ELECTRON MICROSCOPE STUDIES OF EXPERIMENTAL SALMONELLA INFECTION

I. Penetration into the Intestinal Epithelium by *Salmonella typhimurium*

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It has long been recognized that pathogens are capable of penetrating the intact intestinal epithelium, but the mechanism of penetration has been very little understood. It was conclusively demonstrated by the fluorescent antibody technique that invasion and penetration of the mucosal barrier by Shigella bacilli initiated the evolution of bacillary dysentery.[1]

In a study of experimental bacilliary dysentery in guinea pigs, Takeuchi, Sprinz, LaBrec and Formal[2] observed intracellular Shigella organisms within absorptive cells of the ileum and reported the sequential morphologic events surrounding the invasion. The initial penetration by Shigella organisms from the gut lumen into the intestinal epithelial cells, however, could not be demonstrated at that time.

Using light microscopy and fluorescent antibody and bacteriologic techniques, Kent, Formal and LaBrec[3] investigated a diffuse acute enteritis in guinea pigs preconditioned by opium injection and orally challenged with *Salmonella typhimurium*. Recently we have produced a similar but more severe intestinal infection in guinea pigs preconditioned by starvation in addition to opium injection and also challenged orally with *S. typhimurium*. In the early stage of this infection, ample numbers of Salmonella uniformly penetrated the intestinal epithelium; this made an electron microscopic study of this process feasible. The present paper attempts to describe how Salmonella organisms pass from the gut lumen into the small intestinal epithelium and how the cytoplasmic components of the epithelium respond to the penetration. The events occurring in the mucosa subsequent to Salmonella invasion will be presented in a forthcoming paper.

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Material and Methods

Culture Preparation. Each animal was infected by 10^8 *Salmonella typhimurium*, strain w 118, obtained from C. V. Seaston, University of Wisconsin. The strain was grown on nutrient agar for 18 hours and harvested in brain–heart infusion broth.

Techniques of Infection. Walter Reed strain guinea pigs of either sex and weighing 300 to 400 gm were assigned at random to 4 groups of 10 animals each. Prior to oral bacterial challenge, all 4 groups were deprived of food for 4 days but allowed water. Three of the groups were infected, each animal receiving 10 ml infectious broth by stomach tube. One ml tincture of opium was administered intraperitoneally immediately after challenge to inhibit peristaltic movement. The latter 3 groups of animals were sacrificed at 8, 12 and 24 hours after inoculation, respectively. Later stages were not examined as we were principally concerned with early lesions. The remaining group of animals served as 'controls'. Each 'control' received sterile broth and opium and was sacrificed shortly after intubation. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Histologic and Electron Microscopic Techniques. Guinea pigs were killed by cervical dislocation and necropsied in a conventional manner. For this study a segment of the ileum was removed and processed as follows:

(1) For fluorescent antibody studies a 5-cm portion was split longitudinally, rolled and dropped into a bath of isopentane at $-73°$ C. The frozen tissues were sectioned in a cryostat, fixed and treated with fluorescein labeled rabbit *S. typhimurium* antiserum. The details of the procedure were those used by LaBrec and Formal.[4]

(2) For light microscopy a second portion was fixed in 10 per cent neutral formalin. Individual sections of paraffin-embedded ileum were stained with one of the following: hematoxylin and eosin, periodic acid–Schiff (PAS) and Giemsa. Thin sections of plastic-embedded material cut at 1 to 2 μ were also stained with azure II and methylene blue.

(3) For electron microscopy a third portion of fresh ileum was cut into small cubes, fixed in 2 per cent glutaraldehyde in *S*-collidine buffer for 2 hours, followed by 3.3 per cent osmium tetroxide in *S*-collidine buffer or osmium solution alone for 2 hours. The tissues were then dehydrated in water soluble epoxy resin [5] and embedded in Araldite. Ultrathin sections were stained with lead hydroxide and examined with JEM 7 and RCA EMU-3G electron microscopes.

Observations

The fine structure of the guinea pig ileal absorptive epithelium under the effect of starvation has been previously described.[2] An extraneous fuzzy layer on the surface of the epithelial microvilli was inconspicuous in guinea pigs, even when double staining with uranyl acetate and lead was used; this was in accord with a recent study by Ito.[6]

At 8 hours after oral challenge, frozen sections treated with fluorescent antibody disclosed a small number of specifically fluorescing Salmonella organisms in the intestinal lumen. Bacilli were absent from ileal epithelium and from the lamina propria. Ultrastructurally, the organisms in the lumen appeared essentially the same as other gram-negative bacteria reported by different workers.[7,8] The epithelium was unchanged at this time.

