Oxidase activities with multiple substrates indicated the dehydrogenases were rate-limiting for the oxidation of single substrates.

The quinones juglone (5-hydroxy-1,4-naphthoquinone), duroquinone (2,3,5,6-tetramethyl-1,4-benzoquinone; see Ruzicka & Crane, 1970) and ubiquinone-1 (Schatz & Racker, 1966; Eilermann *et al.*, 1970) were used as electron acceptors to investigate the nature and energy-conservation properties of these dehydrogenases. Anaerobic electron transfer from NADH to ubiquinone-1 yielded a P/2e ratio (Site 1) of 0.18 [when calculated from P/O ratio (NADH) minus P/O ratio (malate) this ratio was 0.34], and was stimulated by ADP in the presence of P_i , which indicated a mitochondrial type of respiratory control. Juglone and duroquinone failed to support either ATP synthesis or respiratory control with NADH, although they readily accepted reducing equivalents from NADH dehydrogenase (3.54 and $0.90 \mu\text{mol}$ of NADH oxidized/min per mg of protein respectively). NADPH dehydrogenase was only weakly coupled to ATP synthesis [P/2e with ubiquinone-1, 0.05; P/O ratio (NADPH) minus P/O ratio (malate), 0.04], but was stimulated by the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone, was highly active with juglone ($2.59 \mu\text{mol}$ of NADPH oxidized/min per mg of protein) and was virtually inactive with duroquinone. In contrast, malate dehydrogenase was completely non-phosphorylating with ubiquinone-1, showed no stimulation by ADP + P_i or carbonyl cyanide *m*-chlorophenylhydrazone, and was virtually inactive with both juglone and duroquinone.

These results indicate that ubiquinone-1 accepts electrons from a carrier located between Sites I and II that is accessible to all three dehydrogenases (probably the endogenous ubiquinone-8; see Klingenberg & Kroger, 1966), of which only the NADH dehydrogenase is strongly coupled to ATP synthesis or reacts significantly with duroquinone. The ability of juglone to accept electrons from the NADH (or NADPH) dehydrogenase, both very rapidly and without concomitant energy conservation, suggests that it reacts with the carrier located on the substrate side of the energy-conservation site.

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The Involvement of Iron in the Respiratory System of *Azotobacter vinelandii*

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Respiratory membranes from *Azotobacter vinelandii* rapidly oxidize L-malate [independent of NAD(P)^+], NADH (Eilermann *et al.*, 1970; Ackrell & Jones, 1971) and NADPH with the concomitant synthesis of ATP. Similar preparations, on reduction with NADH, yield an electron-paramagnetic-resonance signal characteristic of a non-haem iron protein (Beinert *et al.*, 1962) and an absorption spectrum typical of an iron-sulphur flavoprotein (Dervartanian & Bramlett, 1970).

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) sulphonate, a water-soluble metal chelator with a high affinity for Fe^{2+} ions (Palmer, 1970), completely inhibits malate oxidase ($[I]_{50}$ 0.21 mM), NADPH oxidase ($[I]_{50}$ 0.88 mM) and electron transfer from NADH to O_2 , juglone or duroquinone ($[I]_{50}$ 3.60 mM), but did not affect the oxidation of ascorbate + 2,6-dichlorophenol-indophenol or ascorbate + *NNN'*-tetramethyl-*p*-phenylenediamine. Hill plots of the inhibition curves yielded slopes of approx. 2 with NADH or NADPH as substrate and of approx. 1 with malate. Kinetically, the chelator acted as a pure competitive (dead-end) inhibitor with respect to NADH, malate or NADPH and also, with NADH as substrate, with respect to juglone or duroquinone.

Low concentrations (≤ 0.25 mM) of bathophenanthroline sulphonate completely uncoupled site I phosphorylation and abolished respiratory control with NADH by stimulating the State 4 rate without affecting State 3. Slightly higher concentrations (≤ 0.62 mM) increased the efficiency of ATP synthesis at Site 2 but failed to induce respiratory control with the Site 2 substrate malate. Bathophenanthroline at 0.15 mM has been shown to inhibit substantially the trypsin-induced adenosine triphosphatase of similar membrane preparations (Eilermann *et al.*, 1971).

