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Nitroblue tetrazolium test in the term small-for-dates newborn

by

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The value of the nitroblue tetrazolium reduction test was studied using granulocytes obtained from 41 small-for-dates and 23 normal term newborns.

The unstimulated test did not show any differences between the two groups, while after latex stimulation a statistically significant decrease was noted in the reduction by granulocytes obtained from small-for-dates newborns in comparison to those obtained from normal newborn infants.

The results showed a wide scattering especially in the small-for-dates newborns. This may indicate that in some of these babies the granulocytic enzymes are defective to reduce the nitroblue tetrazolium to purple formazan.

Dystrophy of the neonate may be accepted to represent a form of malnutrition responsible for many metabolic disturbances. In some cases it may be connected with depressed immunological reactions such as a decreased phagocytic index [7], a low IgG level [1, 5, 10], a low number of leukocytes and especially of granulocytes, with a decreased serum lysozyme activity [8], and involution of the thymus [5].

The important role of granulocytes in antibacterial mechanisms is well known. It has been shown to diminish in small-for-dates newborns [17] and may be assumed to play a part in their susceptibility to infections [8, 19].

The nitroblue tetrazolium (NBT) test described by Park et al [15] has been recommended as an additional test of the bactericidal activity of granulocytes. We have therefore studied this activity of small-for-dates newborns by the NBT test.

Material and Method

The tests were carried out with the peripheral blood granulocytes obtained on the third day of life from 41 small-for-dates newborns and 23 normal term newborn infants. These latter served as the control. All newborns were free from signs of infections and had had no asphyxia.

The NBT test was performed according to Park et al [15] with resting granulocytes and with granulocytes stimulated with latex particles. Capillary blood without anticoagulant was immediately mixed with nitrotetrazolium blue. Results are expressed in the percentage of purple formazan cells.

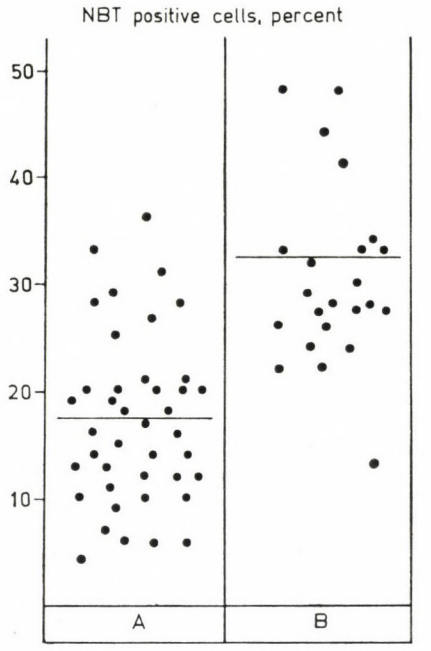

FIG. 1. Unstimulated nitroblue tetrazolium test: A — small-for-dates newborns; B — normal newborn infants

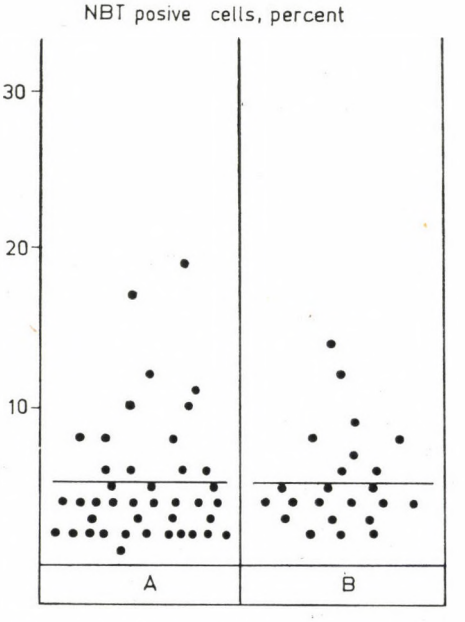

FIG. 2. Stimulated nitroblue tetrazolium test: A — small-for-dates newborns; B — normal newborn infants

Results

The NBT test performed with resting granulocytes did not show any difference between the two groups (Fig. 1). The mean value was 5.2% for both groups, with a wider scatter (2—14%) in the dystrophic group.

Figure 2 shows the results after stimulation with latex particles. The mean value for the dystrophic group was 17.4% (range, 4—40%), and for the control group 32.3% (range, 22—48%), with a significant difference ($p < 0.001$) between the two groups. Such a difference in the percentage of purple formazan cells between resting and stimulated granulocytes may be due to the latex action facilitating the penetration into the cells of nitrotetrazolium blue [12].

Discussion

According to the above results, latex stimulated granulocytes obtained from small-for-dates newborns fail to reduce the redox dye NBT to purple formazan within the region of the phagocytic vacuole.

