A LIGHT- AND ELECTRON-MICROSCOPIC STUDY OF BACTERIAL INVASION IN WHIPPLE'S DISEASE

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In the original description of the disease bearing his name, Whipple placed emphasis upon the accumulation of fat within the intestinal mucosa and within mesenteric lymphatics.[1] "Lipodystrophy," the term suggested by Whipple, was considered to be the outstanding morphologic feature of this disease until 1949 when Black-Schaffer showed that macrophages found within the intestinal mucosa were vividly stained by the periodic acid-Schiff (PAS) method.[2] The presence of prominent PAS-positive macrophages within the intestinal mucosa became pathognomonic for this disease until the recent verification with electron microscopy of Whipple's original observation that rod-shaped organisms are found in affected tissues.[3–15] However, some observers have failed to find or recognize these organisms.[16–19]

The presence of dilated lymphatics and lipid retention within the intestinal mucosa has been mentioned briefly or not at all in the electron-microscopic studies, and there has been no attempt to explain, on the basis of fine structural observations, the mechanism of malabsorption in Whipple's disease. We recently oftained intestinal biopsy specimens from an untreated patient with Whipple's disease and were surprised to find bacterial invasion of intestinal absorptive cells. Because invasion of the epithelum had not been previously noted in this disease, we reviewed all material prepared for both light and electron microscopy, which had been obtained from patients with Whipple's disease at Duke University Medical Center. Findings in a portion of this material were reported previously.[5]

MATERIAL AND METHODS

Prior to treatment of a patient who had a characteristic clinical history of Whipple's disease, small-bowel biopsy specimens were obtained perorally. The patient fasted overnight prior to biopsy. Specimens for light microscopy were fixed in Bouin's solution, embedded in paraffin, and stained with hematoxylin and eosin, PAS, and the McCallum-Goodpasture gram stain. These sections showed typical changes of Whipple's disease and will not be described further.

Specimens for electron microscopy were fixed in 3.3% osmium tetroxide buffered in 0.05 M cacodylate, dehydrated, and embedded in epoxy resin.[20] Other specimens

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were fixed in 1.25% glutaraldehyde buffered in 0.067 M cacodylate, postfixed in cacodylate-buffered osmium, dehydrated, and embedded as above. Thick sections for light microscopy were cut at 1–1.5 μ and stained with methylene blue-azure II.[21] The blocks of tissue were then trimmed so that single villi could be thin-sectioned parallel to their length. Thin sections were collected on carbon-coated copper grids and stained with aqueous uranyl magnesium acetate[22] and lead citrate.[23] Photomicrographs were taken with an RCA 3F electron microscope at original magnifications of 1300–16,400 times and enlarged photographically.

Available for our review were small-bowel biopsy specimens from 4 patients with Whipple's disease who were either untreated or in relapse. Specimens were also obtained from these 4 patients 5–8 weeks after the beginning of continuous antibiotic therapy. A small-bowel and a rectal biopsy specimen were obtained after successful antibiotic therapy and prior to relapse in 1 of these patients. An inguinal node was available from 1 untreated patient with active disease. The above tissues were fixed in osmium and embedded in Vestopal as previously reported.[5] Thick and thin sections of this tissue were prepared as outlined above.

Seven patients died, prior to the advent of antibiotic therapy, with clinically active Whipple's disease, while under observation at the Medical Center. Necropsy was performed on 5 of these patients, and in 4 instances formalin-fixed tissues were still available for additional studies. The necropsy protocols of these 4 patients were carefully reviewed, and additional light microscopic sections were prepared for hematoxylin and eosin and PAS staining after paraffin embedding. Portions of small intestine and lymph nodes of 2 of the patients were postfixed in osmium and embedded in epoxy resin for subsequent electron microscopy.

Results

Many of our findings confirm those of previous electron-microscopic studies[8–15] and thus will not be repeated in detail. Bacteria were found to be scattered throughout the lamina propria of intestinal biopsy specimens of the 5 patients with clinically active disease. The bacteria were in greatest concentration at villous tips just below the epithelial-cell basal lamina. The lamina propria was packed with macrophages that contained presumably ingested bacteria in various stages of disintegration. Polymorphonuclear cells were frequent within the lamina propria and often contained bacteria, a finding also reported by Trier *et al.*[10] Many polymorphonuclear cells containing bacteria were present in intercellular spaces of intestinal absorptive cells. Intestinal epithelial cells were generally normal, though they contained numerous lysosome-like bodies in the apical third of their cytoplasm (Fig. 1). These lysosome-like bodies were similar to those described by Trier *et al.*[10] and were circular to irregular in shape, containing vesicles, myelin-like figures, and granule-studded membranes.

