Azarnoff and Tucker[6] found in rat liver homogenates that ethyl *p*-chlorophenoxyisobutyrate at certain concentrations had minimal inhibition of the incorporation of mevalonic acid into squalene and maximal inhibition into lanosterol and cholesterol. In the present system *p*-chlorophenoxyisobutyric acid appeared to have the same effect on cholesterol and nonsaponifiable material at various levels of the compound; the data seem to indicate a similar inhibition at some point before squalene and between squalene and cholesterol in the reaction sequence.

The results reported in the present paper suggest that in the cell-free system from the bovine aorta there is a direct inhibition of the syntheses of NSF and cholesterol by *p*-chlorophenoxyisobutyric acid.

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REFERENCES

1. J. THORP and W. S. WARING, *Nature (Lond.)* **194**, 948 (1962).
2. L. HELLMAN, H. L. BRADLOW, B. ZUMOFF, O. K. FUKUSHIMA and T. P. GALLAGHER, *J. clin. Endocr.* **19**, 936 (1959).
3. C. DUFAULT, G. TREMBLAY, W. NOWACYNSKI and J. GENEST, *Canad. med. Ass. J.* **85**, 1025 (1961).
4. W. D. COHEN, N. HIGANO, R. W. ROBINSON and R. J. LEBEAU, *J. clin. Endocr.* **21**, 1208 (1961).
5. J. THORP, *Lancet I*, 1323 (June 1962).
6. D. L. AZARNOFF and D. R. TUCKER, *Fed. Proc.* **23**, 552 (1964).
7. L. D. WRIGHT and M. CLELAND, *Proc. Soc. exp. Biol. (N.Y.)* **96**, 219 (1957).
8. H. D. WYCOFF and J. PARSONS, *Science* **125**, 347 (1957).
9. W. M. SPERRY and M. WEBB, *J. biol. Chem.* **187**, 97 (1950).

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Effect of chronic sympathetic denervation on subcellular distribution of some sympathomimetic amines*

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CONVERSION of tyramine to octopamine has been demonstrated in tissue slices,[1] perfused organs,[2–4] and in intact animals.[5–7] This transformation is impaired by chronic sympathetic denervation,[5, 6] and presumably occurs in the sympathetic nerves. Tyramine and α-methyltyramine (p-hydroxyamphetamine) rapidly enter the norepinephrine storage granules, where their β-hydroxylated derivatives are formed,[8] selectively retained,[8] and released by sympathetic nerve stimulation.[4] These storage granules have been separated from the heart and salivary glands.[9]

Although binding is markedly impaired, some administered norepinephrine[10] or tyramine[5, 6] is taken up into chronically denervated salivary glands, probably at extraneuronal binding sites. Norepinephrine formation from dopamine-^{14}C, however, cannot be demonstrated after chronic denervation.[11] Absence of demonstrable norepinephrine-^{14}C may be due to inability of the denervated salivary gland to take up dopamine, to form norepinephrine, or to retain norepinephrine which is formed. Dopamine may not be present because it can be destroyed by monoamine oxidase or catechol-O-methyl transferase. These enzymes do not destroy α-methyltyramine, and destruction of tyramine can be prevented by pretreatment with a monoamine oxidase inhibitor. These amines, therefore, have been used to examine the effects of chronic sympathetic denervation on the subcellular distribution and β-hydroxylation of amines in the salivary gland.

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Male Sprague-Dawley rats, weighing 180–200 g, were anesthetized with ether and the right superior cervical ganglion removed. All animals developed persistent ptosis on the operated side. Two weeks later six of these animals were treated with a monoamine oxidase inhibitor, pheniprazine (10 mg/kg, i.p.); 1 hr later 20 μc tyramine-^{3}H (1,500 mc/mmole) was injected i.v. Another group of rats, not treated with pheniprazine, were given 10 μc α-methyltryamine-^{3}H (5,000 mc/mmole). The animals were killed 1 hr after administration of the labeled amine. Submaxillary salivary glands were homogenized immediately in ice-cold isotonic sucrose solution. The homogenate was centrifuged at 20,000 *g* for 10 min at 4°. The sediment, containing unbroken cells, cell debris, nuclei, and mitochondria, was discarded. The resultant supernatant was centrifuged at 100,000 *g* for 1 hr at 4°; a high-speed sediment ("microsmal" fraction) and a supernatant ("soluble" fraction) were obtained. The supernatant was decanted and the sediment homogenized in 5 ml distilled water. Proteins of both fractions were precipitated with 0·4 ml 60% perchloric acid and radioactive amines determined as previously described.[5]

The only radioactive amines found in the intact salivary glands were the β-hydroxylated derivatives of the administered compounds (Figs. 1, 2). The partition of the β-hydroxylated compounds between

