Studies of the fluidity (Cogan et al., 1973; Shinitzky & Inbar, 1974) of E. carotovora membranes using fluorescent probes gave results underlining their dissimilarity. Membrane preparations were treated with perylene or 1,6-diphenylhexa-1,3,5-triene separately, and the polarization of fluorescence was recorded at the appropriate wavelengths (see Table 1). The results obtained with each probe indicated a higher polarization value, suggesting lower micro-viscosity and higher fluidity, in the inner as opposed to the outer membrane. In both cases total membrane preparations have intermediate polarization values. The proportion of phosphatidylethanolamine in each membrane together with the lower protein to lipid ratio (see Table 1) are consistent with the greater fluidity of the inner membrane.

From the results reported here a tentative model showing the spatial distribution of inner and outer membrane components can be constructed.

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Mitochondrial Reduced Nicotinamide–Adenine Dinucleotide Dehydrogenase is Transmembranous

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Transmembranous organization of the electron-transport components of mitochondrial NADH dehydrogenase has been proposed from experiments showing inhibition of the enzyme in both mitochondria and submitochondrial particles by impermeant inhibitors (Harmon & Crane, 1974). Looping of the pathway of reducing equivalents across the membrane has also been proposed to account for proton translocation linked to NADH–ubiquinone oxidoreductase (Lawford & Garland, 1972; Ragan & Hinkle, 1975).

As described below, we have found that certain of the constituent polypeptides of the enzyme are exposed on either side of the membrane, which would lend support to claims for transmembrane electron or hydrogen transfer.

Complex I (NADH–ubiquinone oxidoreductase; Hatefi et al., 1962) is a lipoprotein enzyme from bovine heart which represents mitochondrial NADH dehydrogenase in substantially unmodified form (Ragan, 1976a). We have raised antibodies in rabbits to intact Complex I and the iron–protein fraction derived from it by chaotropic resolution (Hatefi & Stempel, 1969). The latter fragment consists primarily of polypeptides of mol.wt. 75000, 49000 and 30000 and is much simpler in composition than the holo-enzyme (Ragan, 1976b; Crowder & Ragan, 1977). The presence of antibodies was established by double immunodiffusion and revealed that both antisera cross-reacted with either antigen. Neither antisera inhibited NADH–ubiquinone oxidoreductase or NADH–ferricyanide oxidoreductase activities at concentrations sufficient to immuno-precipitate the enzyme completely. Antibody titres were therefore measured by
Iodination of mitochondria and submitochondrial particles (5 mg of protein/ml of sucrose/Tris buffer) was performed with carrier-free $^{125}$I at a concentration of 10 $\mu$Ci/mg of protein as described by Ragan (1976b). The reaction was arrested by addition of 25 mM-EDTA and the labelled membranes were collected and washed by repeated centrifugation and resuspension in 0.67 M-sucrose/50 mM-Tris/HCl, pH 8.0. Submitochondrial particles (a and b) or mitochondria (c and d) (2 mg of protein/ml) were solubilized with 0.8% sodium deoxycholate and 0.8 M-KCl at 4°C. After centrifugation at 50000g for 40 min, the supernatants were treated with Triton X-100 to a final concentration of 1% (w/v). After incubation overnight at 4°C, the extract was re-centrifuged, and 3.0 ml portions were incubated with 1.2 ml of anti-(Complex I) serum (a and c) or control serum (b and d) for 16 h at 4°C. The precipitates were collected by centrifugation at 40000g for 10 min and washed three times by resuspension in sucrose/Tris buffer containing 1% (w/v) Triton X-100 and recentrifugation. Samples (1 mg of protein/ml) were dissociated by incubation at 100°C for 2 min with 1% (w/v) sodium dodecyl sulphate and 1% (v/v) 2-mercaptoethanol. Discontinuous gel electrophoresis was performed on 12 cm gels containing 12.6% (w/v) acrylamide and 0.34% bisacrylamide, pH 9.5, as described by Crowder & Ragan (1977).
titrating Complex I in 0.5% Triton X-100 with antiserum and determining the residual NADH–ferricyanide oxidoreductase activity in the supernatant after centrifugation. Control sera gave no precipitation. Under our conditions, 1 mg of Complex I protein was quantitatively precipitated by 0.15 ml of anti-(Complex I) serum or 0.75 ml of anti-(iron–protein) serum, agglutinated submitochondrial particles. NADH–ferricyanide oxidoreductase activity was also precipitated by either antiserum from detergent-solubilized mitochondria or submitochondrial particles to a maximum extent of 80%. The immunoprecipitates from such experiments were analysed by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. The profiles of the immunoprecipitates of detergent-solubilized Complex I, bovine heart mitochondria and submitochondrial particles cross-reacted with either anti-(Complex I) serum or anti-(iron–protein) serum were very similar to each other and to Complex I. Apart from the interference with the profile by the heavy and light chains of immunoglobulin G, the consistent differences were the complete absence of the 87000-mol.wt. polypeptide from some gels and the large decrease in the relative content of the 42000-mol.wt. polypeptide. The former has been shown to be nicotinamide nucleotide transhydrogenase which co-purifies to some extent with Complex I (Höjeberg & Rydström, 1977). The latter polypeptide may be the same as that which can be removed from Complex I by differential centrifugation (Dooijewaard et al., 1978). Although this polypeptide is not required for NADH–ferricyanide oxidoreductase, we cannot exclude the possibility that it may be required for ubiquinone reductase activity, since the detergent treatments used cause loss of ubiquinone reductase activity. Minor bands of high molecular weight in the immunoprecipitates were also found in the very small precipitate formed with control serum. Since all the polypeptides of purified Complex I (except those noted) were co-precipitated by anti-(iron–protein) serum, we conclude that they are all subunits of the enzyme.