Possibly, the most striking finding, and one not previously reported, was the presence of myriads of bacteria within intestinal absorptive cells at villous tips (Fig. 2 and 3). These bacteria were enclosed within large circular-to-irregular–shaped membrane-bound structures that are probably best called heterophagic vacuoles.[24,25] These heterophagic vacuoles

contained bacteria in varying stages of degeneration, myelin figures, numerous vesicles, glycogen, and irregular dense masses (Fig. 2 and 3). The smooth and granular endoplasmic reticulum (ER) of most epithelial cells containing these heterophagic vacuoles was markedly dilated, and many bizarrely shaped mitochondria were present. Some epithelial cells appeared to be morphologically normal, apart from the heterophagic vacuoles containing bacteria. Bacterial invasion of intestinal absorptive cells at villous tips was clearly seen in biopsy specimens from 3 of the 5 patients with clinically active disease. In absorptive cells at villous tips and along the sides of villi in specimens from all 5 untreated patients were prominent lysosome-like bodies, many of which appeared to contain bacteria in terminal stages of digestion (Fig. 3). More-viable appearing bacteria were seen only at the base of absorptive cells and within intercellular spaces (Fig. 4). This suggested that bacterial invasion of absorptive cells occurred from the lamina propria and that the bacteria were then "attacked" by the lysosomal digestive system; this apparent transition was often seen within a single absorptive cell. Many epithelial cells contained lipid within profiles of the ER and within Golgi vacuoles, and chylomicrons were frequently present in the intercellular spaces even though all the biopsy specimens were obtained after an overnight fast. This apparent retention of lipid has been reported in previous studies.[6,9,10] Also, the normally dilated, basal intercellular spaces often contained an excess of a finely granular material, presumably precipitated protein. Intestinal crypt cells were normal, although there appeared to be an excess of argentaffin cells. Bacteria were not present in any of the biopsy specimens obtained during antibiotic therapy; nor were bacteria present in the small-bowel and rectal specimens obtained from the 1 treated patient prior to relapse. Lysosomes within absorptive cells of biopsy specimens from treated patients were still prominent but did not contain bacterial remnants.

The lamina propria of specimens from untreated patients was packed with macrophages, but there was a paucity of plasma cells. In addition, the extracellular spaces of the lamina propria were packed with large lipid droplets (Fig. 5). Many macrophages also contained lipid inclusions as noted by Kent *et al.*[6] Virtually all villi contained a dilated central lacteal (Fig. 6). Macrophages and other cellular elements were closely packed about the lymphatic endothelium (Fig. 6). The lymphatic lumens contained dense, finely granular material, presumably precipitated protein. On 1 occasion, a bacterial form was identified within the lumen of an intact, apparently untraumatized lymphatic. Adjacent to the nuclei of lymphatic endothelia were numerous lysosome-like bodies, many of which contained apparently degenerating bacteria (Fig. 7). Most endo-

thelial-cell junctions showed close attachment, though some junctions were open and contained macrophages and polymorphonuclear leukocytes that appeared to be entering the lymphatic lumens. The lymphatic endothelia were otherwise morphologically normal, except for occasional dilatation of ER. Lymphatics were still dilated in post-treatment biopsy specimens, but they did not obtain identifiable bacteria.

The lysosome-like bodies observed within the lymphatic endothelia of untreated patients were similar to the "electron-dense granules" noted by Kent *et al.* within capillary endothelium and muscularis of their patient.[6] We did not find similar bodies in capillaries in this study, but bacterial remnants within lysosomes were seen within the smooth muscle of the muscularis mucosae of 1 patient. Numerous bacteria were found within macrophages and lymph channels of the single inguinal node obtained from an untreated patient. Bacteria were identified in the tissue removed at necropsy, but the tissue preservation was so poor that we did not pursue this approach any further.

Discussion

Trier *et al.* have clearly shown in intestinal biopsy specimens from patients with untreated Whipple's disease that the bacillary structures seen within the lamina propria possess a fine structure characteristic of bacteria.[10] It is difficult to avoid the conclusion that these bacteria are, at least in part, the cause of this disease, though this is by no means unequivocally established.[26,27] To date, bacteria have not been identified within intestinal biopsy specimens obtained from normal controls or patients with other diseases. Kent *et al.* have pointed out that the predominance of this disease in middle-aged men suggests an unusual host susceptibility to the bacteria. The paucity of plasma cells within the lamina propria and the marked infiltration by macrophages suggest a defective immunologic response in these patients. It has been established that plasma cells of the gastrointestinal tract largely produce immunoglobulin antigen (IgA), the antibacterial antigen.[28] A deficiency of this antigen within the intestinal lamina propria may account for the unusual susceptibility of these patients to a bacterium that may well be common to our environment. Nevertheless, this does not explain the finding that generally the lamina propria of the small intestine is involved in Whipple's disease while there is sparing of the lamina propria of the stomach and colon.[6] Also, there is no apparent association of Whipple's disease with the hypogammaglobulinemias.

