with lipids structurally different from those associated with proline transport. Further, the disparities between the transition temperatures obtained from X-ray diffraction of membranes and the temperatures of discontinuities in the Arrhenius plots for proline transport and succinate dehydrogenase lead us to conclude that the distribution of lipids within the membrane is heterogeneous.

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Envelope of Semliki Forest Virus as a Membrane Model

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The different membranes of eucaryotic cells are difficult to isolate in a high state of purity. In addition, their structures are very complex, as evidenced by the multitude of the different constituent molecules. On the other hand, some enveloped viruses contain relatively simple membrane structures. As some of these viruses can be isolated easily in a high state of purity, their use in membrane research has drawn increasing attention (Pfefferkorn & Hunter, 1963; Strauss *et al.*, 1968; Friedman & Pastan, 1969; Klenk & Choppin, 1970; Simons & Kaariainen, 1970; Tiffany & Blough, 1970; Compans, 1971; Harrison *et al.*, 1971).

We have chosen Semliki Forest virus as a membrane model. This virus consists of a nucleocapsid surrounded by a lipoprotein membrane. Electron microscopy shows that the virus obtains its membrane when the nucleocapsid buds through the host-cell plasma membrane (Acheson & Tamm, 1967).

The membrane is isolated from the purified virus by treatment with detergents and subsequent sucrose-density-gradient centrifugation. It contains only one glycoprotein species (mol.wt. 50000), which is synthesized during the virus infection. One virus particle contains about 500 molecules of this glycoprotein. The host (baby-hamster kidney cell line BHK21) plasma membrane, on the other hand, is very complex and contains more than 15 different proteins.

The lipids of the virus membrane consist mainly of free cholesterol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphingomyelin and sialolactosylceramide. The cholesterol/phospholipid molar ratio is 1:1. One virus particle contains about 10000 cholesterol-phospholipid pairs. Each class of the polar lipids is characterized by a specific fatty acid chain composition that is nearly identical in Semiliki Forest virus and host plasma membrane. Virus lipids are synthesized in the cell both before and during the infection. The virus membrane resembles in its lipid composition the host plasma membrane but is different from the endoplasmic reticulum.

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The Role of Lipids in Membrane Structure

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Cytochrome oxidase from mitochondria contains two molecules of haem, a protein (mol.wt. 26000) and one core protein IV (mol.wt. 56000). When free of lipid it forms pentameric clusters of rods 9.0nm (90Å) in diameter made up of 5.0–6.0nm (50–60Å) subunits and has no enzyme activity. On addition of phospholipids it disperses into a membrane sheet with 5.0–6.0nm (50–60Å) subunits separated by dark regions (negative stain). Sections show unit membrane structure 5.0nm (50Å) thick. Freeze-etch

surfaces have a rough appearance, and globular units appear in the sheet structure in cross-section. Membrane formation and activity restoration are saturated at 65 g-atom of phosphorus/mol of haem *a*. At high concentrations excess of lipid can be removed by centrifugation. As lipid content is increased the subunits show increased separation until saturation is reached, indicating that the white globular subunits (negative stain) are the oxidase. Association of lipid and protein in membrane is accompanied by a loss of the i.r. rocking band of lipid at 720cm⁻¹, increase of the thermal stability of enzyme activity, and increase of negative circular dichroism at 225 nm and increase of positive circular dichroism at 200 nm as when a protein is transferred from water to 2-chloroethanol. 1-Anilino-naphthalene-8-sulphonate-fluorescence induced by Triton-dispersed oxidase is 30% decreased by the addition of phospholipid. The results are consistent with a mosaic membrane formed by insertion of protein into the hydrocarbon phase of a phospholipid bilayer. A stabilizing influence of protein on the bilayer is shown, since oxidase plus diolefin form membrane, whereas diolefin alone does not form a bilayer. Formation of a diolefin-oxidase membrane does not restore oxidase activity. Specificity of protein in lipid association to form membrane is shown, since isolated haem *a* protein will form membrane, which shows smaller 3.0–4.0nm (30–40Å) subunits, but core protein IV will not form membrane. Addition of core protein IV to haem *a*-lipid membrane does, however, increase oxidase activity. Thus cytochrome oxidase provides a simple model mosaic membrane in which hydrophobic association of lipid with a functional protein can be studied.

A Soluble Mitochondrial Adenosine Triphosphate Synthetase Complex and its Stimulation by the Membrane Fraction

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This communication reports that a highly purified, soluble, energy-transfer factor preparation carries out an oligomycin- and uncoupler-sensitive ATP-P_i exchange reaction. It has been named the ATP synthetase complex. The exchange activity (up to 35 nmol/min per mg of protein) is dependent on Mg²⁺ and is stimulated threefold by dithiothreitol. The reaction is linear for at least 2 h. The activity is inhibited by low concentrations of oligomycin or carbonyl cyanide trifluoromethoxyphenylhydrazone.