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.

A.K.S. is grateful to the Medical Research Council for a Research Studentship.

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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 & Tatyenenko, 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.

G. G. S. C. and M. B. H. are grateful to the Medical Research Council and Hoescht Pharmaceutical Co. for financial support.

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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 re-oxidation 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