Sickle cell anaemia and the NBT test

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SYNOPSIS Patients with sickle cell anaemia have an increased susceptibility to bacterial infections. Previous reports of false-negative nitro blue tetrazolium (NBT) tests in the presence of bacterial infection and of a faulty phagocytic response following stimulation *in vitro* have suggested the possibility of polymorphonuclear dysfunction in certain patients with sickle cell anaemia.

In the present study an unstimulated, histochemical NBT technique was used to evaluate the test in patients with sickle cell anaemia. There was a significant difference between the results in the group of patients with infection (mean NBT-positive cells 42.7%) compared to those without infection (mean 9.4%). There was no significant correlation between the total white blood cell count, absolute number of polymorphonuclear cells, and infectious complications.

These findings indicate an appropriate polymorphonuclear cell response, as evaluated by the NBT test, in patients with sickle cell anaemia and bacterial infection. The NBT test may be used as an additional parameter in the differentiation of those patients with sickle cell anaemia with bacterial infection.

Patients with sickle cell anaemia have an increased susceptibility to bacterial infections. Although infections such as salmonella osteomyelitis (Hook, Campbell, Weens, and Cooper, 1957) and pneumococcal meningitis (Robinson and Watson, 1966) have been most commonly reported, in a review of bacterial infections in patients with sickle cell anaemia numerous other organisms were identified (Barrett-Connor, 1971). The reduction of nitro blue tetrazolium (NBT) to formazan by polymorphonuclear cells has been previously reported as a finding suggestive of systemic bacterial infection (Park, Figrik, and Smithwick, 1968; Feigin, Shackelford, and Choi, 1971). Reports of a false negative NBT test, that is, absence of reduction of NBT in the presence of bacterial infections in patients with sickle cell anaemia (Park, 1971; Humbert, Marks, Hathaway, and Thoren, 1972), suggested the possibility of a defect in polymorphonuclear function in these patients as an additional predisposing defect related to the increased susceptibility to bacterial infections. A report of an inappropriate response *in vitro* following phagocytic stimulation of polymorphonuclear cells in certain patients with sickle cell anaemia supported this possibility

(Dimitrov, Douwes, Bartolotta, Nochumson, and Toth, 1972). These findings suggested that the predictive value of the NBT test would be limited in patients with sickle cell anaemia. The purpose of the present study was to evaluate the NBT test in patients with sickle cell anaemia and to determine its possible usefulness in identifying the presence of bacterial infections in these patients.

Methods

A modified spontaneous dye reduction test by peripheral blood neutrophils was used (Park, Figrik, and Smithwick, 1968). Heparinized blood, 10 units/100 ml, was drawn through a scalp vein catheter. One hundred lambda of fresh whole blood was incubated in a plastic cup with 100 lambda of 0.2% NBT suspended in 0.9% saline for 15 minutes at 37° and for 15 minutes at room temperature. Coverslip slides were made and 200 polymorphonuclear cells were evaluated. Polymorphonuclear cells were interpreted as NBT positive if black-blue-reduced formazan was present within the cells. When the test was markedly positive, ie, greater than 25%, clumping of NBT-positive cells was noted, a finding previously reported (Neuwirtová, 1973). These clumps were disregarded and only single polymorphonuclear cells were included in the differential count. The results were evaluated

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separately by two investigators without knowledge of the patient's condition or the results of other laboratory procedures. There was a consistent agreement in the interpretation of the morphological findings. The data were then correlated with the clinical, radiological, and bacteriological findings. Haematological studies were performed by standard techniques. The Student *t* test was used to calculate *P* values.

Results and Discussion

There is an increased susceptibility to bacterial infections in patients with sickle cell anaemia. Several predisposing defects have been described: (1) splenic dysfunction has been demonstrated in children with sickle cell anaemia, which is reversible by transfusion of haemoglobin A blood (Pearson, Spencer, and Cornelius, 1969; Pearson, Cornelius, and Schwartz, 1970); (2) an impaired antibody response following the intravenous injection of sheep erythrocytes has further incriminated splenic dysfunction (Schwartz and Pearson, 1972); and (3) a deficiency in heat-labile serum opsonizing activity (Winkelstein and Drachman, 1968), and an abnormality in the alternate pathway of complement activation have also been reported (Johnson, Newman, and Struth, 1973).

