MOVEMENTS OF H^+, K^+, AND NA^+ DURING ENERGY-DEPENDENT UPTAKE AND RETENTION OF CA^{++} IN RAT LIVER MITOCHONDRIA

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The energy-linked accumulation of one molecule of Ca^{++} by respiring mitochondria is accompanied by extrusion of one molecule of H^+ to the suspending medium (Saris, 1963; Chappell, Cohn, and Greville, 1963; Engstrom and DeLuca, 1964; Drahota, Carafoli, Rossi, Gamble, and Lehninger, 1965). The question arises as to whether this energy-dependent Ca^{++}-H^+ exchange is accompanied by net stoichiometric movements of Na^+ or K^+ to preserve intramitochondrial electroneutrality. K^+ and Na^+ are already known to influence markedly the massive accumulation of Ca^{++} and phosphate (Vasington and Murphy, 1962; Carafoli, Rossi and Lehninger, 1964); furthermore, K^+ may be accumulated by mitochondria under some circumstances (cf. Moore and Pressman, 1964; Rasmussen, Fischer, and Arnaud, 1964; Chappell and Crofts, 1965).

This communication reports measurements on the effect of external Na^+ and K^+ on the rate and stoichiometry of the respiration-dependent uptake of Ca^{++} and linked extrusion of H^+ by rat liver mitochondria, the changes in intramitochondrial Na^+ and K^+ occurring during the energy-dependent Ca^{++}-H^+ exchange, and the effect of Na^+ and K^+ on the efflux of Ca^{++} during the dynamic steady-state retention of Ca^{++} which follows pulsed uptake of Ca^{++} (cf. Drahota, et al, 1965). The experiments were carried out in the absence of added phosphate and adenine nucleotides in the medium, under limited-loading conditions in which respiratory control was retained and oxidative phosphorylation mechanisms unimpaired (cf. Rossi and Lehninger, 1964; Drahota, et al, 1965).

Experimental details. Respiration was measured with the oxygen electrode and Ca^{++} uptake by an isotopic method (Carafoli, Rossi and Lehninger, 1964). H^+ movements were followed with a sensitive recording pH meter; internal standards of NaOH or HCl were employed (Drahota, et al, 1965). Na^+ and K^+ were determined by flame photometry. Rat liver mitochondria were isolated and washed in media of 0.25 M sucrose containing no added Na^+ or K^+.

Results. Data in Fig. 1 show that neither the rate of respiration activated by Ca^{++} nor the stoichiometry between Ca^{++} uptake and extra oxygen uptake (Rossi and Lehninger, 1964) is significantly different

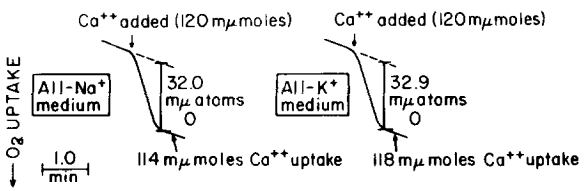

Fig. 1 Effect of all-Na^+ vs. all-K^+ media on Ca^{++} accumulation. Media contained 128 mM NaCl or KCl, 4.0 mM K- or Na-succinate, 2.0 mM tris.HCl pH 7.4 and rat liver mitochondria (4.0 mg protein) in 2.0 ml. T=20°.

in all-Na^+ vs. all-K^+ media. Furthermore, as is shown in Table 1, the molar ratio between Ca^{++} uptake and H^+ output in such experiments is essentially identical at 1.13 in both all-Na^+ and all-K^+ media. The data also show that there is no net stoichiometric efflux (or influx) of K^+ during Ca^{++} uptake by mitochondria in an all-Na^+ medium; conversely, there is no net efflux or influx of Na^+ when Ca^{++} uptake occurs in an all-K^+ medium. It may be concluded that neither Na^+ nor K^+ is required in the medium for the active, respiration-linked uptake of Ca^{++} and the coupled output of H^+, and that there is no significant net movement of

Table 1. Ion movements during active Ca^{++} accumulation. Details exactly as in Fig. 1, except that Ca^{++} was added at 45 mμmoles per mg protein.

	All-Na^+ medium mμmoles per mg	All-K^+ medium mμmoles per mg
Ca^{++} uptake	42	44
H^+ output	37	39
K^+ output	<3	-
Na^+ output	-	<1

Na^+ or K^+ between mitochondria and the medium that is stoichiometrically coupled to the uptake of Ca^{++}.

These findings thus show that if the intramitochondrial milieu is maintained essentially at electroneutrality following active uptake of Ca^{++}, then this condition must be brought about by stoichiometric efflux of some mitochondrial cation(s) other than Na^+ or K^+ (such as Mg^{++}) or by stoichiometric influx of some other anion from the medium, such as Cl^-. Preliminary measurements indicate that mitochondrial Mg^{++} remains essentially constant during Ca^{++} uptake and H^+ output. Inorganic phosphate, when it is present in the medium, is known to enter mitochondria during uptake of Ca^{++} (cf. Chappell et al, 1963; Rossi and Lehninger, 1964); similarly, adenine nucleotides, when present, are accumulated with Ca^{++} (Carafoli and Lehninger, 1964; Carafoli, Rossi and Lehninger, 1965). However, phosphate and adenine nucleotides were not added to the medium in these experiments, and thus uptake of these anions cannot account for balancing of electrical charges during the energy-linked Ca^{++}-H^+ exchange.

