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A Hypothesis for the Pathogenesis of Crohn's Disease Based on an Ultrastructural Study

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Summary. The epithelioid cells forming the granulomata of Crohn's disease show striking vacuolation from the coalescing of pinocytotic vesicles with double membrane-bound bodies and their subsequent fusion with lysosomal dense bodies. The fine detail of the granulomata in each individual is uniform but varies from case to case in such a way as to suggest an episodic stimulation of pinocytosis and vacuole formation.

The production of such granulomata with these cyclical appearances is consistent with an intermittent stimulus by exogenous antigen or antigen antibody complexes arriving in the diet.

Key words: Crohn's Disease — Electron Microscopy — Granuloma.

Introduction

The most easily recognisable histological feature of Crohn's disease, the sarcoid granuloma, is found only in 59–77% of cases (Williams, 1964; Morson, 1971; Cook, 1972) and even then may be sparsely and patchily distributed. It is probably for this reason that previous reports of the ultrastructure of Crohn's disease have made little mention of the details of the granuloma itself (Gonzales-Licea and Yardley, 1966; Aluwihare, 1971; Ranlov *et al.*, 1972; O'Connor, 1972). Albot *et al.* (1970) described in detail a granuloma in a single case of Crohn's disease. Aluwihare (1971) also commented on the finding of some bacteria in the lamina propria which he suggested were an aetiological agent in Crohn's disease.

We considered that the granuloma, being the distinctive feature of Crohn's disease, should be studied in greater detail and in more cases with the electron microscope to assess, as far as possible with this method, the relative importance of the various known stimuli to granuloma formation. We also wished to confirm or refute the published observations on the ultrastructure of other tissues in Crohn's disease.

Materials and Methods

Studying the ultrastructure of Crohn's disease presented a special sampling problem in that the pattern of this disease is patchy and the granulomata, one of its most distinctive features, are usually scattered and sparse. Since only small pieces of tissue can be processed for electron microscopy the probability of including one of these granulomata within a block by chance is very low. To overcome this problem we adopted a method of fixing large ($3 \times 2 \times 0.5$ cm) pieces of tissue in glutaraldehyde, embedding them in wax and cutting 5 μm sections for examination with the light microscope. By this means an area of interest can be identified on the wax block by matching it with the corresponding section. This area can be

Table 1. Details of patients with Crohn's disease

No.	Age yr.	Length of history yr.	Disease site	Treatment	Comments
1	46	4	Rectum	None	Very many granulomata
2	55	1	Colon and Rectum	Antibiotics Steroids	Very many granulomata
3	60	6 mth	Colon	Antibiotics	Many granulomata
4	34	2	Jejunum	Antibiotics Steroids	Many granulomata
5	42	10	Ileum	Antibiotics	Few granulomata
6	63	16 mth	Ileum	Antibiotics Steroids	Ill-defined granulomata with isolated giant cells
7	22	1	Ileum	Antibiotics Sulphonamides	Granulomata in the nodes only
8	16	17 mth	Rectum	Steroids	Granulomata in the nodes only
9	26	4 mth	Ileum	Antibiotics	No granuloma
10	40	13	Ileum	Antibiotics Sulphonamides	No granuloma

cut out, de-waxed and re-embedded in araldite (Watson *et al.*, 1972). Although the resolution was not as good, we felt it acceptable for a pilot study when the chances of examining significant lesions was much increased. Tissues obtained from 17 colectomy or ileal resection specimens with Crohn's disease were examined in this way, giving us a basic understanding of the fine detail of this disease. However, it was desirable that these preliminary observations should be based only on the higher standards obtainable with conventional electron microscopy techniques.

The lesions tissues in this definitive series were taken from surgical specimens received within minutes of excision and immediate dissection enable tissue samples to be taken from diseased areas, adjacent macroscopically normal areas, and the mesenteric lymph nodes in each case. A total of 23 cases was investigated in this way and although the fine structure was studied in every case, those showing ultrastructural evidence of ischaemic damage to the tissues were not included in the final series. In the 10 cases of Crohn's disease on which the detailed conclusions are based, 48 distinct sites were examined electron microscopically. Clinical information concerning the patients is given in Table 1.

