amounts of oligomycin (0.1 $\mu\text{g}/\text{mg}$ of protein) the rate of succinate oxidation progressively decreases without reaching a constant value even after 9 min. The Q_{10} for the progressive inhibition by oligomycin is relatively high (about 4).

The titration curves of the inhibition by oligomycin of the 2,4-dinitrophenol-induced adenosine triphosphatase (measured as P_i or H^+ formation) shows apparent sigmoidicity (contrast Ter Welle, 1968). The inhibition is time-dependent. The steep part of the titration curves is located at about 0.1 μg of oligomycin/mg of protein. The inhibition is incomplete, even at 4 μg of oligomycin/mg of protein.

The titration curve of the inhibition by oligomycin of the P_i -ATP exchange reaction is linear, 100% inhibition being obtained at 0.10–0.12 μg of oligomycin/mg of protein (cf. Groot, 1969). No time-dependency of the inhibition of the P_i -ATP exchange reaction has been observed.

The differences in the characteristics of the inhibition by oligomycin of these reactions will be discussed.

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Rate of Uncoupler-Induced Hydrolysis of Adenosine Triphosphate in Mitochondria

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The rate of the uncoupler-induced adenosine triphosphatase reaction in rat liver mitochondria was studied at different temperatures in order to obtain information on the role of the adenine nucleotide translocator on the rate of hydrolysis of added ATP and on the mechanism of action of uncouplers.

The rate of hydrolysis of endogenous ATP was found to be insensitive, not only to atractyloside, but also to mersalyl, the inhibitor of phosphate transport (Tyler, 1969; Fonyo, 1969), and to bongkrekic acid, shown by H. A. Lardy (personal communication) to be an atractyloside-like inhibitor.

The rate of hydrolysis of endogenous ATP was measured in the presence of the following uncouplers (in decreasing order of effectiveness): carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, carbonyl cyanide *m*-chlorophenylhydrazone, dicoumarol, 4-iso-octyl-2,6-dinitrophenol and 2,4-dinitrophenol. With carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and carbonyl cyanide *m*-chlorophenylhydrazone the rate of hydrolysis of endogenous ATP exceeded that of added ATP at 0°C by at least tenfold, in good agreement with Heldt & Klingenberg

(1968), whereas with 2,4-dinitrophenol these rates approached each other. The K_m for 2,4-dinitrophenol for the breakdown of endogenous ATP at 0°C was about 5 μM . The inhibition by 2,4-dinitrophenol of the carbonyl cyanide *m*-chlorophenylhydrazone-induced hydrolysis of endogenous ATP (Heldt & Klingenberg, 1968) was of the competitive type, with K_i about 5 μM . The breakdown of added ATP induced by carbonyl cyanide *m*-chlorophenylhydrazone, was not influenced by 2,4-dinitrophenol.

The rate of adenine nucleotide translocation was measured with a modified rapid centrifugation technique (Kraayenhof, Tsou & Van Dam, 1969). The rates at 0°C (in nmol/min per mg of protein) of the back-exchange of added ADP with intramitochondrial [^{14}C]adenine nucleotides were: with no additions, 11; with added Mg^{2+} , 11; with added carbonyl cyanide *m*-chlorophenylhydrazone, 18. With ATP instead of ADP these values were 4.5, 9 and 15 respectively. These rates will be compared with those of the different reactions concerned with oxidative phosphorylation.

The influence of the adenine nucleotide translocator and of the transport of uncouplers on the rate of the adenosine triphosphatase reaction will be discussed.

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Inhibition of Mitochondrial Adenosine Triphosphatase by Adenosine Diphosphate: Release of Adenosine Diphosphate Inhibition by Aurovertin

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Both inhibitory and stimulatory actions of aurovertin on mitochondrial ATPase* have been described by Lardy, Connelly & Johnson (1964), Robertson, Holloway, Knight & Beechey (1968) and Lardy & Ferguson (1969).

Using aurovertin (generously supplied by H. A. Lardy) with the object of inhibiting proton-translocating ATPase activity in rat liver mitochondria, we found (methods of Mitchell & Moyle, 1968) that the time for half hydrolysis of ATP pulses was decreased by aurovertin, and proton translocation was not inhibited. The simplest explanation appeared to be that aurovertin prevents ADP from inhibiting the ATPase. This has been confirmed by studies of the effect of ADP on the rate and apparent K_m of the

* Abbreviation: ATPase, adenosine triphosphatase.