neous to discuss the mode of action of endonucleases in terms of single internucleotide bonds. From the present results, a stretch of at least three internucleotide bonds appears to be necessary for the enzyme action to occur. In polynucleotides of chain length longer than tetranucleotide, multiple possibilities for degradation by the enzyme will exist, and the preference for attachment at certain segments and consequent degradation may be determined by the total sequence of bases in those segments. Quantitative definition of preferential specificity should be possible only from study of a variety of oligonucleotides in which initially a single base is varied. Compounds of the type de-pTpTpTpApT, de-pTpTpApTpT, and de-pTpApTpTpT are being therefore prepared in this laboratory, and the study of quantitative mode of degradation of these substrates should be informative.

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Studies on the Mechanism of Action of Monoamine Oxidase: Metabolism of *N,N*-Dimethyltryptamine and *N,N*-Dimethyltryptamine-*N*-Oxide

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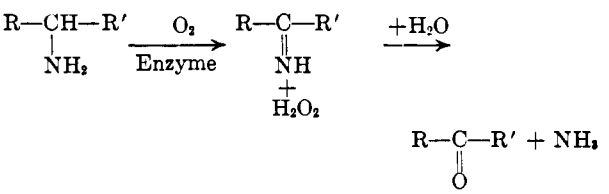
N,N-Dimethyltryptamine and *N,N*-dimethyltryptamine-*N*-oxide were found to be metabolized by a solubilized and partially purified monoamine oxidase preparation from guinea pig liver mitochondria. In contrast to the rate of reaction with other substrates for this enzyme, the rate of reaction with the *N*-oxide is highest under anaerobic conditions. The possibility that monoamine oxidase is an oxygenase and the *N*-oxide an intermediate in the deamination of *N,N*-dimethyltryptamine was investigated with H_2O^{18} and tritium-labeled dimethyltryptamine. The results of these experiments indicate that the *N*-oxide, although a unique substrate, is not an intermediate in the deamination of *N,N*-dimethylamines by monoamine oxidase. It appears that the deamination of these *N,N*-dimethylamines proceeds through an imino compound as in other enzymatically catalyzed deaminations.

Of the four most widely studied enzymes catalyzing oxidative deaminations, monoamine oxidase is the only one which catalyzes the deamination of *N,N*-dimethylamines. The other enzymes, D- and L-amino acid oxidase (Bender and Krebs, 1950) and diamine oxidase (Zeller *et al.*, 1956) require at least one hydrogen atom on the amino group of the substrate for activity. All of these enzymes have a common feature in that the carbon bearing the amino group is converted to a carbonyl function, an aldehyde in the case of the amine oxidases and an α-keto group in the case of the amino acid oxidases. Peroxide and ammonia are also formed.

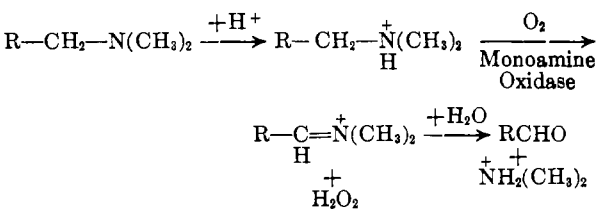
The general mechanism of action of these enzymes has been described by the following equation (Krebs, 1951; Zeller, 1951):



where R′ may be a carboxyl in the case of the amino acid oxidases, or a hydrogen in the case of the amine

oxidases. The rate-determining step is thought to be the dehydrogenation yielding an imino compound which, on (nonenzymatic) hydrolysis, gives ammonia and a carbonyl compound. Oxygen is required to reoxidize the enzymes' prosthetic group, which is a flavin for the amino acid oxidases (Warburg and Christian, 1938; Wellner and Meister, 1960) but has not yet been determined for the amine oxidases.

In spite of the similarities between these enzymes, the observation that monoamine oxidase catalyzes the oxidation of *N,N*-dimethylamines while the others do not, indicates that monoamine oxidase may have a different mechanism of action. This property of monoamine oxidase was first studied by Richter (1937), who showed that monoamine oxidase catalyzes the deamination of *N,N*-dimethyltyramine (hordenine) with the formation of *p*-hydroxyphenylacetaldehyde and dimethylamine. He proposed that the protonated amine was the true substrate for the enzyme. The protonated amine would then be dehydrogenated by a removal of hydrogen from both the nitrogen and the adjacent carbon atom. The imine formed would yield an aldehyde and dimethylamine upon hydrolysis.



