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A Further Application of the Nitroblue Tetrazolium Test

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Summary

The nitroblue tetrazolium (N.B.T.) test was performed on patients in whom a differential diagnosis of pulmonary thromboembolism or lobar pneumonia existed. The mean N.B.T. score in healthy subjects was 6.4% (range 1%-15%). Patients with uncomplicated pulmonary thromboembolism showed a mean N.B.T. value of 7.5% (range 3%-12%). In patients with lobar pneumonia the mean N.B.T. score was 42.4% (range 21%-85%). These results suggest that the N.B.T. test is of value in the differential diagnosis of pulmonary thromboembolism and lobar pneumonia.

Introduction

It is widely known that patients with pulmonary thromboembolic disease often present with chest pain, pyrexia, leucocytosis, and radiographical opacity in the lung fields, with or without haemoptysis, thus simulating lobar pneumonia. The differentiation of infective from non-infective pulmonary lesions can therefore be extremely difficult in the absence of specialized techniques. A rapid and easily performed test facilitating this distinction would clearly be of considerable diagnostic value.

The nitroblue tetrazolium (N.B.T.) test is now established as a useful screening procedure for the detection of systemic bacterial infection and for its differentiation from pyrexial syndromes of non-bacterial origin (Park *et al.*, 1968; *Lancet*, 1971; Gordon and Rowan, 1973 a; Gordon *et al.*, 1973).

We performed N.B.T. tests on blood samples from patients in whom a differential diagnosis of lobar pneumonia or pulmonary thromboembolism existed. We report here our experience of this test as a means of differentiating these conditions.

Subjects and Methods

Three groups of people were studied: 71 normal controls of

age range 17-79 years (35 men, 36 women), 14 patients with pulmonary thromboembolism, and 10 patients with lobar pneumonia. The principal presenting features in all patients were pyrexia, haemoptysis, and pleuritic pain. Clinical and radiographical evidence of pulmonary consolidation was present in all patients. Total and differential white cell counts were performed. Certain patients were subjected to pulmonary angiography. Among the criteria for selection for angiography were sudden onset of chest pain without previous respiratory symptomatology, history of deep vein thrombosis, recent surgery, recent pregnancy, or other predisposing factors to venous thrombosis; one patient was taking an oral contraceptive. The angiographic diagnosis of pulmonary thromboembolism was made on some or all of the following criteria: (a) filling defects in pulmonary arteries, (b) sharply cut-off vessels, (c) avascular opacities, (d) asymmetrical or asynchronous blood flow through arteries and veins (Simon and Sasahara, 1965). Additional evidence was obtained from detection of increased pressures in the pulmonary arterial circulation, usually in the absence of raised "wedged" pressure (indirect left atrial pressure). In some patients re-measurement of pressures after an interval of days showed a fall or a return to normal values.

The collection and processing of blood for the N.B.T. test was carried out by the method previously described by Gordon *et al.* (1973).

Whenever possible samples were processed immediately. When delay was unavoidable specimens were stored at 4°C (for a maximum of 12 hours) to prevent spontaneous enhancement of N.B.T. dye reduction (Winchester *et al.*, 1973). The criteria of neutrophil positivity were intracytoplasmic block deposits of formazan equalling or exceeding the size of individual neutrophil lobes (Segal *et al.*, 1973; Gordon and Rowan, 1973 b). Stippled cells were disregarded. Only intact neutrophils with clearly defined cell membranes were included in the count.

Results

The diagnosis of pulmonary thromboembolism was substantiated by angiography in 11 patients. In the three remaining patients the diagnosis was made on clinical and radiographical grounds. A satisfactory response to a continuous infusion of heparin occurred, however, in each patient in the absence of antibiotic therapy. Two patients with lobar pneumonia were subjected to angiography. No angiographic abnormality was found in either case. All patients with lobar pneumonia responded completely to antibiotic therapy.

One patient (case 14) with angiographically proven pulmonary embolism was in addition shown to be suffering from *Mycoplasma pneumoniae* infection (complement fixation titres obtained on paired sera: 1/256, 1/64).

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The N.B.T. scores and total leucocyte counts are shown in the table according to a diagnosis of pulmonary thromboembolism or lobar pneumonia. Eight of the 14 patients with pulmonary embolism showed a polymorphonuclear leucocytosis while six of the 10 patients with lobar pneumonia showed a similar response.

