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This Week's Citation Classic

Nachlas M M, Tsou K C, De Souza E, Cheng C S & Selingman A M.
Cytochemical demonstration of succinic dehdrogenase by the use of a new *p*-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* **5**:420-36, 1957.
[Sinai Hospital of Baltimore, Baltimore, MD, Johns Hopkins Univ. Sch. Med. Baltimore. MD, Dajac Labs., Chem. Div., Borden Co.]

This paper introduces the use of 2,2'-di(-p-nitrophenyl)-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)-di(tetrazolium chloride) (Nitro BT, or NBT) as the ideal substrate for the cytochemical demonstration of DPN and TPN diaphorase systems, as well as the succinic dehydrogenase system. The 'NBT' method has since become the most popular method for the demonstration of dehydrogenases in histochemistry. Diagnostic tests based on the use of NBT can be found today in most clinical laboratories. [The *SCI*® indicates that this paper has been cited over 1,150 times since 1961.]

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"The development of nitroblue tetrazolium, or NBT, for enzyme histochemistry, was indeed an important episode in our long-term effort to develop new and useful substrates for this still relatively young field of enzyme histo- and cytochemistry. In those days, as is even true today, many of us thought the route to studying respiratory enzymes should be through histochemistry. A. M. Rutenburg, R. Gofstein, and A. M. Seligman had earlier developed a method known as BT for succinic dehydrogenase, but it required the addition of potassium cyanide (KCN).[1] I asked Rutenburg how he could demonstrate oxidative phosphorylation in the presence of cyanide, but he was not pleased with my question. Seligman, on the other hand, encouraged me to look for better electron acceptors for succinic dehydrogenase. As I had already learned tetrazolium chemistry from earlier work at Harvard, I though it would be a cinch to find one with the right redox potential by putting electron withdrawing groups on the phenyl groups.

"In fact, it took a serious effort of almost three years to accomplish this phase. Today, I have a cryostat, a good microscope and other equipment to do better histochemistry. In those days, I used a very crude testing method of a chicken liver extract and looked for blue precipitate in the absence of KCN. Dr. C. S. Cheng, who was my postdoctoral associate then, and an extremely meticulous organic chemist, could never quite understand why I wasn't too happy with the beautifully crystallized compounds he prepared, until NBT came along.[2] I do not remember when, but I know it was some time in the fall of 1955, when I called Seligman one evening in Baltimore to ask if he was ready to test this preliminary sample which formed a beautiful blue precipitate with no trace of color in the supernatant on my bench. Mrs. Seligman told me that Arnold had gone out to pick the corn on his 'farm.' Nachlas called me back next morning and obtained this first sample the next day. A few days later, he called back to say that NBT worked! Our job, however, was not done, as we still had to improve the purification procedure so that he could do an extensive series of studies.

"This work stimulated my interest in the synthesis of substrates for electroncytochemistry and converted my career from organic to cytochemistry. The applications of NBT to isozyme research and medical diagnosis are still active even today, in spite of the fact that few users appreciate our difficulties in the development of this versatile reagent. The *SCI* citations remind me of many associates who contributed to the chemistry of this development and of my friendship with the late A. M. Seligman, whose name in histochemistry will be remembered by more than myself."

1. **Rutenburg A M, Gofstein R & Seligman A M.** Preparation of a new tetrazolium salt which yields a blue pigment on reproduction and its use in the demonstration of enzymes in normal and neoplastic tissue. *Cancer Res.* **10**:113-21, 1950.
2. **Tsou K C, Cheng C S, Nachlas M M & Seligman A M.** Synthesis of some p-nitrophenyl substituted tetrazolium salts as electron acceptors for the demonstration of dehydrogenases. *J. Amer. Chem. Soc.* **78**:6139-44, 1956.