We have noted for the first time the presence of bacteria within intestinal absorptive cells and lymphatic endothelia. Our finding of a bacterium within the lumen of an intestinal lacteal, of bacteria within an

inguinal node, and the finding of similar bacilli within a retroauricular lymph node of a patient with Whipple's disease by Kojecký *et al.*[11] suggest that intestinal lymphatics serve as the portal of entry to systemic involvement. Kent *et al.* noted bacilli in the intestinal lumen, in proteinaceous material attached to surface epithelial cells,[6] and Trier *et al.* observed organisms in intercellular spaces of epithelium but considered this finding to be a probable traumatic artifact.[10] Our observations suggest that bacteria enter the intestinal absorptive cell from the intestinal lamina propria because the more-viable appearing bacteria were seen only at the base of absorptive cells. The bacteria seen at the apex of absorptive cells were always contained within lysosome-like bodies and always appeared to be degenerating as did the bacteria seen within macrophages of the lamina propria. In view of the above observations, it appears unlikely that bacteria are able to traverse the absorptive cell and remain viable. Hence, the original portal of entry of the bacteria remains to be identified. Although it is possible that the original portal of entry was the intestinal lumen, it is equally possible that entry may have been from a break in the skin or some other site. The intestinal lamina propria does appear to be uniquely susceptible to these small rod-shaped bacteria and, along with mesenteric nodes, is the major site of involvement.

Clearly identifiable bacteria were seen only within absorptive cells at villous tips, though what appeared to be bacterial outlines within lysosomes were seen in many other absorptive cells. It is plausible that villous-tip cells, at the end of their life span and about to be extruded from villi,[29] were more susceptible to bacterial invasion than cells along the sides of villi. The dilated ER seen in many of the cells invaded by bacteria was probably a manifestation of injury, although dilated ER may also be seen in the extrusion zone. Bacterial invasion of intestinal epithelium of experimental animals has been observed.[30-33] Hampton and Rosario noted attachment of *Streptobacillus moniliformis* to villous epithelial cells in the distal ileum of the mouse.[30] The area of attachment was restricted to the luminal surface of the epithelial cell, and no evidence of phagocytosis of the organisms by the epithelial cells was found. Takeuchi *et al.*, found penetration of the intact epithelial lining by dysentery and salmonella bacilli in starved guinea pigs.[31,33] The bacilli appeared to be transported through the epithelium in the form of membrane-enclosed vesicles resembling "phagosomes." These phagosomes are quite similar in appearance to the heterophagic vacuoles seen within intestinal absorptive cells of patients with Whipple's disease in the present study.

Fat malabsorption in patients with Whipple's disease has not been

satisfactorily explained.[34,35] One possibility is that malabsorption may be related merely to a reduction of epithelial-cell mass. However, among our patients, fat absorption returned to normal after 4–6 weeks of antibiotic therapy, while the intestinal biopsy specimens remained unchanged in appearance from those taken earlier, except for the disappearance of bacteria.[5,34,35] Thus, reduction in epithelial-cell mass does not appear to be a significant etiologic factor in fat malabsorption. It seems unlikely that the bacteria themselves utilize sufficient fat to be of significance. There is evidence that the epithelial cells themselves are functionally abnormal, in that there is decreased in-vitro amino acid uptake and fatty acid esterification by intestinal mucosa from patients with Whipple's disease although these functional alterations could have been determined by the macrophage and bacterial composition of the lamina propria.[35] Our finding of bacterial involvement of intestinal epithelial cells lends further support to the possibility that the epithelial cells indeed are functionally abnormal.

We have further confirmed the observation of Trier *et al.*[10] that bacteria are absent from antibiotic-treated patients in clinical remission, that the bacteria reappear with clinical relapse, and that the bacteria again disappear with additional antibiotic therapy.

This study also suggests that additional mechanisms may be involved in fat malabsorption seen in Whipple's disease. The presence of chylomicrons within intercellular spaces of epithelium and in the extracellular spaces of the lamina propria and the presence of larger lipid droplets within absorptive cells and in the extracellular spaces of the lamina propria suggest that there is a mechanical block to chylomicron absorption. We suggest that the great numbers of macrophages within the lamina propria physically impede the progression of chylomicrons to the central lacteal. Also, dilatation of central lacteals suggests that there is obstruction to lymphatic flow, probably secondary to enlarged mesenteric nodes. A similar though far greater degree of lymphatic dilatation is seen in intestinal lymphangiectasia, a disease in which malabsorption may be purely "mechanical" in nature.[36] The rather marked involvement of lymphatic endothelium by lysosomes containing bacteria suggests that there may be functional damage to the lymphatic endothelium, which may be a further factor in fat malabsorption.[37] Even though chylomicrons probably enter lymphatics via intercellular gaps, they may in part be transported across the lymphatic endothelium in pinocytotic vacuoles.[38,39] Nevertheless, obstruction to flow of chylomicrons through the lamina propria and through lacteals can hardly be a major factor in malabsorption, because the biopsy specimens taken a few weeks after antibiotic therapy are unchanged in appearance from

those taken earlier, except for the absence of bacteria; yet malabsorption is no longer present.[34]

We have shown in necropsy material kept for as long as 20 years that probable bacterial outlines are present. We would further like to record some observations concerning the postmortem examination of a patient with Whipple's disease[40]: "There are important points, that seem to me to be brought out in this case, which have not been emphasized or, in fact, paid any particular attention to, in the previously studied cases that are on record. There is widespread, chronic infection or chronic inflammation and certainly there is infection, that is bacterial infection, of the valve of the heart. This infection element in Whipple's disease is something that has been noted in all of the cases reported to date, but no one seems to have paid very much attention to it from the point of the study of the etiology of the disease. In view of the fact that in all of the cases of Whipple's disease reported to date, there is a very definite infectious element, it is very difficult for me to avoid referring the whole disturbance back to a general intoxication, indeed of a peculiar sort, related to the infectious process."

