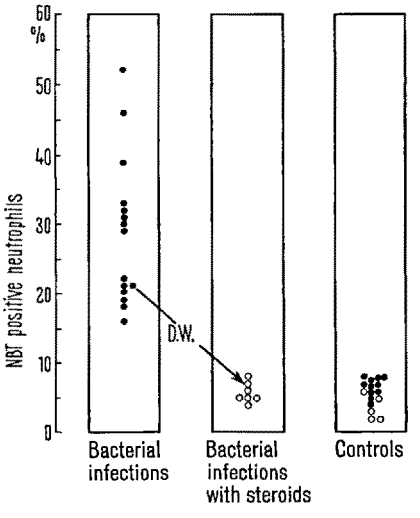
Suppressed Reduction of Nitroblue Tetrazolium by Polymorphonuclear Neutrophils from Patients Receiving Steroids

Most patients with acute bacterial infections demonstrate an increased reduction of nitroblue tetrazolium (NBT) by their polymorphonuclear neutrophils[1]. The metabolic events of bacterial phagocytosis and intracellular killing result in conversion of colorless NBT to easily identified black formazan deposits within the cytoplasm of a large proportion of the neutrophils, and the percentage of 'positive' cells can be counted on a peripheral blood smear. Therefore, the NBT test has been proposed as a rapid method for distinguishing between patients with acute bacterial infections and those who have fever or leukocytosis from other causes.

Work in our laboratory confirms these observations. However, steroid therapy, even of short duration, is found to suppress the NBT test of infected patients to the level of uninfected controls.

Methods. Subjects studied were hospitalized adult patients with both clinical and laboratory evidence of acute bacterial infection. Of the 21 patients with bacterial infection, 6 were receiving steroids when they became infected, and 1 patient was treated with steroids after infection developed. The severity of infection as well as the presence of underlying disease was similar in the steroid and non-steroid groups. A control group of 16 individuals was comprized of similar aged non-infected patients or patients with viral illness. Fever and leukocytosis were common in all groups.



Percent of neutrophils reducing NBT. Closed circle = no steroids. Open circle = steroids. The arrow connects the NBT scores of patient D.W., before and after receiving steroid therapy.

Venous blood was collected in heparinized tubes and processed within 30 min. 0.1 ml of blood was added to 0.1 ml of NBT mixture (General Biochemicals) according to the method of PARK[1], then incubated at 37 °C for 1 h. Cover slip preparations were made and counterstained with Wright-Giemsa stain. 100 neutrophils were counted, and the percent containing black deposits of reduced NBT noted as 'NBT score'.

Results. Patients with acute bacterial infections had NBT scores ranging from 16% to 52% (Figure). Those with bacterial infections who were on steroid therapy had NBT scores ranging from 4% to 8%. The scores of control or non-infected patients ranged from 2% to 8%. (Several of the non-infected patients were also on steroid therapy, noted by open circles in the Figure). The actual suppression of the NBT score by steroids is illustrated in 1 patient who falls into both groups, bacterial infection with and without steroids (arrow in the Figure).

Discussion. Phagocytosis is accompanied by a number of metabolic events within the polymorphonuclear neutrophil: increased oxygen consumption, stimulation of the hexose monophosphate shunt, oxidation of reduced nicotinamide adenine dinucleotide (NADH), and the production of hydrogen peroxide[2]. The production of hydrogen peroxide has been linked to the intracellular killing of bacteria[3]. During these processes the dye NBT is reduced, and the resulting black deposits in the neutrophil cytoplasm can thus serve as a marker for the metabolic events.

The increased NBT score which results from the metabolic activities of the neutrophils in bacterial infection has been suggested as a diagnostic aid to differentiate such infected patients from those with fever or leukocytosis due to other causes[1]. Both normal controls and patients with nonbacterial illness have been found to have resting NBT scores below 10%, while bacterial infection usually results in over 15% of the neutrophils containing black formazan deposits.

False positive results have been reported in normal new-borns[4], in Chediak Higashi Syndrome[5], and after typhoid vaccine[5]. False negative results have been found in chronic granulomatous disease of childhood, lipochrome histiocytosis, and after antibiotic therapy[6]. To this list we can now add the false negative score found in infected patients receiving steroid therapy. Since hydrocortisone inhibits NADH oxidase in vitro[7], this is a suggested mechanism for in vivo NBT suppression.

Zusammenfassung. Die Neutrophilen von Patienten mit Infektionskrankheiten zeigten verringerte Reduktion von NBT, wenn die Patienten zur gleichen Zeit Steroidhormone erhielten.

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