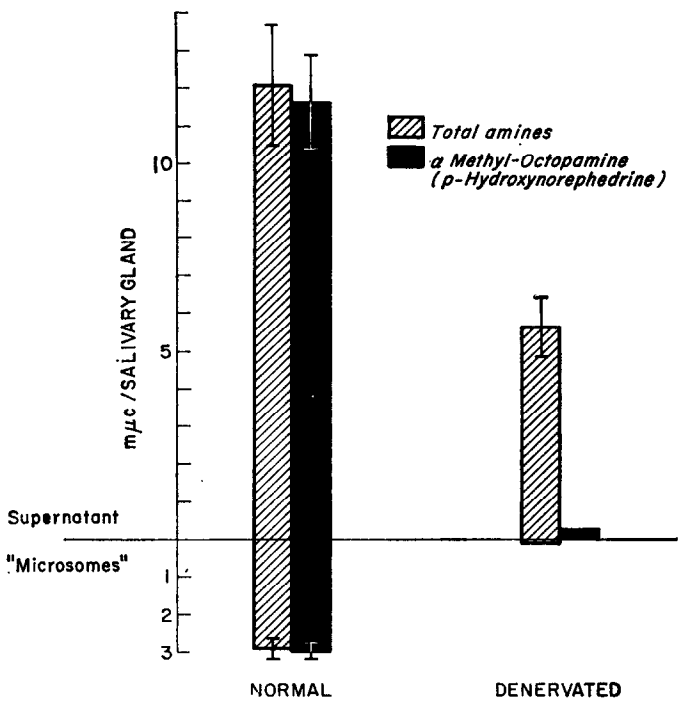

FIG. 1. Effect of denervation on content and subcellular distribution of labeled amines in the salivary gland after administration of α-methyltyramine-^{3}H. The amount of α-methyltyramine-^{3}H is indicated by the difference between the total amines and the α-methyloctopamine. Results are expressed as mμc ^{3}H/salivary gland and are the means ± S.E.M. for groups of 6 animals.

the soluble and microsomal fractions obtained from salivary glands was almost identical with that reported in rat heart.[8] Tyramine-^{3}H or α-methyltyramine remaining in the denervated glands was neither particle bound nor β-hydroxylated to a significant extent. These results are consistent with the view that β-hydroxylation occurs in sympathetic nerves.[5, 6]

Considerable amounts of tyramine and α-methyltyramine were found in denervated, but not in intact salivary glands. It appears that appreciable extraneuronal binding of amines administered in small doses occurs only in the absence of intact sympathetic nerve endings. When uptake into the

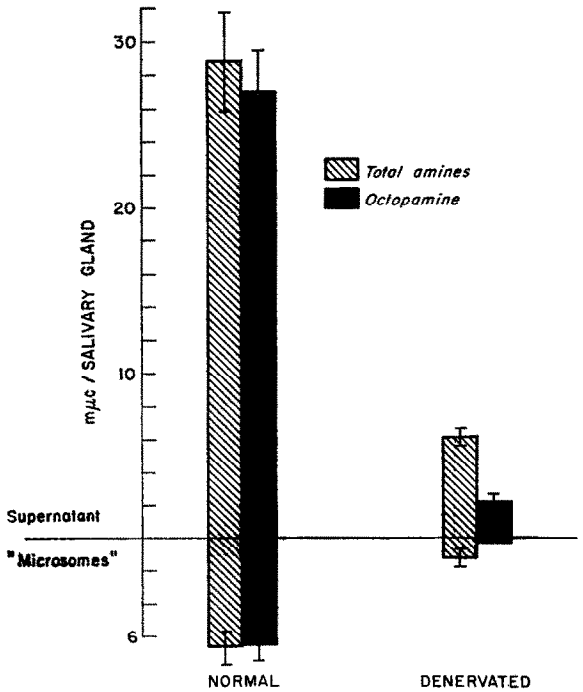

FIG. 2. Effect of denervation on content and subcellular distribution of labeled amines in the salivary gland after administration of tyramine-^{3}H. The amount of tyramine-^{3}H is indicated by the difference between the total amines and the octopamine. Results are expressed as mμc ^{3}H/salivary gland and are the means ± S.E.M. for groups of 6 animals.

nerve endings can occur, little of the amine delivered to the tissue is found in extraneuronal sites. These observations suggest that the sympathetic nerve endings efficiently concentrate and retain amines. When large doses of amines are administered, however, extraneuronal binding can occur in normal salivary glands.[5] Amines which are bound to extraneuronal sites appear in the soluble portion of tissue homogenates and are not associated with the "microsomal" fraction.

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REFERENCES

1. J. J. PISANO, C. R. CREVELING and S. UDENFRIEND, *Biochim. biophys. Acta* **43**, 566 (1960).
2. J. M. MUSACCHIO and M. GOLDSTEIN, *Biochem. Pharmacol.* **12**, 1055 (1963).
3. C. A. CHIDSEY, G. A. KAISER and B. LERR, *J. Pharmacol. exp. Ther.* **144**, 393 (1964).
4. J. E. FISCHER, W. D. HORST and I. J. KOPIN, *Brit. J. Pharmacol.* In press.
5. J. E. FISCHER, J. M. MUSACCHIO, I. J. KOPIN and J. AXELROD, *Life Sci.* **3**, 413 (1964).
6. A. CARLSSON and B. WALDECK, *Acta pharmacol. (Kbh.)* **20**, 371 (1964).
7. I. J. KOPIN, J. E. FISCHER, J. M. MUSACCHIO, W. D. HORST and V. K. WEISE, *J. Pharmacol. exp. Ther.* In press.
8. J. M. MUSACCHIO, I. J. KOPIN and V. K. WEISE. *J. Pharmacol. exp. Ther.* In press.
9. L. T. POTTER and J. AXELROD, *J. Pharmacol. exp. Ther.* **142**, 291 (1963).
10. J. E. FISCHER, I. J. KOPIN and J. AXELROD, *J. Pharmacol. exp. Ther.* In press.
11. J. M. MUSACCHIO, J. E. FISCHER and I. J. KOPIN. Unpublished observation.