For comparison, material from 4 operative specimens of ulcerative colitis, and normal colonic and rectal mucosa obtained by biopsy from 7 patients with completely normal sigmoidoscopic appearances who presented with simple anal lesions or diverticulitis, were also studied.

All the specimens were fixed by glutaraldehyde (Epstein and Holt, 1963), post-fixed in 1 per cent osmium tetroxide in 0.2 M sodium cacodylate buffer, dehydrated in ethanol and embedded in Araldite. Alternate sections were examined by light microscopy after staining with toluidine blue, and with a Philips EM 300 electron microscope after staining with lead citrate (Millonig, 1961). Thiocarbohydrazide was used as by Seligman *et al.* (1966) to stain sections from one case (3) to demonstrate the presence and localisation of any lipid in the cells of the granulomata.

Results

In Crohn's disease intestinal epithelium away from ulcerated areas was normal, with fully differentiated goblet cells and absorptive cells and without inflammatory cell infiltration (Fig. 1). Areas of epithelium adjacent to ulceration resembled that at the base of the crypts in normal mucosa in being less differentiated (Nagle and Kurtz, 1967). The connective tissue of the lamina propria and submucosa contained inflammatory cells but the fine structure of the blood vessels, lymphatics and muscle was normal. Similarly, nerve fibres and ganglion cells, although apparently increased in number as previously reported by Davis *et al.* (1955) were normal in appearance (Fig. 2).

The fine structure of the lymphocytes, plasma cells, macrophages, eosinophil granulocytes and mast cells was normal. In the cases with granulomata some activated lymphocytes were occasionally present (Fig. 3). Mast cells in various stages of degranulation were common in some areas. Neutrophil granulocytes were uncommon and there were markedly fewer moribund cells than were noted in ulcerative colitis. Differential counting of plasma cells and lymphocytes with the light microscope showed that lymphocytes outnumbered plasma cells by a factor of around 3 in granulomatous cases. However, non-granulomatous cases showed these cells present in roughly equal numbers whilst in ulcerative colitis the plasma cells were predominant.

