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THE DIRECTION OF POLARITY OF THE MITOCHONDRIAL TRANSMEMBRANE POTENTIAL

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SUMMARY

The concentration of various anions in the sucrose impermeable space of rat-liver mitochondria relative to the applied concentration can be used to calculate an equilibrium potential if one supposes that the anion distribution is passive. Values so calculated for anions of different net charge are about 30 mV, with the interior positive. The relative merits of the hypotheses that an internal positivity is generated by active cation movement or MITCHELL's chemiosmotic generation of an internal negativity coupled with hydroxyl-for-anion exchanges are examined.

INTRODUCTION

It has been proposed that both oxidative phosphorylation[1–3] and ion transport[1–4] in mitochondria are driven by an energy-linked proton pump which keeps the mitochondrial interior both negative and basic with respect to the suspending medium. Most of the supporting data are equally compatible with an energy-linked electrogenic cation pump which renders the mitochondrial interior positive and secondarily basic by the electrostatically propelled ejection of protons down an electrochemical gradient[5,6]. Both mechanisms predict a metabolically-linked internal basicity, and this has been supported by a number of methods, although each of the techniques employed[7–9] remains open to criticism (*cf.* MITCHELL, MOYLE AND SMITH[10]), but the 'chemiosmotic' hypothesis of MITCHELL has also been much criticised[11].

Although MITCHELL, MOYLE AND SMITH[10] have assumed that K^+ equilibrates passively across the membrane of valinomycin-treated mitochondria and have calculated the internal potential as —130 mV, it seems equally valid to consider that it is the anions which are actually in passive equilibrium with the membrane potential. This possibility was examined for a selection of anions of varied charge, n, by seeing whether their intra–extramitochondrial equilibrium gradients would give rise to similar membrane potentials, ΔE, as predicted by the Nernst equation:

$$\Delta E = RT/nF \ln \frac{[A^{n-}]_{\text{internal}}}{[A^{n-}]_{\text{external}}}$$

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METHODS

These were exactly as described by HARRIS AND VAN DAM[13].

RESULTS

Regardless of charge, the various anions in Table I give rise to similar values for ΔE of ca. +30 mV which is consistent with the conditions of equilibrium with the membrane potential. It is interesting to note that the appreciable gradient increase upon adding valinomycin in the presence of triply charged citrate corresponds to a relatively small change in membrane potential.

TABLE I

MITOCHONDRIAL ACCUMULATION OF REPRESENTATIVE IONS

Mitochondria (5 mg protein/ml) were incubated at room temperature in a medium consisting of: 5 mM KCl, 20 mM KCl, 20 mM Tris chloride, 250 mM sucrose, and 1 mM Tris salt of the indicated ^{14}C-labelled anion. After 2 min incubation the mitochondria were separated from the medium by centrifuging through silicone in a microcentrifuge. The non-specific space + adhering medium was estimated from parallel experiments conducted with ^{14}C-labelled sucrose[13].

Anion	*Concn. in sucrose-impermeable space (mM)*	*Calculated equilibrium membrane potential (mV)*
Monovalent		
Acetate	4.1	+35
Glutamate	4.4	+37
Divalent		
Succinate	9.1	+27.5
α-Oxoglutarate	9.8	+29
Trivalent		
Citrate	19	+23
Citrate + valinomycin	22	+26

According to the proton pump mechanism (*i.e.* interior negative), the anions could not be in passive equilibrium with the membrane potential, but must accumulate against a concentration gradient[12,13] *via* a diffusion exchange mechanism driven by the transmembrane hydroxyl gradient (*cf.* CHAPPELL AND HAARHOF[14]). Such an exchange would not be affected by the transmembrane potential, since at the final steady state both species of like charged ions would be distributed according to the relationship:

$$\frac{[OH^-]_{internal}}{[OH^-]_{external}} = \left(\frac{[A^{n-}]_{internal}}{[A^{n-}]_{external}}\right)^{1/n}$$

which may be derived from the Gibbs equation, assuming both charged species are in equilibrium. It is interesting to note that nigericin has been described to act in analogous fashion as a cation–proton diffusion exchange carrier in a variety of biological and model systems[15].

