activity with increasing pH, presumably due to a shift in the equilibrium of the tautomeric system resulting in the increased formation of the sodium salt of β -hydroxyacrolein. Evidence supporting the coupling of phosphorylation to the oxidative metabolism of malondialdehyde has been obtained from experiments using radioactive (^{32}P)phosphate.

A sex difference, in which the rate of aldehyde-stimulated oxygen uptake by mitochondria isolated from female rat livers was much greater, confirms previously observed effects with hepatic aldehyde oxidase preparations (Deitrich, 1966).

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Malate Oxidation by Tomato Fruit Mitochondria

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Intact particles from the outer walls of tomato fruit have been shown to retain the property of respiratory control during the oxidation of tricarboxylic acid-cycle intermediates. In these circumstances the oxidation of these substrates is stimulated by ADP (State 3 according to Chance & Williams, 1955) and then decreases as ADP becomes limiting (State 4). A gradual improvement in both respiratory control and ADP/O uptake ratios has been demonstrated up to incipient ripeness; thereafter the ratios decrease with advancing senescence of the fruit (Hobson, 1970).

Green tomatoes at any stage during development yield particles that oxidize malate rapidly after the addition of a small quantity of ADP, but subsequent additions of the nucleotide are progressively less effective. Exogenous supplies of thiamin pyrophosphate increase the rate of malate oxidation on ADP addition, and constant respiratory control ratios are

produced as successive amounts of phosphate acceptor are made available.

Mitochondria from tomatoes during ripening become progressively less dependent on exogenous thiamin pyrophosphate for the production of constant respiratory control ratios after a series of ADP additions, but added cofactor continues to stimulate the oxidation of malate. In addition, the State 4 rate immediately after the display of respiratory control slowly increases with time to a constant value. It has been found that in the presence of added CoA this initial inhibition in State 4 disappears and the rate remains linear.

The progressive decrease in the stimulation of malate by ADP on mitochondria from green fruit is probably due to oxaloacetate accumulation, which is counteracted by the presence of thiamin pyrophosphate. The higher rate of malate oxidation by particles from ripening tomatoes leads to greater oxaloacetate production, the further metabolism of which is dependent on the availability of thiamin pyrophosphate. It appears likely that oxaloacetate concentrations reach such a value at the end of the State 3 oxidation period that the initial part of the subsequent State 4 is inhibited. Since CoA prevents this, it would appear that this cofactor is present in limiting quantities when malate oxidation is rapid. Thus not only the concentrations of the various cofactors but possible changes in susceptibility of the oxidative system to both oxaloacetate and thiamin pyrophosphate (Hulme & Rhodes, 1968) may have important bearings on the onset of the climacteric respiration rise and the ripening processes.

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Phosphorylation Associated with Cyanide-Insensitive Respiration in Plant Mitochondria

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Isolated plant mitochondria possess a variable proportion of their respiration that is not inhibited by cyanide or antimycin A (Bonner, 1961). It has been suggested that the cyanide-insensitive respiration utilizes an alternative oxidase system that bypasses phosphorylation sites II and III (Storey & Bahr, 1969). Reversal of electron transport but not phosphorylation has been observed in plant mitochondria with succinate as substrate in the presence

of cyanide (Bonner & Bendall, 1968; S. B. Wilson & W. D. Bonner, unpublished work).

By using the firefly luciferase assay phosphorylation of ADP to ATP has been investigated with succinate as substrate in the presence of potassium cyanide at concentrations greater than that required to inhibit respiration maximally. Mitochondria were prepared from young suspension cultures of sycamore (*Acer pseudoplatinus*) and *Arum maculatum* spadices. These were 50–60% and 100% insensitive to cyanide respectively.

ATP production occurred in the presence of cyanide when succinate was substrate. Phosphorylation was inhibited by low oxygen partial pressure, by high concentrations of cyanide and by 1799 [bis(hexafluoroacetyl)acetone], a potent uncoupler of phosphorylation. Phosphorylation was observed with submitochondrial particles prepared from *Arum* mitochondria. These particles lack malate dehydrogenase, suggesting that the phosphorylation did not result from utilization of products of succinate oxidation.

These experiments show that the energy-conservation system associated with the cyanide-insensitive respiration is complete and the P/O ratios obtained (0.2–0.6) indicate that only one phosphorylation site is associated with the cyanide-insensitive alternative oxidase.

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ratios rarely exceeding 0.5 (see Gel'man, Lukoyanova & Ostrovskii, 1967; Pandit-Hovenkamp, 1966).

As part of an investigation into the electron-transfer system of the Gram-negative nitrogen-fixing aerobe *Azotobacter vinelandii* we have devised an isolation procedure yielding respiratory membranes of consistent and relatively high phosphorylation efficiency. Inherent in this procedure, which will be described in detail, is the necessity to control closely both culture conditions and the extent of cell growth, to modify previously described fractionation procedures and to pay particular attention to the nature, pH and ionic composition of both preparation and assay media.

Unsupplemented respiratory membranes prepared by this procedure are fully active for several hours and exhibit the following P/O ratios: NADH, 1.10; malate, 0.63; lactate, 0.82; succinate, 0.83; ascorbate–2,6-dichlorophenol-indophenol, 0.17. These values suggest that there is some degree of energy conservation at all three phosphorylation sites. They also agree with previous conclusions (Jones & Redfearn, 1966; Pandit-Hovenkamp, Eilermann & Kolk, 1969) that this organism oxidizes malate and lactate via NAD⁺-independent pathways that, in consequence, also lack site I phosphorylation.

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Phosphorylating Respiratory Membranes from *Azotobacter vinelandii*

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Oxidative-phosphorylation efficiencies of isolated bacterial respiratory membranes are in general very much lower than those exhibited by intact mitochondria. Gram-negative bacteria are especially poor in this respect; their cell envelope is considerably more complex than that of the Gram-positive bacteria and hence rather harsh methods of disruption must of necessity be employed during the preparation of respiratory membranes. The result has been a series of membrane preparations from Gram-negative bacteria that, with the exception of some chemosynthetic organisms, have oxidized NADH with P/O

The Effect of the Energetic State on the Glutaminase Activity of Kidney Mitochondria

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In view of the location of glutaminase within kidney mitochondria (Klingman & Handler, 1958) it is desirable to investigate the activity of this enzyme in intact mitochondria in relation to the energetic state. This is particularly so because with the isolated enzyme it has been shown that relatively high phosphate concentrations are required to obtain maximal activity (see Roberts, 1960).

Rat kidney mitochondria isolated by conventional techniques were incubated in a medium containing tris-HCl buffer, pH 7.4 (20mM), bovine plasma albumin (0.1%), EDTA (1–2mM) and either choline