By using lactoperoxidase-catalysed iodination (Phillips & Morrison, 1971), intact mitochondria and submitochondrial particles were labelled with $^{125}$I. The distribution of radioactivity between the polypeptides of intact mitochondria was essentially the same as that which can be removed from Complex I by differential centrifugation (Dooijewaard et al., 1978). Although this polypeptide is not required for NADH–ferricyanide oxidoreductase, we cannot exclude the possibility that it may be required for ubiquinone reduction, since the detergent treatments used cause loss of ubiquinone reductase activity. Minor bands of high molecular weight in the immunoprecipitates were also found in the very small precipitate formed with control serum. Since all the polypeptides of purified Complex I (except those noted) were co-precipitated by anti-(iron–protein) serum, we conclude that they are all subunits of the enzyme.

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A Salt-Induced Reversible Co-operative Transition in the Pig Thyrotropin Receptor

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The binding of thyrotropin ('TSH') to membrane-bound and soluble receptors is inhibited by NaCl, and we have investigated this effect by using 125I-labelled bovine thyrotropin and pig thyroid membranes (Dawes et al., 1978). Membrane preparations and labelled and unlabelled thyrotropin were dissolved in 10mM-Tris/o.1 % bovine serum albumin, pH7.4. NaCl was the only variable in the incubation, which proceeded for 16h at 23°C. Bound and free hormone were separated by centrifugation.

In the range 0-200mM-NaCl, the specific binding of thyrotropin was maximal and constant below 40mM, and fell from 26 to 6 % at the higher concentrations. The midpoint of the transition occurred at approx. 90mM-NaCl. Membranes that had been resuspended and allowed to remain for 90min in 200mM-NaCl in the final stage of their preparation, before being diluted to 20mM, gave a nearly identical curve for specific thyrotropin binding at different NaCl concentrations, indicating that the NaCl-induced change from high thyrotropin binding to low binding is fully reversible. Analysis of the binding of thyrotropin at NaCl concentrations in the range 33-117mM (Scatchard, 1949) showed that the salt does not affect the association constant for thyrotropin ($K_{ass} = 2.8 \times 10^9 \text{M}^{-1}$), but that the binding capacity of the membrane preparation is diminished at higher concentrations. There was no evidence for a lower affinity site at any concentration within this range.

This reversible effect of NaCl on the thyrotropin-binding capacity of thyroid membranes can be considered as a salt-induced transition between active and inactive forms of the receptor:

$$A (\text{active}) \Rightarrow I (\text{inactive})$$

where thyrotropin binds solely to the active form of the receptor with $K_{ass} = 2.8 \times 10^9 \text{M}^{-1}$. The equilibrium constant for the transition is obtained from the specific % binding ($B$) and

$$K_{eq} = \frac{B_A - B}{B - B_I}$$

where $B_A$ and $B_I$ are the binding ability of the membrane preparations measured from the plateau regions on either side of the thyrotropin-binding transition.