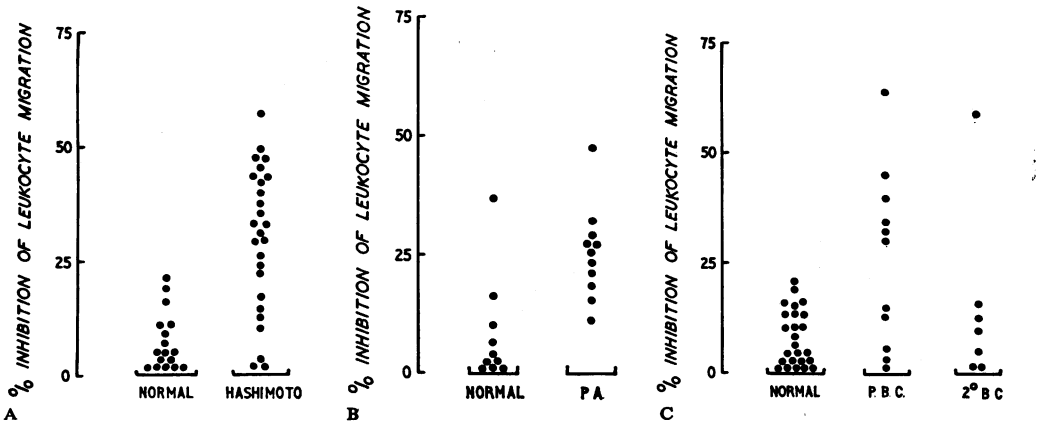

Fig 1 *Migration inhibition:* A, *with thyroglobulin in patients with Hashimoto's disease.* B, *with intrinsic factor in patients with pernicious anæmia.* C, *with mitochondria in patients with liver disease.* PBC=*primary biliary cirrhosis.* 2°BC=*secondary biliary cirrhosis; patients lacking circulating mitochondrial antibodies*

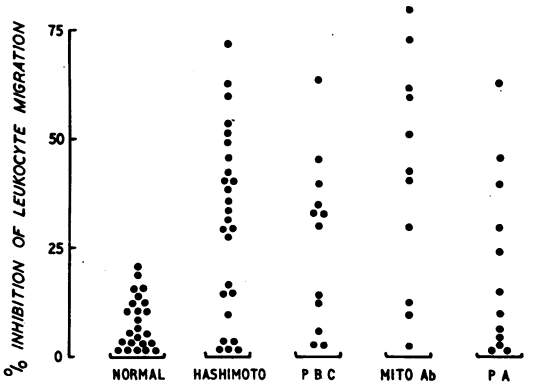

Fig 2 *Migration inhibition with mitochondria in patients with auto-allergic disease.* PBC=*primary biliary cirrhosis.* MITO Ab=*patients with circulating mitochondrial antibodies, but no overt liver disease.* PA=*pernicious anæmia*

antigen (Fig 1A). Patients with thyrotoxicosis show no inhibition of migration.

Similar results are obtained in patients with pernicious anæmia, where 73% of the patients show inhibition using intrinsic factor as antigen (Fig 1B).

The pathognomonic finding in patients with primary biliary cirrhosis is the presence of circulating antibody to mitochondria. Using rat liver mitochondria as antigen, migration inhibition was demonstrated in 55% of these patients (Fig 1C). Patients with secondary biliary cirrhosis, in whom circulating antibodies were not found, were negative in this test.

In the control experiments with the mitochondrial antigen, some patients with Hashimoto's thyroiditis were tested. Surprisingly, two-thirds of these cases showed migration inhibition with mitochondria. When further groups of patients with auto-allergic disease were tested, similar results were obtained (Fig 2). It would seem, therefore, that patients with a variety of auto-allergic diseases show migration inhibition to this particular antigen. It has been postulated that in idiopathic Addison's disease the mitochondrial antigen is organ-specific. In the light of these findings this would seem to be unlikely.

In conclusion, the migration inhibition test appears to be of value in studying the role of cell-mediated immunity in different types of human disorder.

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Delayed Hypersensitivity in Crohn's Disease

Crohn's disease, or regional enteritis, is a focal and frequently multiple inflammatory lesion of the alimentary tract with a granulomatous histological appearance reminiscent of experimental cellular immunity. No evidence so far exists to confirm such a pathological mechanism either by the examination of cellular immunity to tissue antigens (Bendixen 1969) or lymphocyte toxicity for intestinal cells (Shorter *et al.* 1968). The present report is of a preliminary study of delayed hypersensitivity in Crohn's disease and ulcerative colitis, with particular reference to possible non-tissue antigens which might be involved.

