The nitroblue tetrazolium (NBT) test in renal transplantation

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SYNOPSIS The nitroblue tetrazolium (NBT) test has been evaluated as a means of differentiating episodes of infection and rejection in 10 cadaver kidney transplant recipients. Normal NBT values were observed during all 11 episodes of acute rejection. A significant elevation of the NBT score was encountered in six of eight episodes of infection after transplantation. A positive NBT result is useful additional evidence in favour of infection in patients in whom the differentiation of infection and rejection is proving difficult.

While renal transplantation has considerably improved the prognosis of patients with end-stage renal failure, the renal allograft recipient remains prone to postoperative episodes of infection and rejection. Infection is the main cause of death following renal transplantation (Gurland, Brunner, Dehn, Härten, Parsons, and Scharer, 1973), while rejection is the most frequent postoperative complication (Briggs, Timbury, Paton, and Bell, 1972). A rapid and easily performed test which would assist the diagnosis of rejection and its differentiation from an infective episode would clearly be of value.

The nitroblue tetrazolium (NBT) test is now well established as a useful screening procedure for the confirmation of systemic bacterial infection and for its differentiation from non-bacterial pyrexial syndromes (Park, Fikrig, and Smithwick, 1968; *Lancet*, 1971; Gordon, Rowan, Brown, and Carson, 1973). It has also been suggested that this test might provide a simple means of distinguishing between episodes of rejection and infection in the immunosuppressed renal transplant recipient (Wollman, Brennan, Stenzel, David, Lewy, Ruben, and Millar, 1972). Prompted by this report we have performed serial postoperative NBT tests on individuals who have received cadaver renal transplants. We report here our experience of the value of this technique as a means of differentiating infection and rejection in these individuals in the postoperative period.

Patients and Methods

Ten patients with end-stage renal failure (six males,

four females: age range 22-42 years) were investigated. Seven of the patients had chronic glomerulonephritis, two had chronic pyelonephritis, and one had hereditary nephritis. All patients were maintained on regular haemodialysis before receiving a cadaver kidney. The operative technique and the postoperative management were as described by Bell, Briggs, Kyle, Dick, Calman *et al* (1972). Postoperative immunosuppression consisted of azathioprine and prednisolone. Azathioprine was administered once daily in a dose sufficient to maintain the total leucocyte count between 5000 and 8000 cells per cmm. At the time of transplantation oral prednisolone was commenced in a daily dose of 200 mg, which was reduced to 15 mg over the first three months. One gram of intravenous prednisolone was given during the transplant operation and this was repeated during acute rejection episodes as described by Bell *et al* (1972). The diagnosis of rejection was made mainly on the basis of biochemical criteria, the most important being a falling urine volume and a rising serum creatinine. Useful clinical guides sometimes seen included swelling of the graft, tenderness, and weight gain. Frequent bacteriological monitoring of the patients was undertaken, including cultivation of sputum, urine, and swabs from nose, throat, and wounds. If a pyrexia developed blood cultures were also performed.

Serial venous blood samples for the NBT test were collected into sequestrene (EDTA). These were obtained daily for the first four weeks following transplantation and twice weekly thereafter for up to three months. During episodes of established or presumed infection or rejection samples were obtained

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on alternate days. All specimens were processed immediately, but where delay was unavoidable they were stored at +4°C (to a maximum period of 12 hours) before testing, in order to minimize spontaneous enhancement of dye reduction (Winchester, Gordon, Rowan, Lindsay, and Black, 1973). The NBT tests were performed on buffy coat preparations by the EDTA/Ficoll technique previously described by Gordon and his coworkers (1973). The preparations were examined by one experienced observer from the investigating group (A.M.G.) who remained unaware of the clinical courses of the patients after transplantation until the end of the study. As a routine 200 neutrophils with intact cytoplasmic membranes were counted under oil immersion. Cells containing reduced nitroblue tetrazolium (formazan) deposits equal to or exceeding the size of individual neutrophil lobes were considered to be NBT-positive and the percentage of NBT-positive cells was recorded as the 'NBT score'. Stippled cells and those exhibiting membrane disruption were not included in the differential count. Patients with more than 14% NBT-positive neutrophils were considered to have elevated NBT scores.