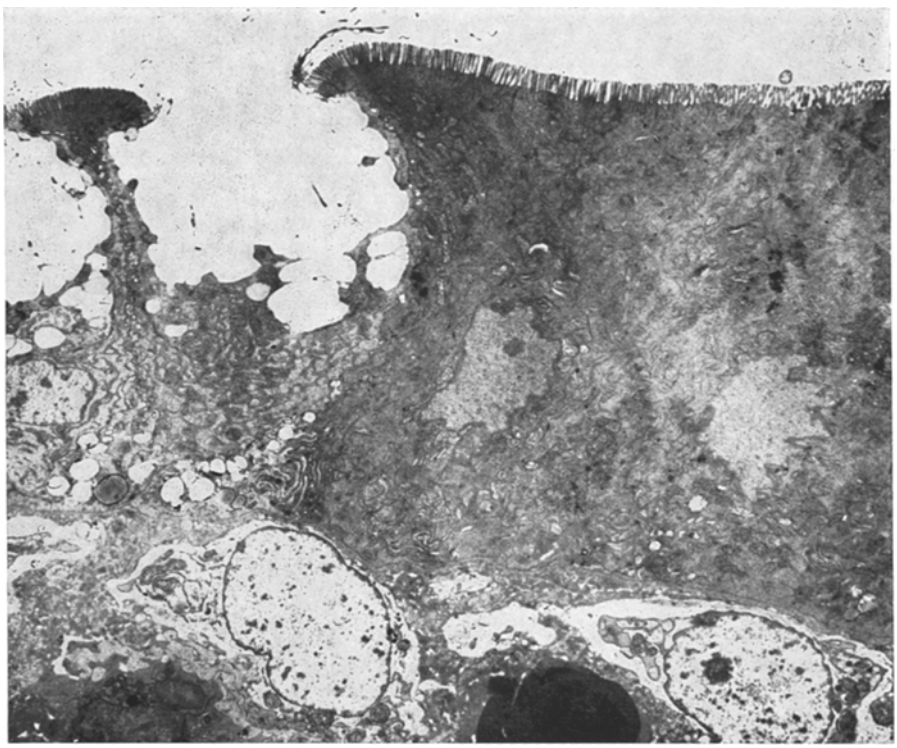

Fig. 1. Electron micrograph (EM). Normal epithelium in a diseased segment of ileum in a case (9) of non-granulomatous Crohn's disease. × 3000

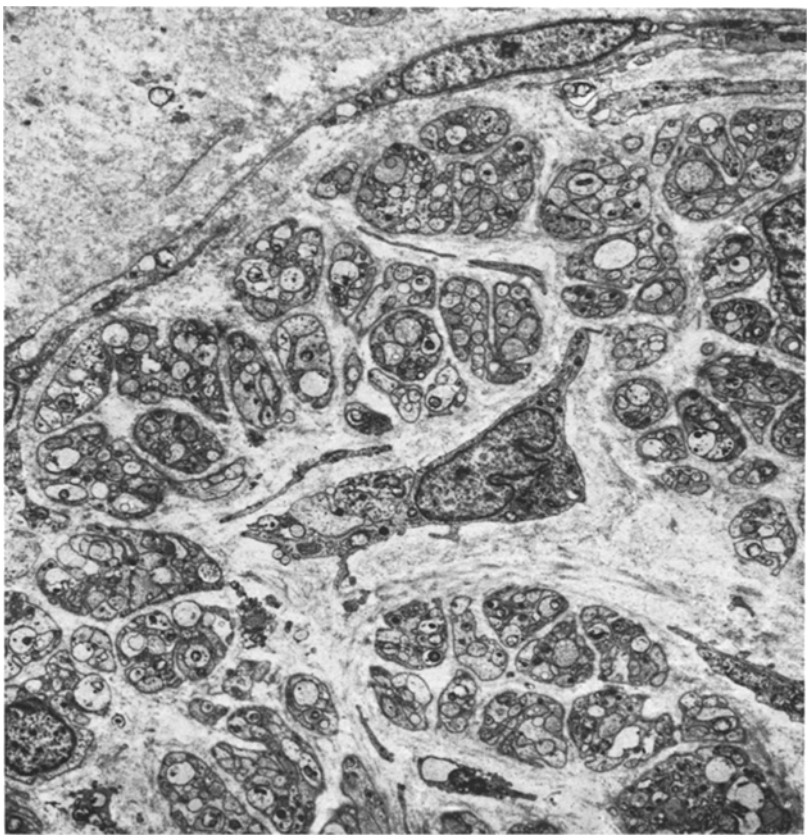

Fig. 2. EM. Prominent but apparently normal nerve bundle in the rectal submucosa of a patient with granulomatous Crohn's disease (2). × 3400

The granulomata comprised epithelioid and giant cells like those in sarcoid lesions and Kveim test granuloma (Hirsch *et al.*, 1967; Wanstrup and Christensen, 1966; Williams *et al.*, 1970). The epithelioid cells were elongated and had large nuclei with a peripheral band of marginated chromatin and prominent nucleoli. The plasma membranes of these cells formed long interdigitating processes but occasionally were smooth and had a varying number of pinocytotic channels leading into the cytoplasm (Fig. 4). The cells of the granulomata in each case, whether in the bowel wall or in the lymph nodes, showed considerable uniformity of features whilst differing from the granulomata of other cases. For example in case 3 all the epithelioid cells contained large vacuoles formed by what appeared to be the coalescence of pinocytotic vesicles with double membrane-bound bodies (Fig. 5). Some of these vacuoles were seen fused with dense bodies often close to the Golgi cisternae. The other cases showed a gradation from marked pinocytosis to a case (1) in which few vesicles were present. In the cases with less prominent pinocytosis the vesicles present either showed a margin of dense material around a pale core, or were smaller and homogeneous (Fig. 6). In one case (2) some of these

