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This Week's Citation Classic

Gleaner G G, Burner H J & Brown G W, Jr. The histochemical demonstration of monoamine oxidase activity by tetrazolium salts. *J. Histochem. Cytochem.* **5**:591-600, 1957.
[Lab. Pathol. and Histochem., National Institutes of Health, and Lab. Biochem. and Metab., NIAMD, National Institutes of Health, Bethesda, MD]

This paper describes a method for the localization of monoamine oxidase (MAO) enzymic activity in tissue sections based on the formation of a colored formazan by reduction of nitro-blue tetrazolium (NBT) using tryptamine as substrate. [The *SCI*® indicates that this paper has been cited over 480 times since 1961.]

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"Much of the clinical excitement at the National Institutes of Health at this time came from the work in the Heart Institute on the carcinoid tumor syndrome and serotonin metabolism. The histochemical methods then available for localizing the tissue sites of the enzyme, MAO, implicated in the inactivation of vasoactive amines, e.g., serotonin, produced either poor or artifactual tissue localization or were ascribed to the activity of MAO on a strictly empirical basis. Dissatisfied with the results of this technique, I went through the list of available substrates with the urging of my colleague, James Longley, and found that tryptamine in the presence of the commercially available tetrazolium salts produced a reasonable cellular localization in frozen tissue sections. Concurrently, George Brown, a staff associate also in the Arthritis Institute, was engaged with me in synthesizing a tetrazolium salt (NBT) which could afford a more precise localization of flavoprotein-linked dehydrogenase activity. With Helen Burtner's technical assistance we applied NBT with tryptamine as substrate to localize MAO activity to cellular sites with astoundingly excellent results. We were pleased when the classic MAO inhibitors, Marsilid (iproniazid) and phenylhydrazine, inhibited the reaction. However, uncertainty set in when other carbonyl reagents such as hydrazine and cyanide ion also acted as inhibitors. This was at variance with known biochemical results. Herbert Weissbach, then a staff fellow in Sidney Udenfriend's group in the Heart Institute, was called in for consultation. Using a purified MAO preparation, he confirmed the histochemical results, but we remained unenlightened as to the anomalous inhibitor reactions, the mechanism producing NBT reduction, and the efficacy in our system of tryptamine, a less than optimal biochemical substrate for MAO. With Herb's biochemical confirmation we went to press with the answers to these questions to await further study. It was only later[1] that we discovered that the mechanism of reduction of the tetrazolium salt was due to instantaneous electron transfer by the labile aldehyde (indolyl-3-acetaldehyde) formed from tryptamine by MAO activity, explaining the inhibitor effect of the carbonyl reagents and the uniqueness of tryptamine as substrate. This was also the first application of a nonenzymatic sequence methodology for localizing enzymes in solid phase systems such as tissue sections and electrophoretic gels.

"This publication has been highly cited for several reasons. The use of newer aqueous-lipid insoluble formazan precursors has made the method applicable for electron microscopic purposes,[2] and its ready adaptability for localization of MAO species in electrophoretic gels has found extensive biochemical use (as have most specific histochemical methods). The accelerating interest in neuropathology, neuropharmacology, and neurophysiology, especially with the focus on MAO inhibitors, has undoubtedly produced a concomitant accelerating interest in this easily reproducible histochemical technique."

1. **Glenner G G , Weissbach H & Redfield B G .** The histochemical demonstration of enzymatic activity by a nonenzymatic redox reaction. Reduction of tetrazolium salts by indolyl-3-acetaldehyde. *J. Histochem Cytochem* **8**:258-61, I960.
2. **Lojda Z, Grossran R & Schiepler T H.** *Enzyme histochemistry.* Berlin: Springer-Verlag, 1979, 339 p.