The natural occurrence of *N,N*-dimethyltryptamine-*N*-oxide (Fish *et al.*, 1955a) and recent reports on the enzymatic oxidation of amines to both *N*-oxides (Chaykin and Block, 1959), (Baker and Chaykin, 1960) and hydroxyamines (Cramer *et al.*, 1960) suggested that compounds of this type may be intermediates in the oxidation of *N,N*-dimethylamines by monoamine oxidase. The experiments reported here, in which a partially purified preparation of monoamine oxidase from guinea pig liver mitochondria was used, show that *N*-oxides can be metabolized by monoamine oxidase. However, isotopic and kinetic studies indicate that *N*-oxides are not intermediates in the oxidation of dimethylamines.

Materials

N,N-dimethyltryptamine, *N,N*-dimethyltryptamine-*N*-oxide, and indoleacetic acid were purchased from the Regis Chemical Company; bufotenine monooxalate·H_2O and tryptamine from the California Corporation for Biochemical Research; hordenine sulfate from Mann Research Laboratories; dimethylamine and dinitrofluorobenzene from Eastman Kodak Company; digitonin and catalase from Nutritional Biochemicals Corporation. Water containing 10.20 atom % excess O^{18} and 0.11 atom % excess O^{17} was obtained from Isomet Corporation; *N,N*-dimethyltryptamine-2′-H^3 hydrogen oxalate, specific activity 109 mc/mmole, from the New England Nuclear Corporation. A mixture of 98.7% helium–1.3% butane was purchased from the Matheson Company, Inc. Tanks of nitrogen containing 0.0051, 0.009, 0.051, 0.10, 0.35, 0.5, 1.0, 2.0, and 3.1% oxygen were specially prepared and assayed for oxygen content by the Southern Oxygen Company. Indoleacetaldehyde was prepared according to the method of Gray (1959).

Methods

Preparation of the Enzyme.—All operations were conducted at 0–5°. Guinea pig liver mitochondria were prepared by standard techniques (Hogeboom *et al.*, 1948). The mitochondria were washed once with sucrose solution and once with 1.15% KCl solution. The volumes used were one third of the homogenizing volume of sucrose. The pellet obtained by centrifugation was resuspended in an equal volume of demineralized water. The protein concentration of the suspension was adjusted to 40 mg/ml and an equivalent volume of 1.0% digitonin (recrystallized) solution was added. After 20 minutes of continuous stirring the mixture was centrifuged at 100,000 × *g* for one hour. The supernatant fluid, which contained a large amount of protein and little enzymatic activity, was discarded. The residue was homogenized in a 1% digitonin solution (1 ml of digitonin for every 20 mg of the mitochondrial protein) and treated as before. This step usually solubilized 40 to 50% of the enzyme activity; however, some mitochondrial preparations required a third extraction. The digitonin extract containing the monoamine oxidase activity was dialyzed overnight against 0.01 M phosphate buffer at pH 6. The specific activity of this preparation, expressed as μmoles of kynuramine metabolized per hour per mg of protein, was about 5.2 as compared to 0.5 for the original mitochondria.

Monoamine oxidase activity was assayed spectrophotometrically during the enzyme purification by following the oxidation of kynuramine according to the method of Weissbach *et al.* (1960). Enzyme activity was also determined by measuring the rate of formation of indoleacetic acid (Weissbach *et al.*, 1959) from tryptamine or its derivatives in the presence of crude aldehyde dehydrogenase and DPN. A typical incubation mixture consisted of approximately 1200 units of crude aldehyde dehydrogenase prepared from hog kidney (Racker, 1949), 250 μmoles of phosphate buffer pH 8 (or pH 7 when the *N*-oxide was substrate), 150 μmoles of nicotinamide, 3 μmoles of DPN, 3 μmoles of ethylenediaminetetraacetate, substrate, and a suitable enzyme preparation in a total volume of 3 ml. Aliquots were taken at various time intervals and assayed for indoleacetic acid. Unless stated otherwise the incubation time was 60 minutes for the *N*-oxide and 30 minutes for *N,N*-dimethyltryptamine.