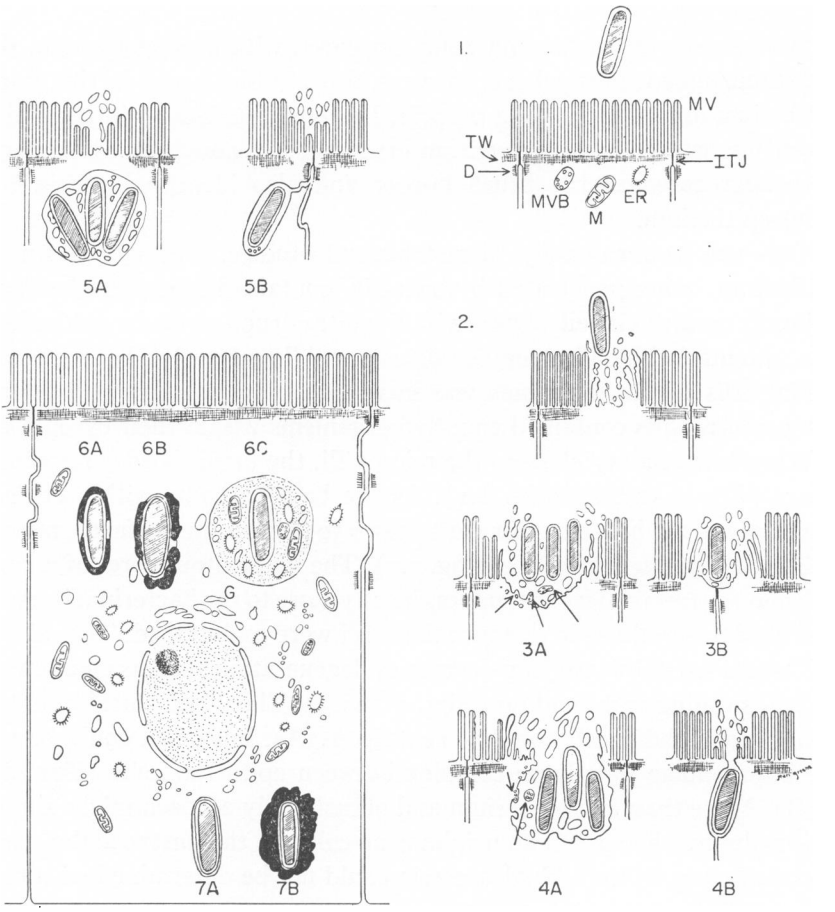
At 12 hours fluorescent antibody treated sections continued to reveal

numerous free organisms but some appeared within phagocytes in the intestinal lumen. Many bacteria were also readily found in the lining epithelium and in the lamina propria. By light microscopy intracellular organisms were not present within crypt cells although many bacteria were aggregated at the brush border and also identified within the villus epithelium.

This was confirmed by ultrastructural studies. The cytoplasm of epithelium, being penetrated by bacteria, contained more vesicles than adjacent unaffected cells. Otherwise the ultrastructure of the epithelium was unremarkable. The number of neutrophils migrating between epithelial cells and in the lumen was increased over that seen at 8 hours. Some neutrophils contained engulfed organisms within their cytoplasm.

When bacteria lay close to the microvilli, the brush border remained intact (Fig. 1 and Text-fig. 1.1). As the bacillus came within critical proximity to the brush border (less than 350 Å from the surface), microvilli began to degenerate (Text-fig. 1.2). The filamentous cores of microvilli and the terminal webs topographically close to the bacterium became obscured, while those in distant microvilli were maintained. The microvilli began to exhibit varying degrees of degeneration such as elongation, fusion, swelling and budding (Figs. 2 and 3). The usual portal of entry was through the brush border (Text-fig. 1.3A), but often could be via the intercellular junctional complex between epithelial cells (Text-fig. 1.3B). More than one bacterium and occasionally a dozen might simultaneously invade one or two neighboring cells. In this instance the exact portal of entry of individual bacteria could not be determined with certainty (Fig. 11).

As the bacterium advanced further through the brush border (Text-fig. 1.3A), in addition to the microvilli the apical cytoplasm in proximity to the bacterium began to degenerate. A cavity was formed around the penetrating organism as it moved through the brush border and into the apical cytoplasm (Figs. 2 to 5, and Text-fig. 1.4A). Cytoplasmic degeneration was characterized by the following process (Figs. 6 and 7): A part of the host cell cytoplasm might bulge and project into the gut lumen or into the cavity surrounding bacteria. These cytoplasmic projections appeared as blebs distinct from the host cytoplasm, and were presumably shed into the lumen or into a cavity surrounding the bacteria. Blebs containing aggregated small vesicles exhibited a striking similarity to a multivesicular body; most blebs, however, lacked such vesicles. As the bacterium advanced into the host cell (Text-fig. 1.4A), the cavity in which it was located expanded accumulating additional degenerated microvilli and parts of the host cytoplasm in the form of blebs or vesicles. The bacterium-containing, membrane-bound vacuole



TEXT-FIG. 1. A schema illustrates the process of bacterial (*Salmonella typhimurium*) penetration into the ileal epithelium.

1. The bacterium in the gut lumen lies beyond critical proximity to the brush border and has no effect on the epithelial cell. The brush border consists of microvilli (MV) and a terminal web (TW) which ends at the terminal bar between the intercellular tight junction (ITJ) and desmosome (D). Mitochondria (M), endoplasmic reticulum (ER) and multivesicular body (MVB) are present in the apical portion of the cell.
2. When the bacterium comes within critical proximity to the brush border, fibrillar cores of the microvilli and tight junction undergo degeneration.
3A. The most frequent mode of invasion is by multiple penetration by the organisms. Degeneration extends to the apical cytoplasm where a cavity is formed before the advancing bacterium (arrows indicate pinched off cytoplasm).
4A. Upon further advance of the bacterium, the cavity expands in size by further pinching-off of the apical cytoplasm (arrows).
5A. The bacterium-containing cavity is transformed into a vacuole and becomes disconnected from the luminal plasmalemma.
3B. The passage of organisms through the intercellular junctional complex is less frequent than that shown in 3A. The advance of the bacterium leads to the degeneration of microvilli and the apical cytoplasm in the proximity of the tight junction.
4B. The bacterium passes through the junctional complex and is enclosed by the lateral plasmalemma of two adjacent epithelial cells.
5B. The bacterium-containing vacuole disconnects from the lateral plasmalemma.
6. In the supranuclear region, regardless of the portal of entry, the bacterium-containing vacuole completely enclosed by a single membrane develops into a smaller vacuole (A) containing the bacterium and dense material. B. The bacterium is surrounded by a discontinuous membrane and dense material. C. A membrane-bound body encloses the bacteria, mitochondria, ER and ribosomes.
7. In the subnuclear region, the bacterium is either tightly enclosed by a single membrane (A) or surrounded by dense material (B).

in the apical cytoplasm eventually became separated from the luminal plasmalemma (Text-fig. 1.5A).