Summary and Conclusions

Electron-microscopic study of the intestinal mucosa of patients with Whipple's disease has shown apparent bacterial involvement of intestinal absorptive cells and of intestinal mucosal lymphatics. These previously unreported findings may explain in part the malabsorption syndrome seen in these patients. In addition, it is suggested that the passage of chylomicrons through the lamina propria may be physically impeded by the sheer numbers of macrophages within the lamina propria and by obstruction to lymphatic flow due to prominent involvement of mesenteric nodes.

Our data and those of others suggest that the lamina propria of the small intestinal mucosa is particularly susceptible to the bacteria of Whipple's disease and that patients with this disease may be deficient in intestinal plasma cells. Epithelial invasion probably occurs from the lamina propria. The route of systemic invasion is probably via lymphatics draining the intestine. The original portal of entry of the bacteria is still a mystery.

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Legends for Figures

With the exception of Fig. 6, which is a photomicrograph, all the figures are electron micrographs.

Fig. 1. Portions of absorptive cells containing prominent lysosome-like bodies (L) and multivesicular bodies (MVB). Microvilli and cell organelles appear to be quite normal. Osmium-fixed, Epon-embedded. × 20,000.

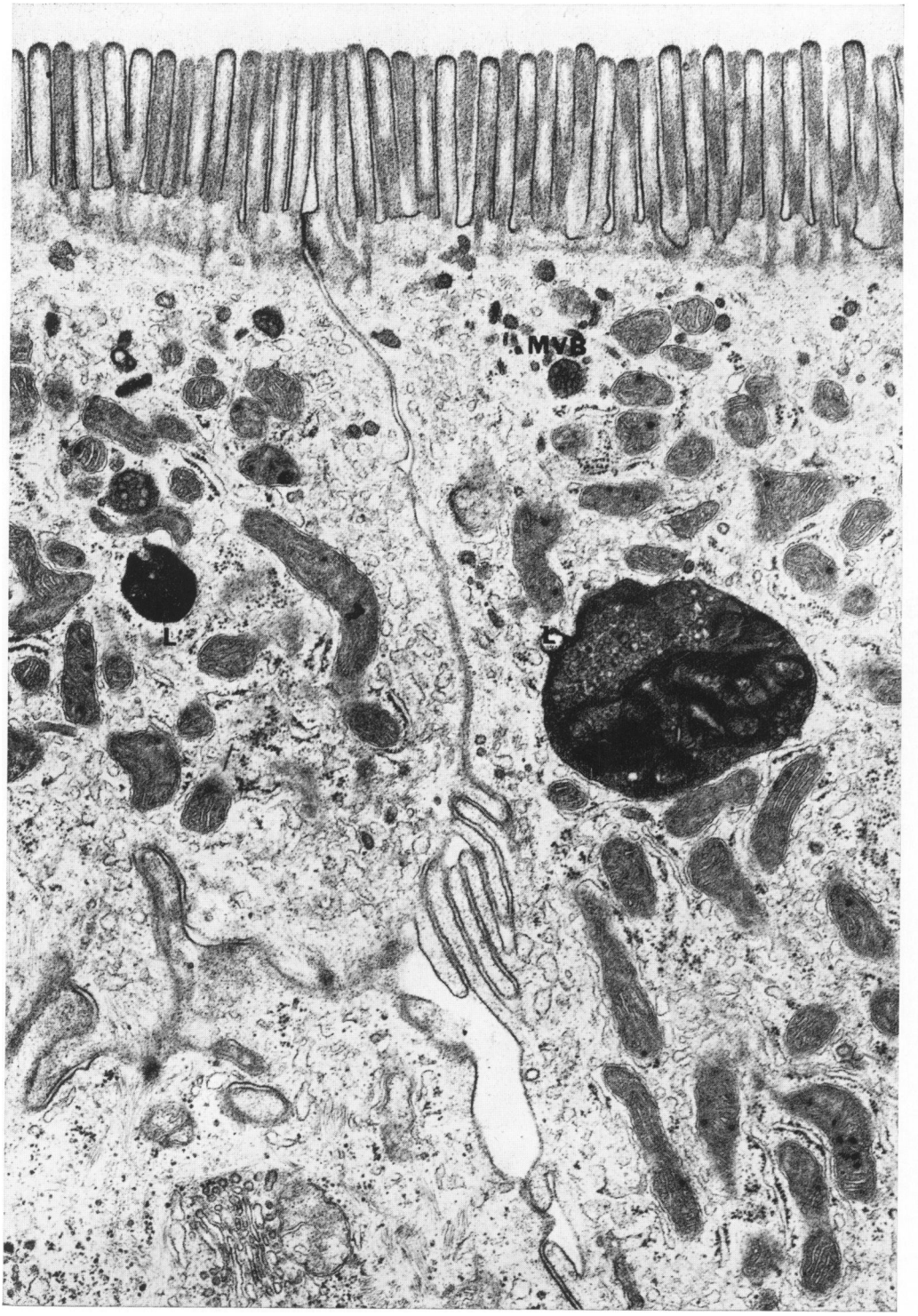
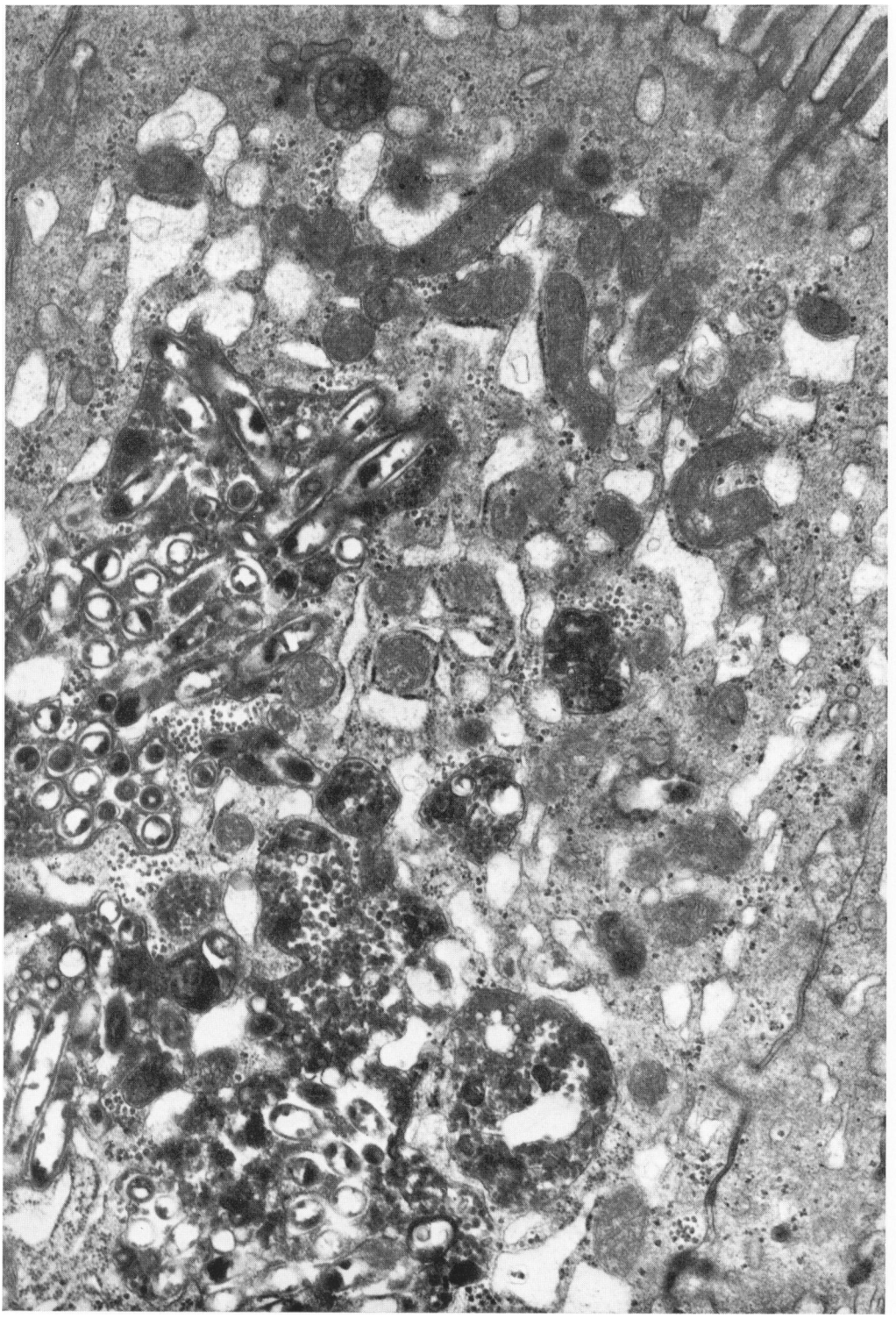
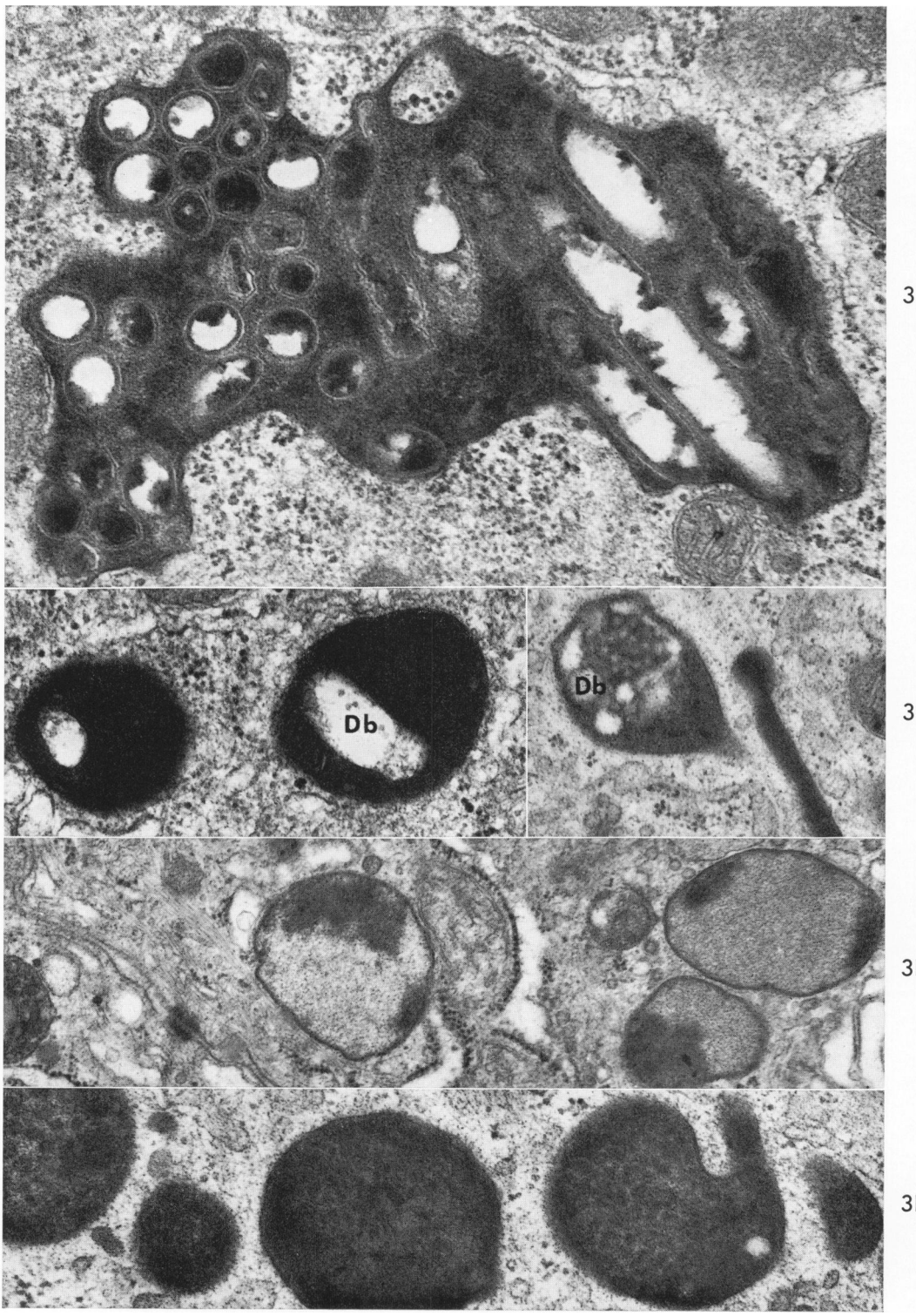

