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# 2,3-DIHYDRO-5-HYDROXYTRYPTAMINE*

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**Abstract**—Serotonin was chemically reduced to 2,3-dihydro-5-hydroxytryptamine, which is extremely labile to oxidation. This substance is a substituted amino phenol which, when oxidized, presumably proceeds through the formation of an iminoquinone. The 2,3-dihydro-5-hydroxytryptamine was attacked enzymatically by amine oxidase and by ceruloplasmin. Quantitatively, 2,3-dihydro-5-hydroxytryptophan behaved similarly to serotonin with respect to its effect on the chromatophore activity of the guppy, the isolated virgin rat uterus, and the isolated ileum of the rabbit.

THE INVESTIGATIONS of the substrate specificity of L-dihydroxyphenylalanine decarboxylase established that this enzyme is responsible for the decarboxylation of 5-hydroxytryptophan.[1, 2] In an effort to determine the structural requirements of the substrates that will allow decarboxylation by this enzyme, we synthesized a number of amino acids related to DOPA and 5-hydroxytryptophan, among them 2, 3-dihydro-5-hydroxytryptophan. The latter amino acid was not decarboxylated by this enzyme, but behaved as a competitive antagonist.[3] This experience led us to a study of the biochemical and pharmacological properties of the corresponding amine related to serotonin, 2, 3-dihydro-5-hydroxytryptamine.

In this report we describe the behavior of this amine as a substrate for two enzymes which attack 5-hydroxytryptamine—i.e., monoamine oxidase and ceruloplasmin—and some pharmacological activities of this amine.

## METHODS

The mitochondrial amino acid oxidase was prepared according to the methods of Creasey.[4] The purified ceruloplasmin was isolated from outdated human blood-bank plasma, according to the procedure of Deutch.[5]

The qualitative inhibition of the effects of lysergic acid diethylamide on the chromatophore activity of the guppy was carried out according to the procedure described by Cerletti and Berde.[6]

The effects of reduced serotonin† on the isolated virgin rat uterus and the isolated ileum of the rabbit were determined by the method of Sinha and West.[7] Solutions of reduced serotonin were bracketed between known standard solutions of serotonin to estimate the relative activities.

2,3-Dihydro-5-hydroxytryptamine (Fig. 1) was prepared by reduction of serotonin creatinine sulfate with hydrogen at low pressure with palladium on charcoal as the

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† Throughout this paper the term "reduced" serotonin is used to indicate 2,3-dihydro-5-hydroxytryptamine.

catalyst: 1 g of serotonin was dissolved in 10 ml of 1 N HCl and placed in a Parr low-pressure hydrogenator with 1 g of 5% palladium on charcoal. With 30 lb of hydrogen pressure, the reduction proceeded within 5 hr. The product was stored in a desiccator over phosphorus pentoxide. The 2,3-dihydro-5-hydroxytryptamine was examined by paper chromatography; serotonin was not detected with either Ehrlich's reagent or diazotized sulfanilic acid. The compound exhibited a single symmetrical absorption peak in 0·01 N HCl at 275 mμ.

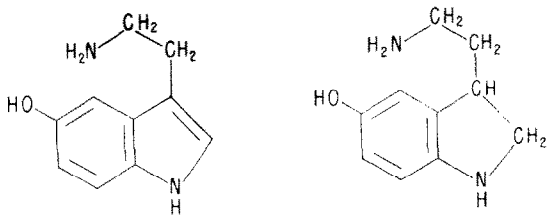


FIG. 1. Chemical structures of serotonin and reduced serotonin.

## RESULTS

### *Amine oxidase*

Serotonin and 2,3-dihydro-5-hydroxytryptamine were oxidized rapidly by the amine oxidase of mitochondria obtained from guinea-pig liver. Corrections in the calculation of uptake of oxygen were needed because of the auto-oxidation of reduced serotonin; these were derived from the use of controls carried out with boiled enzyme preparations. There appeared to be no significant differences between the substrates with respect to uptake of oxygen; however, some difference between the $K_m$ values was apparent (Table 1).