The entrance of bacteria in proximity to the junctional complex (Text-fig. 1.3B) led to the degeneration of microvilli and the apical cytoplasm including the terminal web (Fig. 8). A cavity was formed in the same fashion shown in Text-figure 1.3A (Fig. 9). At the same time the underlying intercellular junctional complex was always displaced. In several instances bacteria were observed passing through an intercellular junctional complex (Text-fig. 1.4B); they were found between tight junctions (*zonular occludens*) and intermediate junctions (*zonula adherens*) or desmosomes (*macula adherens*) (Figs. 10, 11 and 12). As the bacterium progressed, it was enclosed in a vacuole formed by the lateral plasmalemma of two adjoining cells, together with degenerated microvilli and pinched off cytoplasmic blebs. The bacterium-containing vacuole seemed to move laterally into the apical cytoplasm (Text-fig. 5B), although the complete sequence of events was not observed. Thus far bacteria have not been found in the intercellular space below the subnuclear region.

Thereafter, the bacterium-containing vacuoles, regardless of the portal of entry, seemed to pursue the same intracellular pathway and transformation. In the supranuclear region, the vacuole diminished in size as its content, composed of blebs and small vesicles, was eventually replaced by dense osmiophilic material (Fig. 16) (Text-fig. 1.6A). At the same time the overlying brush border including the terminal web was gradually reconstituted (Figs. 13 and 14). In some instances the bacteria in the supranuclear region were further enclosed with various cytocomponents including endoplasmic reticulum, mitochondria and ribosomes into a membrane-bound body identical to that observed in Shigella enteritis (Fig. 15 and Text-fig. 1.6C).[2]

In the subnuclear region the bacterium was either surrounded by dense osmiophilic material (Figs. 18 and 19 and Text-fig. 1.7B), or tightly enclosed by a single membrane (Figs. 17 and 19 and Text-fig. 1.7A). At 24 hours, penetration of the intestinal epithelium by organisms was infrequent. Fewer bacteria were noted within altered epithelial cells. On the other hand, more organisms were present within phagocytes in the lamina propria. The bacteria within both epithelium and phagocytes were tightly enclosed by a single membrane or were included with other cytoplasmic components in phagosomes. Occasional bacteria were surrounded by dense osmiophilic material. Rarely they were non-circumscribed and lay free in the cytoplasm of both cell types.

Organisms within epithelium during intracellular passage always appeared morphologically intact. Even within phagocytes, most of engulfed

organisms seemed unaltered as far as could be determined with the technique employed. Only on rare occasions were organisms within phagocytes undergoing degeneration identical to that seen in phagocytosed Shigella organisms.[2]

Discussion

Florey, in his histologic studies of *Salmonella typhimurium* infection in guinea pigs, suggested that salmonellas passed through or between epithelial cells, possibly being transported by phagocytes.[9,10] The present study has further clarified Florey's concept and has demonstrated the mechanism of epithelial penetration. With the method employed here, most of the organisms identified at 12 hours lay free in the gut lumen or were in the process of penetrating the epithelium. In contrast, only a small number of organisms appeared within phagocytes which were found mainly in the lumen and only rarely in the mucosal lining and in the lamina propria. The relative importance of phagocytes as transport vehicles for the pathogens could not be assessed. In particular, it could not be determined whether phagocytes were capable of transporting bacteria from the lumen to the lamina propria. At 12 hours, the number of bacteria engulfed by the phagocytes present in the mucosal lining was small when compared with the number of bacilli within or passing between epithelial cells.

During the period of our experiment, the crypt epithelium was free of bacterial invasion, although many organisms were present in the crypt lumens. In contrast, the villus epithelium contained many bacteria. Similar observations have also been made in the early stages of experimental Shigella infection[2] and in Cryptosporidium enteritis in guinea pigs.[11,12] This would appear to indicate that crypt epithelium is more resistant to invasion by pathogens than the villus counterpart.

A characteristic tripartite junctional complex between adjacent epithelial cells in the intestinal mucosa has been described by Farquhar and Palade.[13] According to them, the *zonula occludens* (tight junction), characterized by fusion of the adjacent cell membranes, acts as a diffusion barrier or seal against concentrated protein solutions. The other two junctions, the *zonula adherens* (intermediate) and *macula adherens* (desmosome), represent intercellular attachment devices. The present study has demonstrated that *S. typhimurium* is capable of penetrating through the intercellular tight junction (*zonula occludens*). During the process the tight junction separated as the organism passed through it (Fig. 9), and seemed to close as the organism moved deeper (Fig. 10), and then resumed its structural integrity. This observation further confirms previous evidence[2,12] that the intercellular junctional complex,

in particular at the tight junction, is not a permanently stable structure, but rather adjusts to various conditions. For instance, in the work cited above [2,12] an epithelial cell in process of extrusion into the gut lumen is occasionally seen detaching from adjacent cells at the intercellular junctional complex. The last component to detach from the adjacent cells is the *zonula occludens*. Transmigrating lymphocytes and neutrophils are occasionally found separating two adjacent epithelial cells and migrating into the lumen, thus separating the tight junction which in this case is barely visible. The phenomenon of migration between adjacent epithelial cells and extrusion of epithelial cells into the gut lumen is, however, a normal event but increases in frequency during bacterial infection.[2]

