

# This Week's Citation Classic

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**Baehner R L & Nathan D G.** Quantitative nitroblue tetrazolium test in chronic granulomatous disease. *N. Engl. J. Med.* 278:971-6, 1968.  
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Chronic granulomatous disease is a hereditary defect in the killing of certain bacteria by peripheral blood granulocytes and can be detected with the nitroblue tetrazolium (NBT) test. The rate and reduction of NBT by normal leukocytes is stimulated by phagocytosis. It also depends on cell number, pH, and temperature. Granulocytes of affected patients failed to reduce NBT to blue formazan during phagocytosis whereas leukocytes of carrier females, of the X-linked form, demonstrated intermediate dye reduction. Parents' leukocytes of the autosomal recessive form had normal dye reduction rather than intermediate values. [The SCI® indicates that this paper has been cited in over 570 publications since 1968.]

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"Almost 15 years have elapsed since David Nathan and I stumbled on the idea that nitroblue tetrazolium (NBT) might be employed to monitor the activation of normal blood neutrophil oxidase. The initial manuscript was published after a series of controversial reviews because some doubted the biological significance and clinical relevance of the test. Nathan had used redox dyes for studies of red cells deficient in glucose-6-phosphate dehydrogenase. Manfred Karnovsky and his group at Harvard Medical School had previously shown that the respiratory burst of phagocytizing PMN was catalyzed by an FAD-enzyme complex which required reduced pyridine nucleotide (NADPH) as substrate.<sup>1</sup> Thus, the stage was set by these scientists to enable me as an inexperienced hematology fellow at the Children's Hospital Medical Center in Boston to walk on. Two samples of blood neutrophils, one from myself and the other from my three-year-old patient, a former patient of Louis K.

Diamond, were studied. The child had lifelong anemia and suppurative infections resulting in liver and lung granulomas and abscesses. Nathan and I observed that my patient's PMNs could not reduce NBT whereas my PMNs became coated with and internalized the purple formazan. In a short time, I found a way to extract the dye and quantify spectrophotometrically the extent of NBT reduction. We identified several other patients with the same PMN response. Not only was the test completely accurate in identifying all subsequent patients with chronic granulomatous disease, but we and Dorothy Winthorst and Robert Good at Minnesota also found it to be useful to identify the female carriers for the X-linked form of the disease.<sup>2</sup>

"Subsequent studies in our laboratory at Indiana University's James Whitcomb Riley Hospital for Children have shown that the biochemical basis for NBT reduction in PMN is the release of superoxide anion, a univalent reduced product of oxygen.<sup>3</sup> Morphometric studies indicated that NBT reduction was in phagolysosomes.<sup>4</sup> Klebanoff reported, about the same time, that the dismutated form of superoxide anion, hydrogen peroxide, was responsible for effective bactericidal activity in PMN.<sup>5</sup> Quie and his group in Minnesota as well as our own studies confirmed the inability of PMNs deficient in both superoxide anion and hydrogen peroxide to effectively kill bacteria employing *in vitro* tests.<sup>6,7</sup>

"Because the NBT test is so simple in design and is easy to perform, its clinical appeal and popularity increased rapidly during the 1970s. Clinicians employed it to evaluate the oxidation capacities of blood PMNs from children and adults in a wide assortment of disease states. Several slide tests were adapted to assess NBT reduction by individual cells and to establish the diagnosis of chronic granulomatous disease prenatally.<sup>8</sup> The test is now standard in many clinical and research laboratories throughout the world. Perhaps our comments on the NBT test will stimulate young investigators who initially receive rejections of their work to continue to refine it and persist in their attempts to have it published especially when they are convinced of its scientific merit."

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