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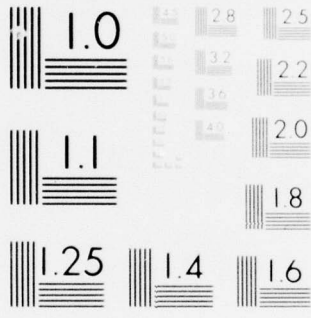
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Interactions of *Entamoeba histolytica* with host cells in the gut mucosa

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Entamoeba histolytica invades the intestinal tissues of its host by mechanisms that remained obscure for almost 100 years. Although this ubiquitous ameba, and the disease it produces, have been studied intensively by eminent investigators throughout the world, and much authoritative information has been forthcoming on the pathology of this parasitic infection, the precise mechanisms of its pathogenesis long remained both elusive and the object of considerable speculation. Studies of amebic ulcerations, in both humans and experimental animals, provided no accurate clues as to how the parasite enters the tissues of its host. Only by intensive study, at the ultrastructural level, of cellular interactions in involved tissue at the very beginning of the disease process, is it possible to observe and identify the complex cytological events that eventuate in tissue penetration and disease production by this pathogen.

We have pursued such a study in an experimental animal model. This was not an ordinary experimental animal, in the usual sense, but a "human flora" animal, a germ-free guinea pig infected via intracecal inoculation with *E. histolytica* and the enteric flora from a human case of acute amebic colitis. We believe that, in this host, the disease process mimics quite accurately that which occurs in man. We have observed sequentially, by electron microscopy, the morphological events that attend the entire process of pathogenesis beginning with the ameba in the colonic lumen, away from the epithelium, then approaching the epithelial lining, and eventually penetrating and migrating through the epithelium into the sub-epithelial tissues and the lamina propria. Particular attention was directed also to the microcirculatory components and changes and interactions therein during these various early stages. Collectively these data, some in review and some original, comprise this presentation. Since we were interested in the earliest cellular alterations, all observations were made on tissues of grossly normal appearance. Although the difficulties of describing a dynamic process from fixed material were fully appreciated, detailed examination of more than 3,000 electron micrographs comprising tissues from 25 infected animals and 5 controls

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(which received only the same enteric flora without amebae), suggests the following sequence of events.

The earliest cytological changes were observed with amebae confined to the gut lumen away from the epithelial lining. Epithelial cells showed reduction of microvilli and an accumulation of lipid droplets in their cytoplasm. At this time other cytoplasmic components remained unchanged. Considerable cells debris was commonly observed around the amebae.

When amebae were observed somewhat closer to the microvilli, a variety of cytoplasmic changes were evident in epithelial cells (figure 1). The microvilli further shortened and the glycocalyx and terminal web were obscured. Lipid droplets were increased in number. Mitochondria were swollen and the matrix had become opaque. The cristae were deranged and both rough and smooth endoplasmic reticulum were dilated. Other cytoplasmic organelles, however, remained unchanged. An electron-dense amorphous material was commonly seen between the ameba and the shortened microvilli.