The brush border of the small intestine is an important region from the physicochemical point of view. The surface of the microvilli is covered by a fuzzy extraneous layer comprising an acid mucopolysaccharide complex.[6] Rich in various enzymes including alkaline phosphatase and adenosine triphosphatase, the brush border is the site of terminal hydrolysis of disaccharides [14,15] and peptides [15] prior to absorption. To what extent this micro-environment may provide a chemical barrier against microbial invasion is unclear. The present study calls attention to the impact of pathogens upon this physicochemically important portion of the intestinal mucosa. The approach of a single pathogen into criticial proximity to the microvilli triggers sudden local degeneration of the brush border. On the other hand, multiple *S. typhimurium* organisms, when present in the lumen but beyond the point of critical proximity appear to have no overall effect on intestinal epithelium. After penetration, the effect of the organisms upon the host cell is localized. The cytocomponents of the cell are surprisingly well preserved despite the enclosure of many organisms in membrane-bound vacuoles. This is also true of organisms not enclosed by a membrane, but surrounded only by osmiophilic material. These observations indicate that after penetration by bacteria, the host cell is capable of confining the organisms and of preventing extensive cellular damage. At the same time the damaged intestinal epithelium is capable of repair along the brush border (Fig. 13).

Alterations such as budding and fusion of microvilli are not specific features of Salmonella infection, but have been observed in the normal intestine of monkeys [12] and human subjects, [16] suggesting that it is not an abnormal condition. These changes in microvilli may also be produced artificially by fixing intestinal mucosa in a hypotonic solution.[17] Symbiotic parasites, for example, *Streptobacillus moniliformis* in mice [18] and Cryptosporidium in guinea pigs, [11] also elicit degeneration of micro-

villi at the site of attachment of the organism to the luminal plasmalemma of the ileal epithelial cell. All of the examples mentioned, however, with the exception of artifacts produced by hypotonic fixatives are much less striking than the destruction of the brush border observed in the present study.

The evolution of the intracellular bacterium-containing vacuole as it descends from the apical to the basal cytoplasm includes a reduction in size and the conversion of the degenerated microvilli and pinched-off cytoplasm into dense osmiophilic material. Hydrolytic enzymes have been described in the intestinal mucosa of rats.[19] Acid phosphatase is present in the guinea pig intestinal epithelium.[20] The manner in which intestinal epithelium handles the bacterium-containing vacuole bears a striking resemblance to the intracellular digestive process by acid phosphatase and nonspecific esterase in mammalian tissue culture cells as reported by Gordon and his colleagues.[21,22] An analogous intracellular response and the mobilization of lysosomal enzymes probably occur in guinea pig intestinal epithelium following bacterial penetration. All Salmonella organisms within epithelial cells and most of those in the phagocytes are morphologically intact. This may signify that in the epithelium, in spite of intracellular segregation by host cell membranes and digestion of cytoplasmic components contained within the vacuoles by acid hydrolases, Salmonella organisms have the capacity to survive and to multiply. It may be postulated that the dense osmiophilic material surrounding intra-epithelial organisms results from host cell products (vacuolar content and enclosing membrane) and metabolites of Salmonella organisms modified by the action of host cell hydrolytic enzymes. This problem is presently under investigation using electron microscopic cytochemistry.

In accordance with their characteristic invasive potential, enteric pathogens may be classified into three groups. The first group is characterized by a pathogen which invades the mucosal barrier, multiplies in the lamina propria, and leads to symptomatic disease. Salmonella and Shigella belong to this group. Members of the second group never invade the mucosal lining but exhibit physical attachment to the luminal plasmalemma of intestinal epithelium. They may or may not produce an inflammatory response and altered intestinal villus architecture. This group includes *Cryptosporidium parvum*[11] and *Streptobacillus moniliformis*.[18] The last group neither penetrates nor attaches to the intestinal epithelium, but elicits symptoms and is exemplified by *Vibrio cholerae*.[23]

When preconditioned guinea pigs are challenged orally with virulent and avirulent strains derived from the same parent strain of *Shigella*

flexneri 2a, only the virulent strain invades the mucosal barrier and multiplies in the lamina propria; the avirulent strain is unable to penetrate the epithelium.[24] From this it appears that strain differences in virulence may play a decisive role in determining invasive capacity. We believe that a confrontation between enteric organisms and the physicochemical barrier of the brush border is successfully overcome only by virulent organisms; this is then followed by epithelial penetration and multiplication in the lamina propria.

Summary

The mechanism of penetration into small intestinal epithelium by Salmonella organisms was studied by electron microscopy, in preconditioned guinea pigs orally challenged with *Salmonella typhimurium*. Most frequently, several bacteria simultaneously invaded a single or two adjoining epithelial cells through the brush border. Bacteria were also capable of penetrating the epithelial lining through the intercellular junctional complex. As bacteria came into critical proximity to microvilli, first the microvilli, and then the apical cytoplasm including the terminal web degenerated and the intercellular junctional complex was occasionally displaced. Next, a cavity was formed in the apical cytoplasm before the advancing organism. The cavity was in open communication with the gut lumen. As the organism further advanced into the host cytoplasm, the cavity developed into a bacterium-containing membrane-bound vacuole incorporating degenerated microvilli and pinched-off apical cytoplasm; meanwhile the brush border was reconstituted. Arriving at the supranuclear region, the bacterium-containing vacuole diminished in size as its vacuolar contents were digested, presumably by lysosome enzymes, and were replaced by dense osmiophilic material. Occasionally a bacterium, together with various cyto-components including endoplasmic reticulum, mitochondria and ribosomes, was enclosed in a membrane-bound body. Eventually the bacterium was either surrounded by dense osmiophilic material or tightly enclosed by a single membrane.

The study has shed light on the mechanism of the initial penetration of the intestinal mucosa by pathogenic bacteria and on the active response of the intestinal epithelium to invasion by enteric pathogens.

