hydrochloride, 2·1s; mol.wt. in 6M-guanidine hydrochloride, 57000.

The enzyme is irreversibly inhibited by NaBH_4 (0·01M) in the presence of substrate (Jeffcoat, 1968). Such inhibition is indicative of the reduction of a Schiff base formed between the carbonyl group of the substrate and the free amino group of a specific lysine residue in the enzyme (Horecker, Pontremoli, Ricci & Cheng, 1961). This has been confirmed by treating deoxyoxo[6- ^{14}C]glucarate and NaBH_4 with enzyme from cells grown in the presence of [4,5(*n*)- ^3H]lysine. The resulting reduced enzyme-substrate complex was dialysed and chromatographed twice on DEAE-cellulose. Co-chromatography of ^{14}C and ^3H was obtained, with the ratio showing that 2 moles of substrate were bound/mole of enzyme. This [^3H]enzyme-[^{14}C]substrate complex was stable to polyacrylamide-gel electrophoresis.

The complex was hydrolysed with 6M-HCl at 110° and the hydrolysate subjected to paper electrophoresis (80 v/cm.) at pH 1·77 for 2 hr. Two radioactive areas were found. The first of these, lysine (82 cm. towards the cathode), contained only ^3H . The second (44 cm. towards the cathode) contained both ^3H and ^{14}C and was therefore judged to be a substrate-lysine derivative. This doubly labelled derivative was stable to further paper electrophoresis (pH 6·5) and to repeated paper chromatography. The ratio of the ^3H in this fragment to ^3H in the free lysine was 1:28. This, together with the amino acid analysis showing 60 μmoles of lysine/ μmole of enzyme, is in agreement with the binding of 2 μmoles of substrate/ μmole of enzyme.

The mechanism of deoxyoxoglucarate dehydratase action thus involves the intermediate formation of a Schiff base between the substrate and a lysine residue in the enzyme. In this respect, the enzyme is similar to acetoacetate decarboxylase (EC 4.1.1.4) (Westheimer, 1963) and to a number of aldolases (class I aldolases; Rutter, 1964) that have this type of mechanism.

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Multiple Polymers of Histidine Ammonia-lyase

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Histidine ammonia-lyase (histidase, EC 4.3.1.3) catalyses the formation of urocanate from L-histidine. It is activated by GSH, mercaptoethanol and thioglycollate, and inhibited by EDTA (Mehler & Tabor, 1953).

We have purified histidase from *Pseudomonas testosteroni* grown in a medium containing L-histidine and succinate as sources of nitrogen and carbon. It was obtained by heat treatment of crude extracts followed by $(\text{NH}_4)_2\text{SO}_4$ fractionation and chromatography on DEAE-cellulose. When prepared in this way, the enzyme was activated some seven- or eight-fold by 3mM-GSH.

When untreated enzyme was subjected to electrophoresis on 7½% polyacrylamide gels at pH 9·5 and stained with Amido Black, as many as nine bands were visible. If unstained gels were assayed for histidase, five and sometimes six bands of activity were detected. The assay method was not sufficiently sensitive to resolve the activities of the slower and closer-moving components. Addition of GSH (3mM) before electrophoresis resulted in only one protein band and one activity band being obtained. These corresponded to the fastest-moving band present when GSH was omitted.

When enzyme without GSH was centrifuged in the analytical ultracentrifuge, five peaks were resolved, sedimenting with coefficients of 9·7, 14·1, 17·1, 19·8 and 21·6s respectively. In the presence of GSH (3mM), a single but much larger 9·7s peak was obtained.

Mercaptoethanol, thioglycollate and dithiothreitol all had the same effect as GSH; substrate and EDTA did not influence the number of enzyme species obtained. The reversal of the effects of thiol treatment was achieved either by $(\text{NH}_4)_2\text{SO}_4$ precipitation at pH 6·0 or by chromatography on DEAE-cellulose.

The molecular weight of the GSH-treated enzyme was found to be 200000 by the sedimentation-equilibrium method of Yphantis (1964); a molecular weight of 33000–36000 was obtained for the enzyme in 6M-guanidine hydrochloride and 0·1M-GSH. These values compare with those of 214000 and 35000 respectively obtained by Rechler (1969) for histidase from an unnamed pseudomonad.

It appears that at least some free thiol groups of histidase are readily oxidized to form disulphide bonds, resulting in decreased enzymic activity. We suggest that these disulphide bonds may be formed either intra- or inter-molecularly, and that when

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 & Tatyanyenko, 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 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