Dimethylamine was determined by the method of Dubin (1960).

Indoleacetaldehyde was determined colorimetrically as its 2,4-dinitrophenylhydrazine derivative. To 0.8 ml of the incubation mixture containing 0.02–0.1 μmole of indoleacetaldehyde was added 0.4 ml of 0.1% 2,4-dinitrophenylhydrazine solution in 2 N HCl. After 20 minutes at room temperature

2.0 ml of 2.5 N NaOH was added. After an additional 10 minutes the optical density of the resultant color was determined at 450 mμ. This assay worked very well in incubation mixtures where the protein content was low, recoveries being on the order of 90%. Internal standards made the assay quantitative.

Protein content was determined by the method of Warburg and Christian (1942) or Lowry *et al.* (1951).

Indoleacetic acid for isotopic determinations was isolated from incubation mixtures by extraction into chloroform from 0.1 N HCl. The chloroform layer was removed and washed two times with 0.1 N HCl. The indoleacetic acid was then extracted into 0.1 N NaOH and the aqueous layer was washed once with chloroform. An equal volume of 0.2 N HCl was added to the aqueous layer, and this was extracted two times with chloroform. Aliquots of the combined chloroform extracts were assayed for indoleacetic acid and for radioactivity. The chloroform was shaken with anhydrous sodium sulfate, filtered, and evaporated. Indoleacetic acid prepared in this manner was chromatographically free of other indoles.

N,N-dimethyltryptamine-*N*-oxide was determined by its absorption at 283 mμ or by a modification (Weissbach *et al.*, 1959) of the xanthydrol reaction for indoles (Dickman and Crockett, 1956). Prior to assay the *N*-oxide was isolated by passage of a 1 to 5 dilution of the incubation mixture through a column containing 1 cm^3 each of Dowex 2 and IRC-50 resins. The columns were prepared as follows: One cm^3 of Dowex 2 (hydroxide form) was put in a small column, about 7 mm in diameter, and washed with 3 to 5 ml of water to remove excess base. One cm^3 of IRC-50 buffered at pH 6.0–6.5 with ammonium acetate was added, and the column was again washed with about 5 ml of water. Two-tenths ml of the incubation mixture was diluted to 1 ml and added to the column. *N,N*-Dimethyltryptamine-*N*-oxide was found to pass through the column, while the dimethyltryptamine and indoleacetic acid were retained. About 60% of the dimethyltryptamine-*N*-oxide could be recovered in the first 3 ml of a water eluate. No counts could be detected in this fraction when 10^5 cpm of dimethyltryptamine was added along with the *N*-oxide. Chromatography of the fraction in 1-propanol–1 N ammonium hydroxide (5:1) gave one spot having an R_F of 0.60 which was identical to that of *N,N*-dimethyltryptamine-*N*-oxide. In some instances the reaction mixture was first chromatographed on paper in 1-propanol–1 N ammonium hydroxide and the *N*-oxide, after elution from the paper, was further purified by the column chromatography method described above.

Tritium measurements were made in a Tri-Carb Scintillation Spectrometer, Packard Model 314-DC. The isotope ratios for the O^{18} studies were kindly performed by Mr. William E. Comstock of the National Institute of Arthritis and Metabolic Diseases.

Results

Studies with N,N-*Dimethyltryptamine.*—Richter's (1937) experiments showed that cell-free preparations were able to catalyze the deamination of hordenine to give *p*-hydroxyphenylacetaldehyde and dimethylamine. These experiments were repeated with the partially purified enzyme to rule out the possibility that an enzyme other than monoamine oxidase catalyzed the reaction. Results similar to those of Richter (1937) were obtained for hordenine, as well as for bufotenine and *N,N*-dimethyltryptamine. It was also observed that the oxidation of the *N,N*-dimethylamines was inhibited by a variety of monoamine oxidase inhibitors. It can be seen in Table I that for each equivalent of dimethyltryptamine metabolized, one equivalent each of indoleacetaldehyde and dimethylamine were formed. These results indicate that the deamination occurs not by a stepwise demethylation but by a process that would involve removal of the amino group with the methyl groups intact.