N.B.T. Scores in relation to Total Leucocyte Counts in Patients with Pulmonary

Case No.	Pulmonary Thromboembolism		Case No.	Lobar Pneumonia	
	N.B.T. Score (%)	W.B.C./mm ³		N.B.T. Score (%)	W.B.C./mm ³
1	8	15,000	15	85	11,300
2	6	9,700	16	40	17,600
3	7	5,200	17	56	9,700
4	7	19,600	18	42	6,500
5	9	12,200	19	23	13,000
6	12	16,900	20	23	12,600
7	6	13,200	21	21	9,600
8	11	12,200	22	67	24,800
9	10	8,800	23	42	16,000
10	6	11,300	24	25	21,800
11	7	12,300			
12	3	12,000			
13	6	19,400			
14	56	10,900			

The results of the N.B.T. test performed on healthy control subjects, patients with pulmonary thromboembolism, and patients with lobar pneumonia are shown in the diagram. The mean N.B.T. score in the healthy control group was 6.4% with a range of 1%-15%. In the uncomplicated thromboembolic group the mean N.B.T. value was 7.5% (range 3%-12%). A mean N.B.T. score of 42.4% (range 21%-85%) was obtained in patients with lobar pneumonia. There was a highly significant difference between the two groups of patients studied ($P < 0.001$), but no significant statistical difference was noted between the control group and those patients with thromboembolism ($P > 0.1$).

FIG. 1—N.B.T. scores in three groups of subjects studied.

Discussion

It is generally recognized that the clinical differentiation of lobar pneumonia from pulmonary thromboembolic disease can be extremely difficult. The symptoms of pleuritic pain, haemoptysis, and pyrexia together with clinical and radio-

graphical features of consolidation and often the presence of neutrophil leucocytosis strongly suggest a diagnosis of pneumonia. Many patients with pulmonary thromboembolic disease present with similar features, however. In neither condition is any one feature pathognomonic, and in fact the combination of these features is a common denominator. A raised total white cell count is usually found in systemic bacterial infection, but it may also occur in a wide variety of non-infective conditions. In this study we failed to show any uniform correlation between the presence of infection and leucocytosis, nor did we find any relation between the severity of infection and the degree of increase in the white cell count. These observations are in accord with those of several other workers (Feigin *et al.*, 1971; Vickers and Hayes, 1973).

Early confirmation of the occurrence of an embolic incident is extremely important so that appropriate therapy may be instituted at an early stage. At the moment pulmonary angiography is the only technique available which will unequivocally confirm or refute a diagnosis of thromboembolism. This procedure has the advantage of permitting accurate definition of the extent of the vascular involvement. Pulmonary angiography, however, is expensive and not generally available. Furthermore, it is a relatively complicated procedure and carries a small but definite risk to the patient.

Enhanced N.B.T. reduction by the circulating neutrophils of subjects during systemic bacterial infection is now well documented (Park *et al.*, 1968; *Lancet*, 1971; Gordon and Rowan, 1973 a; Gordon *et al.*, 1973) though false-positive and false-negative results are often recognized (Gordon and Rowan, 1973 a; Ashburn *et al.*, 1973). We have previously suggested that the N.B.T. test might usefully be applied to the differentiation of infective and embolic pathology of the lung (Gordon *et al.*, 1973). The results of the present study have confirmed this hypothesis. We found that thromboembolic episodes are associated with normal N.B.T. scores except where other pathological processes known to produce raised values coexist. Our patient with serologically confirmed *M. pneumoniae* infection and angiographically proved embolism showed a 56% N.B.T.-positive neutrophil score. This is consistent with the previously reported observation that *M. pneumoniae* infection may be the cause of false-positive N.B.T. results (Freeman and King, 1972). In addition, all our patients with clinically or radiographically based diagnoses of lobar pneumonia showed raised N.B.T. responses (as high as 85% in one instance).

Segal *et al.* (1973) have recently undertaken a re-valuation of the N.B.T. test. Their experience contrasts with that of most previous workers. In their hands the test proved relatively disappointing as a means of distinguishing between infective and non-infective pyrexial disorders. These views were based principally on the interpretative difficulties which they considered might be encountered by inexperienced observers and the poor performance of the test with heparinized samples. We have consistently achieved clear-cut and reproducible differentiation between inflammatory and non-inflammatory conditions using venous blood anticoagulated by edetic acid (Gordon *et al.*, 1973; Gordon and Rowan, 1973 b). We therefore disagree with their view that the technique is of little value.