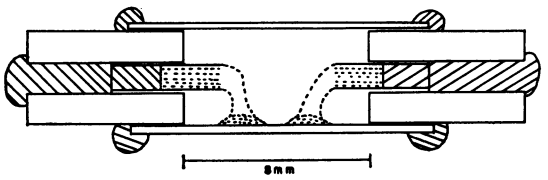

Fig 1 *Cross-sectional drawing of incubation chamber (cross-hatching indicates vaseline-wax seal)*

The major problems in setting up such a study are the preparation of a suitable antigen and modification of existing methods of study (Thor *et al.* 1968) to enable significant results to be obtained from reasonable volumes of patients' peripheral blood. With standard techniques for the measurement of macrophage migration inhibition combining human lymphocytes and guinea-pig macrophages, some 50–100 ml blood is normally required.

A simple new chamber has been designed, with internal dimensions of 8 mm diameter and 3 mm depth, giving a contained volume of 0·15 ml (Fig 1). It is thus possible to harvest enough lymphocytes from 20 ml blood to test against more than one antigen, sometimes at a variety of doses, migration tests being carried out in quadruplicate.

To 20 ml defibrinated blood was added 10 ml of 2½% gelatine, the red cells being left to sediment for an hour. The supernatant was aspirated and centrifuged at 800 rev/min for five minutes. The harvested cells were washed and resuspended in medium 199 (Burroughs Wellcome Ltd) before being divided into three or more equal lots. To each was then added 0, 0·1 or 0·3 ml antigen solution together with 0·3 ml fœtal calf serum, and the suspension then made up to 3 ml, containing between 6 and 10×10^6 white cells of which the majority were lymphocytes. They were placed in small bijou bottles in an incubator at 37°C for three days. The supernatant fluid was aspirated and kept for subsequent incubation with macrophages. The macrophages were prepared by preliminary injection of young guinea-pigs with 20 ml liquid paraffin intraperitoneally three days previously. The cells were washed three times in Eagle's solution, and finally suspended in the test solution and centrifuged in wax-plugged microhæmatocrit tubes of 1 mm internal diameter. The capillary tubes were cut and broken below the upper level of cells, and the small cell-containing pieces inserted into the side arms of the chamber. The chamber was filled with the test solution, and sealed. After incubation for 24 to 48 hours, the cellular migration on to and across the floor of the chamber was measured by projection on paper, cutting out and weighing the pieces. The effect of antigen on migration was expressed as percentage inhibition of the area of migration of cells in the non-antigen-containing solution. On all occasions parallel studies were done using lymphocytes from a normal individual and if the antigen proved to have a nonspecific inhibitory effect, the degree of inhibition of normal cells was subtracted from that obtained with the patient's cells to give the final value.

Antigen was prepared from fresh human fæces by homogenization and ultrasonication, followed by centrifugation and filtration, the final filtration being through sterile Seitz filters. All procedures were carried out as far as practicable at 4°C. Antigen solutions were then dialysed against running tap water overnight. Most of the antigen was obtained from normal subjects, and the remainder from Case 1 (Crohn's disease); from the latter both fæcal material (Cr fæces) and the luminal contents of a resected specimen of involved ileum (Cr lumen) were used as antigen (Table 1). All antigenic solutions were concentrated by ultrafiltration to a protein concentration of 0·8–1·2 g/100 ml.

Results

As a test of antigenicity, an antigenic solution (Cr fæces) was injected with Freund's adjuvant into the foot pads of young guinea-pigs, four pigs each receiving 50 or 5 μg antigen, and four receiving either nothing or Freund's adjuvant alone. Skin testing ten days later showed small reactions in the 5 μg animals, and much larger reactions in those injected with 50 μg. Later macrophage migration inhibition was demonstrated with the peritoneal macrophages of all immunized animals.

