of cyanide (Bonner & Bendall, 1968; S. B. Wilson & W. D. Bonner, unpublished work).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

chloride (100mm) or KCl (120mm). Oxygen uptake, NAD(P)⁺ reduction and mitochondrial swelling were followed and internal spaces were determined as described by Chappell (1964, 1968). Ammonia produced as a result of glutaminase activity was followed by using an ion-selective glass electrode (Crofts, 1967) or by the Conway microdiffusion technique.

When mitochondria were suspended in a medium containing glutamine, inorganic phosphate and rotenone the rate of production of ammonia was stimulated by the addition of an energy source, namely succinate. Addition of an uncoupler or antimycin A abolished this stimulation. The effects of succinate and uncoupler were especially pronounced in the presence of glutamate, which is an inhibitor of glutaminase activity by competition with phosphate.

At low phosphate concentrations (below 10mm) the increase in enzyme activity resulting from the provision of an energy source most probably resulted from an energy-dependent uptake of phosphate (see Hansford & Chappell, 1968). However, this cannot be the only explanation, for at high phosphate concentrations succinate still stimulated and this effect was reversed by addition of an uncoupling agent.

Apparently glutamate penetrates feebly the kidney mitochondrial membrane as revealed by experiments with oxygen uptake, NAD(P)⁺ reduction and swelling. During glutaminase activity large amounts of glutamate are accumulated within the mitochondrion.

A study of the pH-dependence of enzyme activity showed an optimum of pH 8 with Triton-treated mitochondria. With intact mitochondria in the presence of succinate the optimum was pH 8.5 and in the presence of antimycin on uncoupler it was pH 9. This displacement of the pH optimum to a more alkaline value was especially pronounced in the presence of both glutamate and uncoupler. If nigericin (Henderson, McGivan & Chappell, 1969) was present in a KCl medium the pH optimum for enzyme activity in intact non-respiring mitochondria was nearly the same as in a broken preparation, whereas its presence in K⁺ free medium displaced the pH optimum for glutaminase activity to a very alkaline value. Nigericin causes a K⁺-H⁺ exchange under these conditions and therefore would be expected in the latter case to cause the interior of the mitochondria to become more acidic.

It thus appears that the activity of kidney mitochondrial glutaminase depends on a number of factors, including the intramitochondrial phosphate concentrations and the internal pH.

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The Activities of Nicotinamide-Adenine Dinucleotide- and Nicotinamide-Adenine Dinucleotide Phosphate-Linked Isocitrate Dehydrogenase in Insect and Vertebrate Muscles

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On the basis of the distribution of NAD- and NADP-linked ICDH* in various tissues, and their relationship to the content of cytochrome *a*, Goebell & Klingenberg (1963, 1964) proposed that only NAD-linked ICDH was important in the operation of the tricarboxylic acid cycle for the oxidation of substrates in mitochondria. However, Pette (1966) showed that in a number of tissues the sum of the activities of NAD- and NADP-linked ICDH provided a more constant relationship to the cytochrome *a* content than either activity alone. In order to investigate the possible significance of these two enzymes in muscle a comprehensive comparative study of these two enzymes has been carried out.

Activities of ICDH were measured by following the reduction of NAD⁺ or NADP⁺ at 340nm at 25°C by using a Gilford recording spectrophotometer; extraction conditions for the NAD-linked enzyme were as suggested by Goebell & Klingenberg (1964), and assays were always performed within 10min of extraction. In all insect flight muscles studied, except that of the cockchafer (*Melolontha melolontha*), the activities of NAD-linked ICDH exceeded those of the NADP-linked enzyme usually by more than tenfold. However, the converse situation was observed in all vertebrate muscles studied except the quadriceps femoris of the mouse; for example activities of NAD- and NADP-linked ICDH for dogfish red, trout red, pigeon pectoral, mallard pectoral, rabbit semitendinosus and rat heart muscles were <0.1 and 25, <0.1 and 32, 1.4 and 40, 0.4 and 33, <0.1 and 9.3, 3.9 and 41 μmol/min per g fresh wt of muscle respectively.

Thus in fish and avian red muscles, which are known to be oxidative (Bone, 1966; George & Berger, 1966), the activity of the NAD-linked ICDH is extremely low in comparison with that of the

* Abbreviation: ICDH, isocitrate dehydrogenase.