acetyl-CoA formed was converted into ketone bodies. With increasing concentrations of inorganic phosphate this percentage was greatly increased. At 30mM-phosphate it was 68%. The amount of palmitoylcarnitine broken down was lowered by 29%; the oxygen uptake was 55% lower; ketone-body production was doubled; the tricarboxylic acid-cycle activity was lowered by 70%. These observations are in good agreement with the inhibition by inorganic phosphate of palmitoylcarnitine oxidation observed by Van Tol, De Jong & Hülsmann (1969). However, when palmitate was oxidized in the presence of dinitrophenol, 10mM-ATP and 10 μ g of oligomycin (Van den Bergh, 1965), no effect of the inorganic phosphate concentration was observed.

When 2 μ mol of palmitate was oxidized in the presence of 30mM-phosphate and a phosphate-acceptor system, the addition of 1.5% bovine serum albumin lowered the percentage of acetyl-CoA converted into ketone bodies from 78% to 17% in a typical experiment. The addition of albumin lowered by 11% the amount of palmitate broken down; oxygen uptake was 70% increased; tricarboxylic acid-cycle activity was increased 3.3-fold; ketone-body production was 80% decreased. The effect of albumin was much smaller, however, when palmitoylcarnitine was the substrate.

A Research Fellowship of the Netherlands Government to H. H. is gratefully acknowledged.

Van den Bergh, S. G. (1965). *Biochim. biophys. Acta*, **98**, 442.

Van Tol, A., De Jong, J. W. & Hülsmann, W. C. (1969). *Biochim. biophys. Acta*, **176**, 414.

Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962). *Biochem. J.* **82**, 90.

Effects of an Acylcarnitine Analogue, 2-Bromomyristoylthiocarnitine, on Mitochondrial Respiration

By P. K. TUBBS and J. F. A. CHASE. (*Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.*)

The CoA thiol esters of long-chain 2-bromo acids are powerful inhibitors of carnitine palmitoyltransferase and hence of the carnitine-dependent mitochondrial oxidation of palmitoyl-CoA, but, presumably because of a permeability barrier, they do not inhibit palmitoylcarnitine oxidation (Tubbs & Chase, 1967). This is, however, very strongly inhibited by 2-bromoacyl derivatives of the new compound thiocarnitine:

S-Acetylthiocarnitine is a good substrate for

carnitine acetyltransferase, and longer-chain esters are rapidly oxidized by liver or heart mitochondria. Thiocarnitine itself can substitute for carnitine in supporting the mitochondrial oxidation of palmitoyl-CoA. Rat liver mitochondria briefly incubated with very low concentrations of S-2-bromomyristoylthiocarnitine cannot oxidize palmitoylcarnitine, although pyruvate (+ malate) or succinate still support rapid respiration. The simple explanation would be that bromoacylthiocarnitine inactivates the carnitine palmitoyltransferase of the mitochondria, by analogy with the effect of bromoacetylthiocarnitine on carnitine acetyltransferase (Chase & Tubbs, 1969). However, when mitochondria are treated with 50 μ M-palmitoyl-L-carnitine after exposure to 3 μ M-2-bromomyristoyl-DL-thiocarnitine they become incapable of oxidizing pyruvate, α -oxoglutarate or hexanoate, although succinate oxidation is unimpaired. The oxidation of these substrates, but not of palmitoylcarnitine or palmitoyl-CoA, is restored by the addition of 2mM-L-carnitine. These observations suggest that some of the carnitine palmitoyltransferase is still active, and that a metabolite of bromoacylthiocarnitine, presumably 2-bromoacyl-CoA generated within the 'carnitine permeability barrier', inhibits the β -oxidation of long-chain fatty acyl groups. Thus the further addition of palmitoyl-carnitine leads to extensive acylation of mitochondrial CoA and prevents the CoA-dependent oxidation of substrates such as pyruvate, unless sufficient carnitine is present to act as an acyl sink.

The apparent immunity of the 'inner' pool of carnitine palmitoyltransferase to bromoacylthiocarnitine is of interest; whether this is an intrinsic property of the enzyme or is due to its environment within the mitochondrion remains to be determined.

We thank the Medical Research Council for an expenses grant and Miss Joyce Holland for her assistance.

Chase, J. F. A. & Tubbs, P. K. (1969). *Biochem. J.* **111**, 225.

Tubbs, P. K. & Chase, J. F. A. (1967). *Abstr. FEBS 4th Meet., Oslo*, p. 135.

Translocation of Protons and Alkali-Metal Cations Accompanying the Uptake of Neutral Amino Acids by Yeast

By A. A. EDDY, K. BACKEN and J. NOWACKI. (*Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.*)

Eddy (1966) drew an analogy between the effects of varying extracellular $[\text{H}^+]$ and $[\text{K}^+]$ on the rate of uptake of glycine by yeast and the effects of varying $[\text{Na}^+]$ and $[\text{K}^+]$ on glycine uptake by mouse ascites-

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.

Conway, E. J. & Moore, P. T. (1954). *Biochem. J.* **57**, 523.

Eddy, A. A. (1966). *Proc. 2nd Symp. Yeast*, p. 457.

Bratislava: Slovak Academy of Sciences.

Eddy, A. A. (1968). *Biochem. J.* **108**, 195.

Eddy, A. A., Mulcahy, M. F. & Thomson, P. J. (1967). *Biochem. J.* **103**, 863.

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.

Christiansen, R. O., Loyter, A. & Racker, E. (1969). *Biochim. biophys. Acta*, **180**, 207.

Cockrell, R. S. & Racker, E. (1969). *Biochem. biophys. Res. Commun.* **35**, 414.

Lee, C. P. & Ernster, L. (1966). In *Regulation of Metabolic Processes in Mitochondria*, p. 218. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.

Mitchell, P. & Moyle, J. (1965). *Nature, Lond.*, **208**, 1205.

Montal, M., Chance, B., Lee, C. P. & Azzi, A. (1969). *Biochem. biophys. Res. Commun.* **34**, 104.

Papa, S. (1969). In *The Energy Level and Metabolic Control in Mitochondria*, p. 273. Ed. by Papa, S., Tager, J. M., Quagliariello, E. & Slater, E. C. Bari: Adriatica Editrice.

Papa, S., Tager, J. M., Guerrieri, F. & Quagliariello, E. (1969). *Biochim. biophys. Acta*, **172**, 184.

Transport of Protons and Univalent Cations in Submitochondrial Particles

By J. M. TAGER, S. PAPA, F. GUERRIERI, G. S. P. GROOT and E. QUAGLIARIELLO. (*Department of Biochemistry, University of Bari, Bari, Italy, and Laboratory of Biochemistry, University of Amsterdam, Amsterdam, The Netherlands*)

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

By A. J. MEIJER, N. E. LOFRUMENTO, S. PAPA, J. M. TAGER and E. QUAGLIARIELLO. (*Laboratory of Biochemistry, University of Amsterdam, Amsterdam, The Netherlands, and Department of Biochemistry, University of Bari, Bari, Italy*)

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