TABLE 1. MONOAMINE OXIDASE ACTIVITY

| | $O_2$ uptake/hr | $K_m$ |
|---|---|---|
| Serotonin | 152 | 0·0009 M |
| Reduced serotonin | 131 | 0·002 M |

Flasks contained washed guinea-pig liver mitochondria, 0·03 M substrate, 0·06 M semicarbazide, and 0·03 M KCN, according to the method of Creasey.[4] $K_m$-values were determined by plotting the reciprocal of the velocity against the reciprocal of the substrate concentration.

### *Ceruloplasmin*

The oxidation of serotonin and 2,3-dihydro-5-hydroxytryptamine by ceruloplasmin is shown in Fig. 2. The data indicate a steady and appreciable nonenzymic oxidation of the reduced serotonin, as observed in the spectrophotometric studies described below. Nevertheless, there was evidence of an enzymic oxidation of this substrate, which at first proceeded more rapidly than with serotonin as a substrate. Presumably, as the reduced serotonin auto-oxidized, the amount of substrate available for enzymic attack was decreased.

There was paper chromatographic evidence that one of the products formed from the oxidation of serotonin by ceruloplasmin[8] was similar to compound III, described below. This substance, previously reported among the ceruloplasmin oxidation products of serotonin, exhibited a sharp absorption peak at 270 mμ and a shoulder at 295 mμ, and reacted with $FeCl_3$ to give a bright blue color.

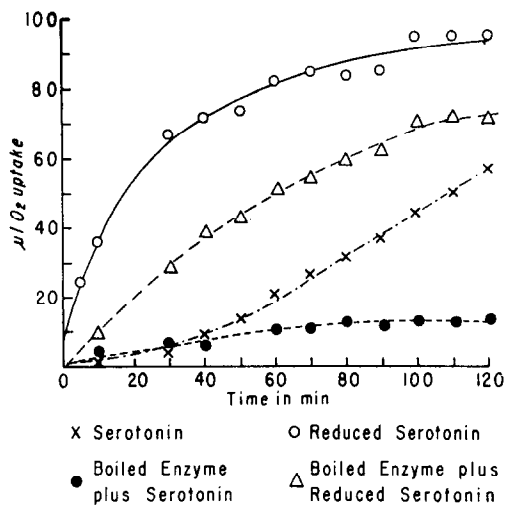


FIG. 2. Each flask contained 1 ml of partially purified ceruloplasmin ($E_{280/610} = 50$), 3 mmoles of substrate, 0·04 M phosphate buffer, pH 6·2. Control flasks contained the additions indicated, in addition to boiled enzyme.

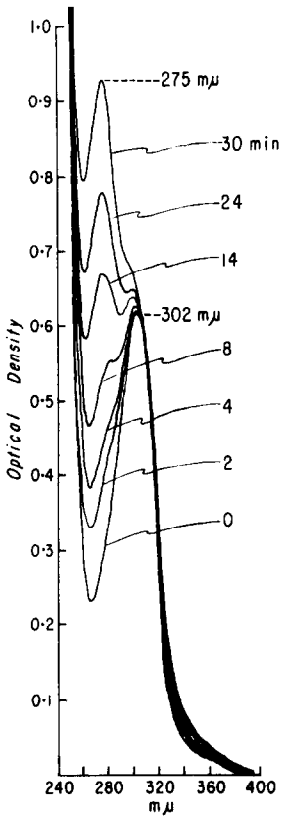


FIG. 3. Spectra were determined with a Cary 14 spectrophotometer. The 2,3-dihydro-5-hydroxytryptamine was dissolved in 0·1 M phosphate buffer, pH 7·0, to an appropriate dilution. The spectra were determined over the period of time indicated.

