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.

NADP-linked enzyme. Moreover calculations based on oxygen uptake during flight (see Tucker, 1968) of the pigeon show that the rate of the cycle is several times higher than the activity of the NAD-linked ICDH (activities having been adjusted for the temperature difference). Thus it is suggested either that vertebrate NAD-linked ICDH is less stable than that from insect flight muscle or that the NADP-linked enzyme must play a role in the oxidation of substrates by the tricarboxylic acid cycle in these muscles.

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The Effects of Calcium Ions and Adenosine Diphosphate on the Activity of Nicotinamide–Adenine Dinucleotide-Linked Isocitrate Dehydrogenase of Muscle

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The NAD-linked ICDH* is considered to be a regulatory enzyme for the tricarboxylic acid cycle in muscle. Previous work has shown that ADP is a specific activator of the enzyme, and therefore changes in the ADP concentration may regulate the rate of the cycle (Chen & Plaut, 1963; Goebell & Klingenberg, 1964). It is known that Ca^{2+} is important in the link between nervous stimulation and mechanical activity in muscle (see Weber, 1966), and that low concentrations of Ca^{2+} can activate the mitochondrial enzyme glycerol 1-phosphate dehydrogenase (Hansford & Chappell, 1967). Therefore effects of Ca^{2+} on NAD-linked ICDH of various muscles were investigated.

The activity of ICDH was measured by following the reduction of NAD^+ by crude extracts or mitochondrial preparations from insect flight muscles (e.g. *Lethocerus cordofanus*, *Locusta migratoria*, *Sarcophaga barbata*) and rat heart muscle. It was found that the activating effect of ADP on ICDH was dependent on the Ca^{2+} concentration, which was controlled by the use of Ca^{2+}–ethanedioxybis(ethylamine)tetra-acetate buffers. At a minimal Ca^{2+} concentration (1nM) the enzyme was maximally active in the absence of any added ADP, so that addition of ADP up to 2mM had no further effect on the enzyme activity. However, at 10μM-Ca^{2+} and in the absence of added ADP the activity was extremely low, but was increased to a maximal rate by addition of ADP. Raising the Ca^{2+} concentration from 10μM to 1mM had no further effect on the activity in the absence of added ADP and did not change the response of the enzyme to increasing ADP concentrations. It is suggested that the effects of Ca^{2+} and ADP on ICDH are independent.

These effects of Ca^{2+} demand that any theory of the control of ICDH activity should include this ion. There is some evidence to suggest that mitochondria may play a complementary role to the sarcoplasmic reticulum in the control of the intracellular distribution of Ca^{2+}; thus mitochondria might absorb Ca^{2+} during relaxation of muscle, and release it into the sarcoplasm during contraction (Weber, 1966; Patriarca & Carafoli, 1969). As ICDH is located in the mitochondrial matrix, a high intramitochondrial Ca^{2+} concentration would inhibit the enzyme whereas a low concentration would activate it. Thus it is suggested that the effects of Ca^{2+} and ADP, which are independent at the enzymic level, provide a concerted regulatory mechanism that ensures that the activity of ICDH is extremely low during rest, yet is maximal when the muscle is mechanically active.

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* Abbreviation: ICDH, isocitrate dehydrogenase.

Control of Respiratory and Fermentative Balance in Yeast

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It has been previously suggested that the fermentative carbon source controls the respiratory capability of the yeast *Saccharomyces cerevisiae* (Tustanoff & Bartley, 1964). To further investigate this hypothesis this species of yeast was grown

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