Human Plasma Monoamine Oxidase

I. PURIFICATION AND IDENTIFICATION

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Amine oxidases that occur in the plasma of mammals have been intensively studied in recent years (1-8). The ability of human plasma and serum to convert benzylamine to benzaldehyde has recently been demonstrated (9). Evidence has been presented to show that this enzymatic activity is different from other human plasma enzymes that catalyze the oxidation of amines (i.e. ceruloplasmin and diamine oxidase) (9, 10). The present paper reports the purification of this monoamine oxidase activity from human plasma. It also defines the substrate specificity of the purified enzyme, the stoichiometry of the reaction it catalyzes, and inhibitors of the reaction.1

EXPERIMENTAL PROCEDURE

Materials

Fresh heparinized whole blood was obtained from normal human donors as previously described (9) or, with the cooperation of Dr. Andrew G. Morrow of the National Heart Institute, from the pump oxygenator apparatus used in surgical cardiopulmonary bypass; the plasma monoamine oxidase preparations from either source behaved identically during purification. Aluminum hydroxide gel Cy was prepared according to Willstätter and Kraut (11) and aged over 6 months before use. Recrystallized beef liver catalase and lyophilized horseradish peroxidase (RZ > 1) were purchased from the Worthington Biochemical Corporation and were freed from ammonium salts before use by dialysis against 0.02 M phosphate2 buffer, pH 7.4. Distilled water, containing less than 10 parts per billion of copper (12), was used throughout.

Methylamine hydrochloride, ethylamine hydrochloride, ethylenediamine dihydrochloride, 2-bromoethylamine hydrobromide, and o-dianisidine, purchased from Distillation Products Industries, were recrystallized twice from absolute ethanol. Ethanolamine, propylamine, butylamine, hexylamine, octylamine, decylamine, N-methylbenzylamine, and N,N-dimethylbenzylamine, purchased as the free bases from Distillation Products Industries, The Matheson Company, Inc., or J. T. Baker Chemical Company, were converted to the hydrochlorides by treatment with hydrogen chloride gas in absolute ethanol and then recrystallized at least twice from absolute ethanol. Benzylamine, purchased as the free base from Distillation Products Industries, was purified by distillation (9). Iproniazid (Marasilid phosphate) was a gift of Hoffmann-La Roche, Inc. All other compounds were obtained from commercial sources.

Methods

Activity of Monoamine Oxidase Preparations—Monoamine oxidase activity was routinely measured, with benzylamine as substrate, by the spectrophotometric assay described by Tabor, Tabor, and Rosenthal (2) except that the assay was carried out at 25° rather than 30°. In this standard assay, 1 unit of enzymatic activity was defined as the amount of enzyme catalyzing a change in absorbance at 250 m/1 of 0.001 per minute in 3.0-ml reaction mixtures containing 0.2 M phosphate* buffer, pH 7.2, and 3.3 mM benzylamine. Under these conditions, rates of benzaldehyde production were constant for at least 30 minutes and were proportional (Fig. 1) to the amount of enzyme added. Specific activities were expressed in units per mg of protein, and protein was determined by the method of Lowry et al. (13), with crystalline bovine serum albumin as the standard.

While the standard assay was suitable for enzyme preparations with specific activities greater than 0.10, the high protein concentrations of cruder preparations prevented the use of this direct spectrophotometric assay. During the initial stages of purification, enzymatic activities were determined by a modification of the standard assay that had been found to be suitable for human plasma and serum (9). The relation of the modified assay to the standard monoamine oxidase assay is given in Fig. 1. From the data of this figure it may be calculated that 1 unit of enzymatic activity (by the standard assay) results in an absorbance change of 0.0042 per minute in the modified procedure.

For the purpose of measuring rates of benzylamine oxidation as well as determining benzaldehyde concentrations, the molar absorptivity of benzaldehyde at 250 mμ was assumed to be 1.2 × 104 M⁻¹ cm⁻¹ (14).

Ammonia Determinations—Ammonia was determined colorimetrically after separation from the sample by microdiffusion according to the method of Brown et al. (15). Since falsely low values occurred in the presence of volatile amines, ammonia determinations were not performed on incubation mixtures con-
taining volatile substrates, except under circumstances in which the amines were fully oxidized. All determinations were corrected for traces of ammonia released from substrates and enzyme preparations.

**Manometric Measurements**—Oxygen consumption was followed in the Warburg constant volume respirometer (16) with air as the gas phase.

