Short Communications

The oxidation of 3,4-dihydroxyphenylacetaldehyde

The pathways of metabolism through which DOPA proceeds in mammalian tissue have been the subject of considerable investigation. These studies have established that an avenue of cardinal importance is the decarboxylation of this amino acid to 3,4-dihydroxyphenylethylamine[1,2]. The oral administration of large doses of DOPA to humans led to the urinary excretion of 3,4-dihydroxyphenylacetic acid as well as homovanillic acid[3]. Furthermore in experiments employing [^{14}C]DOPA, 3,4-dihydroxyphenylethylamine and 3,4-dihydroxyphenylacetic acid were demonstrated to be among metabolic products isolated from the urine of rats injected with the labeled amino acid. More recently, evidence has been presented which demonstrates that soluble monoamine oxidase from mammalian liver was capable of oxidizing a variety of phenylethylamines, including the 3,4-dihydroxyphenylethylamine, to what was presumed to be its corresponding aldehyde and that the aldehyde formed may then undergo further oxidation to the cognate acid[4,5].

We have been investigating the metabolism *in vitro* of catechol amines, among them 3,4-dihydroxyphenylethylamine, in mammalian tissue and wish to present evidence that this material is oxidatively deaminated to 3,4-dihydroxyphenylacetaldehyde, and, moreover, that the aldehyde is oxidized to 3,4-dihydroxyphenylacetic acid.

The whole brain (3.5 g) of adult guinea pigs was homogenized with 10 ml 0.1 *M* phosphate buffer, pH 6.8, in a Potter homogenizer at 0°–5° and 3-ml aliquots were placed in the Warburg flasks. Sufficient semicarbazide was added to bring the final concn. to $5 \cdot 10^{-3}$ *M*. Flasks containing all additions except substrate were run along with boiled-enzyme controls in all experiments. Certain experimental flasks were equilibrated with nitrogen, while others were equilibrated with air. The flasks were incubated at 37°. At zero time the substrate, 5 mg 3,4-dihydroxyphenylethylamine, was tipped into the flask. The final flask vol. was 5 ml. After incubation for 3 h the contents of the flasks were transferred to tubes, acidified to pH 5.1 with dil. HCl, and placed in a boiling water bath for 5 min. The precipitated protein was removed by centrifugation, washed with 5 ml 0.001 *N* HCl and the combined supernatant was extracted 4 times with 5-ml aliquots of ethyl acetate. The combined extracts were evaporated to dryness under a stream of dry nitrogen and the residue dissolved in 0.2 ml ethyl acetate. This material was placed on Whatman No. 3 paper and developed with *n*-butanol–1*N* HCl or *n*-butanol–acetic acid–water. The metabolites were detected with diazotized sulfanilic acid (Table I). The 3,4-dihydroxyphenylacetaldehyde and its semicarbazone were prepared as previously described[6].

Having established that 3,4-dihydroxyphenylacetaldehyde is an intermediate of dihydroxyphenylethylamine, we studied the rate of oxidation of this material by

Abbreviations: DOPA, 3,4-dihydroxyphenylalanine; DPN, diphosphopyridine nucleotide.

TABLE I

	Contents				Isolated metabolites	
	Brain homogenate	*3,4-dihydroxyphenylethylamine*	*Semicarbazide*	*Gas phase*	R_F** *Values*	
Flask 1	3.0 ml	5.0 mg	$5 \cdot 10^{-3}$ *M*	air	0.58	0.72
Flask 2*	3.0 ml	5.0 mg	$5 \cdot 10^{-3}$ *M*	air	—	—
Flask 3	3.0 ml	5.0 mg	—	air	—	0.71
Flask 4	3.0 ml	5.0 mg	$5 \cdot 10^{-3}$ *M*	N_2	—	—
					Standards	
Semicarbazone of 3,4-dihydroxyphenylacetaldehyde	—	—	—	—	0.59	—
3,4-dihydroxyphenylacetic acid	—	—	—	—	—	0.71

* Boiled brain homogenate control.
** R_F determined by ascending paper chromatography. Solvent: *n*-butanol–1*N* HCl.