Several reports have suggested a defect in polymorphonuclear cell dysfunction in these patients. False negative NBT tests have been reported in patients with sickle cell anaemia and bacterial infections such as pneumococcal meningitis (Park, 1971) and salmonella osteomyelitis (Humbert *et al*, 1972). In addition, a subgroup of patients with sickle cell anaemia has been reported to have a defect in polymorphonuclear cell response following phagocytic stimulation *in vitro* (Dimitrov *et al*, 1972). Eight of 13 patients having this abnormality had a history of repeated infections. The five who had an appropriate response were adult patients who had no history of infection.

In contrast to these reports we found a mean of $39.7\% \pm 4.1\%$ NBT positive polymorphonuclear cells in nine patients with sickle cell anaemia and

pneumonia (table I). This was significantly greater than the occurrence of NBT-positive cells in normal individuals without a haemoglobinopathy or infection ($11 \pm 1.1\%$, $P < 0.01$). Although pulmonary infarction is reported as a frequent complication in patients with sickle cell anaemia (Diggs, 1965), our results do not differentiate between a primary bacterial pneumonia and a secondary infection following infarction. In five patients with sickle cell anaemia and septicaemia the mean value of NBT-positive polymorphonuclear cells was $45.7 \pm 6.6\%$ ($P < 0.01$).

In contrast to these findings, in 22 patients in sickle cell crisis without evidence of bacterial infection, the NBT test showed a mean value of 7.8% positive $\pm 1.1\%$. These results were similar to the normal controls. Chest x-rays were done in nine of these patients and were negative as were blood cultures in all.

Controversy exists in the value of the NBT test with both false negative and false positive results (Park, 1971; Humbert *et al*, 1972). It was most recently reported that the use of the total white blood cell count, the number of immature polymorphonuclear cells, cytoplasmic granulation and vacuolization, and the presence of Döhle bodies were more sensitive indicators than the NBT test of the presence of systemic infection (Steibig, Johnson, and Remington, 1974). However, white blood cell counts in normal ambulatory children with sickle cell anaemia have been reported to average 20000 with a range of 12 to 35000 (Pearson and Diamond, 1971). As shown in table II, the total white blood

Clinical Status	No. of Patients	Mean WBC/mm ³	Mean PMN/mm ³
Crisis	22	16 246 $\pm$ SE 4868	10 317 $\pm$ 3800
Pneumonia	9	22 587 $\pm$ 12 820 (<i>P</i> = NS)	14 271 $\pm$ 7823 (<i>P</i> = NS)
Septicaemia	5	31 360 $\pm$ 15 191 (<i>P</i> = NS)	23 535 $\pm$ 17 969 (<i>P</i> = NS)

Table II Total white blood cell and absolute polymorphonuclear count in patients with sickle cell anaemia

The *P* value was calculated to compare the findings in pneumonia and septicaemia to episodes of painful thrombotic crisis without bacterial infections.

Sickle Cell Anaemia	No. of Patients	Mean Percentage Positive PMN Cells	<i>P</i> Value ¹
Controls	24	11.0 $\pm$ SE 1.1	
In crisis	22	7.8 $\pm$ 1.1	NS
With pneumonia	9	39.7 $\pm$ 4.1	<0.01
With septicemia	5	45.7 $\pm$ 6.6	<0.01

Table I Sickle cell anaemia and the NBT test

¹The *P* value was used to compare the results with the haematologically normal controls, who had no evidence of infection.

cell count and absolute number of polymorphonuclear cells were not significantly different between patients in crisis without infection and those with infection, either pneumonia or septicaemia. These results were not helpful in evaluating the presence of infection.

Our findings indicate that polymorphonuclear cell response to infection when evaluated by the

NBT test is appropriate in certain patients with sickle cell anaemia. They also suggest that the NBT test may be a useful adjunct to a comprehensive history and physical examination and other laboratory tests in the evaluation of bacterial infections in these patients.

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