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.

- Bone, Q. (1966). *J. mar. biol. Ass. U.K.* **46**, 321.
 George, J. C. & Berger, A. J. (1966). *Avian Myology*, p. 115. New York and London: Academic Press.
 Goebell, H. & Klingenberg, M. (1963). *Biochem. biophys. Res. Commun.* **13**, 209.
 Goebell, H. & Klingenberg, M. (1964). *Biochem. Z.* **340**, 441.
 Pette, D. (1966). In *Regulation of Metabolic Processes in Mitochondria*, p. 28. Ed. by Tager, M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.
 Tucker, V. A. (1968). *J. exp. Biol.* **48**, 67.

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(ethyl-

amine)tetra-acetate buffers. At a minimal Ca^{2+} concentration (1mM) 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 10 μM - 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 10 μM 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.

- Chen, R. F. & Plaut, G. W. E. (1963). *Biochemistry, Easton*, **2**, 1023.
 Goebell, H. & Klingenberg, M. (1964). *Biochem. Z.* **340**, 441.
 Hansford, R. G. & Chappell, J. B. (1967). *Biochem. biophys. Res. Commun.* **27**, 686.
 Patriarca, P. & Carafoli, E. (1969). *Experientia*, **25**, 598.
 Weber, A. (1966). In *Current Topics in Bioenergetics*, vol. 1, p. 203, Ed. by Sanadi, D. R. New York and London: Academic Press.

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

* Abbreviation: ICDH, isocitrate dehydrogenase.

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