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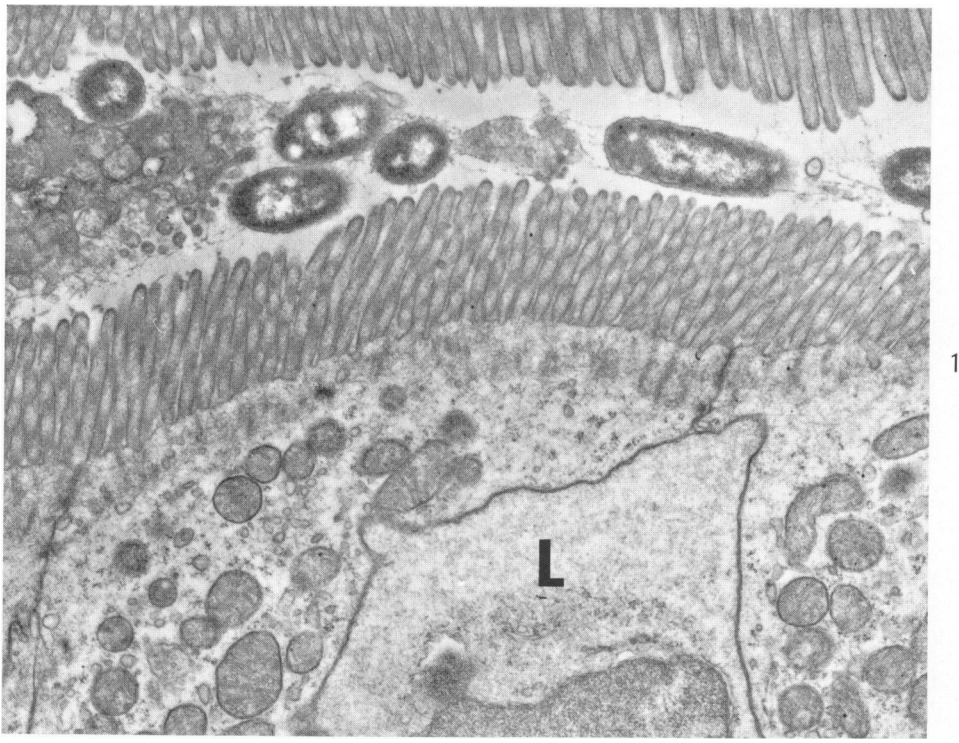
[*Illustrations follow*]

Legends for Figures

All electron micrographs are of absorptive epithelial cells, unless otherwise specified, at the mid-villus portion of the guinea pig ileum 12 hours after challenge with *S. typhimurium*.

Fig. 1. Several organisms lie close to the brush border which is still intact. Cytoplasmic components are well preserved. A transmigrating lymphocyte (L) appears between epithelial cells. Corresponds to Text-figure 1.1. × 14,000.

Fig. 2. Degeneration of the brush border and the apical cytoplasm with cavity formation occurs near a bacterium (arrow). Budding, swelling and elongation of microvilli are evident. Corresponds to Text-figure 1.3A. × 10,000.



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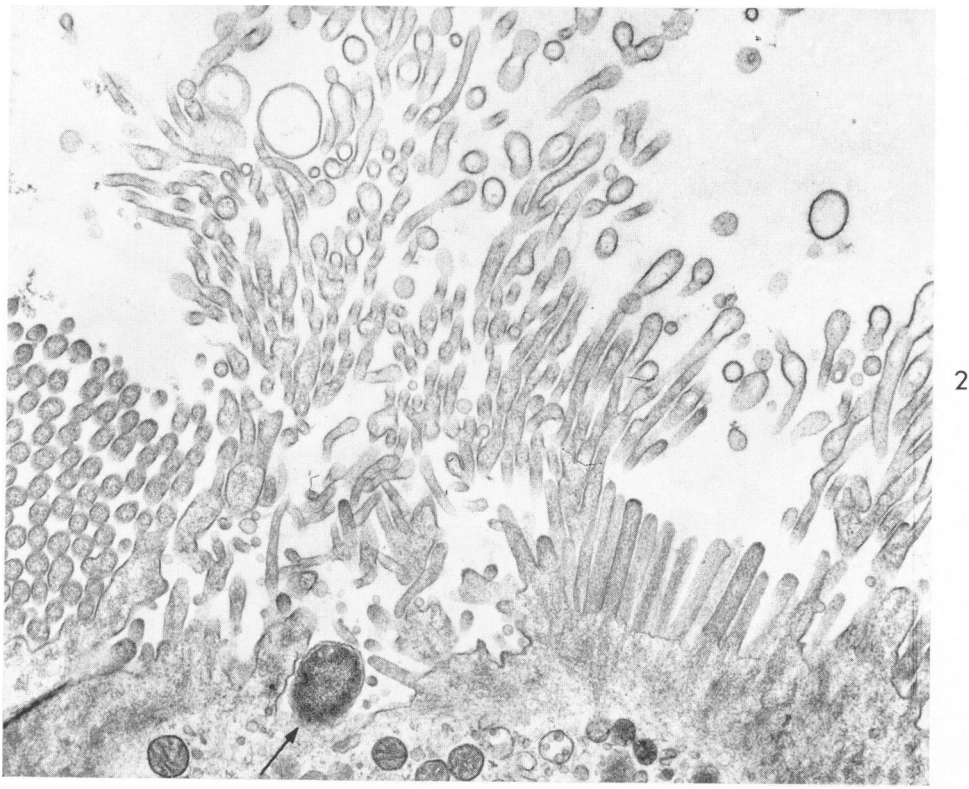