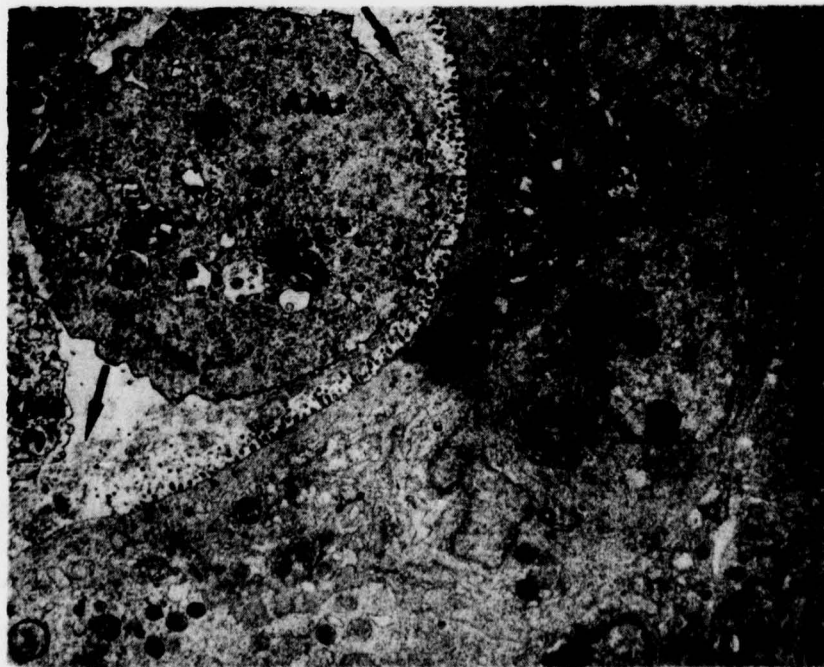


Figure 1. Two amebae (AM 1 and 2) in the gut lumen are close to the surface epithelial cells of the cecum. Dense material (large arrows) is localized between amebae and the short, irregular microvilli. Lipid droplets (LD) are evident in the epithelial cells. Small arrows mark the basal lamina of the epithelium. $\times 6,500$.

When amebae were very close to the epithelium, the microvilli had completely disappeared and often the cytoplasm of host cells projected into the lumen contacting the amebae. These cytoplasmic projections were completely devoid of cell organelles. In some instances a cell, or group of cells, had become detached from their basement membranes and adjacent epithelial cells and were in the process of shedding into the lumen thus producing spaces in the epithelial lining. When this occurred polymorphonuclear leukocytes had often emerged and filled these spaces. The interepithelially migrating leukocytes, likewise, showed various degenerative processes including degranulation, condensation of cytoplasm and sometimes lysis of the cell wall (figure 4). The amebae, using their pseudopodia, were observed in the various stages of passing through the interepithelial spaces and disrupted basement membranes and reaching the lamina propria.

The earliest penetrations of epithelium appeared to occur at the surface with greater frequency (figure 2), but occasionally penetration of crypt epithelium was observed (figure 3). Most often, several amebae were in the process of penetrating the epithelial lining at multiple sites. When this occurred, epithelial cells were observed to have desquamated excessively into the gut lumen thus resulting in the formation of microerosions.

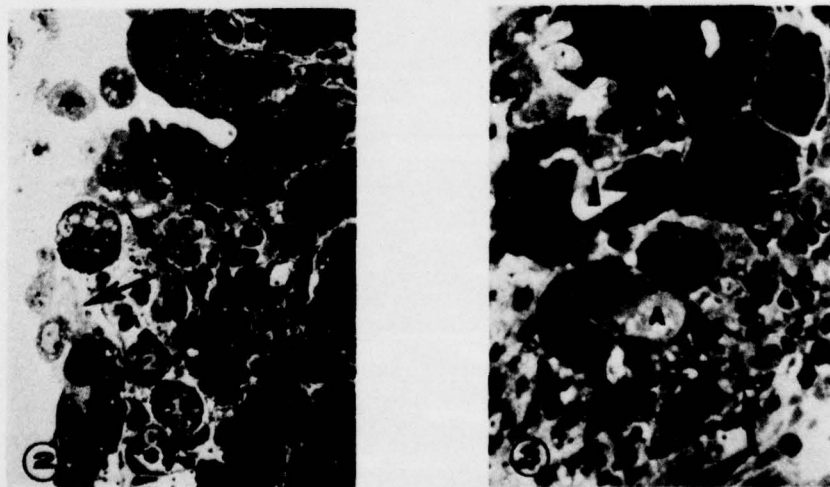


Figure 2. Invasion of the cecal mucosa by trophozoites at interglandular site. Two amebae (1 and 2) are in the lamina propria and one (3) separates degenerating pale epithelial cells which are in process of desquamating into the lumen (arrows). Two other amebae (4 and 5) are in the lumen. C, capillary. $\times 650$.

Figure 3. Invasion by trophozoites of the crypt gland epithelium. Two amebae (A) have penetrated through the crypt epithelium and are surrounded by cellular infiltrate (arrow). An ameba (arrowhead) is in the crypt lumen. In the lamina propria many extravasated red blood cells are present. Capillary lumen (C) is filled by red blood cells. $\times 510$.

