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Intestinal Fine Structure in Crohn's Disease

Lysosomal Inclusions in Epithelial Cells and Macrophages

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Summary. Resected intestines from eight patients with Crohn's disease and three control cases were investigated by transmission electron microscopy. Characteristic changes were observed in the mucosa of all Crohn's disease specimens, most typically an infiltration of numerous macrophages into the propria. These cells displayed large lysosomes with inclusions which were mainly dense, irregularly shaped and composed of aggregated particles and bizarre-shaped, myelin-like figures. Similar inclusions were also found in the lysosomes of the surface epithelial cells and the macrophages in the submucosa. This latter layer otherwise consisted of an oedematous, collagenous connective tissue. The muscularis appeared structurally unaffected. Qualitatively, the findings were almost identical in all patients with Crohn's disease, but varied quantitatively without any clear correlation with the clinical histories. Moreover, in all cases typical alterations were found not only in the macroscopically most clearly affected parts of the intestines, but also in grossly normal regions, close to the margins of resection.

The analogy of the fine structural findings with those of granulomas produced by injection of bacteria into experimental animals suggests that a microbial invasion of the intestinal wall may have initiated the disease. It therefore seems reasonable to assume that the lysosomal inclusions we observed represent partly degraded bacteria. More occasionally, virus-like particles were found within the lysosomes of the epithelial cells and the macrophages in the underlying propria. In view of the diffuse spread of the alterations it seems possible that there exists a generalized defect in the barrier function of the intestine in Crohn's disease. This could lead to passage of bacteria and/or other agents into the mucosa, followed by an influx of inflammatory cells from the blood. Storage of non-degradable microbial components in macrophages could then be responsible for the initiation and propagation of a chronic inflammatory process, similar to that of other granulomatous disorders.

Key words: Crohn's disease – Transmission electron microscopy – Macrophages – Lysosomal inclusions

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Introduction

Crohn's disease is a chronic inflammatory disorder of the gastrointestinal tract which usually involves the distal ileum and colon. Clinically, it is a diarrhoeal disease associated with fever, abdominal masses, and anal/perianal fissures and fistulas. Morphologically, its most characteristic lesion is the epitheloid or sarcoid-type granuloma. The aetiology of Crohn's disease is still unclear. In recent years the main interest has been directed toward autoimmunity, cellular immune deficiency, and transmissible infection (for reviews see Present et al. 1966; Janowitz and Sachar 1976; Martini and Malchow 1979).

So far, only relatively few electron microscopic studies dealing with Crohn's disease have been published. Detailed fine structural observations could help to further elucidate the aetiology and pathogenesis of the disease. In the present report we present electron microscopic observations of intestinal tissue of eight patients with Crohn's disease and three control patients. Special interest is paid to the occurrence of lysosomal inclusions in epithelial cells and in macrophages in the propria and submucosa.

Materials and Methods

Sampling of Tissue. Small pieces of the intestinal wall were taken in connection with operation (resections) and immediately immersed in fixative. Specimens were usually taken from macroscopically clearly affected parts of the resected intestine and from surrounding normally appearing areas. A short summary of the case histories of the eight patients with Crohn's disease and the three control patients is given in Table 1.

Electron Microscopy. Primary fixation was done in 3% glutaraldehyde in 0.1 M sodium cacodylate-HCl buffer, pH 7.3. After postfixation for 2 h in 1% osmium tetroxide prepared in the same buffer, the specimens were dehydrated in ethanol and embedded in low viscosity epoxy resin (Spurr 1969). Before dehydration some of the specimens were stained en bloc for 1 h at room temperature with 0.5% uranyl acetate in Veronal acetate buffer, pH 5.0 (Farquhar and Palade 1965). Thin sections were cut on an LKB Ultrotome I, double-stained with uranyl acetate followed by lead citrate (Reynolds 1963) and examined in a Philips EM 300 electron microscope.

Observations

Control Tissue

The specimens from the control patients demonstrated a fine structure which conformed to previous descriptions of the normal small and large intestine (Toner 1968; Rhodin 1974). The epithelial cells contained only few lysosomes, which usually had a dense homogeneous content. Likewise, the lysosomes of the macrophages in the propria and submucosa were relatively few in number and mostly lacked distinct inclusions. Only in one of the controls (case no. 9) were some lysosomal inclusions resembling those found in the Crohn specimens noted.

Crohn's Disease Tissue

The epithelial layer was generally intact and consisted of columnar cells connected to each other by junctional complexes (Farquhar and Palade 1963) and

Table 1.

