

## Development of delayed hypersensitivity to dinitrochlorobenzene in patients with Crohn's disease

J. VERRIER JONES, J. HOUSLEY, P. M. ASHURST, AND C. F. HAWKINS

*From the United Bristol Hospitals, the Department of Experimental Pathology, Birmingham, and the United Birmingham Hospitals*

Blackburn, Hadfield, and Hunt (1939) were stimulated to study the immunological reactivity of patients with Crohn's disease because of histological features which it shares with sarcoidosis. Both diseases are characterized by the presence of granulomata, with multinucleate giant cells and epithelioid cells surrounded by aggregates of mononuclear cells. Caseation is usually absent in both conditions. In Crohn's disease the granulomata are less numerous, less well formed, and less cellular than in sarcoidosis: they are usually confined to the gut and its draining lymph nodes, though they have occasionally been reported in the liver.

Boeck (1916) noted that many patients with sarcoidosis had a depression of tuberculin sensitivity. Friou (1952) and Citron (1957) showed that this was part of a generalized depression of delayed hypersensitivity, and that the response to oidiomycin, tricophytin, and mumps antigen was also impaired.

The tuberculin response in patients with Crohn's disease was first studied by Blackburn *et al* (1939). Out of 17 patients, 12 (70%) failed to show delayed hypersensitivity to 0.01 mg of tuberculin. Phear (1958) found that 24 patients (69%) out of 35 failed to respond to 10 TU of old tuberculin, and Williams (1965) reported that 32 (64%) out of 50 cases failed to respond to 10 TU. None of these workers studied control groups; however, in 1939 it would have been most unusual to find a population of healthy young adults of whom 70% were tuberculin negative. Fletcher and Hinton (1967) compared the tuberculin sensitivity of 71 patients with Crohn's disease with 71 controls matched for age, sex, and race, and taken from a population of hospital inpatients. They were unable to demonstrate any impairment of the response to tuberculin in those with Crohn's disease.

Binder, Spiro, and Thayer (1966) studied the response of 16 patients with Crohn's disease and 19

controls to oidiomycin, tricophytin, and mumps antigen, and found no significant difference between the two groups. They concluded that 'patients with regional ileitis react normally to antigens which demonstrate delayed hypersensitivity'. There are, however, disadvantages in using natural antigens to test for delayed hypersensitivity. A variable number of normal subjects will fail to respond because they have never previously encountered the antigen, and it may be necessary to compare large groups of patients and controls in order to show a significant difference. With the fall in tuberculin positivity in the adult population, Mantoux testing now presents similar difficulties.

It is possible, however, to study directly the ability of an individual to develop delayed hypersensitivity to a new antigen, dinitrochlorobenzene (DNCB). This is a hapten which, after combining with proteins in the cells of the dermis, acts as a potent antigen and will produce delayed hypersensitivity in 90% of normal subjects (Kligman and Epstein, 1959). Aisenberg (1962) used DNCB to study the ability of patients with Hodgkin's disease to develop delayed hypersensitivity, and discussed in detail the criteria for distinguishing a positive (specific) hypersensitivity reaction from non-specific chemical irritation. Dinitrochlorobenzene has also been used extensively in the United States in normal subjects (Kligman and Epstein, 1959; Aisenberg, 1962; Gross, 1965) and in children with immunological deficiencies (Young, Austen, and Moser, 1964) as well as in patients with cancer (Gross, 1965) and with leprosy (Waldorf, Sheagren, Trautman, and Block, 1966). There have been no reports of any dangers or complications. Testing with DNCB in patients with sarcoidosis, Jones (1967) showed impairment of the response in patients in whom the disease was active. The object of the present work was to assess the ability of patients with

Crohn's disease to develop delayed hypersensitivity to DNCB, and to relate this to their response to tuberculin and to their clinical condition.

#### PATIENTS

Twenty-six patients with Crohn's disease were studied. The diagnosis was based on histological examination of surgical specimens or on typical clinical and radiological findings. Details of the patients are given in Table I. Only one (C.T.) was receiving corticosteroid treatment when the skin tests were performed.

#### METHODS

For the Mantoux test 100 units of tuberculin were injected intradermally. The injection site was inspected at 72 hours, and the test was regarded as positive if there was an area of induration of 4 mm or more in diameter.

