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

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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

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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 $\Delta\text{NADPH}/\Delta P_i$ ratio increases. At concentrations above 0.2 $\mu\text{g}/\text{mg}$ of protein oligomycin inhibits the adenosine triphosphatase and the transhydrogenase equally; the $\Delta\text{NADPH}/\Delta P_i$ 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 $\Delta\text{NADPH}/\Delta P_i$ 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).

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

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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