To explain the increased susceptibility to infection of neonates, poor development of the immunological system, e.g. opsonization, chemotaxis and bacteriolysis, cell mediated immunity, etc., have been suggested [3, 4, 9, 11]. The role of the intracellular leukocyte has also been assumed to represent a factor to the increased susceptibility to infection. This function may to some extent be evaluated by the NBT test which is somewhat dependent on metabolic activity e.g. oxygen consumption, HMPS activity, glycolysis of granulocytes during particle uptake, and intracellular digestion. The precise mechanism of NBT reduction to purple formazan is not clear. According to some authors [2, 10] the proportion of NBT positive neutrophils is increased in healthy newborns. This increased activity of phagocytes in newborns, a false positive NBT test, is connected with the stimulation of hexose monophosphate metabolism and the production of hydrogen peroxide [16].

Stimulated granulocytes of dystrophic babies showed sometimes a tenfold difference and those of the controls a twofold difference. This indicates a wide range of tetrazolium blue reduction in small-for-dates newborns, raising the hope that the test may prove useful in clinical practice, allowing to identify the newborns especially susceptible to infection, which cannot be done on the basis of clinical examinations. Statistically significant differences in the NBT test in small-for-dates newborns may indicate an insufficiency of the enzymatic system responsible for the reaction. This insufficiency may be one of the factors responsible for the dramatic course of infections in small-for-dates newborns.

References

1. Berg, T.: Immunoglobulin levels in infants with low birth, weights. Acta paediat. scand. **57**, 369 (1968).
2. Cocchi P., Mori S., Becattim A.: Nitroblue-tetrazolium reduction by neutrophils of newborn infants: an in vitro phagocytosis test. Acta paediat. scand. **60**, 475 (1971).

3. Fireman, P., Zuchowski, D. A., Taylor, P. M.: Development of the human complement system. J. Immunol. **103**, 25 (1969).
4. Gitlin, D., Rosen, F. S., Michael, J. G.: Transient 19-S gamma-globulin deficiency in the newborn infant Pediatrics **31**, 197 (1963).
5. Gruenwald, P.: Chronic fetal distress. Clin. Pediat. **3**, 141 (1964).
6. Hobbs, J. R., Davis, J. A.: Serum gamma-globulin levels and gestational age in premature babies. Lancet **1**, 757 (1967).
7. Iwaszko-Krawczuk, W., Prokopowicz, J.: Phagocytosis in the small-for-dates newborn. Acta paediat. Acad. Sci. hung. **14**, 47 (1973).
8. Iwaszko-Krawczuk, W.: Serum lysozyme activity in the small-for-dates newborn. Acta paediat. Acad. Sci. hung. **14**, 135 (1973).
9. Klein R. B., Rich K. C., Biberstein M., Stiehm E. R.: Defective mononuclear and neutrophilic phagocyte chemotaxis in the newborn, Clin. Res. **24**, 180A (1976).
10. Kolankowska H.: The nitroblue tetrazolium reduction test in evaluation of the phagocytic activity of neutrophilic granulocytes, Acta med. pol. **18**, 123 (1977).
11. McCracken, G. H., Eichenwald, H. F.: Leukocyte function and the development of opsonic and complement activity in the neonate. Amer. J. Dis. Child., **121**, 120 (1971).
12. Nathan, D. G., Baehner, R. L., Weaver, D. K.: Failure of nitroblue tetrazolium reduction in the phagocytic vacuoles of leukocytes in chronic granulomatous disease. J. Clin. Invest. **48**, 1895 (1969).
13. Orlowski, J. P., Sieger, L., Anthony, B. F.: Bactericidal capacity of monocytes of newborn infants., J. Pediat. **89**, 797 (1976).
14. Papadates, C., Papaevangelou, G. J., Alexiou, D., Mendris, J.: Serum immunoglobulin G levels in small-for-dates newborn babies., Arch. Dis. Childh. **45**, 570 (1970).
15. Park, B. H., Fikrig, S. M., Smithwick, E. M.: Infection and nitroblue-tetrazolium reduction by neutrophils. A diagnostic aid. Lancet **2**, 532 (1968).
16. Park, B. H., Good, R. A.: NBT test stimulated. Lancet **2**, 616 (1970).
17. Prokopowicz, J., Ziobro, J., Iwaszko-Krawczuk, W.: Bactericidal capacity of plasma and granulocytes in small-for-dates newborns. Acta paediat. Acad. Sci. hung. **16**, 267 (1975).
18. Wagner, M. G.: An epidemiologic analysis of dysmaturity. Biol. Neonat. **6**, 164 (1964).
19. Wigglesworth, J. S.: Foetal growth retardation. Brit. med. Bull. **22**, 13 (1966).

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