**DNCB SENSITIZATION** The method used was described by Jones (1967) and was essentially the same as that used by Aisenberg (1962). A circular area of skin 2 cm in diameter on the volar surface of the forearm was surrounded by a barrier cream, and 0.1 ml of a 10% solution of 2,4-dinitro-1-chlorobenzene (DNCB) in acetone was dropped slowly on to the skin. The solution was evaporated in a stream of air, and the area was covered for 24 hours. The presence of DNCB sensitization was tested three weeks later by applying 0.1 ml of a 0.1% solution of DNCB in acetone to the opposite forearm in the same way. The test was read at 72 hours, and if necessary, after one week. In normal individuals the lesion became raised, with vesiculation and underlying induration. This was regarded as a positive reaction (Fig. 1).

In some patients there was erythema without vesiculation or induration, and this was regarded as a negative response.

TABLE Ia

PATIENTS WITH CROHN'S DISEASE WITH NEGATIVE REACTIONS TO DNCB AND MANTOUX TESTING								
Patient	Age (yr)	Sex	Mantoux (100 TU)	DNCB	Activity <sup>1</sup>	ESR	Clinical History	Corticosteroid Treatment
P.I.	28	M	-	-	+	4	1963 proximal and terminal ileum involved. Dec. 1967 Mass in R. iliac fossa. Involvement terminal ileum with obstruction: resected in part	None
A.M.	32	M	-	-	+	30	Jan. 1967 Terminal ileum and colon involved, diarrhoea	Off steroids 2 months
G.P.	60	F	-	-	+	6	1950 Colostomy. 1963 Involvement terminal ileum and transverse colon demonstrated. Diarrhoea × 6/day	None
R.P.	36	M	-	-	+	33	1966 Terminal ileum and right hemi-colon removed. Possible recurrence in duodenum. Diarrhoea × 4/day	Steroids for 1 yr Stopped before testing
J.R.	27	F	-	-	-	6	1962 Extensive disease ileum and right hemicolon; no surgery	Off steroids 12 months
J.H.	36	F	-	-	-	10	1962 Terminal ileum and right hemicolon removed	None
C.P.	23	F	-	-	+	30	1963 Normal follow through; no symptoms	None
S.C.	23	F	-	-	-	15	1966 Terminal ileum and right hemicolon removed; diarrhoea continues	None
R.H.	41	M	-	-	+	43	1963 Excision anal fissure. 1966 Terminal ileum and right hemicolon removed; occasional diarrhoea	Off steroids 9 months
V.W.	53	F	-	-	+	48	1962 Terminal ileum and caecum removed; occasional diarrhoea since operation	Steroids in 1962
							1954 Ileo-transverse anastomosis. 1958 Terminal ileum and right hemicolon removed; diarrhoea (× 6/day) and anaemia continue	None

TABLE Ib

PATIENTS WITH CROHN'S DISEASE WITH NEGATIVE REACTIONS TO DNCB AND POSITIVE MANTOUX REACTIONS								
Patient	Age (yr)	Sex	Mantoux (100 TU)	DNCB	Activity <sup>1</sup>	ESR	Clinical History	Corticosteroid Treatment
M.I.	43	F	+	-	-	5	1957 Colectomy	Off steroids for 3 yr
							1961 Normal follow through; intermittent abdominal pain	
M.G.	42	F	+	-	-	25	Sarcoid lesion leg (childhood)	Steroids until 5 weeks before testing
							1955 Terminal ileum and right hemicolon removed	
							1959 Further ileal resection; few bowel symptoms but developed uveitis	
B.W.	35	M	+	-	+	7	1963 Crohn's, terminal jejunum and ileum; bypass operation	ACTH in 1963
							1966 Ileo-ileal anastomosis, with resection of some of affected ileum; diarrhoea × 3/day	
C.W.	22	M	+	-	+	30	1960 Laparotomy for 'appendicitis'	None
							1965 Laparotomy for obstruction; mass in R. iliac fossa, caecum and terminal ileum; bypass operation	
B.P.	23	M	+	-	+	36	1967 Resection of terminal ileum and right hemicolon and closure of ileorectal fistula; ileo-transverse colostomy	None

<sup>1</sup>This is an arbitrary assessment based on current symptoms and biochemical evidence of activity.

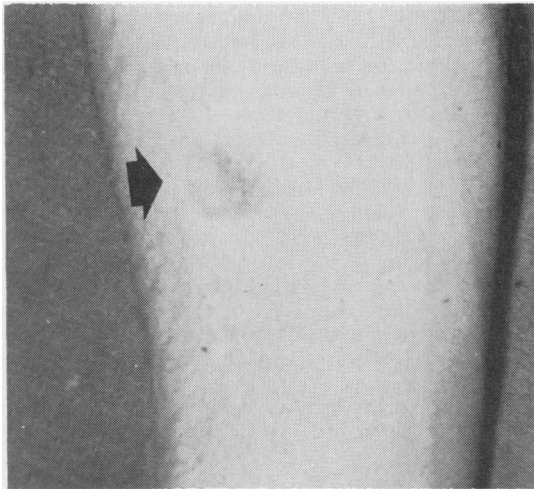


FIG. 1. A positive response to the sensitizing dose of dinitrochlorobenzene. The lesion is raised above the surrounding skin with bullae and is indurated.