TABLE I

STOICHIOMETRY OF THE REACTION OF *N,N*-DIMETHYLTRYPTAMINE WITH MONOAMINE OXIDASE

Incubation Time (min)	Dimethyltryptamine Metabolized (μmoles)	Indoleacetaldehyde Formed (μmoles)	Dimethylamine Formed (μmoles)
30	0.33	0.33	0.39
60	0.58	0.56	0.66

The incubation mixture contained 2.1 mg of enzyme protein, 250 μmoles of phosphate buffer pH 8, and 2.5 μmoles of *N,N*-dimethyltryptamine in a final volume of 4.0 ml.

Metabolism of N,N-*Dimethyltryptamine*-N-*Oxide.*—When *N,N*-dimethyltryptamine-*N*-oxide was incubated with the partially purified monoamine oxidase, aldehyde dehydrogenase, and DPN, indoleacetic acid was formed. The indoleacetic acid was extracted from acidified incubation mixtures into chloroform and identified by its fluorescence and absorption characteristics. The R_F of the extracted indole in 1-propanol–1 N ammonium hydroxide (5:1) was the same as that of authentic indoleacetic acid. The only other indole observed was the *N*-oxide; no dimethyl- or methyltryptamine could be detected. Because the rate of metabolism of *N,N*-dimethyltryptamine-*N*-oxide was slow in comparison with that of the parent amine (Table II), additional criteria were necessary to show that the metabolism observed was, in fact, due to monoamine oxidase.

Several structurally different compounds which inhibit monoamine oxidase to varying degrees were tested for their effect on the metabolism of both

TABLE II

DEAMINATION OF VARIOUS *N*-SUBSTITUTED TRYPTAMINES BY MONOAMINE OXIDASE

Substrate	Relative Rate
Tryptamine	100
N-methyltryptamine	80–90
N,N-dimethyltryptamine	5–15
N,N-dimethyltryptamine-*N*-oxide	0.2–0.4

The reaction mixture consisted of 0.5 ml of the solubilized enzyme preparation (1.25 mg of protein) and 8 μmoles of substrate. The other constituents were as described under Methods. Under these conditions tryptamine yielded about 4.5 μmoles of indole-3-acetic acid per hour per mg of protein.

TABLE III

EFFECTS OF MONOAMINE OXIDASE INHIBITORS AND SUBSTRATES ON THE METABOLISM OF *N,N*-DIMETHYLTRYPTAMINE AND ITS *N*-OXIDE

Inhibitor	% Inhibition: *N*-Oxide	% Inhibition: *N,N*-Dimethyltryptamine
1-Isopropyl-2-isonicotinyl hydrazide (iproniazid)	94	86
β-Phenylisopropylhydrazine	86	96
Harmaline	71	77
β-Phenylcyclopropylamine	77	96
N-Methyl-*N*-benzyl-2-propynylamine	88	97
Tyramine	28	37
Isoniazid	0	0
Potassium cyanide	0	0

Incubation conditions were as described under Methods. Inhibitor concentration was 3.3×10^{-4} M. There was no preincubation except in the case of iproniazid, where the enzyme was incubated aerobically for 20 minutes with the inhibitor before addition of the substrate.

dimethyltryptamine and its *N*-oxide. Table III shows that the metabolism of these two compounds was inhibited to about the same extent by the various monoamine oxidase inhibitors used. Tyramine also inhibited the metabolism of both compounds, presumably by competition. Cyanide and isoniazid, known inhibitors of diamine oxidase, were both ineffective.

The effects of enzyme concentration and *N*-oxide concentration on the rate of indoleacetic acid formation are shown in Figures 1 and 2. The pH optimum for the *N*-oxide was about 7 as compared to 8 for bufotenine and dimethyltryptamine. Because of the low rate of metabolism of the *N*-oxide, the stoichiometry of the reaction could not be evaluated. The requirement for DPN and aldehyde dehydrogenase (Fig. 3) indicates that an aldehyde is formed as a product of the monoamine oxidase reaction. The volatile amine obtained by diffusion, or by steam distillation of the final reaction mixture, reacted with 2,4-dinitrofluorobenzene to give a product having spectrophotometric characteristics of the dinitrophenyl derivative of dimethylamine as described by Dubin (1960), *i.e.*, the 350:390 ratio was less than one, as would be expected for dimethylamine. However, the sensitivity of the method did not permit quantification of the small amounts of amine formed.