Case no.	Age (yr)	Sex[a]	Clinical diagnosis[b]	Earlier resection of	Current resection of	Light microscopy[c]	Electron microscopy[d]
1	23	F	diarrheas 6 yr, CD	–	small and large intestine	ulcerative entiritis, lgl = CD	+
2	26	M	diarrheas 14 yr, CD	small and large intestine	small intestine	chronic inflammation lgl = CD	+
3	33	M	intestinal symptoms 16 yr, CD	–	small and large intestine	sclerosing, ulcerative enteritis, lgl = CD	+
4	33	F	CD 12 yr	small and large intestine	small and large intestine	ulcerative inflammation = CD	+ +
5	41	F	CD 13 yr	large intestine	small intestine	unspecific inflammation, lgl = CD	+ +
6	41	F	diarrheas 5 yr, CD	–	small and large intestine	sclerosing, stenosing entiritis = CD	+ + +
7	46	M	CD 12 yr	small and large intestine	small and large intestine	sclerosing enteritis + abscesses = CD	+ + +
8	64	M	CD 12 yr	small and large intestine	small intestine	unspecific inflammation	+ + +
9	14	F	adenomatosis coli heriditaria	–	large intestine	adenomatous colonpolyposis	±
10	52	M	adherence ileus	(Billroth II)	small intestine	diverticulum	–
11	69	M	colon-polyposis	–	small and large intestine	adenomatous colonpolyposis	–

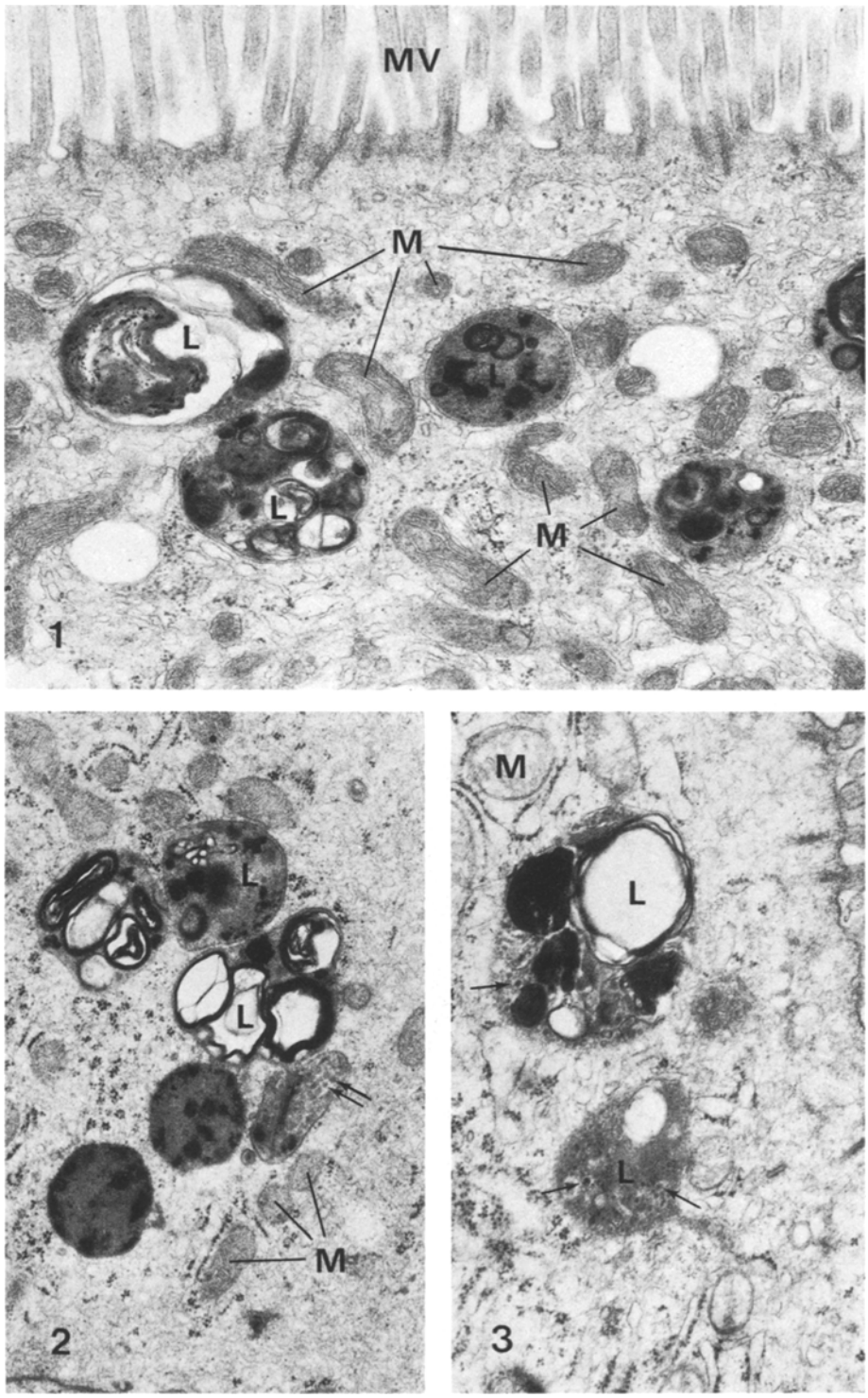
Short summary of case histories and light and electron microscopical changes of the intestine.