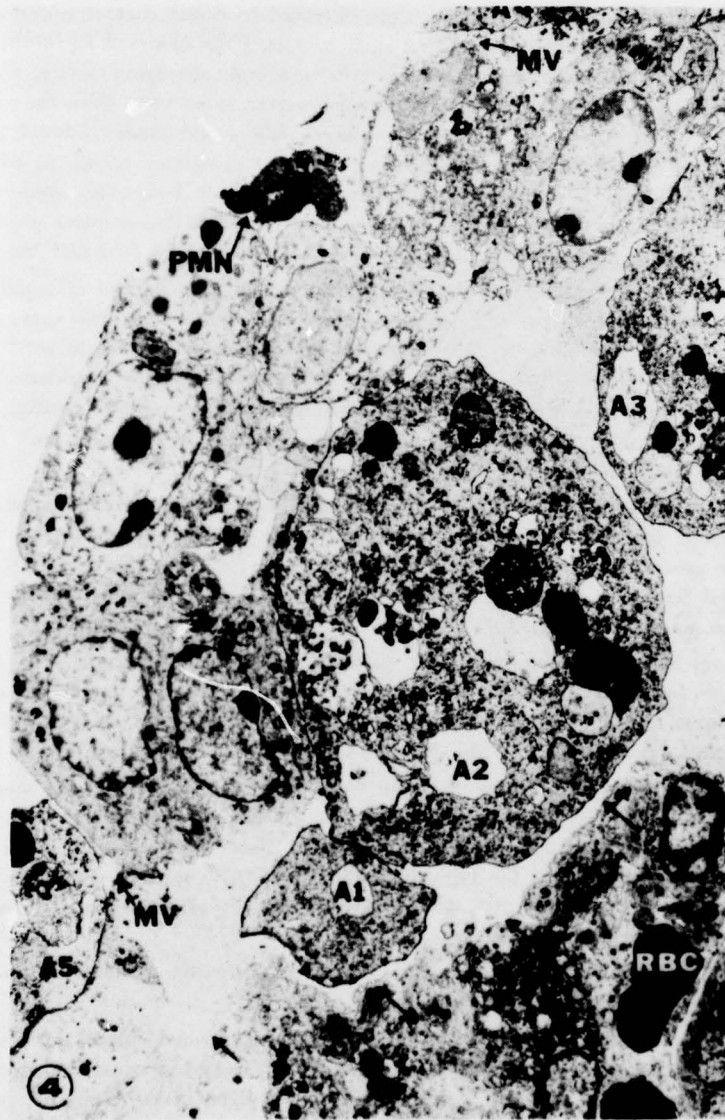


Figure 4. Amebae in process of penetrating the interglandular portion of the cecal epithelium. Amebae (A2 and 3) are between epithelial cells and basal lamina (arrows). A1 appears to be pseudopodium of A2. Luminal amebae (A4 and 5) are close to altered microvilli (MV) of degenerating epithelial cells. Polymorphonuclear leukocyte (PMN) shows degeneration. Extravasated erythrocyte (RBC) is present in the subepithelial lamina propria. Arrows mark the basal lamina of the epithelium. $\times 3,800$.

In the lamina propria some amebae were observed in direct contact with mesenchymal cells, whereas others were without such contact. Cells observed in the lamina propria included fibroblasts, smooth muscle cells, macrophages, lymphocytes, plasma cells, eosinophils, red blood cells and polymorphonuclear leukocytes. With the exception of the last, all other cells, as well as collagen fibers and nonmyelinated nerve bundles, showed no significant changes when in close proximity to, or in contact with amebae (figures 5 and 6). When polymorphonuclear leukocytes were topographically close or contacting amebae, they showed the same degenerative processes observed in these leukocytes at the interepithelial location (figures 5, 8 and 10).

When amebae were present in the subepithelial region, the lumina of capillaries and venules were often dilated and contained large numbers of red blood cells, polymorphonuclear cells, lymphocytes, thrombocytes and fibrin suspended in condensed plasma. When the amebae were deep in the lamina propria, many capillaries and venules showed various degrees of endothelial damage including cellular swelling, loss of cytoplasmic density, dilatation and swelling of endoplasmic reticulum and mitochondria (figure 7).

