CC/NUMBER 5
FEBRUARY 4, 1980

This Week's Citation Classic

Nagatsu T, Levitt M & Udenfriend S. Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis. *J. Biol. Chem.* **239**:2910-17, 1964.
[Lab. Clin. Biochem., National Heart Institute, National Institutes of Health, Bethesda, MD]

This paper describes the presence and properties of a new pteridine-requiring enzyme tyrosine hydroxylase in brain, adrenal medulla, and sympathetically innervated tissues. [The *SCI*® indicates that this paper has been cited over 645 times since 1964.]

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September 4, 1979

"It is most gratifying to learn that our tyrosine hydroxylase paper has been so frequently cited. The work in this paper was started in 1963 at the laboratory of Sidney Udenfriend in the NIH, where I was a NIH international postdoctoral fellow and Morton Levitt was a graduate student. At that time, among the four enzymes involved in the catecholamine biosynthesis, only the enzyme responsible for converting tyrosine to dopa was elusive. Therefore, assuming that such an enzyme may exist in catecholamine-containing tissues, we first developed a highly sensitive isotopic assay for the enzyme activity L-(^{14}C), tyrosine was used as a substrate, and L-(^{14}C) dopa, enzymaticaliy formed, was isolated on an alumina column and assayed. We started our initial work to discover the enzyme in tissue slices and minces which should contain necessary cofactors with the enzyme, and we found a substantial formation of dopa from L-(^{14}C) tyrosine. However, we also found a significant nonenzymatic hydroxylation of tyrosine to dopa in heated tissue slices and minces. This may be the reason why the presence of this enzyme went unnoticed for so long. Fortunately, we found the absolute stereospecificity of this enzyme which permitted the use of D-(^{14}C) tyrosine as a control, and we became convinced that we were really detecting a new enzyme.

"We found bovine adrenal medulla contained a large amount of the enzyme activity in the soluble fraction, and we could isolate the enzyme by ammonium sulfate fractionation. After testing many probable cofactor sub stances, the preparations were shown to require for activity a tetrahydropteridine and molecular oxygen. The requirement of a tetrahydropteridine as a cofactor of the hydroxylating process was first discovered with rat liver phenylalanine hydroxylase by Seymour Kaufman in the NIH in 1959.[1]

"Another significant finding in this work was the inhibition of the enzyme by the products catecholamines, and we proposed the possibility of the feed back inhibition *in vivo*, which is now of great interest for the short term regulation of catecholamine biosynthesis.

"The reasons why our publication is so frequently cited may be the great physiological significance of this enzyme, its widely-applicable assay pro cedure, and its interesting properties as a pleridine-dependent monooxygenase.

"Sidney Udenfriend is the director of Roche Institute of Molecular Biology, and Morton Levitt is at the New York State Psychiatric Institute. We had another chance to collaborate again in 1972 at Roche Institute.[2]

"I recall distinctly how much we were pleased to see the enzyme activity of tyrosine hydroxylase in front of the liquid scintillation counter at mid night. The best reward for scientists may be the pleasure and excitement of new findings."

1. **Kaufman S.** Studies on the mechanism of the enzymatic conversion of phenylalanine to tyrosine. *J. Biol. Chem.* **234**:2677-88, 1959.
2. **Nagatsu T & Udenfriend S.** Photometric assay of clopamie β-hydroxylase activity in human blood.*Clin. Chem.* **18**:980-3, 1972.