## RESULTS

**HISTOLOGY OF DNCB RESPONSE** A biopsy was taken from the positive DNCB test produced in J.V.J. This showed dense infiltration of the superficial dermis with lymphocytes and endothelioid cells, with perivascular cuffing of lymphocytes. There were foci of giant cells in the deeper layers (Fig. 2). This was regarded as fully consistent with a delayed hypersensitivity response.

A negative test was also biopsied. This showed inflammatory changes in the superficial dermis but no evidence of delayed hypersensitivity.

**CONTROLS** Dinitrochlorobenzene sensitization was attempted on 20 control subjects. These were all adults, who volunteered for the test after its nature had been explained to them. The group comprised six healthy adults, four patients with spastic colon, two with duodenal ulcer, five with rheumatoid arthritis, one with nervous dyspepsia, one with ulcerative

TABLE Ic

PATIENTS WITH CROHN'S DISEASE WITH POSITIVE REACTIONS TO DNCB AND NEGATIVE MANTOUX REACTIONS								
Patient	Age (yr)	Sex	Mantoux (100 TU)	DNCB	Activity <sup>1</sup>	ESR	Clinical History	Corticosteroid Treatment
E.Y.	40	F	-	+	-	22	1966 Terminal ileum (2 cm) affected; no operation; no symptoms	Off steroids for 2 yr
M.H.	20	F	-	+	+	16	1964 Short segment terminal ileum narrowed; laparotomy, gland biopsy Crohn's; no resection; diarrhoea	None
M.P.	35	F	-	+	+	60	1962 Terminal ileum and right hemicolon removed 1966 Follow-through, Crohn's disease present in terminal ileum; diarrhoea and perianal abscess	None
L.S.	61	F	-	+	+	60	1942 Bypass operations 1962 Two blind loops with active Crohn's, one excised; diarrhoea and anaemia persist	None

TABLE Id

PATIENTS WITH CROHN'S DISEASE WITH POSITIVE REACTIONS TO DNCB AND THE MANTOUX TEST								
Patient	Age (yr)	Sex	Mantoux (100 TU)	DNCB	Activity <sup>1</sup>	ESR	Clinical History	Corticosteroid Treatment
D.B.	37	M	+	+	-	11	1954 Terminal ileum and right hemicolon removed; no subsequent symptoms	None
E.P.	39	F	+	+	-	24	1962 Short segment terminal ileum narrowed; no surgery; no symptoms.	Pred. 1962-63. Off 4 yr when tested
M.Y.	34	F	+	+	+	35	1964 Ileum (27 cm) removed; anaemia and diarrhoea now recurring	Off steroids 12 months when tested
P.R.	48	F	+	+	-	5	1937 Ileal resection 1958 Ileo-transverse anastomosis 1959 Removal of part of jejunum and transverse colon; no symptoms	Off steroids 8 yr
D.H.	35	M	+	+	-	8	1962 Short segment terminal ileum narrowed; no surgery; no recent symptoms	None
T.H.	36	M	+	+	-	9	1962 Terminal ileum (15 cm) resected; occasional obstructive symptoms subsequently	Prednisone 1962 and 1565-1966. Off steroids 9 months when tested
C.T.	21	F	+	+	+	1	1966 Barium enema, Crohn's disease of ascending and descending colon; intermittent diarrhoea	On Prednisone 10 mg/day when tested

<sup>1</sup>This is an arbitrary assessment based on current symptoms and biochemical evidence of activity.