In severely altered vasculature the intercellular tight junctions of endothelial cells were separated more frequently, and their fenestrae occasionally developed gaps. Thrombotic processes appeared to occur, simultaneously, close to the separated cell junction and fenestrae (figure 8). The space between altered endothelium and the basal lamina was often widened.

When amebae were topographically close to endothelial cells, blebs or small vesicles of uniform size appeared to have developed from the endothelial cell membrane. In some instances, blebs of different sizes were seen in an apparent process of pinching-off from the endothelial cytoplasm into the lumen (figure 9).

Isolated fragments and clumps of fibrin were observed not only in the vascular lumen (figures 7 and 10) but also were seen frequently, in abundance, in the extravascular space of the lamina propria (figure 10). Most fibrin in both intra and extravascular locations lacked the typical striation of 220 A. Indeed, very little such striation was observed (figure 10). There was no evidence of extravasated thrombocytes in the lamina propria.

None of the cellular changes reported herein as occurring in infected animals were observed in the control animals.

The cellular events observed in early lesions in intestinal amebiasis in our studies, was a remarkably consistent and orderly process. There was no physical evidence that associated microorganisms were in any way directly involved in the occurring pathologic changes. Although a variety of bacterial species were abundantly present in the enteric lumen, bacteria were never found in the mucosal tissue at the early stage of the mucosal invasion by amebae.

Degenerative changes in epithelial and polymorphonuclear leukocytes organelles, in cells in close association with amebae, provide unequivocal proof for the inherent pathogenicity of *E. histolytica*.



Figure 5. Amoeba (AM) in the subepithelial region. Degenerating PMN leukocytes (black letters) are close to or in contact with amoeba (AM) and with what appears to be an amoebic pseudopodium (AM?). Unaltered PMN leukocyte (white letters) is present in capillary lumen (CAP). Other host cells surrounding amoeba remain unchanged. White arrows mark the basal lamina of epithelial cells; MV, microvilli. $\times 5,700$.