Incubation of respiratory membranes with bathophenanthroline sulphonate yielded a weak absorption spectrum that was greatly intensified after the addition of NADH, malate or NADPH (λ_{max} 539 nm, shoulder at 495 nm). An identical spectrum was also obtained with dithionite-reduced spinach ferredoxin and with Fe^{2+} ions; Fe^{3+} ions were less effective, and all other metal ions tested were completely ineffective.

These results suggest that, in *A. vinelandii* respiratory membranes, iron may function both as an electron-transfer component (at the dehydrogenase level) and also in an unknown capacity in energy conservation at Sites 1 and 2.

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The Allosteric Properties of the Reduced Nicotinamide-Adenine Dinucleotide Phosphate Dehydrogenase of *Azotobacter vinelandii* Respiratory Membranes

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Phosphorylating respiratory membranes of *Azotobacter vinelandii* oxidize NADPH via a rate-limiting NADPH dehydrogenase that exhibits only very weak oxidative phosphorylation ($P/2e$ ratio ≤ 0.05) and sigmoidal saturations kinetics ($s_{0.5}$ 0.44 mM-NADPH; Hill coefficient, $n_{\text{NADPH}} \approx 2$) when assayed at pH 6.8 (Erickson *et al.*, 1972).

NAD⁺ acted as an allosteric inhibitor (partially competitive kinetics) of the dehydrogenase (no change in V_{max} , K_m 1.28 mM-NADPH, K_i 7 μ M-NAD⁺) and changed the sigmoidal saturation plot to a rectangular hyperbola ($n_{\text{NADPH}} \approx 1$). The dehydrogenase was inhibited in a purely competitive (dead-end) manner by AMP (K_i 0.73 mM), ADP (K_i 1.2 mM) and ATP (K_i 4.5 mM), and was therefore subject to control by the adenylate energy charge (as defined by Atkinson, 1968). No activators were found for the dehydrogenase. When assayed at pH 8.5 the general regulatory properties of the dehydrogenase were largely unchanged, but at pH 5.5 the saturation curve was hyperbolic ($n_{\text{NADPH}} \approx 1$) and NAD⁺ no longer had any inhibitory effect.

These results suggest that the respiratory-chain NADPH dehydrogenase of *A. vinelandii* contains a catalytic site, which binds NADPH or adenine nucleotides, and an acid-labile regulatory site, which binds NADPH (positive homotropic effector) or NAD⁺ (negative heterotropic effector). The control properties of NADPH dehydrogenase are thus clearly different from those of NADH dehydrogenase and malate dehydrogenase, which are subject to classical ADP-dependent respiratory control and product inhibition by oxaloacetate respectively.

The ability of low energy charge or very low concentrations of NAD⁺ to inhibit NADPH dehydrogenase, together with the presence in this organism of a highly active NADPH-NAD⁺ transhydrogenase (Chung, 1970; Van den Broeck & Veeger, 1968), suggest that at the whole-cell level NADPH may be preferentially oxidized indirectly via NADH and the more efficiently phosphorylating NADH dehydrogenase. The homotropic kinetics suggest that significant direct oxidation of NADPH via NADPH dehydrogenase may occur only at relatively high NADPH concentrations, e.g. under highly aerobic conditions when nitrogen fixation, a major consumer of NADPH (Benemann *et al.*, 1971), is switched off (Yates, 1970). Under these conditions NADPH oxidation may contribute to the respiratory protection of nitrogenase (Dalton & Postgate, 1969) and to the maintenance of the requisite intracellular NADPH/NADP⁺ concentration ratio.

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Determination of the Molecular Weights of Actins from Different Species by Gel Chromatography

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Recent studies (Spudich & Watt, 1971; Adelstein & Kuehl, 1970; Rees & Young, 1967) on rabbit actin indicate a molecular weight of 45000–48000. In all cases a purified protein was necessary for molecular-weight determination, gel chromatography often being used for purification.

Gel chromatography separates substances according to molecular size; Andrews (1965) has shown that, on Sephadex G-200, for many globular proteins over a considerable molecular-weight range (25000–200000) elution volumes are a linear function of the logarithm of the molecular weight.

Our studies show that actins from different sources obey this relationship, and that gel chromatography, as well as being a useful preparative tool,