MVB
L
L

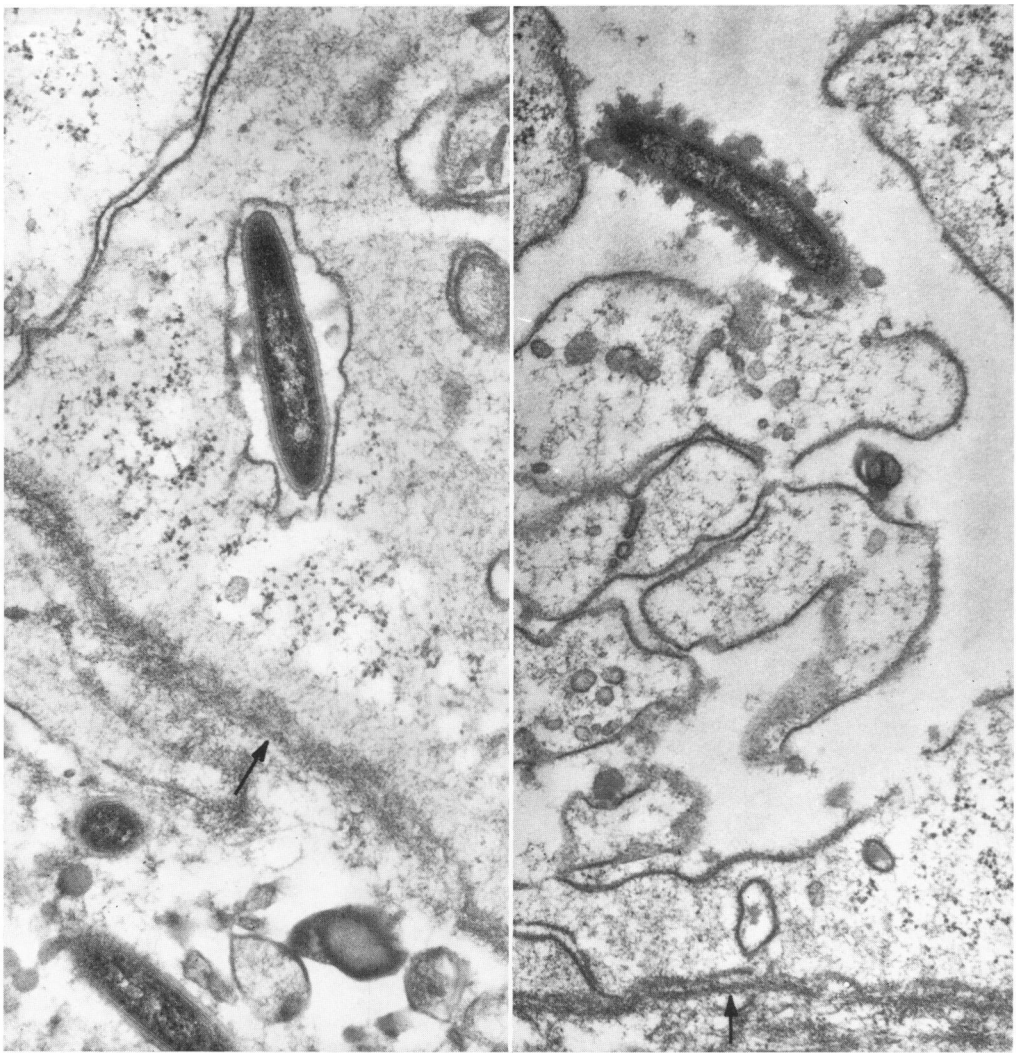
FIG. 2. Apical portion of an intestinal absorptive cell showing heterophagic vacuoles containing degenerating bacteria and numerous vesicles. Note prominent dilatation of endoplasmic reticulum and modest increase in glycogen within absorptive cell. Glutaraldehyde-osmium–fixed, Epon-embedded. × 20,000.