**Hydrogen Peroxide Determinations**—Hydrogen peroxide was measured by a modification of the peroxidase assay (17, 18) used for the colorimetric estimation of glucose (Glucostat). Since purified preparations of the monoamine oxidase contained catalase activity (see below), the amine oxidase reaction was coupled to the peroxidase reaction as indicated in the legends of Figs. 4 and 5. Under these conditions, the chromophore resulting from added (or enzymatically produced) hydrogen peroxide was stable for 1 hour and was not affected by the amines used as substrates for the monoamine oxidase. Its absorbance at 440 m\(\mu\) was proportional to concentrations of (added) hydrogen peroxide in the range of 5 to 75 m\(\mu\). The concentrations of hydrogen peroxide solutions used as standards for the coupled assay were determined by the change in optical density at 230 m\(\mu\) after the addition of catalase, and the molar absorptivity of hydrogen peroxide was assumed to be 61 m\(\mu\)H per liter. The absorbance at this wave length (19). With benzylamine as the substrate of the monoa-

**Results**

**Purification of Plasma Monoamine Oxidase**

Except where otherwise noted, all operations were carried out at 4°. All centrifugations were done at 1300 \(\times\) g for 45 minutes.

**Step 1: Collection of Plasma**—Plasma was obtained by cen-
trifugation from human whole blood, containing 4500 U.S.P.
units of heparin per liter. The plasma was used immediately or stored for not more than 2 weeks at -20° before use.

**Step 2: First Ammonium Sulfate and Ethanol Fractionations**—To each liter of plasma were added 400 mg of disodium EDTA and then, with stirring, 300 g of solid ammonium sulfate. The precipitate was collected by overnight filtration and extracted with 200 ml of distilled water. After removal of any insoluble material by centrifugation, the extract was diluted to a volume of 400 ml with distilled water and cooled to the point of freezing. The extract was treated with 40 ml of a 0.1 M aqueous solution of manganese chloride and then, with stirring, with 500 ml of 95% ethanol that had been cooled to -10°. After immediate cen-
trifugation at -10°, the precipitate was discarded and an additional 200 ml of precooled 95% ethanol were added. The mix-
ture was centrifuged in the same manner and the supernatant solution was discarded. The precipitate was extracted into 200 ml of a 0.2 M dipotassium hydrogen phosphate solution containing 1 mM EDTA. Insoluble material was removed by centrifugation, and the extract was dialyzed against three changes of 2 liters of 0.02 M dipotassium hydrogen phosphate solution containing 0.1 mM EDTA.

**Step 3: Second Ammonium Sulfate Fractionation**—To each liter of the dialyzed solution were added 250 g of solid ammonium sulfate. The precipitate was removed by centrifugation, and an additional 100 g of solid ammonium sulfate were added to the supernatant solution. The resultant precipitate was collected by centrifugation and dissolved in 10 ml of 0.2 M phosphate buffer, pH 7.9. If the specific activity of this solution was greater than 2.0, it was used directly in Step 4. If it was not, the solution was fractionated with solid ammonium sulfate before the subsequent step. On fractionation, the enzyme precipitated from solutions containing 1.3 to 1.5 M ammonium sulfate.

**Step 4: Adsorption with Alumina C\(\gamma\)**—The product of the above ammonium sulfate step was dialyzed for 4 hours against hourly changes of 0.01 M phosphate buffer, pH 7.4. The dialyzed solution was then treated with aluminum hydroxide gel C\(\gamma\) (sus-
pended in distilled water) to absorb inert protein. This “negative adsorption” step gave results that varied from preparation to preparation and required the use of pilot experiments. In general the following procedure was used: 2.5 ml of a 50 g/100 ml (dry weight) suspension of alumina C\(\gamma\) were added per 100 mg of protein and the mixture was centrifuged; 0.5-ml (per 100 mg of protein) aliquots of the suspension were then added until the intensely blue color of the supernatant solution (due to contami-
nating ceruloplasmin) was completely gone; at this point, further additions of alumina C\(\gamma\) resulted in marked increases in specific activity but at the expense of losses in total activity; for this reason, addition of alumina C\(\gamma\) was usually not continued after 30% of the enzymatic activity was adsorbed. Specific activities after adsorption varied from 10 to 120. In all cases the enzym-
atic activity in the final supernatant solution was concentrated (without change in specific activity) by the addition of solid ammonium sulfate to a final concentration of 2 M and by dis-
solving the precipitate in a minimal volume of 0.2 M phosphate buffer, pH 7.2. Before use this enzyme solution was freed of detectable ammonium ions by dialysis against the same buffer.

