findings (Lee & Ernster, 1966, 1968): (1) that the energy expenditure of the energy-dependent reaction is 1 high-energy bond/molecule of NADP^+ reduced by NADH; (2) that ADP and P_i do not influence the ATP-driven reaction; (3) that the reaction from right to left is incapable of generating ATP.

It therefore appeared that the extent of the overall reaction is governed by the ratio $v_{\text{ETH} \rightarrow} / v_{\text{NTH} \leftarrow}$. Evidence that this indeed is the case was obtained in experiments in which the $v_{\text{ETH} \rightarrow} / v_{\text{NTH} \leftarrow}$ ratio was altered by means of variations in pH and Mg^{2+} concentration from 0.2 (pH 6, no Mg^{2+}) to 74 (pH 9, 8 mM- Mg^{2+}), with a resulting increase in the steady-state $[\text{NAD}^+][\text{NADPH}] / [\text{NADH}][\text{NADP}^+]$ ratio of about 30-fold.

Another, more unexpected, factor of importance for the final extent of the overall reaction is the initial $[\text{NADH}] / [\text{NADP}^+]$ ratio. For example, increase of this ratio from 1 to 2 resulted in a decrease of the steady-state $[\text{NAD}^+][\text{NADPH}] / [\text{NADH}][\text{NADP}^+]$ ratio from about 500 to 100. Although the nature of this effect is not clear, it appears that, at a high $[\text{NADH}] / [\text{NADP}^+]$ ratio, NADH may compete with NADP^+ for the NADP^+ -binding site of the enzyme and thereby lower the rate of the energy-dependent transhydrogenase reaction.

The available evidence suggests that the mitochondrial nicotinamide nucleotide transhydrogenase system operates as a cyclic process, where the same enzyme, the transhydrogenase, transfers hydrogen from NADH to NADP^+ in an energy-dependent reaction, linked to the energy-transfer system of the respiratory chain, and from NADPH to NAD^+ in a non-energy-dependent reaction. The resulting steady state ratio, $[\text{NAD}^+][\text{NADPH}] / [\text{NADH}][\text{NADP}^+]$, is determined by factors governing the rates of the individual reactions.

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Oligomycin-Induced Energization of Sub-mitochondrial Particles

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Oligomycin has been shown to induce a state of respiratory control in 'EDTA-particles' derived from ox heart mitochondria that is relieved by uncouplers (Lee & Ernster, 1966, 1968). The oligomycin-induced respiratory control is characterized by biphasic reduction kinetics of the cytochromes, a rapid initial phase and a slow second phase, which is particularly striking when respiration is partially suppressed by an electron-transport inhibitor (Lee, Ernster & Chance, 1969). To further investigate this phenomenon we have taken advantage of the fluorochrome 8-anilino-naphthalene-1-sulphonic acid, which has been shown to serve as a sensitive indicator of the oligomycin-induced energized state of EDTA-particles (Montal, Chance, Lee & Azzi, 1969).

When EDTA-particles were incubated in the absence of substrate and oligomycin, an increase in fluorescence occurred on the addition of 8-anilino-naphthalene-1-sulphonic acid, the extent of which was dependent on the pH and ionic strength of the medium. Subsequent additions of succinate and oligomycin caused further increases in fluorescence, but the extents of these 'energy-dependent' responses were virtually independent of changes in pH and ionic strength that strikingly altered the extent of the first, 'non-energy-dependent', fluorescence increase. Further, the energy-dependent fluorescence increase reached half-maximal extent at a markedly lower 8-anilino-naphthalene-1-sulphonic acid concentration than did the non-energy-dependent increase. Both the succinate- and the oligomycin-induced fluorescence increases were reversed by uncouplers and by anaerobiosis.

Addition of increasing concentrations of malonate had no effect on the non-energy-dependent increase in fluorescence, but lowered, as expected, both the initial rate and the steady state of the succinate-induced increase. On the other hand, the further increase occurring on the subsequent addition of oligomycin diminished only in initial rate whereas its final steady state was virtually the same as in the absence of malonate. In accordance with these findings, when succinate was added after the prior addition of oligomycin a biphasic response was obtained in the presence of malonate, similar to that earlier observed with cytochrome reduction in the presence of electron-transport inhibitors (Lee *et al.* 1969).

These preliminary findings suggest that the effect of oligomycin in promoting the maintenance of an energized state proceeds by way of an autocatalytic

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 $\Delta\text{NADPH}/\Delta\text{P}_i$ ratio increases. At concentrations above $0.2\text{ }\mu\text{g}/\text{mg}$ 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).

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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- KH_2PO_4 medium, supplemented with gramicidin or valinomycin. The redox state of $15\text{ }\mu\text{M}$ -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