In most patients studied, purified tuberculin was used in parallel with the antigen under study, and one such study is illustrated (Crohn's disease,

Table 1

Macrophage migration inhibition in patients with inflammatory bowel disease

		Percentage inhibition	
Case No.	*Antigen*	*0·1 ml antigen*	*0·3 ml antigen*
1 (Crohn's disease)	Cr lumen	0	22
2 (Crohn's disease)	Cr fæces	0	0
3 (Crohn's disease)	Cr fæces	47	37
4 (Crohn's disease)	Normal (1)	0	36
4 (Crohn's disease)	Normal (2)	21	16
5 (Crohn's disease)	Normal (2)	0	22
6 (Crohn's disease)	Normal (3)	29	0
7 (ulcerative colitis)	Normal (1)	0	0
8 (ulcerative colitis)	Normal (3)	0	0

Cr antigen obtained from Case 1, normal antigen from fæces of normal subjects

(1), (2), (3): Three different preparations of antigen, from 3 separate 'donors'

Case 3, Fig 2). Both patient and normal control were tuberculin-positive by intradermal testing, but whereas only tuberculin induced migration inhibition through the normal lymphocytes, fæcal antigen affected the patient's but not normal cells. Further study of a small number of patients with Crohn's disease and of 2 with ulcerative colitis produced the results listed in Table 1. Inhibition was demonstrated at one or both antigenic concentrations measured in 5 of the 6 patients with Crohn's disease, but in neither of the 2 with ulcerative colitis. The degree of inhibition induced when allowance had been made for the effect on normal cells was not great, but was still well beyond the limits of variation expected from examination of comparable control groups.

A segmental resection of diseased ileum was carried out in Case 2 (Crohn's disease) and the opportunity was taken to obtain lymphocytes from draining lymph nodes together with those from peripheral blood. Unfortunately, a migration inhibition experiment on these cells failed for technical reasons, but 1 μCi ^{3}H-thymidine was added to the lymphocytes for the last 24 hours of culture. After removal of the supernatant for the migration experiment, the remaining cells were washed three times with Eagle's solution, and then suspended in liquid scintillation fluid for counting. Presence of the antigen induced considerable inhibition of thymidine uptake in all cell groups, but 50% greater uptake occurred in the patient's lymphocytes than in the normal, and when luminal antigen was used the uptake was twice that of the control. Comparable numbers of cells were present in each culture.

Discussion

It is not surprising that such a fæcal antigen should have inhibitory properties of its own on normal cell function, and the present studies represent an attempt to demonstrate the presence of cellular immunity in these patients to a naturally occurring non-tissue antigen. Once this has been convincingly demonstrated, further purification and subdivision of the antigen solutions can be carried out to examine a number of subfractions in a purer state. It is possible that specific inhibition might then be demonstrated by a non-toxic material, which could be more precisely identified.

The fæcal solution by this technique was strongly antigenic as demonstrated by guinea-pig foot-pad injection, and must contain a wide range of antigenic substances. Penetration of minute quantities of this material beneath the mucosa might well induce cellular immunity and lesions similar to those seen in Crohn's disease.

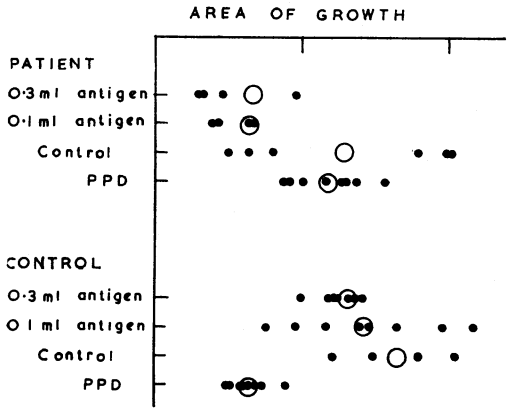

Fig 2 *Macrophage migration in the presence of supernatant solution from lymphocyte cultures of Case 3 (Crohn's disease)*

The degree of macrophage migration inhibition induced in this preliminary study is suggestive of a specific phenomenon, but is by no means conclusive. The degree of utilization or adsorption of antigen on to cells in culture has yet to be determined, and similar studies must be carried out adding antigen to the migration chambers to equalize the concentrations. It is also necessary to extend the comparison with tuberculin sensitivity, but to date, there has been no evidence of spuriously positive macrophage tests in the presence of negative skin tests. The experiment on lymphocyte transformation is also inconclusive, but points in the same direction, and it was interesting that the cells taking up the most thymidine from antigen-containing solutions were being exposed to the luminal antigen obtained from the segment of Crohn's ileitis. Further studies using such antigens are clearly indicated, particularly if more than one measure of cellular immunity is examined.

Acknowledgment: This work was greatly dependent on expert technical assistance from Mrs Jean Leonard.

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