Results

POSTOPERATIVE COURSE

Three of the cadaver kidneys underwent irreversible rejection, necessitating nephrectomy and a return to maintenance dialysis after six weeks in two of the patients, and after three months in one case. One graft exhibited primary non-function and was removed six weeks after transplantation. The remaining six patients were clinically well with stable renal function nine to 12 months after receiving their transplants.

COMPLICATIONS

Table I summarizes NBT scores in relation to all postoperative complications occurring in the patients studied.

Eleven episodes of graft rejection were observed in eight patients. The diagnosis of rejection was based

Episode	Number	NBT Score	
		Normal	Elevated
Rejections	11	11	0
Infections	8	2	6
Dialysis pyrexias	5	5	0

Table I NBT values in relation to episodes occurring following renal transplantation (10 patients)

on standard clinical and laboratory criteria, the main emphasis being placed on urine volume and serum creatinine (Bell *et al*, 1972). Three of the rejections were irreversible, leading to loss of the transplanted kidney. The remaining eight episodes were less severe and were successfully reversed by the intravenous administration of prednisolone. Eight of the 11 episodes of rejection were associated with the development of a pyrexia or a leucocytosis, thus simulating systemic bacterial infection. However, serial NBT values within the normal range were recorded during all rejections, the scores being less than 5% in six episodes and between 5% and 10% in the other five instances (table I).

Eight episodes of infection complicated the post-transplantation course of six patients. The nature of the infections and the range of NBT values encountered during each episode are recorded in table II. Multiple elevated NBT responses (as high as 54% in one instance) were found during six of the infective episodes despite the concurrent administration of immunosuppressive agents. The NBT scores within the normal range were noted in the two remaining infective episodes. These were two clinically mild respiratory infections occurring in a patient who remained apyrexial during both episodes.

Five episodes of pyrexia occurred in three patients during haemodialysis after transplantation, but NBT tests performed during the pyrexial phases yielded normal scores (less than 10%) in all instances.

Discussion

Infection remains the most serious complication

Infection	Pathogen	NBT Values (%) (range and mean % score)	No. of Tests
Perinephric abscess	None isolated	17-54 (32.6%)	8
Perinephric abscess	None isolated	18-34 (26.4%)	7
Respiratory tract infection	<i>Staph. aureus</i>	22-38 (28.1%)	4
Respiratory tract infection	<i>Haemophilus influenzae</i>	18-22 (19.6%)	4
Urinary tract infection	<i>Proteus</i> species	17-23 (20.3%)	5
Wound infection	<i>Staph. aureus</i>	16-40 (30.9%)	6
Respiratory tract infection	<i>Haemophilus influenzae</i>	4-8 ¹ (6%)	3
Respiratory tract infection	<i>Esch. coli</i>	3-6 ¹ (4.5%)	4

Table II NBT responses during episodes of infection

¹'False-negative' NBT scores

following renal transplantation and in a recent report accounted for 39% of deaths in cadaver kidney recipients (Gurland *et al*, 1973). Diagnosis of the presence and site of infection is complicated by the use of immunosuppressive drugs which tend to mask clinical signs as well as interfering with helpful diagnostic features such as leucocytosis. The differentiation of infection from rejection is often difficult as some features such as pyrexia and deteriorating renal function can be common to both. It is recognized that delay in the diagnosis and hence the treatment of an acute rejection episode adversely affects the fate of the transplanted kidney. At present the diagnosis of acute rejection is made mainly on the basis of clinical signs such as pyrexia, weight gain, graft swelling and tenderness, and biochemical changes such as a falling urine volume and creatinine clearance. However, the biochemical changes are non-specific and are of no value in the early postoperative period before the graft has started to function. New diagnostic aids which have been advocated include techniques for studying the immunological process associated with rejection. Of these the leucocyte migration test shows greatest potential (Wood, Gray, Briggs, and Bell, 1973) while further evaluation of the rosette inhibition test has not confirmed its earlier promise (Wood and Gray, 1973). Other tests which have been advocated include measurement of urinary fibrinogen derivatives (Naish, Peters, and Shackman, 1973) and estimation of the enzyme N-acetyl- β D-glucosaminidase (Wellwood, Ellis, Hall, Robinson, and Thompson, 1973).