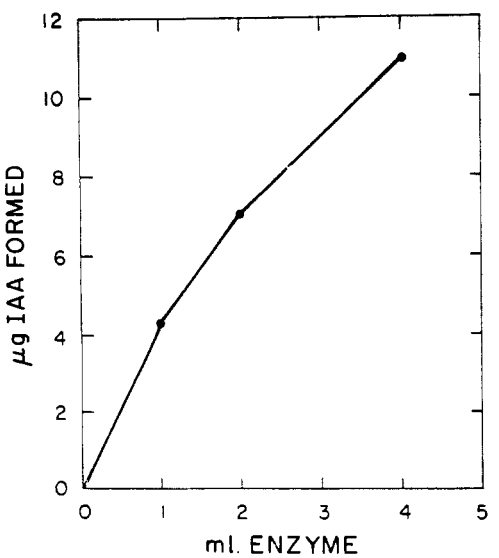


Fig. 1.—Effect of enzyme concentration on the rate of formation of indoleacetic acid (IAA) from *N,N*-dimethyltryptamine-*N*-oxide. Incubation conditions are described under Methods. The enzyme solution contained 1.05 mg of protein per ml.

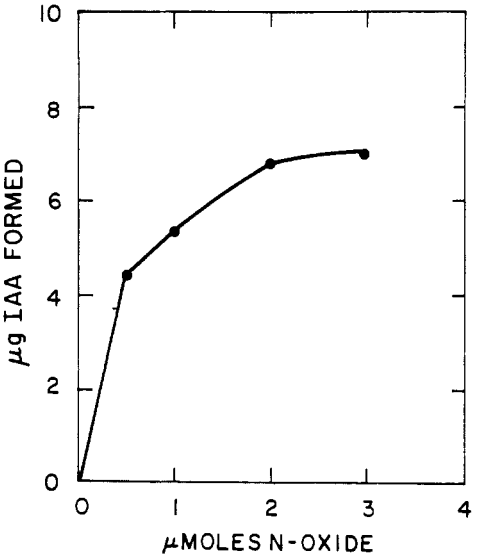


FIG. 2.—Effect of *N,N*-dimethyltryptamine-*N*-oxide concentration on the rate of formation of indoleacetic acid (IAA). Each incubation vessel contained 0.5 ml of enzyme containing 0.52 mg of protein.

The Influence of Anaerobiosis on the Rate of N,N-*Dimethyltryptamine*-N-*Oxide Metabolism.*—The fact that the *N*-oxide is in a more highly oxidized state than the other known substrates for this enzyme makes it unique in this respect and suggested that additional oxygen may not be required for the conversion of *N,N*-dimethyltryptamine-*N*-oxide to

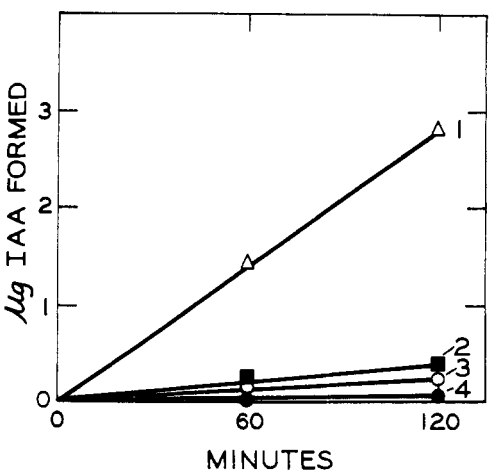


FIG. 3.—Requirements for *N,N*-dimethyltryptamine-*N*-oxide metabolism. Micrograms of indoleacetic acid (IAA) formed from *N,N*-dimethyltryptamine-*N*-oxide. (1) Complete mixture, as described under Methods, incubated in air at 37°. (2) DPN was omitted. (3) β-Phenylisopropylhydrazine in a final concentration of 3.3×10^{-4} M was included. (4) The enzymes were heated at 100° for 3 minutes prior to incubation.

indoleacetaldehyde. When *N,N*-dimethyltryptamine-*N*-oxide was incubated with monoamine oxidase under commercial nitrogen, which contains about 0.1% oxygen, it was found to be metabolized at a rate several times faster than in the presence of oir (Table IV). By contrast, metabolism of tryptamine and *N,N*-dimethyltryptamine was markedly inhibited under the same conditions.

