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The Synthesis of Adenosine Triphosphate by Transmembrane Ionic Gradients

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Further studies have confirmed the synthesis of ATP by a protonmotive force (Reid, Moyle & Mitchell, 1966). Rat liver mitochondria in which electron transport is blocked by rotenone and antimycin or by anoxia synthesize ATP on rapid lowering of the external pH. This synthesis is dependent on well-coupled mitochondria (respiratory control quotient > 6 at pH 7.2) with intact membranes, and is abolished if the permeability barrier is damaged by butan-1-ol, Triton X or freeze-thawing. Agents that increase the membrane permeability to protons (gramicidin, 2,4-dinitrophenol) also inhibit ATP synthesis.

The extent of ATP synthesis is influenced by: (1) valinomycin; (2) the size of the transmembrane pH gradient established; (3) the transmembrane K⁺ gradient. Mitochondria preincubated at 25°C in high-K⁺ medium, pH 8.5 (sucrose, 80mM; glycylglycine, 14mM; KCl, 78mM; KOH, 13mM; K₂HPO₄, 3mM; ADP, 6.6mM) synthesize 520 ± 42nmol of ATP/g of protein in the first second after depression of the external pH to 4.2–4.3, provided that valinomycin is added before the acid. However, under these conditions the mitochondria rapidly burst and the ATP synthesized is hydrolysed by oligomycin-sensitive adenosine triphosphatase activity within 10s. Omission of valinomycin decreases the ATP pulse by over 50%, suggesting that it acts by collapsing any potential opposing the net translocation of protons through the membrane (Mitchell, 1966). In this high-K⁺ medium the pH depression must be over 3 units before synthesis occurs.

In contrast, if the K⁺ in the medium is replaced by Na⁺ or sucrose, valinomycin alone induces ATP synthesis (maximum of 215 ± 35nmol/g of protein

after 4s), supporting findings by Cockrell, Harris & Pressman (1967). This, however, is greatly stimulated if the mitochondria are subjected to a small pH jump (8.5–6.5) 1s after valinomycin addition, when it can approach 500nmol/s per g of protein before declining to zero after 3–4s. Experiments to date have been unable to establish any relationship between K⁺ efflux and ATP synthesis, which might support the idea that valinomycin makes K⁺ accessible to an energy-linked carrier that is reversed by the subsequent downhill movement of the ions. At present, the simplest explanation is that of a proton-translocating reversible adenosine triphosphatase.

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Factors Governing the Steady State of the Mitochondrial Nicotinamide Nucleotide Transhydrogenase System

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Submitochondrial particles catalyse two types of nicotinamide nucleotide transhydrogenase reaction: a 'non-energy-dependent transhydrogenase' (NTH) reaction:

and an 'energy-dependent transhydrogenase' (ETH) reaction:

where I~X is a hypothetical high-energy intermediate of the respiratory chain (Danielson & Ernster, 1963). The non-energy-dependent transhydrogenase reaction has an equilibrium constant near unity ($K = [\text{NAD}^+][\text{NADPH}]/[\text{NADH}][\text{NADP}^+] = 0.8$), although its maximal initial velocity is considerably lower from left to right ($v_{\text{NTH} \rightarrow}$) than from right to left ($v_{\text{NTH} \leftarrow}$) (Kaplan, Colowick & Neufeld, 1953). The energy-dependent transhydrogenase reaction exhibits a left-to-right initial velocity ($v_{\text{ETH} \rightarrow}$) greatly exceeding that of the non-energy-dependent reaction, and its 'apparent equilibrium constant' ($K' = [\text{NAD}^+][\text{NADPH}]/[\text{NADH}][\text{NADP}^+]$) has been reported to be about 500 (Lee & Ernster, 1964).

However, the latter value represents not a true equilibrium but a steady state, as indicated by the