mechanism, which involves a pre-existing energized state as a requisite for the initiation of the oligomycin action.

This work was supported by the Swedish Medical and Natural Science Research Councils and Cancer Society.

Lee, C. P. & Ernster, L. (1966). In *Biochim. biophys. Acta Library Vol. 7: Regulation of Metabolic Processes in Mitochondria*, p. 218. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.

Lee, C. P. & Ernster, L. (1968). *Eur. J. Biochem.* **3**, 391.

Lee, C. P., Ernster, L. & Chance, B. (1969). *Eur. J. Biochem.* **8**, 153.

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

Comparison of the Stimulatory Effects of Oligomycin and Factor F_1 -X on the Adenosine Triphosphate-Driven Transhydrogenase in A-Particles from Ox Heart Mitochondria

By G. S. P. GROOT (introduced by J. M. TAGER). (Laboratory of Biochemistry, University of Amsterdam, Amsterdam, The Netherlands)

Tager, Groot, Roos, Papa & Quagliariello (1969) have shown that the ATP-driven energy-linked transhydrogenase in Mg-ATP-particles elicits no 'extra' ATP hydrolysis in comparison with that occurring in the absence of the transhydrogenase. The relationship between the rate of ATP hydrolysis and of NADP⁺ reduction has now been studied in A-particles. The stimulatory effects of low concentrations of oligomycin (Lee & Ernster, 1965) and of coupling factor F_1 -X (Vallejos, Van den Bergh & Slater, 1968) on the transhydrogenase in these particles have been compared. Low concentrations of oligomycin inhibit the adenosine triphosphatase and stimulate the reduction of NADP⁺. Consequently the $\Delta\text{NADPH}/\Delta\text{P}_i$ ratio increases. At concentrations above 0.2 $\mu\text{g}/\text{mg}$ of protein oligomycin inhibits the adenosine triphosphatase and the transhydrogenase equally; the $\Delta\text{NADPH}/\Delta\text{P}_i$ ratio remains constant. When factor F_1 -X is added to the particles the adenosine triphosphatase activity of factor F_1 -X becomes latent, whereas the transhydrogenase is stimulated. In this case the $\Delta\text{NADPH}/\Delta\text{P}_i$ ratio increases. The mechanisms of action of oligomycin and factor F_1 -X will be discussed, taking into account that X in factor F_1 -X is identical with oligomycin-sensitivity-conferring protein or factor F_c (Groot & Meijer, 1969).

Groot, G. S. P. & Meijer, M. (1969). *Biochim. biophys. Acta*, **180**, 575.

Lee, C. P. & Ernster, L. (1965). *Biochem. biophys. Res. Commun.* **18**, 523.

Tager, J. M., Groot, G. S. P., Roos, D., Papa, S. & Quagliariello, E. (1969). In *The Energy Level and Metabolic Control in Mitochondria*, p. 453. Ed. by Papa, S., Tager, J. M., Quagliariello, E. & Slater, E. C. Bari: Adriatica Editrice.

Vallejos, R. H., Van den Bergh, S. G. & Slater, E. C. (1968). *Biochim. biophys. Acta*, **153**, 509.

Phase Relationships of Mitochondrial Oscillations Energized by Adenosine Triphosphate: the Effect of Propranolol

By M. K. F. WIKSTRÖM (introduced by D. E. GRIFFITHS). (Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital Laboratory, L2 49, Haartmaninkatu 4, Helsinki 29, Finland)

Caswell (1966) has shown that the redox potential of cytochrome *c*, measured with tetramethyl-*p*-phenylenediamine, follows closely the phosphorylation potential of mitochondria, and is thus also a measure of the high-energy state or compounds of oxidative phosphorylation, or, alternatively, a measure of the protonmotive force of the chemiosmotic theory (Mitchell, 1966).

The aim of this work has been to study the behaviour of this parameter during the oscillatory state (Pressman, 1965; Packer, Utsumi & Mustafa, 1966) of rat liver mitochondria, energized by ATP. The oscillations were produced in a sucrose-KH₂PO₄ medium, supplemented with gramicidin or valinomycin. The redox state of 15 μM -tetramethyl-*p*-phenylenediamine was measured with a dual-wavelength spectrophotometer (650–620 nm), and mitochondrial volume changes at 520 nm single-ended.

The redox state of tetramethyl-*p*-phenylenediamine was found to oscillate with inflexion points preceding those of the volume changes by 3–7 s. Under these conditions it might be of interest to study the phase relationship between the redox changes of the respiratory chain and the oscillatory change in the pH of the suspension medium, because, according to the chemiosmotic theory, the pH changes should precede the redox changes. Preliminary work has indicated that the reverse might be true, i.e. the redox changes of tetramethyl-*p*-phenylenediamine seem to precede the changes in pH.

The addition of propranolol (0.05–0.50 mM) to the ATP-supported steady state produced a considerable amount of extra swelling and a simultaneous increase of reduction of tetramethyl-*p*-phenylenediamine, indicating an increase of the phosphorylation potential. Thus propranolol acts primarily on the energy-transfer mechanism rather than on salt transport. Further, the redox change induced by propranolol preceded the change in volume. Propranolol does not inhibit the activity of the respiratory chain and shows no oligomycin-like effects. The

effects of propranolol are completely sensitive to uncouplers, and are not produced in the absence of an energy source.

