Serial nitroblue tetrazolium tests in the management of infection

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SYNOPSIS Two cases are described in which extensive use was made of the nitroblue tetrazolium (NBT) test. In the first case the advantages of using this technique to diagnose and control infection is shown; in the second the considerable advantage of the speed of the technique. In both of these cases the test made a material contribution to the management of the patient, and it is concluded that the test brings bacteriological control of the patient within the immediate clinical area, thus overcoming one of the principal disadvantages of the classical bacteriological methods.

The nitroblue tetrazolium (NBT) test has recently been put forward as an aid to the diagnosis and management of infection (Park, Fikrig, and Smithwick, 1968; *Lancet*, 1971). The test can also be used to monitor treatment and to reveal phagocytic defects in the host (*Lancet*, 1971).

We present two selected cases in which the NBT test was used extensively and made a material contribution to the recovery of the patients concerned.

Materials and Methods

In both cases to be described the NBT tests were performed by the method of Park *et al* (1968), with the subsequent modification of Freeman and King (1971). The test was considered positive if the value obtained exceeded 11%. Any value below 4% led to the performance of a stimulated test, which was carried out with freshly prepared endotoxin after the method of Park and Good (1970). Failure to produce a rise in the test value to at least 20% was interpreted as suppression of the normal phagocytic function.

Case Histories

CASE 1

This young woman was admitted to the maternity unit for delivery. Apart from two lower urinary tract infections, both of which had been successfully treated in early pregnancy, her antenatal and previous medical history were unremarkable. On 6 February she was delivered of a healthy infant. After

delivery she became shocked and hypovolaemic, and a diagnosis of uterine rupture was made. This was confirmed at laparotomy, and hysterectomy was performed. She received a total of 25 pints of blood, much of it fresh blood, in the 24 hours following delivery. Therapy with hydrocortisone (500 mg six hourly) and ampicillin was initiated. At this stage monitoring with the NBT test began. Samples were sent daily or more often if the clinical situation required.

Almost immediately the NBT test could be shown to be suppressed (Fig. 1), and it was thought that this was due to the high dosage of steroids (Matula and Paterson, 1971). The steroids were rapidly reduced in dosage and withdrawn, and the results of the NBT test rose to more normal levels, the suppressive effect disappearing simultaneously. The patient remained well and the NBT test normal until 14 February, when she developed a painful, tender area in the right upper quadrant associated with a temperature and x-ray evidence of a right pleural effusion. The NBT test was now positive (Fig. 1) and a subphrenic abscess was considered likely. The abdominal wound was leaking purulent fluid, from which a heavy growth of *Escherichia coli* resistant to ampicillin but sensitive to kanamycin, was isolated.

Treatment with kanamycin produced a rapid clinical recovery, and the results of the NBT test fell to normal levels. Kanamycin was discontinued on 20 February. On 24 February the patient again fell ill, complaining of muscle pains and malaise, and she was once more febrile. She was ambulant, and no abnormal physical signs were elicited. She was given cephaloridine with no effect. The NBT test

Fig. 1 Nitroblue tetrazolium test results and relevant clinical data from case 1. * indicates that suppression was found in a stimulated test.

was again showing very low values, and suppression could again be demonstrated (Fig. 1).

Recalling the previous blood transfusion, and reports that viral infection can suppress phagocytosis (Clein, 1972), we suggested that the patient might be suffering from infection with cytomegalovirus. Smears of the urine deposit contained the typical inclusion bodies, and the peripheral blood showed a lymphocytosis. These changes were recognized on 1 March, and antibiotics were then withheld. The patient recovered clinically two days later, and the results of the NBT test again rose to normal levels. Subsequently, seroconversion to cytomegalovirus was shown, the titre rising from 16 to 1024. The patient remained well and was subsequently discharged home.

CASE 2

This 43-year-old man was admitted for aortic valve replacement. A preoperative NBT test scored 12%, but no overt cause for the score could be found, and since this was a minimal rise, no action was taken. Monitoring with the NBT test was commenced on the day of operation, and samples were sent daily or more often if necessary. The operation and the immediate postoperative period were uneventful, but on the sixth postoperative day the NBT level rose from normal to 16%. The urine was shown to be infected with *Escherichia coli*. No treatment was given, and two days later the patient collapsed, with all the typical features of 'Gram-negative shock'.

