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

By FREDERICK L. CRANE (*Division of Biological Sciences, Purdue University, Lafayette, Ind. 47907, U.S.A.*)

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

By D. RAO SANADI, R. J. FISHER, S. S. KAPLAY, J. C. CHEN and B. P. SANI (*Department of Cell Physiology, Boston Biomedical Research Institute, 20 Stamford Street, Boston, Mass. 02114, U.S.A.*)

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.

These results imply that the binding sites for oligomycin and carbonyl cyanide trifluoromethoxyphenylhydrazone are on the ATP synthetase. *p*-Chloromercuribenzenesulphonate also inhibits the exchange reaction, which points to the involvement of a thiol group in the exchange reaction. The energy-coupling factor D resolved from the complex is also inhibited by *p*-chloromercuribenzenesulphonate in its activity (stimulating ATP-dependent NAD⁺ reduction by succinate on addition to the urea-depleted submitochondrial particle).

The ATP-P_i exchange activity and 95% of the protein remain in the supernatant fraction when the synthetase is centrifuged at 105000g for 60min in 60mM or higher concentrations of phosphate buffer. In 10mM-phosphate buffer a significant fraction of the protein sediments and the activity in the supernatant liquid is considerably diminished. This

suggests aggregation of the ATP synthetase in solutions of low salt concentration.

The ATP synthetase stimulates the ATP-P_i exchange activity of urea-depleted submitochondrial particles. The specific activity is increased more than tenfold in the presence of urea-depleted submitochondrial particles. This may be due to allosteric activation of the ATP synthetase when it is bound to the membrane or to some necessary component that is provided by the membrane.

The ATP-ADP exchange rate is at least twice the ATP-P_i exchange rate, and this would indicate the formation of X~P as an intermediate in ATP synthesis. These data suggest that the soluble ATP synthetase complex contains X~C. This represents the first report of a soluble ATP-P_i exchange activity related to mitochondrial oxidative phosphorylation.

SYMPOSIUM ON PLANT SCIENCES

The Ecological Significance of 'Unusual' Amino Acids in Plants

By E. A. BELL (*Department of Botany, University of Texas at Austin, Austin, Tex. 78712, U.S.A.*)

Twenty amino acids are found universally distributed as protein constituents in all living organisms. In addition to these a few others occur frequently as metabolic intermediates. The great majority of natural amino acids are more limited in their distribution, however, and relatively little is known of their metabolism or significance in the organisms that synthesize them.

The higher plants are a rich source of such 'unusual' amino acids: 60 for example have been isolated from or identified in species of one single family, the Leguminosae (Bell, 1971). Certain of the 'unusual' amino acids that have been isolated from this and other plant families are known to be toxic to other species of plants, to micro-organisms, to insects or to animals. Bell & Janzen (1971) have suggested that the high concentrations of L-3,4-dihydroxyphenylalanine (L-dopa) and 5-hydroxy-L-tryptophan that occur in species of *Mucuna* and *Griffonia* may serve to protect the seeds of these species from insect attack. This and other possible roles of 'unusual' amino acids will be discussed.

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Studies on the Metabolism of *sym*-Homospermidine in Sandal (*Santalum album* L.)

By R. KUTTAN and A. N. RADHAKRISHNAN (*Wellcome Research Unit, Christian Medical College Hospital, Vellore-4, Tamil Nadu, India*)

Although the occurrence of polyamines, spermine and spermidine, in the animal kingdom has been established for a long time (Tabor & Tabor, 1964), only limited reports are available on their occurrence in plants (Smith, 1970). A new polyamine, *sym*-homospermidine, has been isolated from sandal leaves and it is present in a free state in high concentrations: 0.5-1.5% of the dry leaves (Kuttan *et al.*, 1971). The present communication deals with the study of the biosynthesis of this polyamine in the sandal plant by using radioactive amino acids as precursors. The procedure for the infiltration of amino acids into the leaves under vacuum has been described previously (Kuttan & Radhakrishnan, 1970). For determination of radioactivity homospermidine was isolated by using a small Dowex 50 (X8; H⁺ form) ion-exchange column and eluting with different concentrations of HCl. With DL-[¹⁴C]ornithine as the precursor nearly 7.5% of the initial radioactivity (assuming only the L-isomer was active) was incorporated into homospermidine. When L-arginine was used nearly 5.5% of the initial radioactivity was in the homospermidine. However, considering the pool sizes of the respective amino acids, arginine was nearly ten times as effective as ornithine. Proline and glutamic acid also served as precursors. With L-[¹⁴C]arginine as the precursor the radioactivity in homospermidine was significantly