Propranolol may exert its effect by binding fixed negatively charged phospholipids of the membrane, as suggested by Seppälä, Wikström & Saris (1969). The increase of the phosphorylation potential with a concomitant extra uptake of salts focuses the interest on the role of fixed charges of the mitochondrial inner membrane in the mechanism of oxidative phosphorylation and ion transport.

Caswell, A. H. (1968). *J. biol. Chem.* **243**, 5827.

Mitchell, P. (1966). *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*. Bodmin: Glynn Research Ltd.

Packer, L., Utsumi, K. & Mustafa, M. G. (1966). *Archs Biochem. Biophys.* **117**, 381.

Pressman, B. C. (1965). *Fedn Proc. Fedn Am. Socs exp. Biol.* **24**, 425.

Seppälä, A. J., Wikström, M. K. F. & Saris, N.-E. L. (1969). *Proc. Sigröd Jusélius Symp. Oscillations and Membrane Instabilities, Hanko*. Philadelphia: Johnson Foundation (in the Press).

Differences between the Phosphorylation Potentials of Adenosine Triphosphate Inside and Outside the Mitochondria

By H. W. HELDT (introduced by D. E. GRIFFITHS). (*Lehrstuhl Physikalische Biochemie, University of Munich, Munich, Germany*)

Heldt & Pfaff (1969) have shown that the ATP/ADP ratio found in the mitochondria reflects a steady-state equilibrium between these compounds.

In the present investigations the relationship between the ATP/ADP ratios in the mitochondria and in the medium was studied by silicon-layer filtering centrifugation (Pfaff & Klingenberg, 1968). In a typical experiment carried out at 12°C, the ATP/ADP ratios measured during the state of respiratory control in the mitochondria and in the medium were 3.2 and 20 respectively.

By taking into account the inorganic phosphate assayed in both fractions, the phosphorylation potential of the ATP in the medium was calculated to be 2.8 kcal higher than in the mitochondria.

In another experiment oxidative phosphorylation, adenosine triphosphatase and substrate-level phosphorylation were inhibited by the addition of oligomycin and rotenone. When the mitochondria were kept aerobic in the presence of succinate, there was a linear relationship observed between the ATP/ADP ratios fixed in the medium by addition of various concentrations of ADP and ATP, and the resultant ATP/ADP ratios found in the mitochondria. Again, the ATP/ADP ratios assayed in the mitochondria were five- to ten-fold smaller than those in the medium. When uncoupler was also added no such

difference was observed. In this case the ATP/ADP ratio in the mitochondria was found to be equal to the ATP/ADP ratio fixed in the medium.

The differences of ATP/ADP ratios between the mitochondrial and extramitochondrial space observed with aerobic coupled mitochondria are explained by the ADP-specificity of the adenine nucleotide transport into the mitochondria (Pfaff & Klingenberg, 1968). ADP is transported against a potential from the extramitochondrial space into the mitochondria, the energy for this process being provided from the respiratory chain (Klingenberg, Heldt & Pfaff, 1969). Thus a small increase in the ADP concentration outside the mitochondria leads to a large increase of the ADP concentration within the mitochondria. In this way a stimulation of oxidative phosphorylation is obtained by a small rise in the ADP concentration in the cytosol. This mechanism may have an important function in maintaining the ATP concentration in the cytosol constant.

This work was supported by the Deutsche Forschungsgemeinschaft.

Heldt, H. W. & Pfaff, E. (1969). *Eur. J. Biochem.* (in the Press).

Klingenberg, M., Heldt, H. W. & Pfaff, E. In *The Energy Level and Metabolic Control in Mitochondria*, p. 237. Ed. by Papa, S., Tager, J. M., Quagliariello & Slater, E. C. Bari: Adriatica Editrice.

Pfaff, E. & Klingenberg, M. (1968). *Eur. J. Biochem.* **6**, 66.

Multiple Effects of Trialkyltin Compounds on Mitochondria

By M. J. SELWYN, M. STOCKDALE and A. P. DAWSON. (*School of Biological Sciences, University of East Anglia, Norwich NOR 88C, U.K.*)

Trialkyltin compounds have been reported to affect mitochondria in a manner similar to that of oligomycin but accompanied by a weak uncoupling effect (Aldridge, 1958; Sone & Hagihara, 1964). Aldridge & Rose (1969) have used these observations and the finding that trialkyltin compounds are bound to a pair of histidine residues in haemoglobin to elaborate a hypothesis for the mechanism of oxidative phosphorylation.

We have confirmed that at low concentrations (1 μ M) tripropyltin chloride produces an immediate inhibition of ADP-stimulated respiration of rat liver mitochondria, which in a potassium chloride medium is associated with swelling of the mitochondria. In a potassium chloride medium with succinate or β -hydroxybutyrate as substrate Ca^{2+} induced an extremely rapid swelling of the mitochondria when tripropyltin chloride was added. Further investigation showed that low concentrations of tripropyltin chloride (2 μ M) allowed rapid swelling of mitochondria suspended in 0.1M-ammonium chloride or 0.1M-