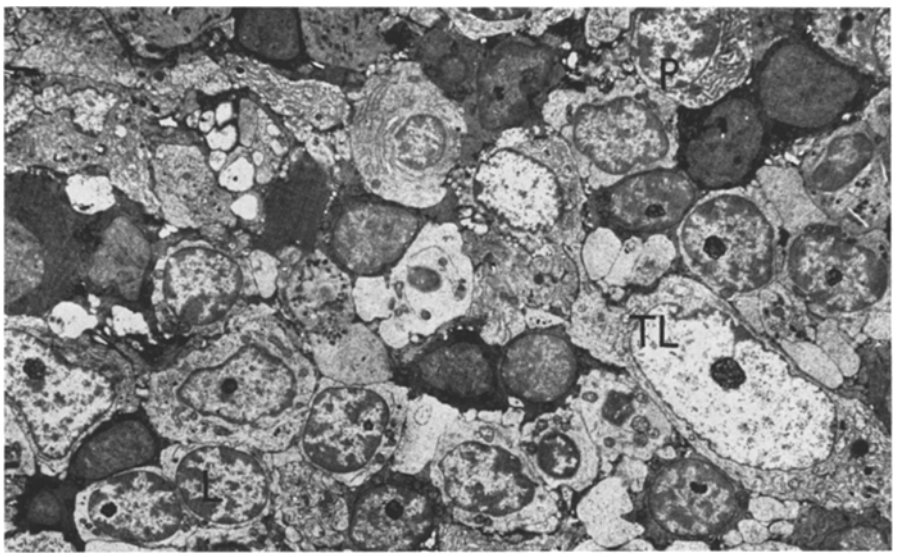

Fig. 3. EM. The inflammatory infiltrate in the submucosa of the colon in a case (3) of granulomatous Crohn's disease. In addition to the numerous small lymphocytes (*L*) there are transformed lymphocytes (*TL*) and plasma cells (*P*). × 2750

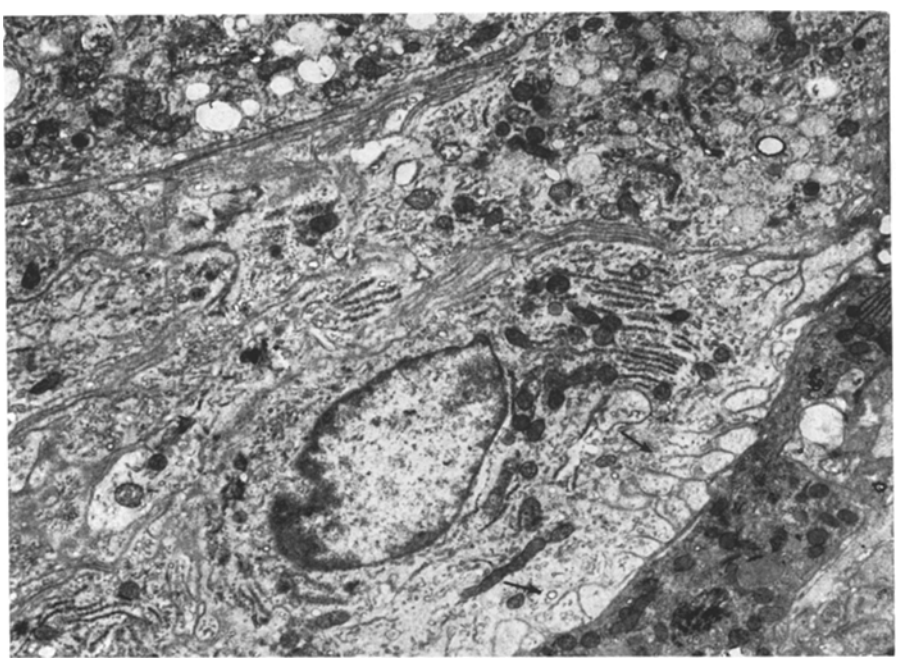

Fig. 4. EM. Epithelioid cell in a mesenteric lymph node in a case (3) with granulomatous Crohn's disease. There is pinocytotic activity along one side of the cell (arrows) and elsewhere the plasma membrane forms long processes interdigitating with adjacent epithelioid cells which are vacuolated. × 6500