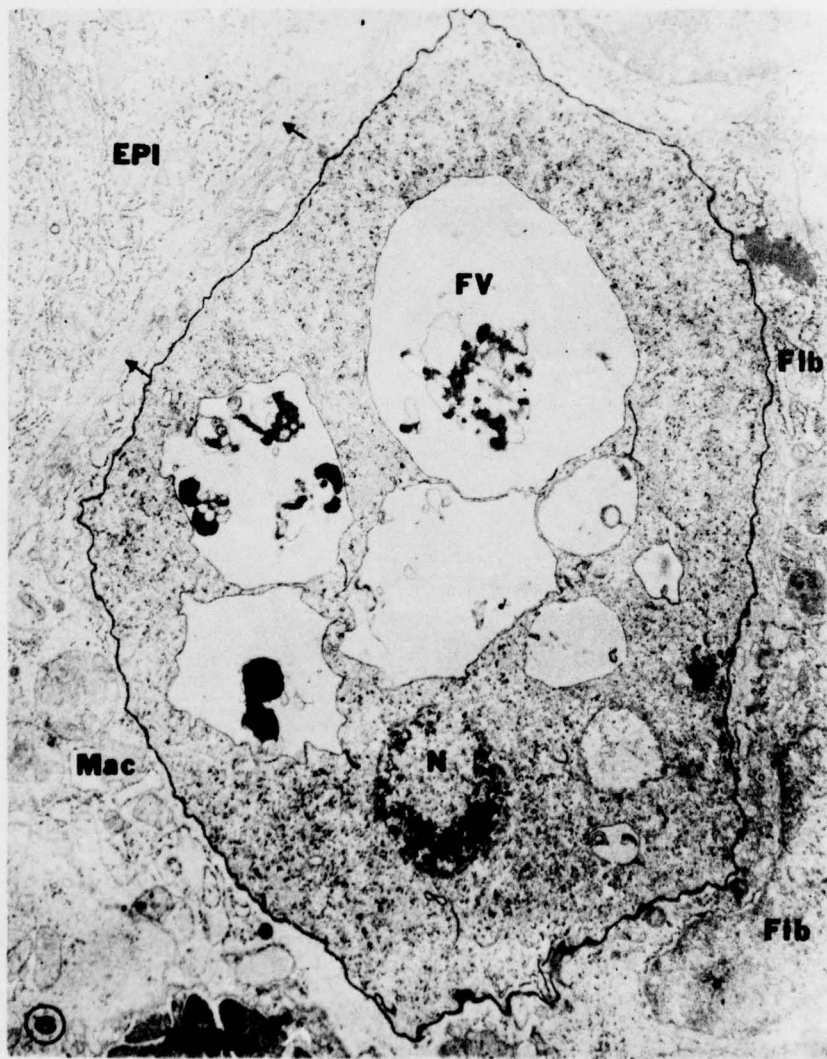


Figure 6. Ameba surrounded by fibroblasts (Fib) and macrophages (Mac) in the lamina propria beneath the epithelium. Degenerating polymorphonuclear leukocyte (PMN) shows condensation of nuclear material. Arrows mark basal lamina of the crypt epithelium. $\times 8,000$.

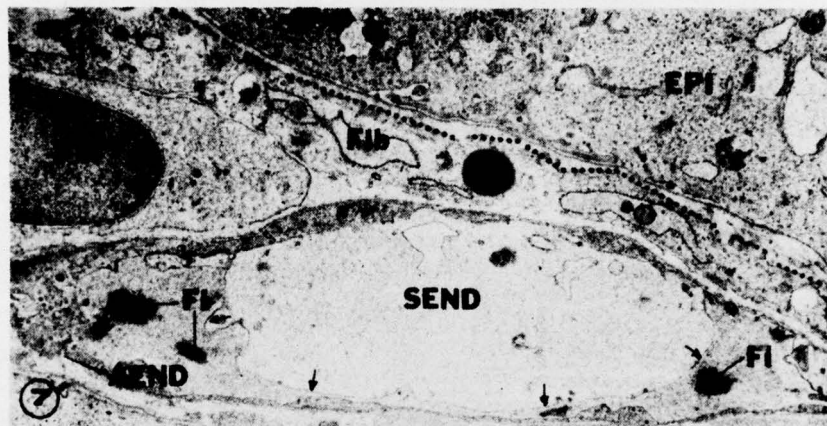


Figure 7. Subepithelial capillary shows swollen endothelial cell (SEND) which obliterates most of the lumen (arrows). The lumen contains fragments of fibrin-like structures (FI) in condensed plasma. The adjoining endothelial cells (AEND) are unaltered. Dotted line follows the basal lamina of the epithelium (EPI). Fib, fibroblast. $\times 7,800$.

The presence of polymorphonuclear leukocytes in appreciable numbers in the early lesions is of special interest. It is generally believed that *E. histolytica* does not provoke any appreciable neutrophilic response in intestinal tissues. This is based upon the almost complete absence of intact neutrophils in the luminal content in acute amebic colitis. Our studies show, however, that there is a significant neutrophilic response which occurs very early during the process of mucosal invasion by amebae. The massive destruction of polymorphonuclear leukocytes by *E. histolytica* precludes their presence in the exudate. This massive destruction of polymorphonuclear leukocytes with release of their cytolytic enzymes into the environment, we believe, may be a very important factor in the ensuing pathological process.

Abstract

Germfree guinea pigs were inoculated intracecally with *Entamoeba histolytica* and the enteric flora derived from patients with acute amebic colitis. The enteric tissues were studied, sequentially, at various postinoculation intervals, with the aid of light and electron microscopy. When luminal amebae were observed close to the cecal epithelium, the apposing epithelial cell was found to have protruded into the lumen and making contact with the amebae. Such cells often had become detached from their basal lamina and intercellular tight junctions affording spaces through which amebae invaded the mucosa. Other epithelial cells in close proximity to amebae showed degenerative changes characterized by swelling of mitochondria and endoplasmic reticulum and disappearance of microvilli. The shedding of these cells in appreciable