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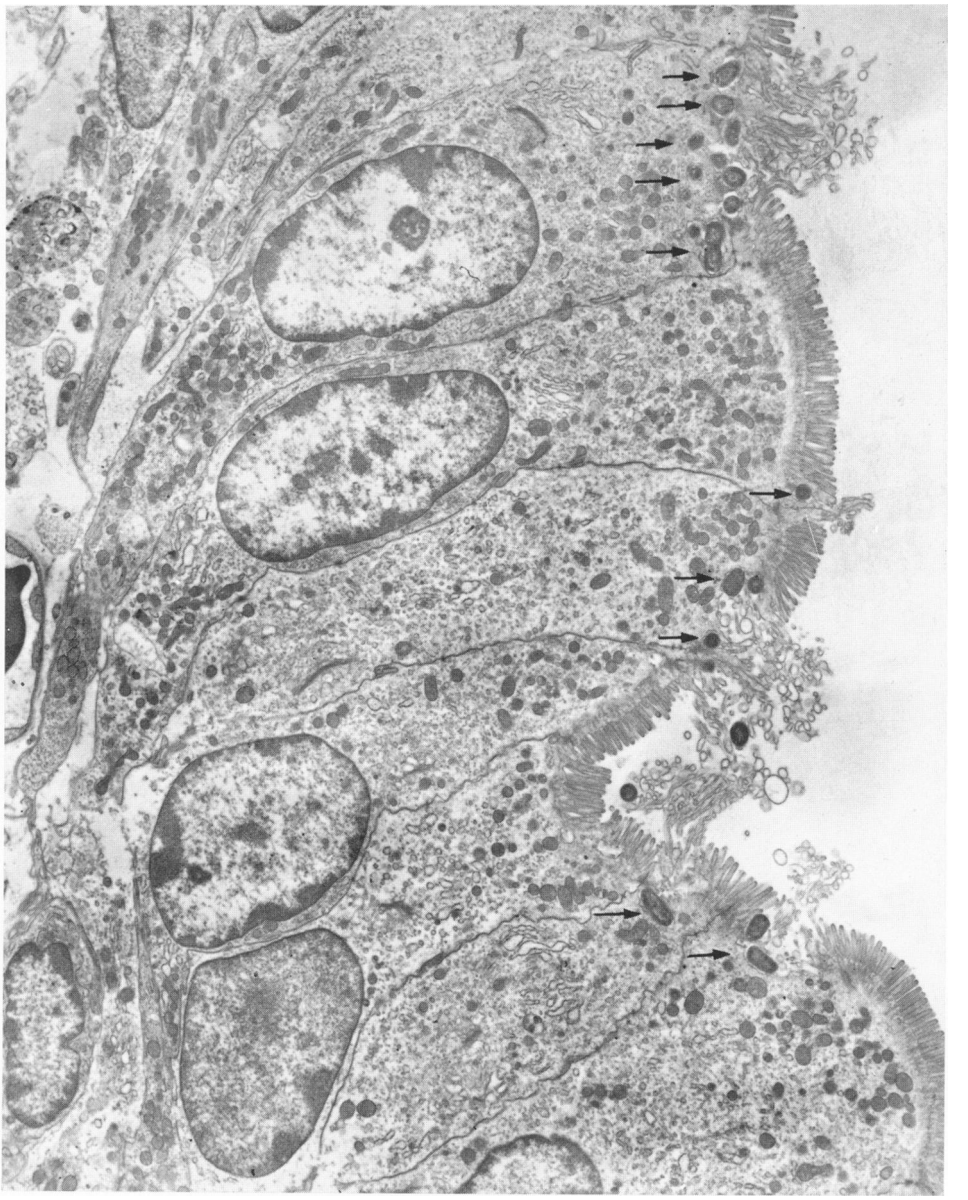
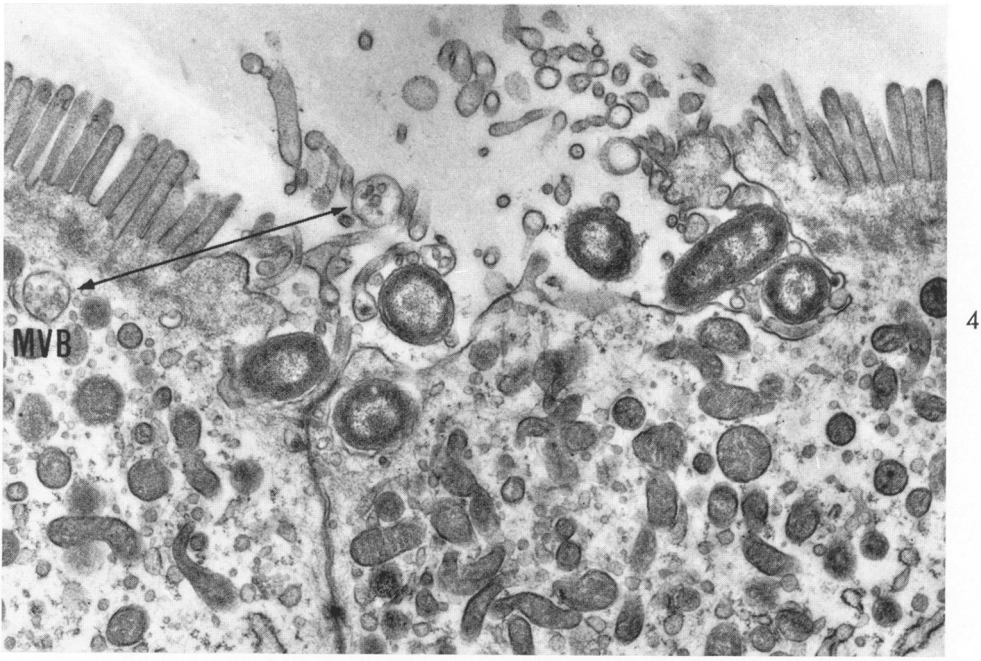


FIG. 3. Extensive degeneration of the microvilli, terminal web and apical cytoplasm appears at sites of bacterial penetration (arrows). Other cytoplasmic organelles and adjacent cells are unaltered. × 3,200.