The NBT level on a sample taken during the collapse was 40% (Fig. 2). This result was available within one hour of the patient collapsing, and, taken together with the clinical evidence, was thought to indicate

Fig. 2 Nitroblue tetrazolium test results and relevant clinical data from case 2.

bacteraemia with the *E. coli* previously found in the urine. Therapy was immediately instituted with penicillin and gentamicin, and in addition hydrocortisone was given (100 mg six hourly). The steroids were rapidly reduced and discontinued. After 24 hours the patient was improving clinically, the NBT test was falling in value, and recovery seemed likely (Fig. 2). At this stage he was catheterized and this led to another collapse. The results of an NBT test taken immediately were again very high, and it was thought that the catheterization had produced a further bacteraemia. Accordingly, treatment was continued, and the catheter was left *in situ*, any further manipulation being carefully avoided. The patient then made an uneventful recovery, and the NBT test showed a progressive fall to normal levels. Treatment was stopped on the tenth day and the NBT test remained normal. The patient remained well. Blood cultures taken during the initial collapse were subsequently reported as containing *E. coli*, the same organism as found in the urine. The organism was found to be sensitive to gentamicin.

Discussion

These two cases illustrate the value of the NBT test to those involved in managing infection.

In case 1 the NBT test allowed detection of the immunosuppressive effect of the steroids and of the bacterial and viral infections. The treatment of the bacterial infection was accurately monitored and, most importantly, all the information was available within one hour of the blood sample being obtained. The results of the test thus influenced diagnosis and treatment daily. The changes in the NBT levels and the relevant clinical events are shown in Figure 1.

Case 2 is an example of the common problem variously called 'Gram-negative shock', endotoxaemia, or Gram-negative septicaemia. The two most urgent clinical points in such cases are, first diagnosis and secondly some method by which treatment can be monitored. Classical bacteriological methods are largely unusable since they take too long to perform. The NBT test, as used here, provided an answer to both these problems. Blood taken during the initial collapse confirmed the presence of

septicaemia, and did this within one hour. Subsequently, treatment was monitored and the second collapse was accurately detected, and confirmed to be due to a further bacteraemia, probably as a result of the catheterization.

It must be emphasized that the clinical problem was well under control by the time that the classical methods were able to provide any information, ie, the blood culture was reported as positive after more than 48 hours. A further 24 hours was needed for sensitivity testing, but since the NBT test has been shown not to fall if therapy is inappropriate, incorrect antibiotic therapy would have been detected within 24 hours by the NBT test (Feigin, Shackelford, Choi, Flake, Franklin, and Eisenberg, 1971). Finally, the NBT test pointed to the source of the infection, namely, the urinary tract, well before the major complication began.

We feel that the NBT test has been shown in these two instances to fill the void that has existed between clinical medicine and bacteriology, that is, the relative slowness by which bacteriological techniques provide answers to clinical problems. It is obviously too early to claim that the technique is firmly established but the possibilities are exciting.

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References

- Clein, G. P. (1972). The neutrophil granulocyte. *Brit. J. hosp. Med.*, 7, 83-88.
- Feigin, R. D., Shackelford, P. G., Choi, S. C., Flake, K. K., Franklin, F. A., Jr., and Eisenberg, C. S. (1971). Nitroblue tetrazolium dye test as an aid in the differential diagnosis of febrile disorders. *J. Pediat.*, 78, 230-237.
- Freeman, R., and King, B. (1971). A modification to the N.B.T. test. (Letter). *Lancet*, 2, 1154.
- Lancet* (1971). Leading article. Nitroblue tetrazolium: a routine test? *Lancet*, 2, 909-910.
- Matula, G., and Paterson, P. Y. (1971). N.B.T. tests in a patient on steroids. *Lancet*, 1, 803-804.
- Park, B. H., Fikrig, S. M., and Smithwick, E. M. (1968). Infection and nitroblue tetrazolium reduction by neutrophils. *Lancet*, 2, 532-534.
- Park, B. H., and Good, R. A. (1970). N.B.T. test stimulated. (Letter *Lancet*, 2, 616.