tumour cells (Eddy, Mulcahy & Thomson, 1967). In the latter system the flow of glycine into the cells is closely coupled to the entry of Na^+ and the efflux of K^+ , a process that occurs in the virtual absence of ATP (Eddy, 1968). If the above analogy meant that similar types of carrier mechanism were involved in both the mammalian and the microbial systems, then the uptake of neutral amino acids by yeast depleted of ATP might be expected to stimulate a stoichiometrical flow of H^+ into the cells accompanied by a loss of K^+ . This novel phenomenon has now been observed as follows.

The yeast was manipulated as described by Eddy (1966) and suspended at 30°C in 5mm-tris solution buffered at pH 4.5–5 with citric acid. Energy metabolism was blocked with 5mm-2-deoxyglucose and $0.2\mu\text{g}$ of antimycin A/mg dry wt. of yeast. Proton movements were monitored with a sensitive glass-electrode system and K^+ efflux was assayed by flame photometry. In a typical experiment at pH 4.8 the addition of small amounts (up to $1\mu\text{mol}/5\text{ml}$ of suspension) of various amino acids (e.g. glycine, L-leucine or L-phenylalanine) stimulated H^+ uptake by the cells (basal rate 1–3equiv./min per mg) by 300%. The induced flow of H^+ (up to about 2equiv./mol of amino acid absorbed) and the stimulated loss of K^+ roughly paralleled the process of amino acid absorption.

In a further series of experiments, both with 'sodium' yeast (Conway & Moore, 1954) and 'potassium' yeast, endogenous energy metabolism continued while the amino acid was absorbed. The complex behaviour of the system under these conditions suggested that the influx of H^+ with the amino acid then led to an ATP-dependent secretion of cations. Various transport models incorporating these observations will be described.

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cyclic transport of the cations. The uncoupling is potentiated by transport-inducing antibiotics (Christiansen, Loyter & Racker, 1969; Cockrell & Racker, 1969; Montal, Chance, Lee & Azzi, 1969; Papa, 1969). To obtain maximal uncoupling (or inhibition of energy-linked reactions) by potassium salts valinomycin + nigericin must be added. With ammonium salts valinomycin on its own gives maximal uncoupling (or inhibition).

In submitochondrial particles obtained by sonication respiratory control may be induced by oligomycin (Lee & Ernster, 1966). Although respiration in these particles is accompanied by an inward translocation of H^+ (Mitchell & Moyle, 1965), Cockrell & Racker (1969) observed that the induction of respiratory control may be accompanied by a slow inward translocation of K^+ .

We have found that oligomycin enhances the respiration-linked proton uptake in submitochondrial particles and depresses the rate of the release of protons on the exhaustion of oxygen. The relationship of these observations to the stimulatory effect of low concentrations of oligomycin on energy-linked reactions in submitochondrial particles will be discussed.

The data to be presented provide further support for the concept that an energy-linked turnover of protons and univalent cations can control the rate of electron flow in submitochondrial particles.

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Transport of Protons and Univalent Cations in Submitochondrial Particles

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Papa, Tager, Guerrieri & Quagliariello (1969) have shown that univalent cations uncouple oxidative phosphorylation in submitochondrial particles, and have proposed that this is due to an energy-dissipating

Properties of some Anion-Exchange-Diffusion Reactions in Rat Liver Mitochondria

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The translocators mediating the transport of certain anions across the mitochondrial membrane (Chappell & Crofts, 1966) by an exchange-diffusion

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

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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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