FIGS. 4 and 5. The microvilli, terminal web and apical cytoplasm are replaced by a shallow (Fig. 4) and a deep cavity (Fig. 5) in which degenerated microvilli, blebs and vesicles are present. The adjacent intercellular tight junctions are laterally displaced. The arrow indicates a striking similarity between a multivesicular body (MVB) and a bleb containing small vesicles. The remaining cytoplasmic organelles are intact. Refer to Text-figure 1.3A. × 12,000.



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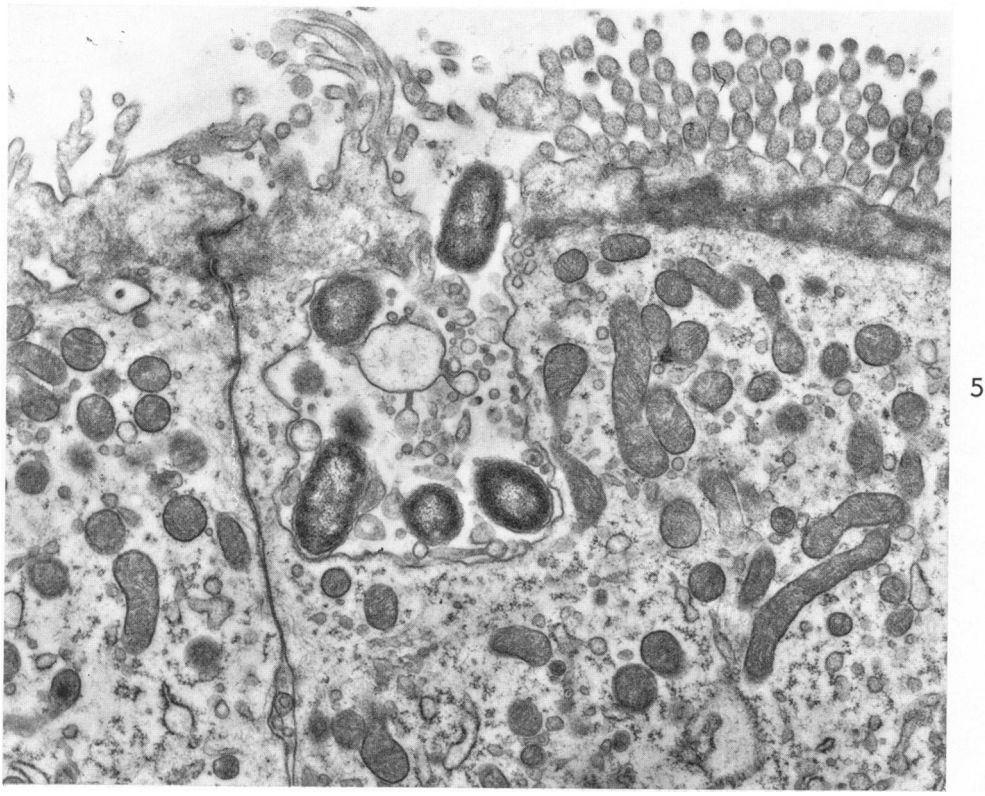

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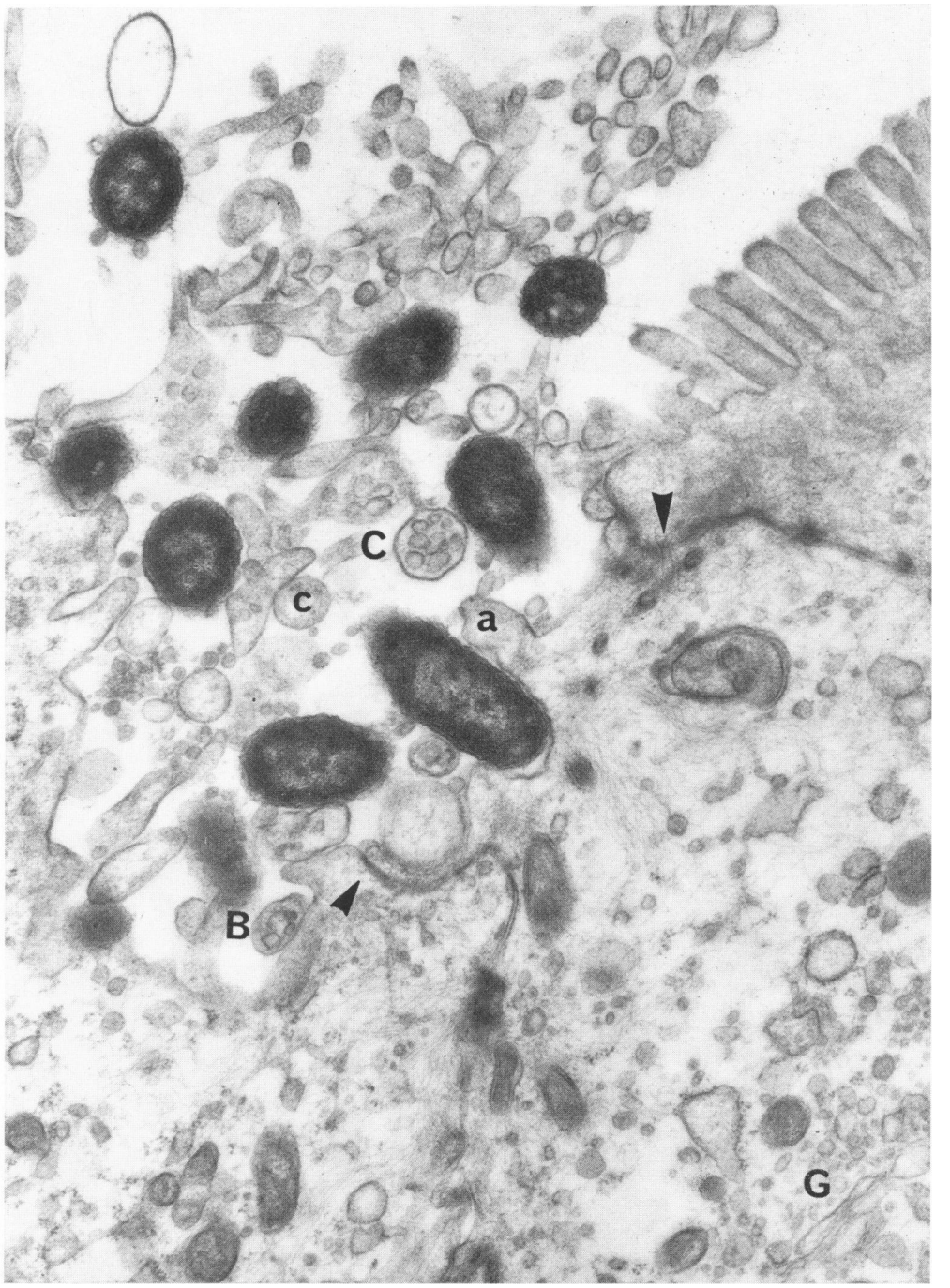

