

# This Week's Citation Classic

**Park B H, Fikrig S M & Smithwick E M.** Infection and nitroblue-tetrazolium reduction by neutrophils: a diagnostic aid. *Lancet* 2:532-4, 1968. [Dept. Pediatrics, State Univ. New York, NY]

This paper described a supravital staining of phagocytes in fresh unstimulated whole blood using nitroblue tetrazolium (NBT) dye, and classified neutrophils as 'NBT positive' and 'NBT negative.' The proportion and absolute number of the former was increased in patients with certain types of bacterial infections. [The **SCI**<sup>®</sup> indicates that this paper has been cited over 500 times since 1968.]

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"In April 1967, at the annual meeting of the American Pediatric Society, I heard Baehner and Nathan's report<sup>1</sup> that neutrophils from chronic granulomatous disease failed to reduce NBT dye, which was attributed to the defective oxidative metabolism and phagocytic defect. I thought that one might extend this finding to other conditions associated with phagocytic dysfunction, such as patients receiving steroid therapy.

"With initial help from Fikrig and Smithwick, I could demonstrate the reduction of NBT dye in normal neutrophils. In July 1967, Fikrig sent me with 'the slide' to Nathan in Boston, who kindly identified the 'NBT-positive' cell. Since I was an acting chief resident, as well as an immunology fellow, most of the experiment was done in the evening hours and on weekends. For the next five months my results were entirely negative.

"My project came to a turning point when I was on call on Christmas Eve 1967. While I was browsing through the journals in the library, I came across a report by Cluck,<sup>2</sup> who used whole blood for testing phagocytic function in newborns. It was already 2 am, and I promptly went to the laboratory,

which was on the seventh floor of King's County Hospital, one floor up from the library. After many 'feeble' attempts to lance my finger, and with some sweat on my forehead, I finally collected a few drops of blood into a plastic tube. Using 'unstimulated' whole blood this time, I found only one NBT-positive cell in the entire slide, which was in contrast to the usual number of about 20 percent. It was 4 am. Feeling tired, I went back to my room in the dormitory.

"Why were there so few NBT-positive cells? Suddenly it occurred to me: 'It might be due to the way the WBC was handled *in vitro*, i.e., minimal stimulation. The one NBT-positive cell might represent a small proportion of neutrophils in activated conditions in the blood of a healthy person. Therefore, one might find an increased number of these cells during natural infection.' At that moment I was seized by a kind of strange feeling, a sort of excitement, hard to describe for lack of proper words.

"For the next two months I improved and standardized the method. I tried to avoid stimulation of WBCs *in vitro* while making the method so simple that it could be performed with minimum equipment in developing countries.

"The reasons for the paper's receiving so many citations are probably due to: 1) a new idea and simplicity of methodology, 2) description of methodology which left many 'critical' aspects undefined, 3) its potential clinical use, and 4) discordant results and heated controversy due to different methodology and sometimes erroneous interpretations based on insufficient data.

"I believe that my hypothesis of activated neutrophils during natural infection is largely correct, with a few exceptions.<sup>3,4</sup> Since the publication of this paper, I have learned a great deal about the art of scientific investigation while the original idea has grown and matured. In this personal sense, this paper may be affectionately called 'a classic' "

1. **Baehner R L & Nathan D G.** Chronic granulomatous disease: an X-linked deficiency of leukocyte NADH oxidase. *Pediat. Res.* 1:306-7, 1967.
2. **Gluck L & Silverman W A.** Phagocytosis in premature infants. *Pediatrics* 20:951-7, 1957.
3. **Park B H.** The use and limitations of NBT-test as a diagnostic aid. *J. Pediatrics* 78:376-8, 1971.
4. **Kite P.** *The evaluation and some applications of the nitroblue tetrazolium test.* PhD thesis. Leeds. England: University of Leeds, 1980.