of cyanide (Bonner & Bendall, 1968; S. B. Wilson & W. D. Bonner, unpublished work).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

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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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