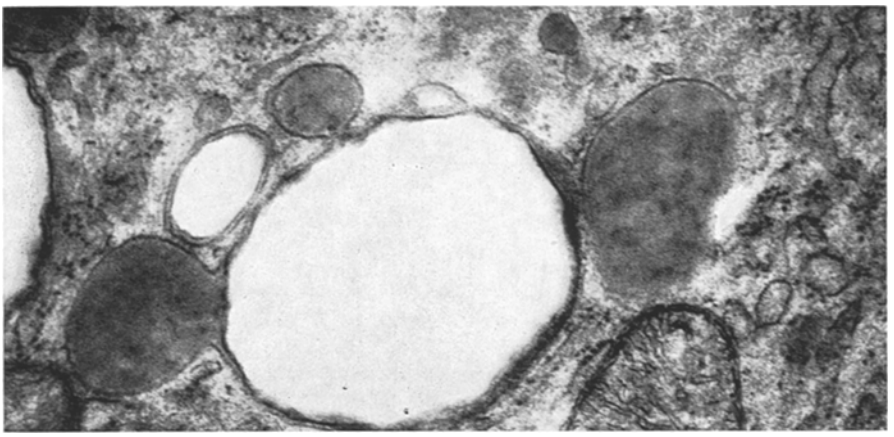

Fig. 5. EM. Part of a giant cell in a mesenteric lymph node in a patient (3) with granulomatous Crohn's disease. The large, double membrane-bound vacuoles have fused with the dense bodies. × 44500

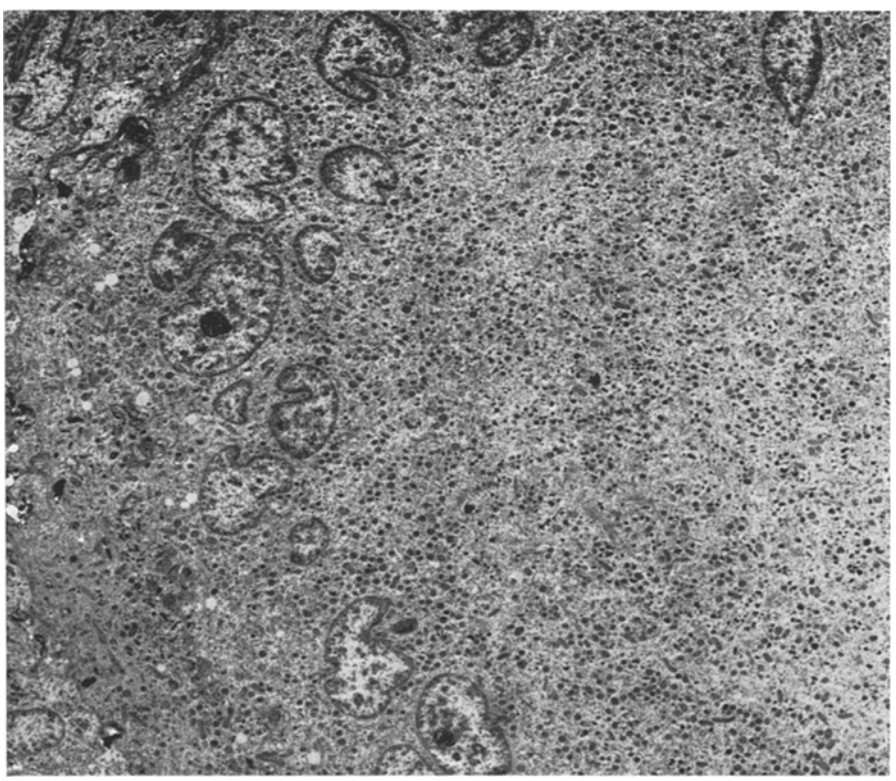

Fig. 6. EM. Part of a giant cell in the rectum of a patient (1) with granulomatous Crohn's disease. The cytoplasm contains many homogeneous dense bodies, few vacuoles and little endoplasmic reticulum. × 2500