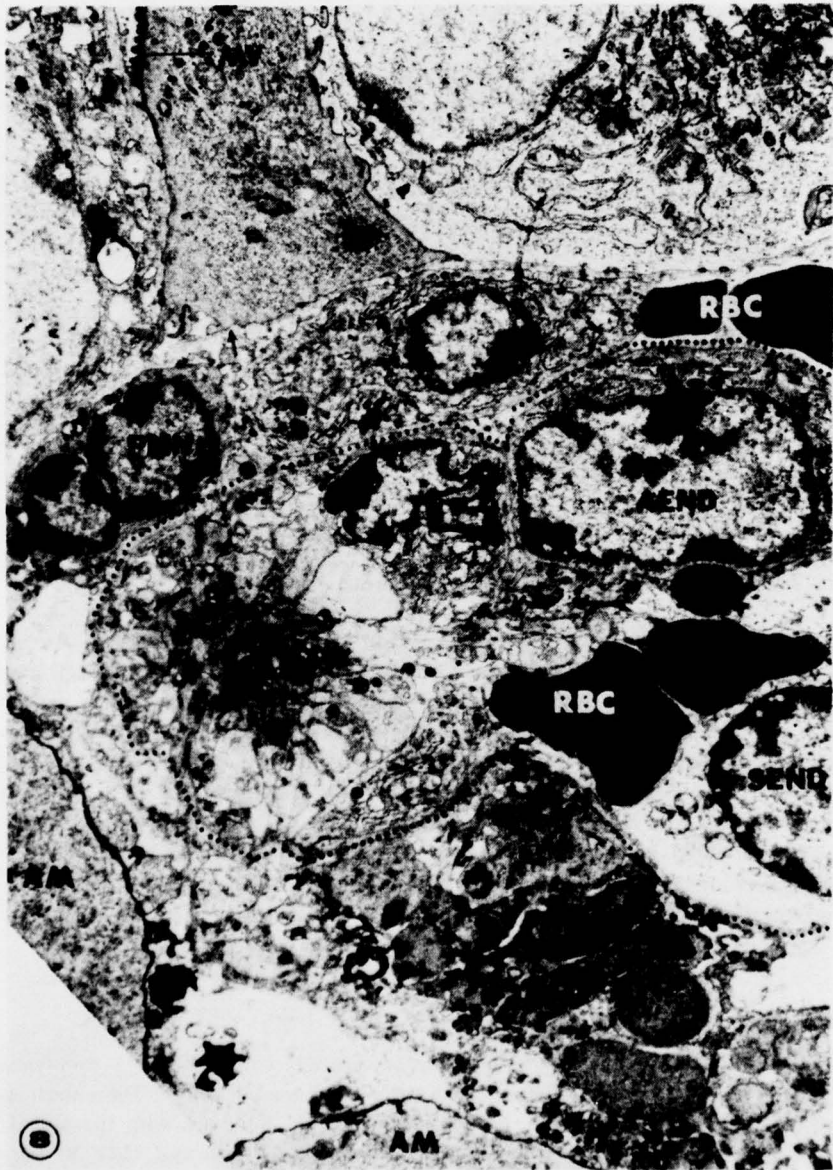


Figure 8. Two amebae below surface epithelium. Lumen of capillary (enclosed by dotted line) contains erythrocytes (RBC) and aggregations of platelets showing signs of degranulation. Endothelial cell is swollen (SEND) while another remains unaltered (AEND). PMN leukocytes close to amebae (AM) show signs of degeneration. Note extravasated erythrocytes (RBC) between basal lamina of the epithelium (arrows) and the capillary. MV, microvilli of epithelial cells. $\times 6,900$.