Na^+ and K^+ do, however, have profoundly different effects on the rate of efflux of accumulated Ca^{++}. In Fig. 2 are shown traces of the H^+ movements and analyses of mitochondrial Ca^{++} in experiments in which the uptake, steady-state retention, and loss of $^{45}Ca^{++}$ were measured in all-Na^+ vs. all-K^+ media. It is seen that in the all-Na^+ medium, which earlier work had shown to be optimal for Ca^{++} uptake in massive Ca^{++}-loading (Carafoli, Rossi and Lehninger, 1964), the Ca^{++} is taken up rapidly and

H^+ appears in the medium. The Ca^{++} is then retained in the mitochondria

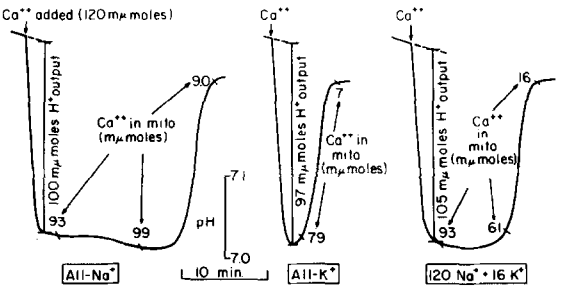

Fig. 2. Effect of Na^+ and K^+ on the efflux of accumulated Ca^{++}. The all-Na^+ and all-K^+ media contained total of 136 mM Na^+ or K^+, 4 mM succinate, 2 mM tris.HCl, and mitochondria (2 mg protein per ml). The mixed medium contained 120 mM Na^+ and 16 mM K^+. Data in mμmoles per mg protein.

for an extended period (20 min) in a dynamic steady-state in which active uptake of Ca^{++} is counter-balanced by constant efflux (Drahota et al, 1965). In this period, the rate of oxygen uptake remains at the resting or State 4 level. The molar ratio Ca^{++} uptake:H^+ output in the Ca^{++} uptake phase was 0.95. In the all-K^+ medium, Ca^{++} is taken up at essentially the same rate as in the all-Na^+ medium, and with the usual stoichiometry with H^+ output, confirming the data in Fig. 1 and Table 1. However, the Ca^{++} is retained for only a very short period in the all-K^+ medium; within 5 minutes it is completely lost to the medium again. Ca^{++} efflux was accompanied by essentially stoichiometric uptake of H^+ from the medium. In the third trace the medium contained 120 meq Na^+ + 16 meq K^+; substitution of only a small fraction of the Na^+ by K^+ greatly decreased the stability of the accumulated Ca^{++}, from over 20 minutes to about 12 minutes. Since the experiments in Fig. 1 and Table 1 have shown that the active uptake of Ca^{++} is not influenced by Na^+ or K^+, the failure of the Ca^{++} to be retained in a steady-state in the presence of K^+ must be due to the specific stimulation of the Ca^{++} efflux rate by K^+.

The efflux of Ca^{++} from mitochondria is not a simple diffusion-controlled process; it has a relatively high temperature dependence and appears to involve enzyme- or membrane-linked changes (Drahota et al, 1965). In the experiments of Fig. 2 the optical density of the initial mitochondrial suspension at 520 mμ remained essentially unchanged after the accumulation of Ca^{++} and during its retention in the State 4 steady-state in the all-Na^{+} medium. However, it abruptly fell to less than half the original value when the rapid loss of Ca^{++} occurred in the all-K^{+} medium, showing that the stimulation of Ca^{++} efflux by K^{+} is accompanied by mitochondrial swelling. This effect of K^{+} may be related or similar to the requirement of K^{+} for swelling of mitochondria in the presence of gramicidin (cf. Neubert and Lehninger, 1962; Chappell and Crofts, 1965), valinomycin (Moore and Pressman, 1964), and parathyroid hormone (Rasmussen et al, 1964).

Summary. There is no influence of Na^{+} or K^{+} on the rate and stoichiometry of energy-linked Ca^{++} accumulation and H^{+}-extrusion by mitochondria, nor is this process accompanied by stoichiometric movement of Na^{+} and K^{+} between mitochondria and medium. However, K^{+} was found to stimulate the efflux of accumulated Ca^{++}, the linked stoichiometric influx of H^{+} and the swelling of the mitochondria, whereas in an all-Na^{+} medium the accumulated Ca^{++} was maintained in a steady-state for long periods, without swelling of the mitochondria.

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References

Carafoli, E. and Lehninger, A. L. (1964). Biochem. Biophys. Research Commun. 16, 66.

Carafoli, E., Rossi, C. S., and Lehninger, A. L. (1964). J. Biol. Chem. 239, 3055.

Carafoli, E., Rossi, C. S., and Lehninger, A. L. (1965). J. Biol. Chem. In press.

Chappell, J. B., Cohn, M., and Greville, G. D. (1963). in B. Chance (ed), Energy-linked mitochondrial functions, Academic Press, New York, p. 219.
Chappell, J. B., and Crofts, A. R. (1965). Biochem. J., In press.
Drahota, Z., Carafoli, E., Rossi, C. S., Gamble, R. L., and Lehninger, A. L., (1965). J. Biol. Chem., In press.
Engstrom, G. W. and DeLuca, H. F. (1964). Biochemistry 3, 379.
Moore, C. and Pressman, B. C. (1964). Biochem. Biophys. Research Commun. 15, 562.
Neubert, D. and Lehninger, A. L. (1962). Biochim. Biophys. Acta, 62, 556.
Saris, N. E. (1963). Soc. Scient. Fenn. 28, 1.
Rasmussen, H., Fischer, J., and Arnaud, C. (1964). Proc. Nat. Acad. Sci. U. S. 52, 1198.
Rossi, C. S. and Lehninger, A. L. (1964). J. Biol. Chem. 239, 3971.
Vasington, F. D. and Murphy, J. V. (1962). J. Biol. Chem. 237, 2670.