TABLE IV

EFFECT OF ANAEROBIC CONDITIONS ON THE METABOLISM OF TRYPTAMINE, *N,N*-DIMETHYLTRYPTAMINE AND *N,N*-DIMETHYLTRYPTAMINE-*N*-OXIDE

	Micrograms of Indole-3-Acetic Acid per Hr.		
Gas Phase	*N*-Oxide	*N,N*-Dimethyltryptamine	Tryptamine
Air	0.37	7.69	171
Nitrogen[a]	1.86	3.99	36.2
Helium[b]	0.53	0.36	0.12

The incubation mixture was as described under Methods. The enzyme added contained 0.16 mg protein. The anaerobic reactions were carried out in Warburg vessels which were flushed with the appropriate gas.

[a] Standard water-pumped nitrogen containing a maximum of 0.1% oxygen. [b] See Materials section.

Even in a helium atmosphere, essentially devoid of oxygen, *N,N*-dimethyltryptamine-*N*-oxide was still metabolized at a greater rate than in air and, more importantly, its rate of metabolism was greater than that of *N,N*-dimethyltryptamine or tryptamine. The monoamine oxidase inhibitors listed in Table III were also found to block the anaerobic conversion of the *N*-oxide to indoleacetic acid.

The role of oxygen was evaluated more carefully by conducting the incubation in a helium atmosphere in double-side-arm Warburg vessels and controlling the oxygen concentrations by adding catalase and measured amounts of hydrogen peroxide. When the reaction between catalase and peroxide was completed and the system was once again at equilibrium the *N*-oxide was added and the reaction rate was determined. The results of such an experiment are shown in Figure 4. The amount of oxygen required for maximal activity was found to approximate 0.1%. Inhibition occurred at both very low and very high oxygen tension. Similar results were obtained with commercially prepared mixtures of nitrogen and oxygen. Analogous experiments with *N,N*-dimethyltryptamine showed that unlike the *N*-oxide its rate of metabolism increased over the entire range of oxygen concentrations tested (Fig. 4).

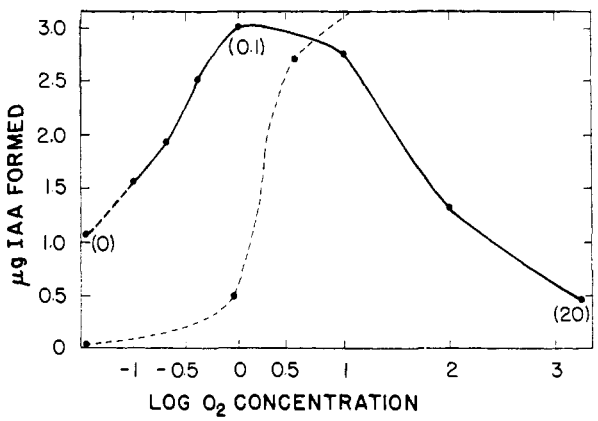


FIG. 4.—Effect of oxygen concentration on the metabolism of *N,N*-dimethyltryptamine and *N,N*-dimethyltryptamine-*N*-oxide. The reaction was carried out as described in the text. The oxygen concentration is expressed in μmoles per flask. The flask used had an average volume of 18.5 ml and contained about 3 ml of fluid. The figures in parentheses represent the approximate percentages of oxygen in the gas phase. .——. *N,N*-dimethyltryptamine-*N*-oxide; ·——· *N,N*-dimethyltryptamine × 10^{-1}.

Many of the experiments reported above for *N,N*-dimethyltryptamine-*N*-oxide were also carried out with *N,N*-dimethyltyramine-*N*-oxide. The results were essentially the same for this tyramine derivative as for the analogous tryptamine compound.

