the latter occurs polymers are obtained. The formation of intramolecular disulphide bonds would explain why there is always some unpolymerized enzyme present even in highly polymerized preparations.

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Further Properties of Multiple Forms of Mitochondrial Monoamine Oxidase

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On polyacrylamide-gel electrophoresis of solubilized human and rat liver mitochondrial monoamine oxidase (Youdim & Sandler, 1967) five bands of activity have been demonstrated (Collins, Youdim & Sandler, 1968; Youdim, Collins & Sandler, 1968). These bands are stable at 4° for at least 1 month and when re-run electrophoretically do not break down into further bands of activity. Their absorption spectra are similar and resemble that of monoamine oxidase purified from rat liver (Youdim & Sourkes, 1966) and bovine kidney (Erwin & Hellerman, 1968) mitochondria. The possibility that the multiple forms represent a polymeric series is discounted, as the molecular weights of the bands as determined by the gel-filtration method of Andrews (1964) all lie within the range 288000–320000. These values are comparable with those reported by Youdim & Sourkes (1966) and Erwin & Hellerman (1968) for the whole enzyme. Observations by Udenfriend (1968) and Hartman, Kloepter & Yasunobu (1969), who used a highly purified ox liver mitochondrial monoamine oxidase preparation, confirm that multiple enzyme forms occur, although these authors, using immunological tests, suggest that multiplicity is due to the aggregation state of the enzyme similar to that of glutamate dehydrogenase (Bitensky, Yielding & Tomkins, 1965). Our findings suggest that the various bands of activity represent conformational isoenzymes resembling those of mitochondrial malate dehydrogenase (Markert & Whitt, 1968). Whether they are separate enzymes or different molecular forms of the same enzyme is still unclear.

Comparison of the effects of inhibitors on the human and rat enzymes shows remarkable similarities, although they differ in stability to heat treatment and in pH optima. The substrate employed strongly affects the activities of the inhibitors against the different enzyme bands. It is unlikely that any of the bands represent a diamine oxidase type of enzyme, for isoniazid and hydrazine are ineffective as inhibitors of enzyme activity (Gorkin & Tatyanenko, 1967).

If multiple forms of monoamine oxidase are actual constituents of the mitochondria, even though catalysing the same reaction, each may function somewhat differently in fulfilling specialized physiological roles in different metabolic sequences. It should be noted that many measurements of monoamine oxidase properties in the past have been made on what we now know to be multienzyme systems, and may have to be repeated on individual isoenzymes or at least on preparations of known composition.

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The Importance of α-Glycerophosphate Oxidase in Oxidation of Extramitochondrial Reduced Nicotinamide–Adenine Dinucleotide in Vertebrate and Insect Muscle

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As mitochondria are impermeable to external NADH, the α-glycerophosphate cycle has been proposed as a mechanism for mitochondrial reoxidation of glycolytic NADH (Klingenberg & Bücher, 1960; Sacktor, 1961). This cycle is important in insect flight muscle (for review see Sacktor, 1965), and it has been suggested that it may be important in vertebrate muscle (see Pette, 1966). However, there has been no attempt to obtain quantitative information on the maximum activity of this cycle in relation to the maximum rate of

glycolysis. In this investigation it has been assumed that the maximum activities of the regulatory enzymes of both glycolysis and the cycle will reflect the maximum rates of these two processes; and therefore maximum activities of α-glycerophosphate oxidase (EC 1.1.2.1) and phosphofructokinase (or hexokinase plus phosphorylase) have been measured in crude muscle extracts.

For insect flight muscles the maximum activities of these two enzymes were almost identical (expressed as μmoles of triose phosphate produced or oxidized/min./g. wet wt. of muscle), whereas in vertebrate muscle phosphofructokinase activity was about 100-fold higher than the activity of the oxidase: the activities were in blowfly (*Calliphora erythrocephala*) 90 and 110, in bumble bee (*Bombus hortorum*) 50 and 54, in cockroach (*Periplaneta americana*) 48 and 96, in locust (*Locusta migratoria*) 33 and 32, in butterfly (*Vanessa urticae*) 24 and 24, in the water bug (*Lethocerus cordofanus*) 8 and 8, in pheasant pectoral muscle 2·8 and 280, in pigeon pectoral muscle 1·2 and 36, in rabbit semitendinosus 0·2 and 16, in rabbit adductor magnus 0·8 and 60, in dogfish red muscle 0·1 and 24, and in dogfish white muscle 0·6 and 62 for the oxidase and phosphofructokinase respectively. It is concluded that for insect flight muscle the α-glycerophosphate cycle is of sufficient activity to account for reoxidation of all the glycolytic NADH, whereas in vertebrate muscle this cycle cannot account for more than about 2% of the required rate of NADH oxidation. In vertebrate white (anaerobic) muscle the conversion of pyruvate into lactate by lactate dehydrogenase is probably the most important mechanism for reoxidation of NADH. However, in red (aerobic) muscle, in which pyruvate is oxidized by the mitochondria, mechanisms other than the conversion of pyruvate into lactate or the α-glycerophosphate cycle must exist for reoxidation of glycolytic NADH.

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The Effects of Calcium Ions on the Activities of Hexokinase, Phosphofructokinase and Fructose 1,6-Diphosphatase from Vertebrate and Insect Muscles

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The coupling between nervous stimulation and mechanical activity in muscle is considered to be mediated by release of Ca^{2+} from the sarcoplasmic reticulum (for review see Weber, 1966): the increased concentration of Ca^{2+} in the sarcoplasm stimulates myofibrillar adenosine triphosphatase activity, which causes contraction; relaxation is brought about by reabsorption of the Ca^{2+} by the sarcoplasmic reticulum. The dependence of muscular contraction on a supply of ATP from catabolic processes (particularly glycolysis) suggested the possibility that the increased sarcoplasmic concentration of Ca^{2+} might stimulate the activities of the regulatory enzymes of glycolysis, or modify the basic control mechanism of glycolysis. A study was made of the effects of Ca^{2+} on the activities of hexokinase, phosphofructokinase and fructose 1,6-diphosphatase, and their inhibitions by glucose 6-phosphate, ATP and AMP respectively. As regulation of intracellular Ca^{2+} concentration may differ between red muscle and white muscle and between insect fibrillar muscle and non-fibrillar muscle (see Smith, 1966), enzymes from these four types of muscle have been used in this investigation.

The results show that increasing the Ca^{2+} concentration from 0·001 to 10 μM (the range that activates myofibrillar adenosine triphosphatase) has no effect on the activities of these enzymes from all types of muscle; nor does it change the inhibitions of hexokinase by glucose 6-phosphate, phosphofructokinase by ATP and fructose 1,6-diphosphatase by AMP. However, high concentrations of Ca^{2+} (1000 μM) inhibited these enzymes, although the inhibitions by glucose 6-phosphate, ATP and AMP were not changed.

Margreth, Cantani & Schiaffino (1967) have suggested that inhibition of frog leg muscle phosphofructokinase by Ca^{2+} might be an important mechanism of glycolytic control, as this enzyme might be located within the sarcoplasmic reticulum. An investigation of the possible location of hexokinase and phosphofructokinase by differential centrifugation showed that in vertebrate muscle a proportion of these enzymes sedimented with the reticulum fraction. However, with insect muscles the activities of these enzymes were found exclusively in the supernatant fraction. Thus in various muscles there is no correlation between the intracellular distribution of these two enzymes and their