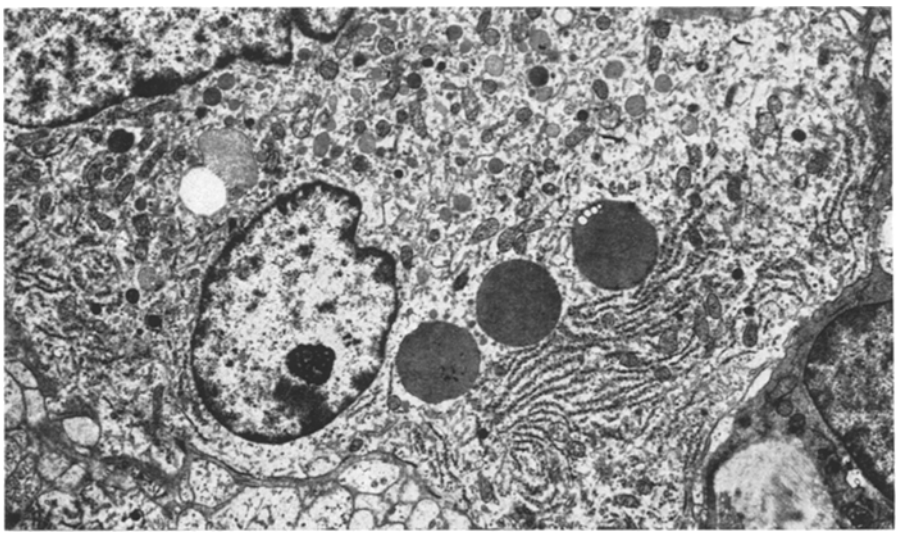

Fig. 7. EM. Part of a giant cell in the colon of a case (2) of granulomatous Crohn's disease. The vacuole in the cytoplasm is fusing with one of the many homogeneous dense bodies and there are a few residual lysosomal bodies. × 5500

homogeneous vesicles were seen fusing with single membrane-bound and presumably newly formed vesicles (Fig. 7).

These observations suggested a synchronised recycling of vesicles and dense bodies. The fact that the stage of this cycle seen in an individual case was the same throughout a specimen and yet varied from case to case was attributed to the variation in timing of the resection of the diseased bowel with reference to this cycle.

The mitochondria in the epithelioid cells were small and evenly distributed while the endoplasmic reticulum varied in amount and usually was rough. The Golgi cisternae were moderately developed. Very few fibrillar structures were present. These cells had the same complement and appearance of organelles as the giant-cells. The distribution of plasma membranes within some of the giant-cells clearly indicated their development by the fusion of epithelioid cells as described by Sutton and Weiss (1966) with the nuclei at first arranged irregularly but in more mature forms distributed peripherally.

Bacteria were observed only in the gut lumen and not in the tissues and there was no other morphological evidence of an infective aetiology such as residual bodies of bacterial origin.

The sections stained with thiocarbohydrazide demonstrated that occasional vacuoles contain lipid but most were devoid of lipid and appeared the same as with conventional staining.

In the lymph nodes, where epithelioid and giant cells were present they reflected entirely the appearances of these cells in the bowel wall and in particular appeared to be in the same stage of vesiculation. The other cells in the lymph nodes appeared completely normal.

There was no correlation between the ultrastructural appearances and the type of pre-operative drug therapy.