2

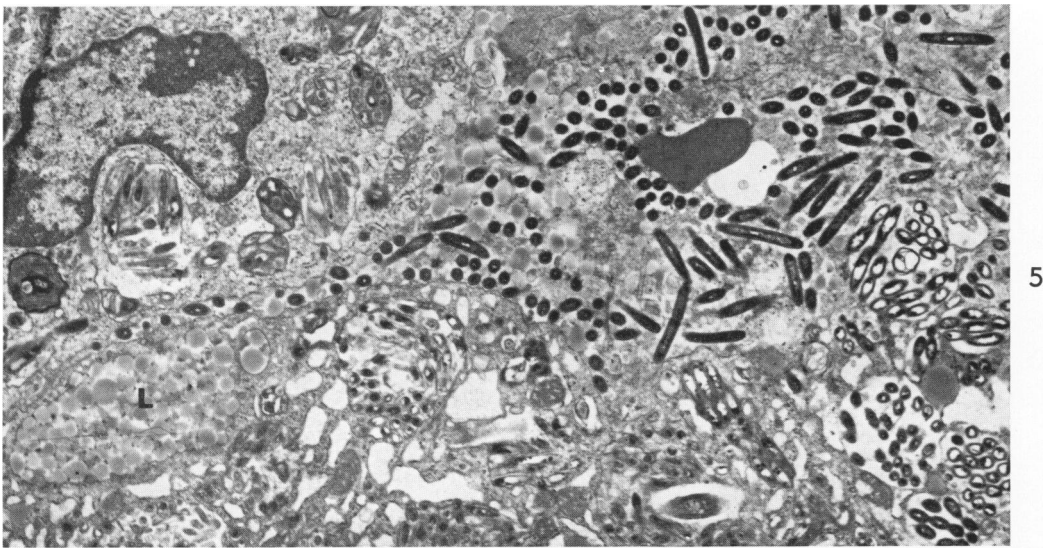
FIG. 3. Glutaraldehyde-osmium–fixed tissue showing variety of lysosome-like bodies within intestinal absorptive cells. A. Heterophagic vacuole containing clearly visible bacterial outlines. × 45,000. B and C. Lysosome-like bodies seen in most absorptive cells. What may represent degenerating bacteria (Db) is outlined by denser material within lysosomes. B, × 45,000. C, × 31,000. D and E. Lysosome-like bodies similar to those described in detail by Trier *et al.*[10] These and the denser lysosome-like bodies (Fig. 3E) may represent final stage of lysis of bacteria by heterophagic vacuoles, and may best be termed residual bodies. D, × 31,000. E, × 45,000.


3A
3B
Db
Db
3C
3D
3E



4A 4B

FIG. 4. Osmium-fixed, Vestopal-embedded tissue. Arrows point to basal lamina of absorptive cells. A. Viable-appearing bacterium within vacuole at base of absorptive cell. × 45,000. B. Viable-appearing bacterium within intercellular space of absorptive cells. Small lipid droplets appear to be attached to bacterium. × 34,100.