Studies with H_2O^{18}.—The finding that *N,N*-dimethyltryptamine-*N*-oxide was a better substrate of monoamine oxidase at low oxygen tensions made this compound an even more attractive possibility as an intermediate in the metabolism of *N,N*-dimethyltryptamine. It was conceivable that the first step in the metabolism of *N,N*-dimethyltryptamine was an oxygenation of the nitrogen to form an *N*-oxide or *N*-oxide-like compound which could then rearrange to an aldehyde and dimethylamine.

$$R{-}CH_2{-}N(CH_3)_2 \xrightarrow[\text{Monoamine Oxidase}]{O_2}$$

$$R{-}CH_2{-}\underset{\underset{O}{\downarrow}}{N}(CH_3)_2 \xrightarrow[\text{Oxidase}]{\text{Monoamine}}$$

$$\text{Intermediate "X"} \longrightarrow \begin{matrix} RCHO \\ + \\ NH(CH_3)_2 \end{matrix}$$

If the *N*-oxide was an intermediate, as shown above, then, in contrast to the classical mechanisms of deamination, oxygen in the aldehyde formed by the action of monoamine oxidase would be derived from molecular oxygen and not from water. To investigate this possibility experiments with H_2O^{18} were carried out.

Indoleacetaldehyde is not stable enough to permit isolation and purification in milligram quantities from an incubation mixture, but its oxidation product, indoleacetic acid, is stable and readily purified. Therefore, the incubations with H_2O^{18} contained the aldehyde dehydrogenase system to convert the indoleacetaldehyde to indoleacetic acid. Aldehyde dehydrogenase utilizes water in the formation of acids as shown:

$$R{-}\underset{\underset{H}{|}}{C}{=}O + H_2O + DPN \xrightarrow[\text{Enzyme}]{}$$

$$R{-}\underset{\underset{OH}{|}}{C}{=}O + DPNH_2$$

If the initial conversion of amine to aldehyde utilized oxygen from water the indoleacetic acid isolated from incubations in H_2O^{18} should contain two equivalents of O^{18}, while if the initial step utilized oxygen from air, the indoleacetic acid should con-

tain one equivalent of O^{18}. The results of such an experiment are shown in Table V. As expected, the

TABLE V
INCORPORATION OF H_2O^{18}

Substrate	Enzyme	Atom % Excess Theoretical[a]	Atom % Excess Experimental
Indoleacetaldehyde	Aldehyde dehydrogenase	0.85	0.61
Tryptamine	Monoamine oxidase and aldehyde dehydrogenase	1.70	1.71
N,N-Dimethyltryptamine	Monoamine oxidase and aldehyde dehydrogenase	1.86	1.84
Tryptophan	L-Amino acid oxidase	0.85	0.82

For incubation conditions see Methods. Tryptophan was incubated with 1 mg of crude snake venom L-amino acid oxidase and additional catalase to destroy H_2O_2 which would convert the keto acid, indolepyruvate, to indoleacetate. All values shown have been corrected for a small nonenzymatic exchange of O^{18}.

[a] If all the oxygen incorporated in the product was derived from H_2O^{18}.

control incubation of indoleacetaldehyde with aldehyde dehydrogenase and DPN showed an incorporation of one equivalent of oxygen from water into indoleacetic acid. With *N,N*-dimethyltryptamine or tryptamine as substrate two equivalents of O^{18} from water were incorporated into the indoleacetic acid formed, indicating that the oxygen from both reactions came from water. In a comparable experiment with L-amino acid oxidase and tryptophan as substrate, in which the keto acid was isolated, there was only one equivalent of O^{18} incorporated, as expected. These results show that, as in other enzymatic deaminations, the oxygen is derived from water, and militate against the direct oxygenation theory as a mechanism for deamination of dimethylamines by monoamine oxidase.

Studies with Tritiated N,N-*Dimethyltryptamine.*— The above H_2O^{18} experiments would not rule out the possibility that other mechanisms of rearrangement of *N*-oxides (Culvenor, 1953; Wenkert, 1954) could result in an imino compound that would then undergo spontaneous hydrolysis to an aldehyde and dimethylamine with the incorporation of O^{18} from water into indoleacetaldehyde. Therefore, another type of labeling experiment was carried out in which 2′-H^3-*N,N*-dimethyltryptamine was incubated with monoamine oxidase in the presence of carrier *N,N*-dimethyltryptamine-*N*-oxide. At the end of the incubation period indoleacetic acid and *N,N*-dimethyltryptamine-*N*-oxide were isolated and their radioactivities determined. As shown in Table VI, there was no significant labeling in the *N*-oxide, which indicated that the *N*-oxide was not a free intermediate in the metabolism of *N,N*-dimethyltryptamine. In addition to this, the specific activity of the indoleacetic acid formed was the same as that of the starting *N,N*-dimethyltryptamine. This indicated not only that there was no equilibration with the *N*-oxide but that there was no loss of hydrogen from the C-2 position of the side-chain.