In the investigation of suspected infection, repeated culture of swabs, blood, urine, etc, is mandatory but a delay is inevitable before full reports are available and cultures are often negative, even in proven infection. In a series of 48 patients in our own unit, organisms were recovered in only 11 out of 16 bacterial infections. A rapid and reproducible test which would confirm the occurrence of infection and thus differentiate infection from rejection would, therefore, be of great value. The nitroblue tetrazolium test has been advocated as such a test (Wollman *et al*, 1972) while its value in the confirmation of systemic bacterial infection and its differentiation from non-infective syndromes has previously been demonstrated (Park *et al*, 1968; *Lancet*, 1971; Gordon *et al*, 1973). Wollman and his colleagues (1972) have studied NBT responses in uraemic patients and in renal transplant recipients during episodes of infection and rejection. The NBT test was shown to be strongly positive in transplant patients with bacterial infections but yielded normal test values during acute rejection episodes, thus permitting a differentiation between infection and

rejection in these individuals. Our findings are in overall agreement with these observations. We have noted enhanced NBT responses during six of the eight infective episodes encountered in this series. It has previously been reported that decreases in NBT dye reduction are induced by immunosuppressive therapy thus producing false-negative NBT scores in the presence of systemic bacterial infection (Miller and Kaplan, 1970; Matula and Paterson, 1971). All of our patients were receiving prednisolone and azathioprine at the time of performing the NBT test. We have nevertheless shown that immunosuppressed renal transplant recipients remain capable of responding to bacterial infections with significant increases in the percentage of NBT-positive neutrophils, thus confirming the value of the technique in the early detection of bacterial infection in such patients. One of our patients yielded NBT values within the normal range during each of two respiratory infections, but these normal scores may be explained by the fact that they were clinically mild infections unaccompanied by a pyrexia. Mild or superficial non-systemic infection and concurrent antibiotic therapy are known causes of a diminished NBT response (Matula and Paterson, 1971; Rubinstein and Pelet, 1973; Gordon *et al*, 1973).

In our experience the NBT test was not influenced by episodes of transplant rejection. We obtained NBT values within the normal range during each of the 11 rejections encountered in the present series despite the occurrence of pyrexia in several of the patients at some stage during the rejection. This confirms the findings of Wollman *et al* (1972) who observed normal NBT scores during four rejection episodes in three patients. Although it has been shown that high serum steroid levels attained shortly after the administration of prednisolone may render neutrophils less responsive in the NBT test (Miller and Kaplan; Wollman *et al*, 1972), the low NBT results encountered in our patients during their rejections cannot be explained on this basis since blood samples for the test were always collected before the intravenous administration of prednisolone. Segal, Trustey, and Levi (1973) have postulated that low NBT scores recorded during transplant rejections may be due to 'a secondary abnormality of neutrophil function induced by circulating immune complexes'. There is as yet, no evidence to substantiate this hypothesis.

The NBT test was originally introduced as a screening procedure to identify patients with the X-linked disorder chronic granulomatous disease. The test was also initially believed to distinguish clearly between bacterial and viral infections and pyrexial syndromes of non-infective origin (Park *et al*, 1968). Indeed, an editorial on this subject

(*Lancet*, 1971) concluded 'its introduction into the repertoire of the routine hospital laboratory seems justified'. More recently an objective reassessment of the NBT test has rendered its status more doubtful (Segal *et al*, 1973; Steigbigel, Johnson, and Remington, 1974; *Lancet*, 1974). It would appear that the early promise shown by the test has not been fulfilled. There may be an overlap between normal and abnormal samples. Steigbigel *et al* (1974) have found routine haematological indices more sensitive than the NBT test as indicators of infection. Segal *et al* (1973) have found the technique relatively disappointing as a means of distinguishing between pyogenic infection and non-infective pyrexial disorders. These views are largely based on the interpretative difficulties which they considered might be encountered by inexperienced observers and the relatively poor results obtained with heparinized blood samples. Furthermore, numerous causes of false-positive and false-negative results in the NBT test have now been identified (Gordon *et al*, 1973; *Lancet*, 1974). However, certain specific clinical indications for using the NBT test remain. Its value for the detection of individuals with chronic granulomatous disease remains undisputed, and it has an important application in the early detection of bacterial infection in patients undergoing intensive care or those who are immunosuppressed (Freeman and King, 1972; Wollman *et al*, 1972; Winchester *et al*, 1973). The renal transplant recipient clearly belongs to the latter category. We believe that the NBT test can usefully be employed to differentiate between infection and rejection in such patients. In circumstances where this distinction is proving difficult, an elevated NBT response is good evidence in favour of infection.

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