to any given $H^+\cdot P$ ratio. If the ratio is 3 and the permittivity 20, the distance is approximately 0.3 nm. Unlike the electrochemical parameters, $\psi_0$ values do not depend on a cumulative ion effect, one proton alone at the right distance from a fixed charge will be at the potential required for synthetic activity. On these terms the rate of synthesis is determined by the number of charge interactions through the turnover rate of the fixed-charge cycle (Archbold et al., 1979, 1980b; Malpress, 1981b) which provides the dynamic link between $\psi_H$ and its conversion into conformational change and intramembranous proton path activation.

The coulombic hypothesis is a ‘local energized proton’ interpretation of mitochondrial energy transduction, and is therefore closer to the views of Williams (1978) than to those of Mitchell (1979). But in many respects it may be radically differentiated from Williams’ prototype model, notably in its acceptance of the evidence for transmembrane proton pump activity as opposed to intermembrane proton release, in its explicit involvement of newly generated negative fixed-charges and of the diffuse double layer, by the electrostatic mechanism which it sees as the first step in the utilization of the energized protons in contrast to the kinetic ‘hydrating-dehydrating’ process of the prototype view (Fig. 3), and in its interpretation of Ca"+ movements.

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A structural basis for mosaic protonic energy coupling

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The chemiosmotic concept of Mitchell (1961) has proved to be of great predictive and practical value in the analysis of free-energy transduction. In order to make maximal use of the predictive value, we have incorporated this concept in a mosaic non-equilibrium description of oxidative phosphorylation (Westerhoff & Van Dam, 1982). This description relates the rates of different processes in mitochondria (or bacteria) to their driving forces (free-energy differences), as well as to the activities and kinetic properties of the enzymes involved.

Some experimental tests of the mosaic non-equilibrium thermodynamic description gave results that fitted with a simple model of chemiosmotic energy transduction in mitochondria, with $H^+$ ions as the central coupling agent. For instance, the prediction that the dependence of the rate of phosphorylation on the rate of oxygen uptake should be linear and steeper in the presence of a protonophore than in its absence, could be verified (Van Dam et al., 1978).

However, some predicted relations did not stand up to experimental test. An important anomaly is the finding that in State 4, i.e. at the maximal degree of phosphorylation of the adenine nucleotides, the ratio $\Delta G_{/\Delta H}$ is not independent of $\Delta H_{/\Delta H}$ (Wischmann et al., 1975; Azzone et al., 1978; Westerhoff et al., 1981). The ratio is consistently found to increase at low values of $\Delta H_{/\Delta H}$. Such a finding cannot be explained by the presence of slips in the proton pumps, since this would actually lead to a decrease in the measured ratio.

A second anomaly is found when so-called double-inhibitor titrations are performed: under some conditions partial inhibition of the oxidative proton pump does not lead to the expected diminished sensitivity of phosphorylation to an inhibitor of the ATPase or vice versa (Baum et al., 1971; Hitchens & Kell, 1982; Venturioli & Melandri, 1982; Westerhoff et al., 1983). Respiratory chain and ATPase complexes behave as if they are functionally coupled in such a way that energy delivered by one complex is available to one other complex only. In terms of the proton as energy-transducing agent, there is no equilibration throughout the bulk phases inside or outside the mitochondria.

A physical counterpart of the functional compartmentalization is seen in the model of the mitochondrial cristae as proposed by Sjöstrand (1978). According to this model, the cristae membranes are closely apposed. We now specify that protons pumped to the (virtual) outside cannot escape, except to a neighbouring complex. We have obtained evidence that there is indeed a correlation between the "localized" behaviour of the protons and the putative dilation of the cristae, for instance by comparing mitochondrial cristae isolated in the presence or absence of a high molecular weight osmotic support, such as polyvinylpyrrolidone. The surprising conclusion that the localized protons are in contact with the external medium is schematically depicted in Fig. 1.

In order to combine these different aspects in a coherent picture of energy transduction, a new model was proposed: mosaic protonic coupling (Westerhoff et al., 1983; cf. H. V. Westerhoff, B. A. Melandri, G. Venturioli, G. F. Azzone & D. B. Kell, unpublished work). This model allows us to make a better quantitative description in terms of mosaic non-equilibrium thermodynamics. As an example we have calculated the expected variation of $\Delta G_{/\Delta H}$ with $\Delta H_{/\Delta H}$ when the proton permeability of the mitochondrial inner
cases, due to the fact that both the protonophorous were added. The same relationship was obtained in both uncoupler and the respiratory inhibitor affect the dis-

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biochemical society transactions

Lateral diffusion, collision and efficiency of oxidation-reduction components in mitochondrial electron transport

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Recent observations from this laboratory (Hackenbrock, 1981), which reveal that the redox components of the mitochondrial inner membrane are freely diffusible of one another, are important for several reasons: they characterize the structure of the inner membrane as a fluid rather than solid state membrane, they characterize the physical

Table 1. Rates of lateral diffusion for the major inner membrane redox components

<table>
<thead>
<tr>
<th>Redox component</th>
<th>Lateral diffusion coefficient (cm²/s)</th>
<th>Theoretical diffusion-controlled collision frequency (collisions/s per redox partner)</th>
<th>Experimental turnover number (turnovers/s per redox partner)</th>
<th>Collision efficiency = Column 2/Column 3 (collisions/turnover) and (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>4 x 10⁻¹⁰</td>
<td>1 36274</td>
<td>3360</td>
<td>11 x (9.1)</td>
</tr>
<tr>
<td>Complex II</td>
<td>4 x 10⁻¹⁰</td>
<td>1 7710</td>
<td>1680</td>
<td>4.6 x (22)</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>3 x 10⁻⁹</td>
<td>1 707</td>
<td>3</td>
<td>26.7</td>
</tr>
<tr>
<td>Complex III</td>
<td>4.4 x 10⁻¹⁰</td>
<td>1 1555</td>
<td>700</td>
<td>2.2 x (45)</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>1.9 x 10⁻⁹</td>
<td>1 1561</td>
<td>60</td>
<td>4.6 x (21.8)</td>
</tr>
<tr>
<td>Complex IV</td>
<td>3.7 x 10⁻¹⁰</td>
<td>1 275</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>