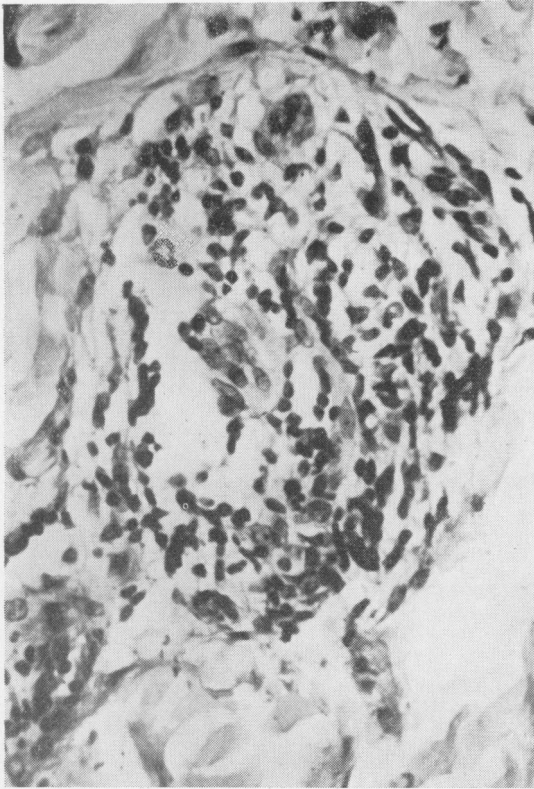


FIG. 2. Histology of a positive DNCB response. This shows a giant cell with surrounding epithelioid cells and dense lymphocytic infiltration.

colitis, and one recovering from a myocardial infarction. All 20 subjects developed hypersensitivity to dinitrochlorobenzene.

**PATIENTS** The results in the patients with Crohn's disease are summarized in Table II. Out of 26, 11 responded normally to DNCB, but 15 failed to develop delayed hypersensitivity. Of the DNCB-negative group, 10 out of 15 also failed to respond to 100 TU while, of the DNCB-positive group, only four out of 11 were Mantoux-negative.

We attempted to correlate the results of DNCB testing with clinical and biochemical features of the disease. There was no correlation with the activity of the disease, judged clinically and from the ESR

and plasma viscosity. In some patients, all of the diseased gut had been removed surgically: in others, affected segments remained at the time of testing. The DNCB results also failed to correlate with these two groupings. There was no correlation with previous steroid treatment. It had originally been intended to exclude from the study patients who were receiving corticosteroid treatment, since this is known to depress delayed hypersensitivity. However, one patient (C.T.), who was receiving 10 mg daily of prednisone, had positive reactions to both tuberculin and DNCB, and was therefore included.

We did, however, note that the four patients (E.Y., M.H., E.P., and D.H.) in whom Crohn's disease was localized to a single segment of ileum less than 5 cm long, were all DNCB positive. With such small numbers, this observation may be fortuitous.

#### DISCUSSION

These results show that a high proportion of patients with Crohn's disease fail to develop delayed hypersensitivity to dinitrochlorobenzene. This appears to correlate to some extent with their failure to react to tuberculin, since a negative Mantoux reaction was found in 10 out of 15 (67%) of those patients who were DNCB negative, but in only four out of 11 (36%) of those who were DNCB positive. In view of the comments of Fletcher and Hinton (1967), who found no difference in tuberculin sensitivity between patients with Crohn's disease and controls, we checked through our Mantoux-negative patients to see if we could find any who were previously known to be Mantoux positive. We did, in fact, find two (C.P. and S.C.) who had both had successful BCG vaccination nine years before we retested them. Neither had been tuberculin-tested after BCG. Enell (1955) found that when a large group of schoolchildren were retested with 100 TU at yearly intervals after being given BCG, between 0.5 and 1.5% became negative each year. At the end of 10 years, one would expect between 5 and 15% of a group of patients to have reverted to a negative Mantoux reaction. We therefore feel that it is at least possible that two of our patients reverted from being Mantoux positive to being Mantoux negative after developing Crohn's disease. We conclude that there is evidence that some patients with Crohn's disease have a generalized depression of delayed hypersensitivity.

This type of immunological abnormality is common to several other diseases. Aisenberg (1962) showed that most patients with active Hodgkin's disease failed to respond to DNCB, and that this correlated well with their tuberculin anergy.

Jones (1967) showed that a group of patients

TABLE II

#### SUMMARY OF RESULTS

	Mantoux Negative	Mantoux Positive	Total
DNCB negative	10	5	15
DNCB positive	4	7	11
Total	14	12	26

with active sarcoidosis also failed to respond to DNCB, and Waldorf *et al* (1966) demonstrated an impaired response to DNCB in a proportion of patients with lepromatous leprosy. Among the patients with sarcoidosis, and those with leprosy, a few individuals were found who retained a positive tuberculin test, although negative to DNCB; this happened in four of our patients with Crohn's disease. This appears at first sight anomalous, since DNCB is a powerful antigen and the ability to develop sensitivity to DNCB is regarded as definitive evidence of the 'state of the delayed hypersensitivity mechanism in the individual' (Aisenberg, 1962). However, there is extensive evidence from animal work (see, for example, de Weck and Frey, 1966) that it is easier to prevent the development of delayed hypersensitivity to a new antigen than it is to abolish an already existing response. Moreover, tuberculin and DNCB are testing different aspects of the immune response since DNCB is testing the induction and expression of hypersensitivity to a new antigen, while the Mantoux reaction tests the ability to express an already established immunity (Jones, 1967).