A typical purification protocol (Table I) shows that the procedure afforded a purification of approximately 5000-fold. In practice, it was found that 90% of the monoamine oxidase ac-
tivity discarded in Steps 3 and 4 could be “reworked” in the same steps (enzymatic activity adsorbed by alumina C\(\gamma\) was eluted with 0.2 M phosphate buffer, pH 7.2), and the over-all yield raised to 30% or greater.
After Steps 3 and 4, enzyme preparations (in 0.2 M phosphate buffer, pH 7.2) could be stored at -20° for 6 months or more without detectable loss of activity. No loss of enzymatic activity was detected after periods of 48 hours at 4° or 4 hours at 25°. Dialysis against repeated changes of 0.2 M phosphate buffer, pH 7.2, for periods of 12 hours led to small (approximately 10%) losses of activity.

While enzyme preparations resulting from Step 4 appeared colorless, absorption spectra of concentrated solutions usually revealed an absorption maximum at 404 mp and a small broad shoulder in the area of 500 mp. Neither of these spectral features could be correlated with monoamine oxidase activity, however, and neither was significantly altered by the anaerobic addition of benzylamine. Since even the most purified preparations contained measurable (20, 21) (but variable) amounts of catalase activity, the visible spectra could be attributed, at least in part, to catalase.

In phosphate and pyrophosphate buffers, 3.3 mm concentrations of benzylamine were optimally oxidized (Fig. 2) at neutral pH values. Identical pH optima have been observed for benzylamine oxidation by crude human plasma (9) and by the amine oxidase purified from beef plasma (2). Rates of benzylamine oxidation in pyrophosphate buffer agreed well with rates obtained in low molarities (1 and 20 mM) of phosphate buffer. At pH values below 8.0, the oxidation of 3.3 mm concentrations of benzylamine was stimulated to a slight degree (Fig. 2) by high molarities (0.2 M) of phosphate buffer. Higher concentrations of this buffer produced no further stimulation, and the stimulation was not related to the cation present in that similar results were obtained in potassium and sodium phosphate buffers. A

<table>
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<th>Step</th>
<th>Volume</th>
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<th>Protein</th>
<th>Specific activity</th>
<th>Yield</th>
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<td>6.8</td>
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Fig. 1 (left). Relation of modified to standard monoamine oxidase assay. In the standard assay (2), the indicated amounts of an amine oxidase preparation of specific activity 4.4 were incubated at 25° in 3.0 ml of 0.2 M phosphate buffer, pH 7.2, containing 3.3 mm benzylamine; the change in optical density at 250 mp (O) was recorded directly. In the modified assay, the indicated amounts of the same enzyme preparation were incubated at 37° in 1.5 ml of phosphate buffer, pH 7.2, containing 0.8 mm benzylamine; after 3 hours of incubation, the reaction mixtures were extracted with 1.5 ml of cyclohexane as previously described (9), and the optical densities of the extracts at 242 mp (O) were recorded.

Fig. 2 (right). Effect of pH and buffers on apparent enzyme activity. Initial rates of benzaldehyde production were obtained spectrophotometrically from reaction mixtures containing 10 units of purified monoamine oxidase (specific activity, 45) and 3.3 mm benzylamine in 3.0 ml of 0.001 M ( ), 0.02 M ( ), or 0.2 M ( ) phosphate buffer, or in 3.0 ml of 0.1 M sodium pyrophosphate buffer (O). All pH values are those of the complete reaction mixtures after 20 minutes of incubation. The substrate, as the free base, was brought to the appropriate pH before use by the addition of hydrochloric acid.
more dramatic, but also unexplained, stimulation of benzylamine oxidation by phosphate buffer at neutral pH has been reported for the amine oxidase purified from beef plasma (4).

As has previously been observed with crude human serum and plasma (9), the addition of catalase had no effect on the initial rates of benzylamine oxidation by purified preparations of the human monoamine oxidase. In keeping with the presence of catalase activity in purified preparations of the enzyme (noted above), initial rates of oxygen consumption (6.25 μatoms per hour per 275 units of enzyme), as measured manometrically under the conditions given in Table II, were the same whether or not the indicated amount of catalase was added to the reaction mixture.