[a] F = female; M = male

[b] CD = Crohn's disease

[c] Pathological anatomical diagnosis made by the clinical pathologist; lgl = lymph glands

[d] Estimation of number of epithelial cells and macrophages with lysosomes containing polymorphous inclusions of characteristic appearance. – no or only occasional cells with lysosomes containing inclusions; + small number of cells with lysosomes containing moderate amounts of inclusions; + + moderate to large number of cells with lysosomes containing plenty of inclusions; + + + large or very large number of cells with lysosomes extensively filled with inclusions



Figs. 1–3. Cases no. 8, 7, and 6, respectively. Apical portions of surface epithelial cells demonstrating large lysosomes (*L*) with inclusions of characteristic appearance. Arrows (Figs. 2 and 3) point out small dense particles surrounded by an electron-lucent ring. *M*, mitochondria; *MV*, microvilli. Fig. 1, ×30,000; Fig. 2, ×25,000; Fig. 3, ×27,000

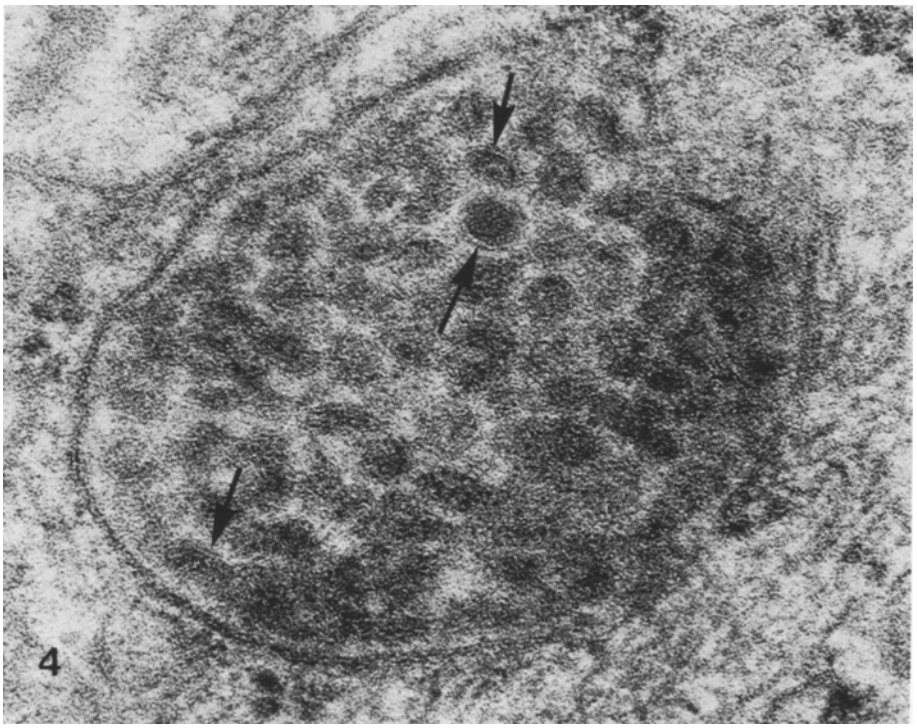
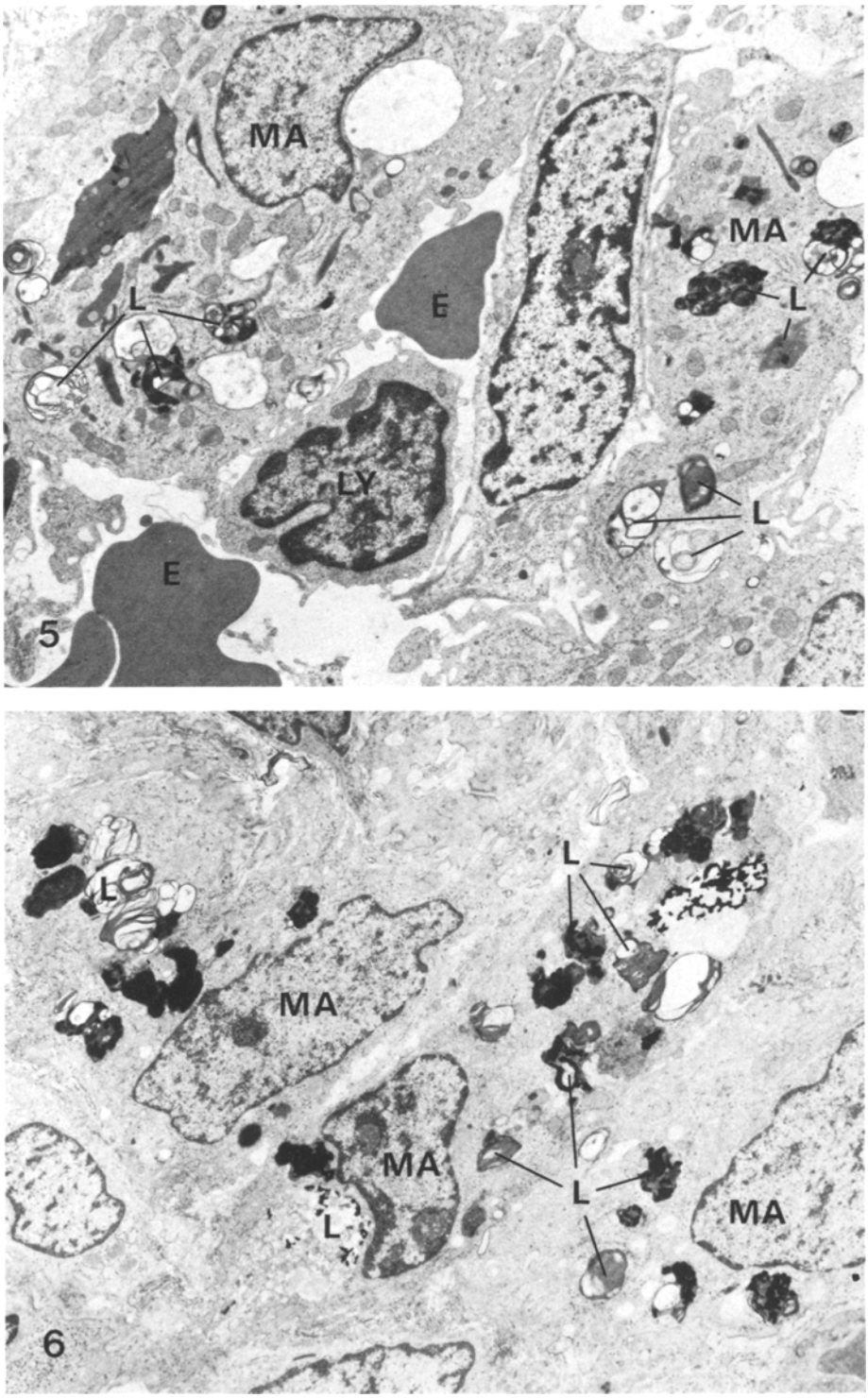