The significance of the impairment of delayed hypersensitivity in Crohn's disease is at present obscure. We believe that it is now a fact which must be taken into account in any reasonable hypothesis of the aetiology of the disease.

#### SUMMARY

The ability to develop delayed hypersensitivity to tuberculin and to dinitrochlorobenzene (DNCB), a potent chemical antigen, has been studied in 26 patients with Crohn's disease. Fifteen out of the 26 patients failed to develop delayed hypersensitivity to dinitrochlorobenzene. Twenty out of 20 control subjects developed delayed hypersensitivity to DNCB in the normal way.

Of the 15 DNCB-negative patients, 10 (67%) also failed to develop delayed hypersensitivity to 100 TU of old tuberculin. Of the 11 DNCB-positive patients, only four (36%) were tuberculin-negative.

There was no correlation between the response to DNCB and the extent or activity of the Crohn's disease.

These results indicate that patients with Crohn's disease show a depression of delayed hypersensitivity reactions.

We are most grateful to Dr J. M. Naish, of the Department of Gastroenterology, Frenchay Hospital, Bristol, to Dr A. E. Read, of the Department of Medicine, Bristol Royal Infirmary, and to the physicians of the United Birmingham Hospitals for permission to study their patients. We wish to thank Professor P. G. H. Gell for his helpful criticism of the manuscript. J. H. is in receipt of a clinical research fellowship from the Medical Research Council. P.M.A. is in receipt of a research grant from the South Western Regional Hospital Board. J.V.J. wishes to acknowledge financial assistance from the Medical Research Committee of the United Bristol Hospitals.

#### REFERENCES

- Aisenberg, A. C. (1962). Studies on delayed hypersensitivity in Hodgkin's disease. *J. clin. Invest.*, **41**, 1964-1970.
- Binder, H. J., Spiro, H. M., and Thayer, W. R., Jr (1966). Delayed hypersensitivity in regional enteritis and ulcerative colitis. *Amer. J. dig. Dis.*, **11**, 572-574.
- Blackburn, G., Hadfield, G., and Hunt, A. H. (1939). Regional ileitis. *St. Bart. Hosp. Rep.*, **72**, 181-224.
- Boeck, C. (1916). Nochmals zur Klinik und zur Stellung des 'benignen Miliärlupoids'. *Arch. Derm. Syph. Berl.*, **121**, 707-741.
- Citron, K. M. (1957). Skin tests in sarcoidosis. *Tubercle (Lond.)*, **38**, 33-41.
- Enell, H. (1955). B.C.G.-vaccination, tuberculin allergy and tuberculosis in school-children. *Acta paediat. (Uppsala)*, **44**, suppl. 101.
- Fletcher, J., and Hinton, J. M. (1967). Tuberculin sensitivity in Crohn's disease. *Lancet*, **2**, 753-754.
- Friou, G. J. (1952). A study of the cutaneous reactions to oidiomycin, trichophytin, and mumps skin test antigens in patients with sarcoidosis. *Yale J. biol. Med.*, **24**, 533-539.
- Gross, L. (1965). Immunological defect in aged population and its relationship to cancer. *Cancer (Philad.)*, **18**, 201-204.
- Jones, J. V. (1967). Development of sensitivity to dinitrochlorobenzene in patients with sarcoidosis. *Clin. exp. Immunol.*, **2**, 477-487.
- Kligman, A. M., and Epstein, W. L. (1959). Some factors affecting contact sensitization in man. In *Mechanisms of Hypersensitivity*, edited by J. H. Shaffer, G. A. LoGrippo, and M. W. Chase, p. 713. Little, Brown, Boston.
- Phear, D. N. (1958). The relation between regional ileitis and sarcoidosis. *Lancet*, **2**, 1250-1251.
- Waldorf, D. S., Sheagren, J. N., Trautman, J. R., and Block, J. B. (1966). Impaired delayed hypersensitivity in patients with lepromatous leprosy. *Ibid.*, **2**, 773-776.
- Weck, A. L. de, and Frey, J. R. (1966). *Immunotolerance to Simple Chemicals*, p. 71. Elsevier, New York.
- Williams, W. J. (1965). A study of Crohn's syndrome using tissue extracts and the Kveim and Mantoux tests. *Gut*, **6**, 503-505.
- Young, R. R., Austen, K. F., and Moser, H. W. (1964). Abnormalities of serum gamma 1A globulin and ataxia telangiectasia. *Medicine (Baltimore)*, **43**, 423-433.