Discussion

We found that in many respects the ultrastructure of Crohn's disease confirms the impression of light microscopy that the nonulcerated epithelium which appears normal on light microscopy shows no abnormalities of fine detail either. Nerve bundles are hypertrophied but show no ultrastructural abnormalities and neither do any of the cells of the inflammatory infiltrate. These observations apply to all the cases studied including those without granulomata. The latter, represented by cases 9 and 10 in our series, did show a proportion of plasma cells in between the low numbers of granulomatous Crohn's disease and the high numbers seen in ulcerative colitis. This may indicate a humoral response perhaps comparable to that in ulcerative colitis where there is evidence of autoimmune activity against the epithelium (Harrison, 1965; Bendixen, 1969; Schofield *et al.*, 1969). The predominance of lymphocytes with occasional transformed lymphocytes in granulomatous Crohn's disease would be expected in cell mediated immunity and according to Ranlov *et al.* (1972) the appearances indicate a state of hyper-immunity.

The abundance of mast cells reported by Ranlov *et al.* (1972) and also by Rao (1973) was apparent only in some of the diseased tissues. We did not find the endothelial thickening described in both Crohn's and ulcerative colitis by O'Connor (1972).

The granulomata in Crohn's disease were ultrastructurally similar to those seen in sarcoidosis by Wanstrup and Christensen (1966) and Williams *et al.* (1970). They also resembled the granulomata produced in positive Kveim tests (Hirsch *et al.*, 1967).

The granulomata of an individual case of Crohn's disease show a uniform appearance in terms of vacuolation and pinocytosis even in widely separated parts of the bowel. This together with the gradation in the state of vacuolation in the series suggests a synchronised sequence of pinocytosis, vacuole formation and lysosomal activity such as migh be stimulated by the episodic arrival in the gut of an antigen or antigen-antibody complex. The absence of lipid from most of the vacuoles tends to exclude this as a possible provoking agent for the granulomata.

In the single case of Crohn's disease studied by Albot *et al.* (1970) there was marked fusion of vacuoles with dense bodies and some pinocytosis. Aluwihare (1971) showed that bodies resulting from this fusion contained acid phosphatase and were therefore lysosomes but, in his series of 16 cases, did not report on pinocytosis or vacuolation of the epithelioid cells. It appears that Albot *et al.* were describing one stage of the process and Aluwihare part of a later stage which, in our series, is more common. It is clear from our observations of all stages of this lysosomal cycle that these previous workers noted only a segment of a progressive and repeated process. Despite the lysosomal activity in the epithelioid cells of Crohn's disease they did not contain many residual bodies suggesting that these cells ingest very little solid material. Papadimitriou and Spector (1972) demonstrated prominent persistent ingested material in the macrophages of granulomata experimentally induced by bacteria but the macrophages of epi-

thelioid cells in Crohn's disease contained no such residues. These observations, in conjunction with the complete absence of morphological evidence of bacteria or viruses, do not support the possibility of an infective agent as suggested by Mitchell and Rees (1970), Aluwihare (1971) and more recently by Cave *et al.* (1973).

Granulomata have been produced in sensitised rats at the site of injected antigen and in unsensitised animals at the site of injected antigen-antibody complex (Spector and Heesom, 1969). The amount of material required to produce this reaction was very small. The granuloma in Crohn's disease may be similarly a local response to the combination of antigen with excess antibody (Spector and Heesom, 1969) within the tissues. Some support for this was given recently by Doe *et al.* (1973) who found that there were circulating antigen-antibody complexes in 57 per cent of a series of patients with Crohn's disease, and by Jewell *et al.* (1972) who also suggested that immune complexes were present in the sera of these patients. Exogenous antigen may sporadically penetrate the tissues from the gut lumen and induce the formation of granulomata containing epithelioid cells and subsequently stimulate synchronous pinocytosis and lysosomal activity in these cells.

The pathogenesis of non-granulomatous forms of Crohn's disease is quite unclear but there may be an autoimmune humoral element. Cell mediated hypersensitivity probably stimulated by the episodic passage from the gut lumen of an exogenous antigen seems likely to be the important factor in the pathogenesis of granulomatous Crohn's disease.

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