Fig. 4. Case no. 6. Detail of surface epithelial cell showing a lysosome limited by a triple-layered membrane and filled with numerous 40–60 nm diameter, virus-like particles. Around some of these a capsid-like structure is observed (*arrows*). ×150,000

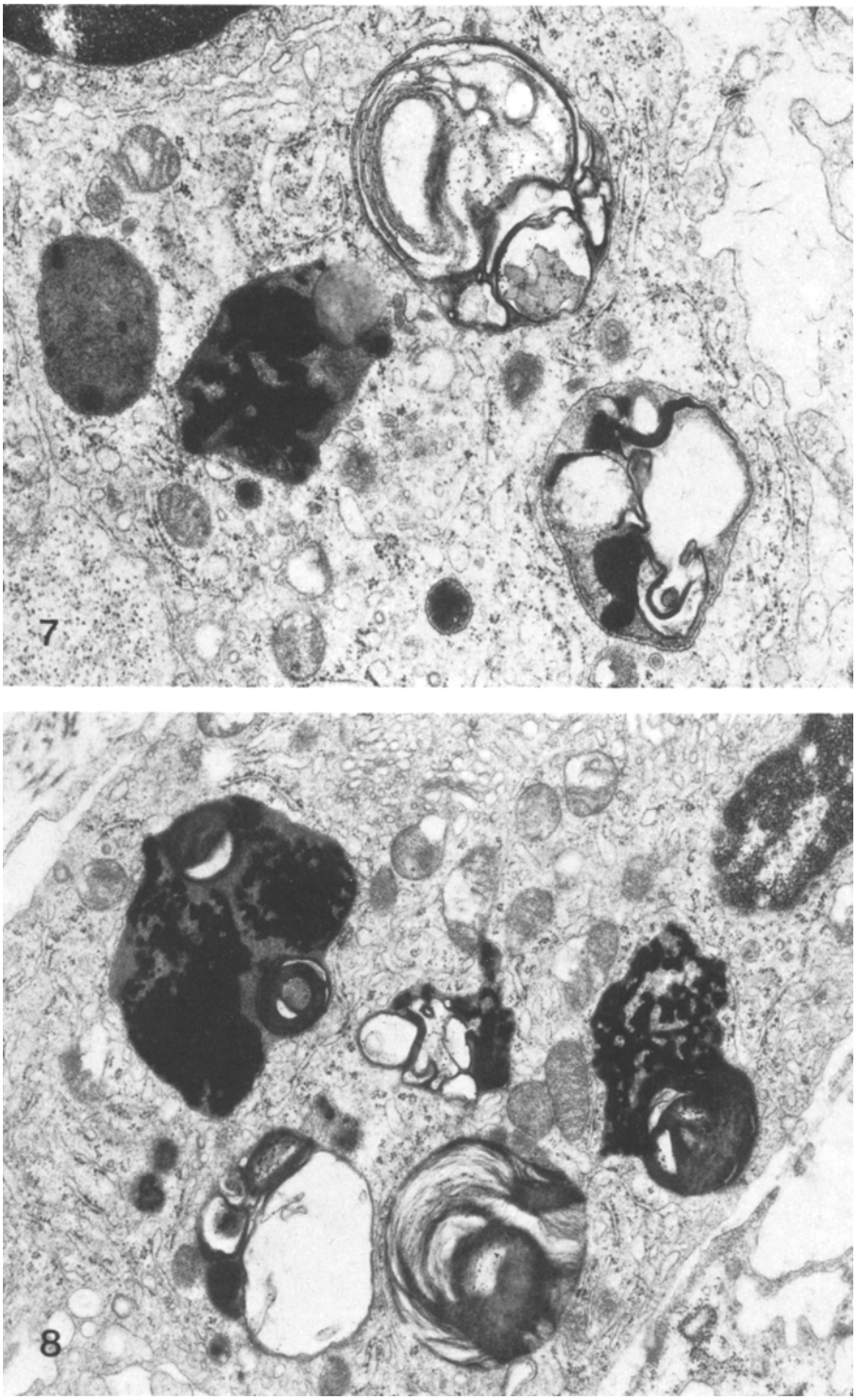
with long microvilli regularly distributed along their luminal surface. Some surface cells with a more cuboidal shape and short, irregularly distributed microvilli were also noted. An increased number of lysosomes was present in the apical cytoplasm of the epithelial cells. They had a heterogeneous content with rounded granules of varying size and density (diameter about 0.1–0.5 μm) and myelin-like figures as major components (Figs. 1 and 2). Rounded, 40–60 nm diameter, dense particles, often surrounded by an electron-lucent ring, were also observed (Figs. 2–4). In addition to the absorptive surface cells described above, numerous large goblet cells were evident in the epithelial layer.