Stoichiometry of Amine Oxidation

Previous work has shown that the conversion of benzylamine to benzaldehyde in the presence of crude human serum requires molecular oxygen (9). Manometric determination of oxygen consumption (Table II) indicated that, with benzylamine as substrate and in the presence of added catalase, 1 μatom of oxygen is consumed for each micromole of benzylamine initially present. An equivalent amount of ammonia was formed. While, at relatively high concentrations, benzaldehyde was lost from incubation mixtures (distillation into the upper portions of the Warburg vessel was noted at 37°), good stoichiometry of benzaldehyde (as well as ammonia) production and benzylamine utilization was observed with incubation mixtures containing small substrate concentrations and maintained (without shaking) at 25° (Fig. 3).

As previously reported for the amine oxidase of steer plasma (2), the human enzyme deaminates both amino groups of long chain diamines. Incubation of 1.0 μmole of 1,8-octanediine with 55 units of monoamine oxidase (specific activity, 113) and 30 μmoles of phosphate buffer, pH 7.6, in a total volume of 0.20 ml for 3 hours at 37° resulted in the production of 2.0 μmoles of ammonia.

Catalase activity found in even the most purified monoamine oxidase preparations prevented a manometric demonstration of hydrogen peroxide production. Hydrogen peroxide production could be demonstrated, however, when the amine oxidase reaction was coupled with the enzymatic peroxidation of o-dianisidine. Manometric determination of oxygen consumption (Table II) indicated that, with benzylamine as substrate and in the presence of added catalase, 1 μatom of oxygen is consumed for each micromole of benzylamine initially present. An equivalent amount of ammonia was formed.

Stoichiometry of Benzylamine Oxidation

Each Warburg vessel contained in a total volume of 3.0 ml, 600 μmoles of phosphate buffer (pH 7.2), 5.0 μmoles of benzylamine, 275 units of monoamine oxidase (specific activity, 113), and 3000 units of catalase. Incubations were carried out, with shaking, at 37° for 1 hour, at which time O₂ uptake was complete.

| Benzylamine present initially | 5.0 μmoles |
| Oxygen consumed                | 4.9 μatoms |
| Benzaldehyde found             | 3.9 μmoles |
| Ammonia formed                 | 5.1 μmoles |

**Fig. 3.** Stoichiometry of benzylamine oxidation. Monoamine oxidase (12 units; specific activity, 113) and the indicated number of millimicromoles of benzylamine were incubated in 3.0 ml of 0.1 M sodium pyrophosphate buffer, pH 9.2, at 25° until benzaldehyde production (A), as indicated by the change in optical density at 250 mμ, was complete. Ammonia production (B) was corrected for traces of ammonia found in controls incubated without substrate and without enzyme.

**Fig. 4.** Stoichiometry of benzylamine oxidation. Monoamine oxidase (50 units; specific activity, 45), the indicated number of millimicromoles of benzylamine, 300 μg of horseradish peroxidase, 60 μg of o-dianisidine in 25 μl of absolute methanol, and 300 μmoles of sodium pyrophosphate buffer, pH 9.0, were incubated in a total volume of 3.0 ml at 25° until hydrogen peroxide production, as indicated by the change in optical density at 440 mμ, was complete. Controls without substrate or without monoamine oxidase gave no change in optical density at 440 mμ. Optical density was converted to millimicromoles of H₂O₂ as described under "Methods."
derived amine. Under conditions (1.0 mM substrate concentration) given in Fig. 5, 15 units of enzyme catalyzed the oxidation of β-phenylethylamine at one-fifth (24 μmole per hour) the rate of the parent compound, ethylamine. Significantly lower rates of oxidation (i.e. less than 10 μmole per hour) were also observed with β-bromoethylamine, ethanolamine, β-mercaptoethylamine, β-aminopropionitrile, and ethylenediamine in this assay system. Under the same conditions, no oxidation of the secondary amine, dimethylamine, could be detected.

While short chain diamines were poor substrates of the plasma enzyme, the long chain homologue, 1,8-diaminoocotane, was actively oxidized. Under the conditions (1.0 mM substrate concentration) given in Fig. 5, 1,8-diaminoocotane was oxidized at a rate (150 μmole per hour) similar to that found for octylamine. The long chain polyamines, spermidine and spermine, on the other hand, were not oxidized under these conditions.