If one transforms the Nernst equation into the exponential form:

$$\exp\frac{F\Delta E}{RT} = \left(\frac{[A^{n-}]_{\text{internal}}}{[A^{n-}]_{\text{external}}}\right)^{1/n}$$

its parallelism with the last equation vis-a-vis the parameter n becomes clearer.

It thus emerges that the data of Table I are equally compatible with the proton-pump, exchange diffusion mechanism of anion accumulation, since both equations predict that for a given gradient the greater the charge, n, of the anion, the greater its concentration within the mitochondria.

A more definitive choice between the outward directed proton pump and the inward directed cation pump can be based on data already published. In contrast to the transient respiratory burst produced by the addition of a limited load of Ca^{2+} (refs. 16, 17), valinomycin-treated mitochondria continue to respire at elevated rates even after net K^+ uptake ceases[5,18]. Tracer studies have indicated that under these conditions, in the presence of the energy source required for maintaining the steady level of accumulated K^+, the influx of K^+ remains approximately at the initial high value[19].

In order to reconcile the energy dissipation of the steady-state flux of K^+ with the proposition of an energy-driven proton pump, MITCHELL has invoked an *ad hoc* cation–proton diffusion exchange system[1,2]. Independent evidence for the existence of this device for coupling an undetectable proton flux to the isotopically established steady-state flux of K^+ has not been provided[19]. A pre-existing cation–proton exchange diffusion system appears incompatible with the known low K^+ permeability of native mitochondria[19] and with their marked response to nigericin, which experimentally does introduce a cation–proton diffusion exchange system analogous to the one postulated[15,20].

It still has to be considered whether the movement of cations is coupled to the movement of anions[15], so that the work load imposed by the steady-state flux of K^+ actually derives from the accompanying energy-driven anion flux (*cf.* VAN DAM AND SLATER[21]). This seems unlikely, since the gradients against which K^+ can be moved[22] greatly exceed those anion gradients which have been measured[12,13]. Further, Fig. 1 shows that the valinomycin-induced uptake of K^+ preceeds the uptake of the associated anion.

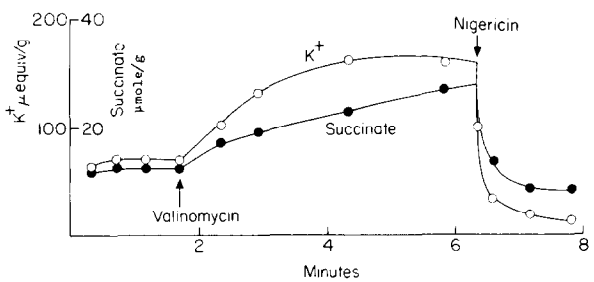

Fig. 1. Mitochondrial contents of K^+ and succinate before, during an induced uptake of K^+ and after discharge with nigericin. The medium contained: Sucrose 300 mM, KCl 10 mM, Tris–HCl (pH 7.4) 20 mM, ascorbate 2 mM, tetramethyl-*p*-phenylenediamine 10 μM, antimycin 1 μg/ml, [^{14}C]succinate and carrier to 1 mM, protein 10 mg/ml. The mitochondria were spun through silicone into $HClO_4$ which was then used for analysis for K^+ and assays of radioactivity. Valinomycin used at 8 μg/g and nigericin at 25 μg/g.

DISCUSSION

A simple explanation for the energy-linked accumulation of K^+ by mitochondria is the existence of an electrogenic, inwardly directed cation pump analogous to the outwardly directed Na^+ pump of nerves and muscles[23]. This pump would translocate several K^+ per equivalent ATP[22] and render the mitochondrial interior positive with respect to the medium. The resulting membrane potential would induce anions to accumulate passively within the mitochondria, or discharge other cations. The proposition that the mitochondrial interior is held positive when energy is available has been mentioned by SKREDE[24] as a possible explanation of his finding that the positively charged cystamine ion is only excluded in the energized state.

According to the arguments offered here, the observed reversibility of the energy-coupled cation pump would be more likely to derive its energy for synthesizing ATP[25] and reversing electron transport[26] directly from the movement of K^+ down the electrochemical gradient set up when the electrogenic process is inhibited rather than from a shift of internal pH (*cf.* GLYNN[27]).

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