The lamina propria contained large infiltrates of inflammatory cells, mainly macrophages and lymphocytes (Figs. 5 and 6). Moreover, there appeared to be an increased number of eosinophilic leukocytes. The macrophages demonstrated large lysosomes filled with inclusions similar in appearance to those in the lysosomes of the epithelial cells. Dense, irregularly shaped, aggregated particles and myelin-like figures were two major constituents among the inclusions (Figs. 7–9). More newly formed phagosomes or lysosomes were filled with moderately dense, rounded granules with a diameter of about 0.1–0.5 μm (Figs.10 and 11). Occasionally such structures were also noted extracellularly.

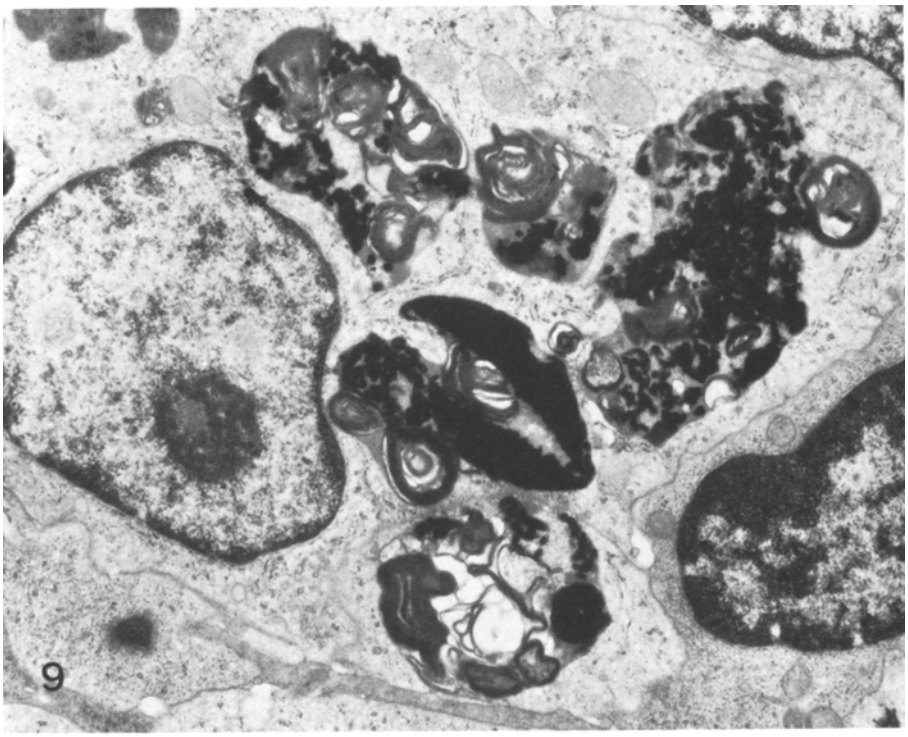
The submucosa was usually oedematous with extravasated erythrocytes. Nevertheless, the capillary walls mostly appeared normal. Otherwise, this layer