The monoamine oxidase of human plasma specifically oxidizes primary aliphatic amines that lack substituents on the α-carbon atom. Compounds related to benzylamine which could not be characterized in this manner (but which could be tested as substrates by the spectrophotometric determination of benzaldehyde) were not oxidized. Under conditions identical with those described in Fig. 2, there was no detectable production of benzaldehyde from reaction mixtures containing 3.3 mM concentrations of the secondary or tertiary amines, N-methylbenzylamine, N,N-dimethylbenzylamine, or dibenzylamine, at pH 7.2, 8.6, or 9.2. Similarly, the conversion of benzyl alcohol or benzyl bromide to benzaldehyde could not be demonstrated under these conditions. By a similar spectrophotometric assay (246 μmole), the oxidation of di-α-methylbenzylamine to acetophenone was not detected.

The ability of the plasma amine oxidase to deaminate primary amines of pharmacological or biological interest was tested at pH 7.6 (Table III). Amines that were unequivocally deaminated (i.e. deamination of greater than 10% of the amine initially present) include tyramine, 3,4-dihydroxyphenylethylamine (dopamine), 3-methoxy-4-hydroxyphenylethylamine, tryptamine, kynuramine, ethanolamine, and β-mercaptoethylamine. Rates of deamination of simple monoalkylamines could not be determined by this assay (see "Methods"). Spectrophotometric and manometric determinations of initial rates of benzylamine oxidation, however, indicated that under the conditions of Table III, 55 units of the monoamine oxidase oxidize 3000 μmole of benzylamine in 3 hours. Although the data of Table III do not furnish truly initial rates, it is apparent that no compound in this table is oxidized as actively as benzylamine. It is also apparent that any compound with ionized substituents in close proximity to the amino group is not a substrate of the enzyme. With particular reference to catecholamines, only those derivatives of tyramine that lack a β-hydroxy group were deaminated. Typical substrates of diamine oxidase (histaminase), histamine and 1,4-diaminobutane, were poor substrates of the plasma monoamine oxidase.

Inhibitors of Amine Oxidation

Semicarbazide and other carbonyl reagents are potent inhibitors of the amine oxidase of steer plasma (2, 6). Like this enzyme, the monoamine oxidase of human plasma was completely inhibited by 0.1 mM semicarbazide (Fig. 6A), and 30 μM concentrations of the reagent produced a 50% inhibition of enzymatic activity. The inhibition (Fig. 6B) was noncompetitive with respect to substrate. As has been previously reported for the steer plasma enzyme, the inhibition occurred instantaneously, and preincubation of the enzyme with semicarbazide for 15 minutes at 25°C did not alter the results.

Isoniazid and iproniazid are also effective inhibitors of the
Fig. 6. Semicarbazide inhibition. A, monoamine oxidase (48 units; specific activity, 113) was incubated in 0.40 ml of 0.1 M sodium pyrophosphate buffer, pH 8.9, containing 0.1 mM kynuramine and the indicated concentrations of semicarbazide; B, an equal amount of monoamine oxidase (specific activity, 113) was incubated in 0.40 ml of 0.1 M sodium pyrophosphate buffer, pH 8.9, containing the given concentrations of kynuramine, with (●) or without (O) 25 µM semicarbazide. Mixtures were incubated at 25°, and rates are expressed as millimicromoles of 4-hydroxyquinoline per hour, measured spectrophotometrically. Under these conditions no interaction of semicarbazide with either kynuramine or 4-hydroxyquinoline could be detected spectrally.

As previously reported for the amine oxidase of steer plasma (2), both compounds inhibited the monoamine oxidase of human plasma (Fig. 7) only after a period of preincubation. While no inhibition of benzylamine oxidation occurred without preincubation of enzyme and inhibitors, 1.0 mM isoniazid and 0.1 mM iproniazid gave more than 50% inhibition after 30 minutes of preincubation. Since rates of benzylamine oxidation determined in the presence of 0.1 mM iproniazid remained constant for at least 30 minutes, it was concluded that substrates of the enzyme may prevent, but not reverse, the time-dependent inhibition. The sensitivity of the human enzyme to isoniazid inhibition was considerably less than that reported for the steer plasma enzyme under similar conditions (2). The sensitivity of the human enzyme to inhibition by iproniazid, on the other hand, was more marked.

The amine oxidase of beef plasma has recently been demonstrated to be a copper protein and to be inhibited by certain chelating agents (5). Although the quantities of available human plasma monoamine oxidase were not sufficient to characterize its metal content, the inhibition of the human enzyme by various chelating agents (Table IV) was studied. In contrast to the beef plasma amine oxidase (5), the human enzyme was not remarkably inhibited by sodium diethylthiocarbamate, 8-hydroxyquinoline, 2,2'-bipyridyl, or 2-phenanthroline. Unequivocal inhibition of the human enzyme was seen only with cuprizone2 and relatively high concentrations (10 mM) of azide.