TABLE VI
STUDIES WITH H^3-*N,N*-DIMETHYLTRYPTAMINE

Product	cpm/μmole
N,N-dimethyltryptamine-*N*-Oxide	11
Indoleacetic acid	5.0×10^6

Included in the incubation mixture was 1 μmole of 2′-H^3 *N,N*-dimethyltryptamine (5.1×10^6 cpm), 3 μmoles of unlabeled *N,N*-dimethyltryptamine-*N*-oxide, and catalase to prevent the nonenzymatic formation of *N,N*-dimethyltryptamine-*N*-oxide by the action of hydrogen peroxide.

DISCUSSION

In the absence of information concerning the nature of the prosthetic group of monoamine oxidase, the idea that this enzyme could act by a direct oxygenation mechanism seemed worthy of further investigation. Although the experiments with O^{18} and tritium clearly rule out such a mechanism, they do not explain how *N,N*-dimethyltryptamine and *N,N*-dimethyltryptamine-*N*-oxide are metabolized by monoamine oxidase. The H_2O^{18} studies showed that the oxygen in the aldehyde came from water and not from molecular oxygen, as would have been expected if the *N*-oxide were an intermediate. The 2′-H^3-*N,N*-dimethyltryptamine experiments utilizing *N,N*-dimethyltryptamine-*N*-oxide as a carrier showed that no free *N*-oxide was formed.

With respect to the *N,N*-dimethylamines, the data presented here are consistent with the protonation mechanism proposed by Richter (1937). However, another mechanism, perhaps more consistent with present information on the prosthetic group(s) of monoamine oxidase, is also possible. Data have been presented which suggest that monoamine oxidase contains a metal (Gorkin, 1959). It has also been suggested that the enzyme is a flavoprotein (Richter, 1937; Hawkins, 1952). The reaction might be pictured, then, as involving removal of either a hydride ion or a comparable group by one equivalent transfers, as occurs with known metalloflavin enzymes (Mahler, 1956). The over-all reaction may be pictured as follows:

$$R-\overset{H}{\underset{H}{C}}-\overset{\curvearrowright}{N}(CH_3)_2 \xrightarrow[\text{Enzyme}]{\text{Oxidized}} R-\underset{H}{C}=\overset{+}{N}(CH_3)_2 \xrightarrow{H_2O}$$

$$RCHO + H_2\overset{+}{N}(CH_3)_2$$

The electrons on the nitrogen could shift, possibly by a concerted mechanism stabilizing the expected ion and resulting in the same intermediate as proposed earlier (Richter, 1937), but without the requirement for a protonated amine. Preliminary experiments with the partially purified enzyme have shown that the reaction rate is fastest at alkaline pH values for all amines tested. The amount of protonated amine present under these conditions would probably be too small to account for the observed rates of amine metabolism.

The ability of *N,N*-dimethyltryptamine-*N*-oxide to serve as a substrate of monoamine oxidase is certainly established. Fish *et al.* (1955b) could detect no metabolism of this *N*-oxide in mouse liver mitochondrial preparations, possibly because their experiments were carried out in air and with a much less sensitive method of assay.

The formation of indoleacetic acid from *N,N*-

dimethyltryptamine-*N*-oxide appears to be due to the metabolism of the *N*-oxide itself and not to that of a rearranged product. Although no free *N*,*N*-dimethylamine could be detected during incubation, the possibility still exists that the *N*-oxide is reduced to the parent amine while attached to the enzyme and, without becoming free from the binding sites, may be oxidized to the aldehyde and dimethylamine.

The unusual requirement for trace amounts of oxygen for maximal activity is difficult to explain. It may be that the *N*-oxide shares the same binding sites that are common to other substrates of monoamine oxidase and to oxygen. That is, the normal complex may be a termolecular one involving enzyme, amine, and oxygen. When the *N*-oxide is a substrate it may occupy the same position as that normally filled by both oxygen and amine. This could be an explanation for the inhibition of *N*,*N*-dimethyltryptamine-*N*-oxide metabolism observed at high oxygen tension. The trace oxygen requirement for maximal activity could be necessary to regenerate a reduced prosthetic group.

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