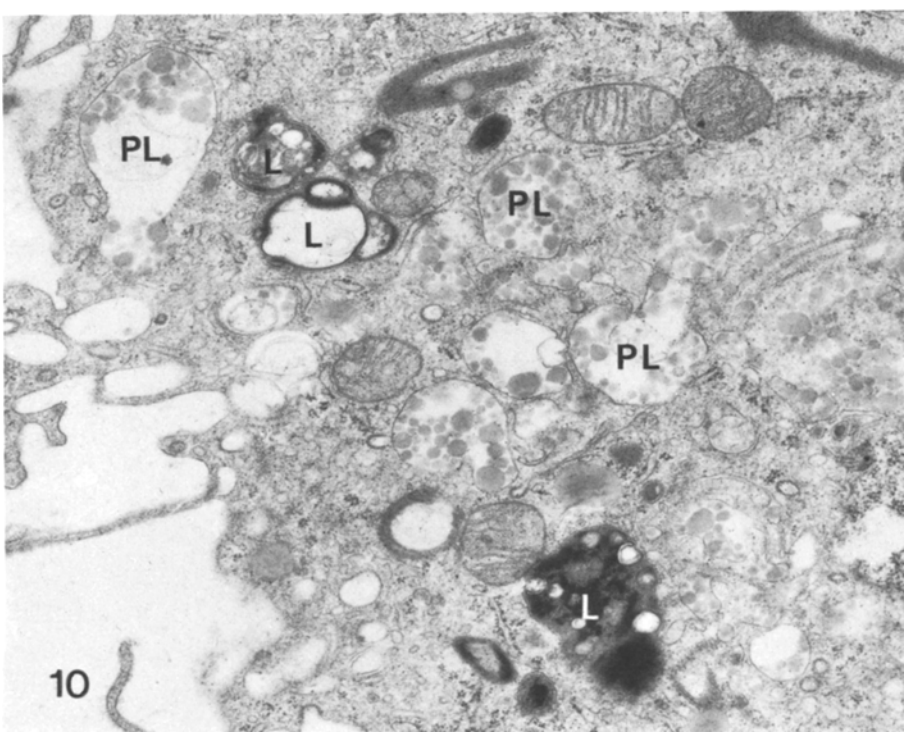


Figs. 5 and 6. Cases no. 8 and 5, respectively. Low power micrographs showing macrophages (*MA*) and lymphocytes (*LY*) accumulated in the lamina propria. The macrophages contain numerous large lysosomes (*L*) with heterogeneous inclusions. E, extravasated erythrocytes. × 5,800



Figs. 7–9. Cases no. 8 (Figs. 7 and 8) and 7 (Fig. 9). Details of macrophages in the lamina propria with large lysosomes containing inclusions of characteristic appearance. Fig. 7, ×26,000; Fig. 8, ×22,000; Fig. 9, ×14,000





Figs. 10 and 11. Cases no. 8 and 5, respectively. Details of macrophages in the lamina propria, demonstrating newly formed phagosomes or lysomes (*PL*) filled with moderately dense, rounded structures. More mature lysosomes (*L*) with inclusions of the type shown in Figs. 7–9 are also seen. Fig. 10, ×24,000; Fig. 11, ×16,000

