Review Letter

ABSENCE OF A METABOLICALLY INDUCED ELECTRICAL POTENTIAL ACROSS THE MITOCHONDRIAL SEMIPERMEABLE MEMBRANE

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It is not generally recognized that the results presented by Mitchell and Moyle [1] on the distribution of K^+ in isolated rat liver mitochondria in the presence of valinomycin constitute clear evidence for the absence of an electrogenic pump. Upon the initiation of metabolism, in the presence of valinomycin, the H^+ efflux corresponds to the uptake of K^+ (figs. 5–8, [1]). Under these conditions, the negative charge generated in the inner mitochondrial phase increases with time and corresponds quantitatively to the K^+ taken up in the electroneutral exchange. At steady state, the internal negative charge, the corresponding high internal concentration of K^+ and the relatively high permeability to K^+ are in harmony with a classical Gibbs-Donnan distribution (e.g., see [2]) as it holds, for example, in muscle. (e.g., see [3]). This situation quantitively explains the distribution of K^+ observed by Mitchell and Moyle [1] and by others in subsequent studies (e.g. [4–6]). They are not consistent with the electrogenic pump model of the chemiosmotic hypothesis.

The principles involved can be most easily presented by an actual example. In the experiments of Mitchell and Moyle (figs.5 and 7 of [1]) the total efflux of H^+ corresponds to about 30 μeq./g mitochondrial protein and hence to the same amount of internal negative charge (X^-) balanced by a corresponding K^+ influx. Using the mitochondrial volume assumed by Mitchell and Moyle (0.4×10^{-3} 1/g mitochondrial protein), this amount corresponds to a concentration of 75×10^{-3} of X^- equivalents/liter. The Donnan ratio (r) or internal, over external K^+ concentration can be readily calculated using the X^- concentration and the external concentration of K^+ $(K^+)_0$ given by Mitchell and Moyle in item III of their table ($(K^+)_0 = 3.4 \times 10^{-4}$ M), since,

$$r = \frac{(X^-)}{2(K^+)_0} + \left[\frac{(X^-)^2}{4(K^+)_0^2} + \frac{(A)_0}{(K^+)_0}\right]^{1/2} \quad (1)$$

In Eqn [1], $(A)_0$ is the external concentration of an anion which follows a Donnan distribution. Under the conditions of the experiments $(A)_0/(K^+)_0$ is negligible and hence the Donnan ratio is expressed by Eqn 2.

$$r = (X^-)/(K^+)_0 \quad (2)$$

The calculated value of r is approximately 220. The value used by Mitchell and Moyle to calculate a presumed potential of −139 mV for these conditions is about 230. The calculation leaves no question that the experimentally obtained distribution is quantitatively explained in the absence of a membrane potential. Other results summarized in table 1 are consistent with this view. The observed distribution of Rb^+ and Ca^{2+} in the presence of valinomycin should result in the same Donnan ratios for these ions as well, as in fact observed [8,9].

Paradoxically, in mitochondria in the presence of valinomycin, the higher internal K^+ concentration and the high K^+ permeability (in this case induced by valinomycin, see [5]) should produce a K^+ diffusion potential, as it does in intact muscle (e.g., see [3]). This potential should be approximated by the Nernst equation. A K^+ diffusion potential was suggested earlier [10] to explain, on the basis of the chemiosmotic hypothesis, the phosphorylation observed in

Table 1

Experiment from [1]	$-59 \times \text{Log}(X^-)/(K^+)_o$	Apparent membrane potential [1]
I	−189	−199
II	−183	−171
III	−142	−139
IV	− 73	− 83

Agreement between the values predicted in the absence of an electric potential and the membrane potentials estimated by Mitchell and Moyle (table, ref. 1). Our calculation assumes a reaction vessel of 3.2 ml, 7 mg of mitochondrial protein per ml and 0.4 ml of internal mitochondrial space per g of mitochondrial protein. X^- was estimated from the pH changes reported by Mitchell and Moyle and the K^+ concentrations are also those reported in table, ref. 1.

isolated mitochondria in the presence of valinomycin and the efflux of K^+ [11]. However, it would not be the result of an electrogenic pump and it would be irrelevant to the mechanism of oxidative phosphorylation.

The results of Bakeeva et al. [12] and Grinius et al. [13] on the exchange of organic cations or anions in mitochondria and submitochondrial particles can also be interpreted on the basis of H^+/ion counter or co-exchange. These results will be discussed in a separate communication.

The misunderstanding of these simple theoretical principles are responsible for the persistance of the idea that mitochondrial membrane potentials play a role in oxidative phosphorylation.

Studies using electrofluorimetric dyes [14,15] will be dealt with in a separate communication.

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