*Oxidation products of 2, 3-dihydro-5-hydroxytryptamine*

A study of the ultraviolet absorption spectra of reduced serotonin and of the products of auto-oxidation of reduced serotonin was carried out in 0·1 M phosphate buffer, pH 7·0. Serotonin exhibited a single sharp ultraviolet absorption peak at 275 mμ and a shoulder at 296 mμ. The 2,3-dihydro-5-hydroxytryptamine in acid solution showed a single symmetrical peak at 275 mμ; in neutral buffer, however, this peak rapidly shifted to 302 mμ and slowly emerged at 275 mμ (Fig. 3). Ultimately the solution appeared blue in color, and finally a black-brown precipitate formed. The nature of the products formed from the auto-oxidation of reduced serotonin in

neutral buffer has not been entirely elucidated. Paper chromatography of the product revealed the presence of at least two substances. The blue-colored material was not identical with the major products (see Table 2). No serotonin could be detected, a finding which indicates that reoxidation at the 2,3-position did not take place.

TABLE 2. PRODUCTS FORMED BY AUTO-OXIDATION OF REDUCED SEROTONIN

| Compound | Color | $R_f$* | Ehrlich's | Fe (III) | Cu (II) |
|---|---|---|---|---|---|
| Serotonin | — | 0·33 | purple red | — | — |
| Reduced serotonin | — | 0·25 | yellow | — | — |
| Product from reduced serotonin | | | | | |
| Major | — | 0·27 | yellow | blue | blue |
| Minor | blue | 0·21 | yellow | — | purple |

* $R_f$ determined on Whatman #1 in *tert*-butanol : acetic acid : water (70 : 5 : 20); descending.

The data suggest that reduced serotonin could auto-oxidize to the quinimine and cyclize, in a manner analogous to the formation of dopachrome from DOPA (Fig. 4).

The substituted ortho-aminophenol structure proposed for compound III was consistent with the observed reactions. The reaction of this substance with ferric and cupric salts to form colored chelates could be explained with this structure.

*Effects of reduced serotonin on certain pharmacological test preparations*

(1). Light-adapted female guppies (*Poecilia reticulatus*) exhibited marked chromatophore activity in the presence of 30 $\mu$g of lysergic acid diethylamide (LSD) per ml.

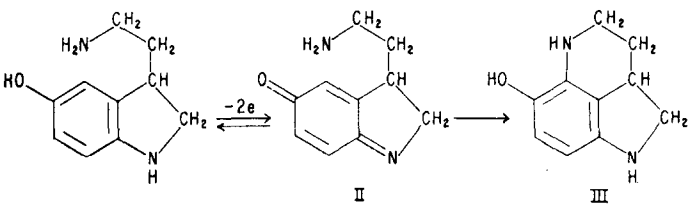


FIG. 4. Suggested auto-oxidation reaction for reduced serotonin.

This effect was totally inhibited by pretreating the fish with either 1000 $\mu$g of reduced serotonin or serotonin per ml (Fig. 5). No attempt was made to quantify the activity of the indolamines. (2.) Reduced serotonin was roughly ten times less effective than serotonin when assayed on the isolated virgin rat uterus. Because of the lability of the 2, 3-dihydro-5-hydroxytryptamine in neutral buffer, we could feel secure only with the qualitative effects observed. (3). on the isolated rabbit ileum, the reduced compound was approximately one-half as active as serotonin.


FIG. 5. The upper beaker in the photograph demonstrates the effect of 30 μg of lysergic acid diethylamide (LSD) on the chromatophore activity of the guppy. The middle beaker shows the inhibition of this effect by pretreatment with 1 mg of reduced serotonin per ml. The lower beaker demonstrates the inhibition of this effect by 1 mg of serotonin per ml.

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## REFERENCES

1. J. H. FELLMAN, *Enzymology* **20**, 366 (1959).
2. A. YUWEILER, E. GEDER and S. EIDUSON, *Arch. Biochem.* **80**, 162 (1959).
3. J. H. FELLMAN (unpublished data).
4. N. H. CREASEY, *Biochem. J.* **64**, 178 (1956).
5. H. F. DEUTCH, *Arch. Biochem.* **89**, 225 (1960).
6. A. CERLETTI and B. BERDE, *Experientia* **11**, 312 (1955).
7. Y. K. SINHA and G. B. WEST, *J. Pharm.* (*Lond.*) **5**, 370 (1953).
8. G. M. MARTIN, E. P. BENDITT and N. ERIKSEN, *Arch. Biochem.* **90**, 208 (1960).