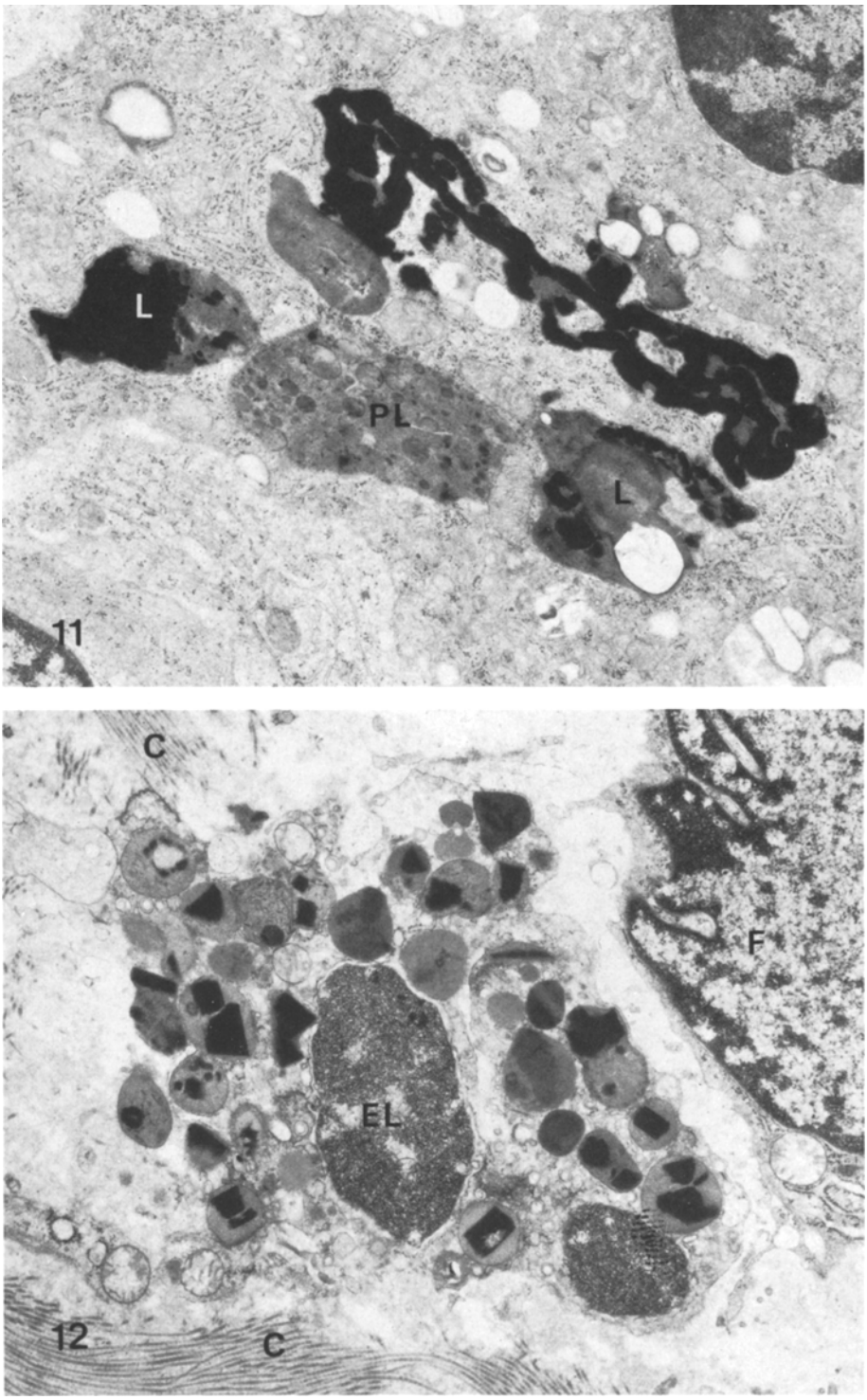


Fig. 12. Case no. 5. Submucosal tissue with a degenerating eosinophilic leukocyte (*EL*) and part of an adjacent fibroblast (*F*). *C*, bundles of collagen fibrils. × 15,000

consisted of a loose, collagenous connective tissue partly infiltrated with inflammatory cells of the same type as in the propria. Disintegrating eosinophilic leukocytes were often noted (Fig. 12). The muscle layer did not show any distinct morphological changes.

Qualitatively, the findings described above were almost identical in all patients (cf. Figs. from different patients). However, as shown in Table 1, the number of cells with lysosomal inclusions and the amount of deposits per lysosome varied considerably. A closer examination of the case histories with respect to age and sex of the patient, duration and severity of symptoms, types of therapy including earlier resection, and extent of affection of the intestine by the disease failed to demonstrate any direct correlation between clinical data and degree of fine structural alterations. Another somewhat surprising finding was that these alterations often were as prominent close to the margin of resection (macroscopically unaffected intestine) as in the macroscopically most clearly affected parts of the intestine.