While effective concentrations of the most potent inhibitor, cuprizone, were similar to those reported for the beef plasma enzyme (5), the kinetics of inhibition of the human enzyme by this copper-chelating agent was different. Unlike the beef
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plasma amine oxidases of lower mammals (2-8). Like all such circulating enzymes, it deaminates only primary amines, and simple aliphatic amines are among the substrates most actively oxidized. Secondary and tertiary amines, as well as amines with \( \alpha \) substituents (e.g. amino acids), are not attacked. Long chain aliphatic diamines are better substrates of the enzyme than the short chain homologues. With respect to its sensitivity to semicarbazide and hydrazine derivatives, the human enzyme is also similar to the plasma amine oxidases of other mammals. The inhibition of the enzyme by these carbonyl reagents, as originally suggested for the beef plasma enzyme (2), allows the conjecture that the human enzyme, like the beef enzyme (6), has an aldehyde as its prosthetic group.

In other respects, however, the substrate specificity of the human plasma monoamine oxidase is different from that reported for any other plasma enzyme. While the plasma amine oxidases of the steer and other ruminants (2, 8) oxidize the polyamines, spermidine and spermine, as actively as simple aliphatic amines, polyamines are not substrates of the human enzyme. Unlike the amine oxidase of pig plasma (7) or the diamine oxidase found in human plasma during normal pregnancy (25), the monoamine oxidase of human plasma does not oxidize histamine at a significant rate. The human enzyme deaminates tryptamine but not serotonin, while the enzyme of horse plasma is reported to oxidize both indole derivatives with equal facility (3). Mescaline is a substrate of all the amine oxidases thus far recognized in the plasmas of lower primates (8); it is not deaminated by the human enzyme.

Qualitative and quantitative differences were found for the inhibition of the human plasma monoamine oxidase by agents known to inhibit the amine oxidase of beef plasma. Unlike the latter enzyme (2), the human monoamine oxidase was found to be more susceptible to inhibition by iproniazid than by isoniazid. Several metal-binding agents that are effective inhibitors of the steer enzyme (5) had no significant effect upon the human enzyme. The copper-chelating agent, cuprizone, was found to inhibit the human enzyme in concentrations identical with those reported to be inhibitory for the beef plasma enzyme (5). The inhibition of the human enzyme by cuprizone, however, depended upon the time of preincubation of the inhibitor with the enzyme, and the inhibition was noncompetitive with respect to substrate. Both of these features of cuprizone inhibition distinguish the human plasma monoamine oxidase from the beef enzyme. Although the experiments with metal-binding agents, including dialysis against diethyldithiocarbamate, suggest that the human plasma monoamine oxidase is not a metallo-enzyme or that it is, in particular, a copper protein. On the contrary, the time-dependent, noncompetitive inhibition by cuprizone is consistent with this possibility.

The monoamine oxidase of human plasma is unlike any enzymatic activity thus far recognized in animal tissues. In contrast to diamine oxidase (histaminase) (25), the monoamine oxidase of human plasma does not actively oxidize histamine or butylenediamine. Unlike mitochondrial monoamine oxidase, (25, 26), the human plasma enzyme does not attack secondary or tertiary amines and is easily inhibited by semicarbazide. On the other hand, mitochondrial monoamine oxidase actively oxidizes all substrates of the human plasma enzyme. The recent
experiments by Gorkin (27) indicate that rat liver mitochondria contain separable monoamine oxidase activities. On this count, the substrate specificity of the monoamine oxidase of human plasma does not exclude the possibility that the plasma enzyme is identical with a component of mitochondrial monoamine oxidase.

SUMMARY

1. A soluble monoamine oxidase has been purified approximately 5000-fold from human plasma by a procedure involving precipitation with ammonium sulfate, fractionation with ethanol, and adsorption on alumina C3.

2. The purified enzyme oxidizes primary amines with the stoichiometric formation of the corresponding aldehyde, hydrogen peroxide, and ammonia.

3. The substrates most actively oxidized by the enzyme are simple aliphatic amines. Less actively oxidized substrates include kynuramine, tryptamine, and tyramine.

4. The enzyme differs from previously described amine oxidases with respect to inhibitors as well as substrate specificity.

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