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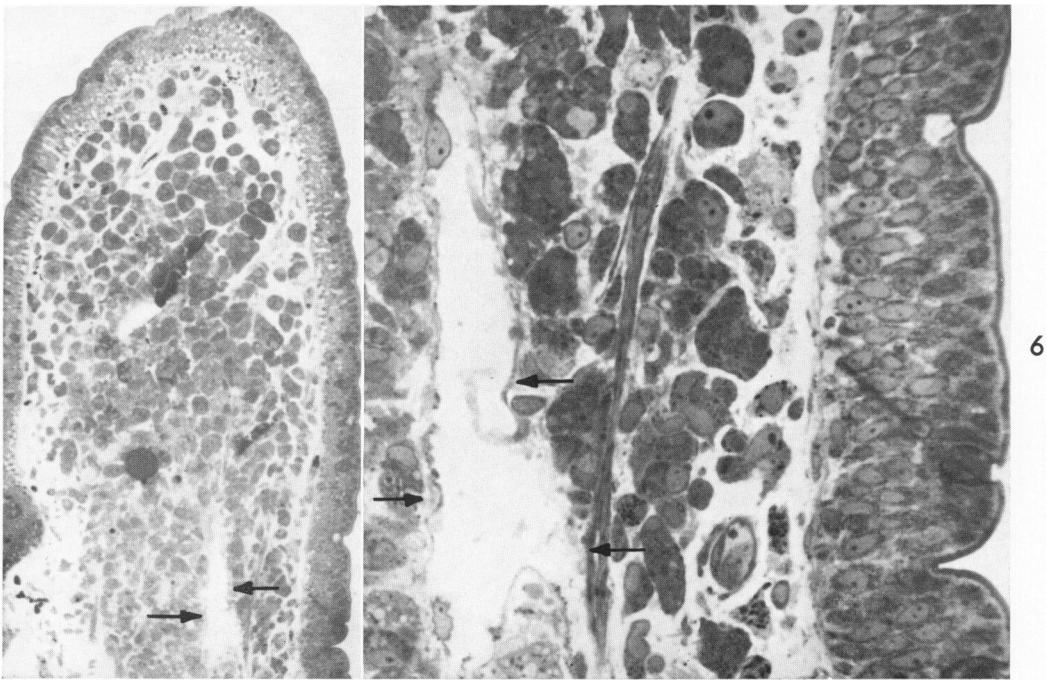

6A 6B

FIG. 5. Large accumulations of lipid droplets (L) and bacteria within extracellular spaces of lamina propria. Osmium-fixed, Epon-embedded. × 6700.

FIG. 6. Osmium-fixed, Epon-embedded tissue illustrating dilated central lacteal (arrows) with numerous macrophages closely packed about lacteal. A, × 250. B, × 1000.

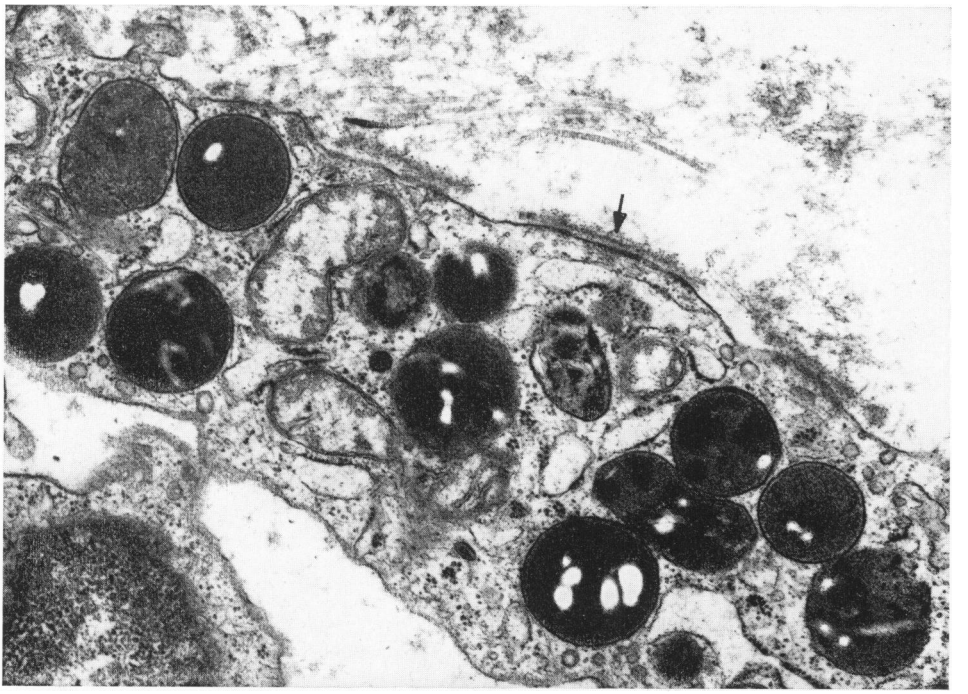

FIG. 7. Osmium-fixed, Epon-embedded tissue illustrating variety of lysosome-like bodies seen within lymphatic endothelia. Less-dense areas in the lysosomes may represent degenerating bacteria. Note incomplete basal lamina (arrow), a characteristic of intestinal mucosal lymphatics. × 20,000.