The results obtained in this investigation indicate that the N.B.T. test is a useful screening procedure for the differentiation of pneumonia from pulmonary thromboembolic disease. The test takes little time to execute, involves no risk to the patient, and results are available within one hour of collection of blood samples. We suggest that the N.B.T. test will prove to be of particular value in circumstances where the technique of pulmonary angiography is not available for the confirmation of thromboembolism. In our experience a normal N.B.T. score in a patient with a pneumonia-like presentation of suspected thromboembolism strongly supports this diagnosis and almost certainly refutes a diagnosis of bacterial pneumonia.

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Retention of Nitrogen, Fat, and Calories in Infants of Low Birth Weight on Conventional and High-volume Feeds

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Summary

Two balance studies were performed on each of five infants of low birth weight. About 230 ml/kg/day of S.M.A. S26 milk was given during one study and 180 ml/kg/day during the other. The proportion of nitrogen, fat, and calories retained was similar in the two studies, suggesting that the larger weight gains on the high-volume feeds were due to growth rather than retention of water or excessive deposition of fat.

Introduction

Large gains in weight have been reported in infants of low birth weight receiving higher than conventional volumes of milk (Valman *et al.*, 1972). Such gains could be owing to excessive salt and water retention or excessive fat deposition. We report here the results of balance studies on infants of low birth weight given both high-intake and low-intake feeds.

Patients and Methods

Five infants weighing between 1,100 g and 1,500 g at birth were studied (table I). The gestational age as assessed clinically (Dubowitz *et al.*, 1970) agreed with that calculated from the mother's last menstrual period. Two metabolic balance studies were performed on each infant, one on 230 ml/kg/day of S.M.A. S26 milk and the other on 180 ml/kg/day. Each volume was given for at least five days and collections of stool and urine were made on the final three days. Infants were weighed on scales weighing to 10 grammes.

The balance studies were performed using disposable napkins and Rayon liners as described elsewhere (Valman and Aikens, 1974). The total energy content of the excreta was determined by ballistic bomb calorimetry and the nitrogen content by Kjeldahl digestion and steam distillation. Faecal fat was estimated by the method of Van der Kamer (1958).

TABLE I—Clinical Results in Five Infants of Low Birth Weight

Case No.	Gestational Age (Weeks)	Birth Weight		Balance Study No.	Age at Start of Study (Days)	Weight at Start of Study (g)
		(g)	Centile			
1	32	1,460	10	1	12	1,480
				2	18	1,620
2	32	1,160	<10	3	21	1,320
				4	26	1,440
3	33	1,500	<10	5	26	1,950
				6	33	2,200
4	28	1,100	50	7	47	1,900
				8	52	2,110
				9	30	1,500
5	29	1,130	25	10	41	1,960
				11	47	2,180

The study was approved by the hospital ethical committee and informed consent was obtained from each mother.

Results

The percentage of the intake of nitrogen, fat, and calories which was retained was similar in the studies performed during the high and low intakes (tables II-IV). The retention of nitrogen is expressed as absolute amounts in the diagram.

TABLE II—Results of Nitrogen Balance Studies in Five Infants of Low Birth Weight

Case No.	Balance Study No.	Approximate Milk Intake (ml/kg/day)	Nitrogen (g/3 days)			
			Intake	Stool	Urine	Retention (%)
1	1	260	2.918	0.531	0.926	50
	2	190	2.371	0.350	0.893	48
2	3	260	2.647	0.493	0.670	56
	4	180	1.970	0.370	0.593	51
3	5	230	3.423	0.466	1.029	56
	6	180	3.20	0.340	0.994	58
4	7	230	3.360	0.517	0.842	60
	8	180	2.877	0.409	0.890	55
	9	200	2.351	0.254	0.724	58
5	10	260	3.955	0.261	1.077	66
	11	200	3.285	0.267	0.972	62

TABLE III—Results of Fat Balance Studies in Five Infants of Low Birth Weight

Case No.	Balance Study No.	Fat (g/day)		
		Intake	Stool	Retention (%)
1	1	13.8	3.28	76
	2	11.2	2.06	81
2	3	12.5	3.6	71
	4	9.3	3.5	62
3	5	16.5	2.22	87
	6	14.8	1.26	92
4	7	16.2	2.93	82
	8	13.6	1.74	87
	9	10.8	1.2	89
5	10	18.1	1.9	90
	11	15.8	0.7	96

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