Discussion

In the original description of regional ileitis Crohn et al. (1932) discussed the possibility of a microbiological aetiology. In an electron microscopic study of Crohn's disease of the colon, Aluwihare (1971) reported intramural bacteria in six of eleven specimens with intact epithelia. Bacteria have also been identified in the jejunal mucosa of patients with Whipple's disease (Trier et al. 1965), a disorder with many similarities to Crohn's disease. In both studies, bacteria were observed extracellularly as well as within the macrophages of the propria. Moreover, Mitchell and Rees (1970), and subsequently several others, have found that classical epitheloid granulomas can be transmitted into experimental animals by inoculation with intestinal or mesentheric lymph node homogenates from patients with Crohn's disease. However, so far it has been difficult to isolate any specific organism(s) from such material (for reviews see Janowitz and Sachar 1976; Martini and Malchow 1979).

In the present electron microscopic study of resected intestines from patients with Crohn's disease, we report on the presence of large lysosomes with inclusions of characteristic appearance in surface epithelial cells and macrophages, particularly those of the propria. Dense, aggregated particles and bizarre-shaped, myelin-like figures were the main constituents of the lysosomes. These organelles thus had a structure almost identical to that of the lysosomes found in tissue macrophages after in vivo injection of bacteria into different sites (for a review and comparison of pictures see Ginsburg, 1979). It therefore seems reasonable to assume that the inclusions we observed represent partly degraded bacteria and/or bacterial cell walls. The nature of the moderately dense, rounded, 0.1–0.5 μm diameter granules noted in some newly formed phagosomes or lysosomes is unknown. A possible microbiological origin remains to be demonstrated. More occasionally, we saw virus-like particles with a diameter of 40–60 nm within the lysosomes, particularly those of the surface epithelial cells (Figs. 2–4). Viral agents have been isolated from intestinal filtrates of patients with Crohn's disease (Aronson et al 1975; Gitnick et al. 1976) and grown in tissue culture to titers sufficient to allow electron microscopic detection (Gitnick and Rosen

1976). Moreover, Das et al. (1980) recently found that a transmissible factor present in Crohn disease lymph nodes, possibly a virus, produced lymphoma in athymic mice. An aetiologic role of viruses in Crohn's disease has not yet been clearly demonstrated, however.

The striking likeness of the findings in all eight patients studied here is suggestive of a common aetiology and pathogenesis. Moreover, the analogy with granulomas produced by injection of bacteria into experimental animals suggests that microbial invasion of the intestinal wall may have initiated the disease. Storage of non-degradable microbial components in lysosomes of macrophages accumulating at the site of invasion could be responsible for the initiation and propagation of a chronic inflammatory process. In this way, the pathogenesis of Crohn's disease would correspond to that of other granulomatous disorders (Ginsburg 1979). In contrast to the findings of Aluwihare (1971) and Trier et al. (1965), we did not observe any intact bacteria intra- or extracellularly, indicating that no acute infection was going on.

The fact that lysosomal inclusions supposedly representing partly degraded bacteria were found both in epithelial cells and macrophages of the propria suggests that the invasion of the intestinal wall had occurred from the lumen. A spreading through the blood cannot be excluded but seems less likely. The infection could be due to a specific pathogen and/or a defect in the local defense mechanisms of the intestine. There have been many discussions, as yet largely inconclusive, concerning a possible cellular immune deficiency in Crohn's disease (Janowitz and Sachar 1976; Martini and Malchow 1979). The findings of Segal and Loewi (1976), indicating a defective acute inflammatory response and an abnormality of neutrophil function in patients with Crohn's disease, may be of particular interest in this context.

A striking finding in the present study was the diffuse spread of the fine structural alterations in the resected intestines. In the specimens from all eight patients with Crohn's disease numerous macrophages with large, inclusion-filled lysosomes were thus found not only in the macroscopically clearly involved parts of the intestine, but also in the apparently normal margins of resection. Recent scanning electron microscopic studies of Crohn's disease have likewise indicated a more widespread involvement than can be appreciated by gross and routine light microscopic examination (Dvorak et al. 1979; Myllärniemi and Nickels 1980). By more detailed light microscopic and cytochemical investigations it is also possible to detect abnormalities in macroscopically normal intestinal mucosa in Crohn's disease (Goodman et al. 1976; Dunne et al. 1977). These observations suggest that Crohn's disease is a generalized disorder of the entire intestinal tract. In view of our findings, it is then tempting to speculate that the disease is associated with a defective barrier function of the intestine. This could then lead to an uncontrolled passage of bacteria – not necessarily of high virulence – and/or other agents into the mucosa, influx of mononuclear phagocytes from the blood, and generation of a chronic inflammatory process as discussed above. This is in agreement with the findings of a stimulation of monocytopoiesis in patients with Crohn's disease (Meuret et al. 1978) as well as the elevation of serum lysozyme (Falchuk et al. 1975; van de Merwe et al. 1980), an enzyme actively secreted by macrophages (Gordon et al. 1974).

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