mechanism (Quagliariello, Meijer, Tager & Papa, 1970) may be distinguished on the basis of their specificity for at least one of the anions transported.

We have tested the effect of *n*-butylmalonate (Robinson & Chappell, 1967) and thiol-blocking reagents such as mersalyl (Fonyo, 1968; Tyler, 1968) on anion-exchange reactions in rat liver mitochondria. The phosphate-hydroxyl exchange is sensitive to mersalyl, but not to *n*-butylmalonate. The phosphate-dicarboxylate exchange is sensitive to both mersalyl and *n*-butylmalonate. The malate-tricarboxylate and dicarboxylate- α -oxoglutarate exchanges are insensitive to both inhibitors. The malate-malonate exchange is insensitive to both inhibitors under conditions where they completely inhibit the dicarboxylate-phosphate exchange. Thus malate may be transported across the mitochondrial membrane by at least four mechanisms, in which the counter-anion is phosphate, tricarboxylate, dicarboxylate or α -oxoglutarate respectively.

The equilibrium constants of the malate-phosphate, malate- α -oxoglutarate and malate-citrate exchange reactions have been determined.

The physiological significance of these results will be discussed.

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Distribution of Anions Across the Mitochondrial Membrane

By F. PALMIERI, E. QUAGLIARIELLO and M. KLINGENBERG. (*Department of Biochemistry, University of Bari, Bari, Italy, and Lehrstuhl für Physikalische Biochemie, University of Munich, Munich, Germany*)

The factors regulating the distribution of anions between the inner and outer mitochondrial phase have been investigated.

To obtain a clear insight in these mechanisms, the distribution of anions across the mitochondrial membrane was studied under conditions where energy-driven ion transport (by respiration or ATP) was excluded, since the energy supply can be considered to be a factor superimposed to the actual anion-distributing mechanisms. For this purpose mitochondria were incubated in the presence of

rotenone, antimycin and oligomycin with labelled anions and then separated from the incubation mixture by centrifugal filtration (Klingenberg, Pfaff & Kröger, 1964).

As the external pH decreased the ratio of the intra- to the extra-mitochondrial concentration of added anion increased with malate, malonate, acetate, P_i and, in the presence of malate, citrate. An increase of succinate accumulation by lowering the pH was observed by Palmieri & Quagliariello (1969). The accumulation of endogenous malate was also higher at lower pH values. With SCN^- , on the other hand, the increase of the ratio between the inner and outer mitochondrial phase by lowering of the pH was negligible.

The slope of the increase of anion uptake by lowering of the pH was higher with malate than with acetate, and, for the same anion, it increased on lowering of the concentration from 1 mM to 0.2 mM. In sucrose medium, on decrease of the external pH, the ratio of the intra- to the extra-mitochondrial concentration of K^+ decreased, and the uptake of anions increased.

By studying the accumulation of anionic substrates at equilibrium at different external concentrations, it was found that the concentration required for half maximal loading of the mitochondria was decreased by lowering the external pH, whereas the maximal loading, i.e. 'the capacity of the system', was unaffected. At the same external pH the ratio of the intra- to the extra-mitochondrial concentration of anions decreased with increasing external concentration of anions, especially at the lower pH values.

The increase of the uptake of acetate and malate by lowering of the pH was nearly unaffected by the addition of nonactin, but strongly decreased by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, by nigericin or by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone + nonactin.

On the basis of these results the anion distribution can be explained essentially as a function of two parameters: (a) the pH difference across the membrane; (b) the capacity of the mitochondria for anions.

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