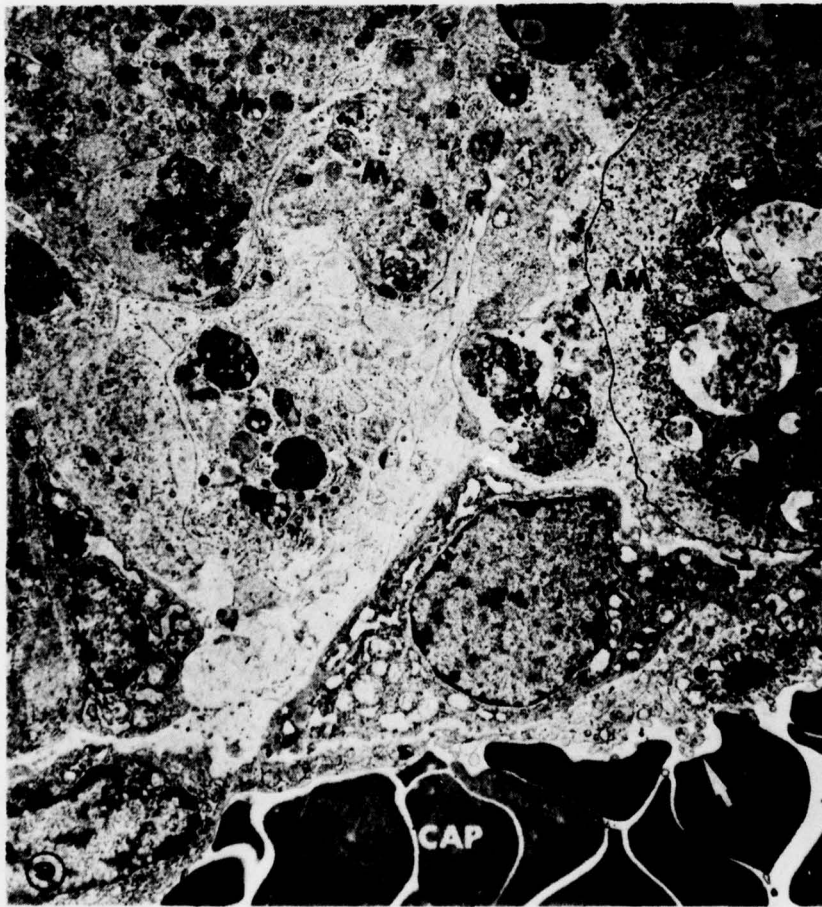


Figure 9. Ameba is close to subepithelial capillary which contains many erythrocytes. Blebs develop from endothelial cytoplasm into the capillary lumen (white arrows) and into the extravascular space (black arrows). Fibroblast (F) and macrophages (M) near ameba are unaltered. $\times 6,500$.



Figure 10. Ameba deep in the lamina propria. Translucent areas in amebic cytoplasm (AM) are artifacts resulting from extraction of glycogen during ethanol dehydration. A PMN leukocyte (white letters), in contrast with the ameba, shows signs of degeneration such as homogenous nucleoplasm and degranulation. Other PMN leukocytes (black letters) are intact. Fragments of fibrin (large arrows) are present within the capillary lumen (CAP) and in the extravascular space. Small arrows mark the basal lamina of the crypt epithelium. L, lymphocyte; M, macrophage; F, fibroblast. $\times 4,700$.

Inset. High magnification of fibrin (square), illustrating striation of 240 \AA . $\times 65,000$.

numbers into the lumen also provided spaces (microerosions) through which mucosal invasion by amebae occurred. Polymorphonuclear leukocytes in proximity to invading amebae also showed marked degenerative changes including condensation of nucleoplasm and cytoplasm and lysis of cell membrane with release into the environment of cytoplasmic components including granules. Other mesenchymal cells in the mucosa remained unaltered even in close association with the amebae.

As amebae reached the lamina propria, changes became evident in capillaries and venules. These included cellular swelling and gap formation at the intercellular junctions of endothelial cells and sometimes at the fenestrae of the capillaries. Vasculature close to amebae showed formation of cytoplasmic blebs on endothelial cells which were subsequently pinched-off into the vascular or extravascular space. Platelet and fibrin thromboses were seen in capillaries and venules which showed gaps at both the intercellular tight junctions and at the fenestrae. Fibrin-like material was observed also in extracellular spaces. The vascular changes appeared to be related to the polymorphonuclear cells degeneration resulting from interaction of these leucocytes with invading amebae.

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