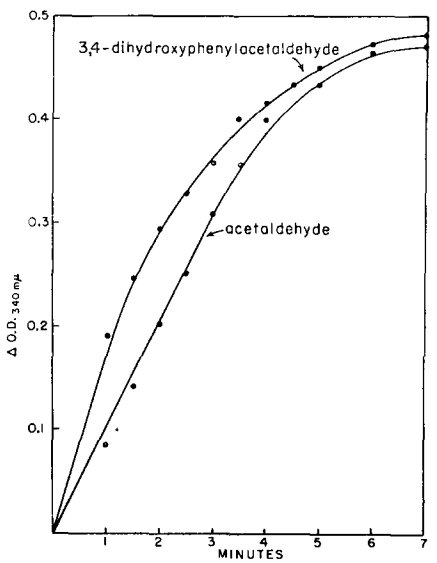

Fig. 1. Cuvettes contained 2.4 ml water, 0.3 ml 0.01 *M* pyrophosphate buffer, pH 9.3, 0.1 ml 0.2% DPN, 0.1 ml enzyme equiv. to 100 units of liver aldehyde dehydrogenase activity, and 0.1 ml of 10 mg/ml 3,4-dihydroxyphenylacetaldehyde or 0.1 ml 0.5% freshly distilled acetaldehyde. Controls containing no enzyme or no substrate were run.

the DPN-dependent aldehyde dehydrogenase described by RACKER[7] and the flavin-linked aldehyde oxidase of MAHLER[8]. DPN-dependent aldehyde dehydrogenase rapidly oxidized 3,4-dihydroxyphenylacetaldehyde as shown in Fig. 1. Acetaldehyde is shown for comparison.

3,4-dihydroxyphenylacetaldehyde was not oxidized by the flavin-linked aldehyde oxidase.

The oxidative metabolism of 3,4-dihydroxyphenylacetaldehyde was further explored with regard to the oxidation of the catechol. For these studies, mammalian tyrosinase was obtained from Harding-Passey melanoma as described[9] and oxidation of the substrate was followed with the conventional Warburg manometric technic. The flask contained 1.0 ml phosphate buffer, pH 6.8, 1.0 ml enzyme equiv. to approx. 6 units, and 1.0 ml of substrate containing either 1 mg/ml DOPA or 1 mg/ml 3,4-dihydroxyphenylacetaldehyde. Boiled enzyme and endogenous controls were run. The

oxidation of DOPA proceeded at a rate of 432 μl O_2/h, while that of 3,4-dihydroxyphenylacetaldehyde progressed sluggishly at 37 μl O_2/h under these conditions. We conclude that 3,4-dihydroxyphenylacetaldehyde is indeed a substrate for this enzyme, but that it is oxidized at an extremely slow rate.

These studies *in vitro* indicate that 3,4-dihydroxyphenylethylamine is metabolized to 3,4-dihydroxyphenylacetaldehyde which may be oxidized by DPN-dependent aldehyde dehydrogenase to 3,4-dihydroxyphenylacetic acid.

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Action of the chondroitinase of *Proteus vulgaris* on hyaluronic acid

Enzymes capable of degrading hyaluronic acid have been classified as follows[1]: endoglucuronidases, yielding a tetrasaccharide as reaction product (*e.g.* leech hyaluronidase); endohexosaminidases, yielding a different tetrasaccharide as the main reaction product and acting by a process of transglycosylation (*e.g.* testicular hyaluronidase), this type of enzyme will also degrade chondroitin sulphates A and C; endohexosaminidases, yielding the Δ 4,5-unsaturated disaccharide, 3-O-(β-D-Δ 4,5-glucoseenpyranosyluronic acid)-N-acetyl-2-deoxy-2-amino-D-glucose (*e.g.* staphylococcal hyaluronidase). All bacterial hyaluronidases which have previously been studied belong to the last group.

Recently DODGSON AND LLOYD[2] showed that the chondroitinase of *Proteus vulgaris* (National Collection of Type Cultures No. 4636) degraded cartilage chondroitin sulphate in a manner analogous to that of testicular hyaluronidase. It was therefore of interest to examine the activity of the Proteus enzyme towards hyaluronic acid.

Several different preparations of a chondroitinase concentrate (the C2 concentrate described by DODGSON AND LLOYD[3]) were used during the course of the work. The activities of these and other enzyme preparations towards hyaluronic acid were followed by determination of liberated reducing material according to the method of SOMOGYI[4], using glucose as the reference standard (see DODGSON, LLOYD AND SPENCER[5]). The optimum pH for chondroitinase activity towards sodium hyaluronate (0.75 mg/ml), in the presence of a mixture (1:1) of 0.2 *M* sodium acetate–acetic acid