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FIGS. 6 and 7. Bleb-like projections with (A) or without (a) vesicles arise from the host cell cytoplasm and are pinched off (B,C and b,c) into a cavity which also contains degenerating microvilli. Blebs with vesicles (A,B and C) are strikingly similar to the multivesicular body seen in Figure 4. There are increasing numbers of small vesicles around the Golgi (G). An intercellular junctional complex is laterally displaced (arrow). Corresponds to Text-figure 1.4A. × 27,000.

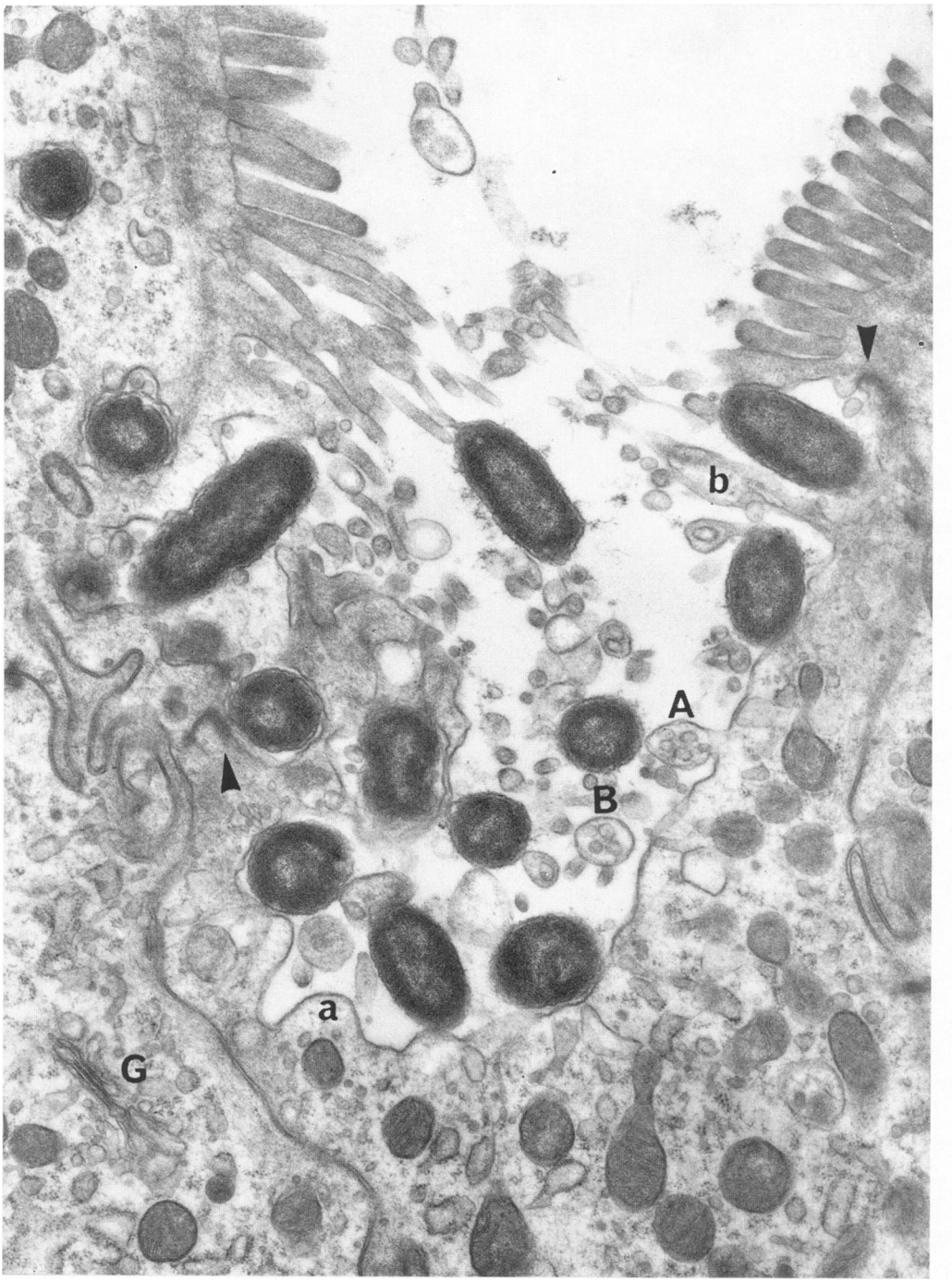

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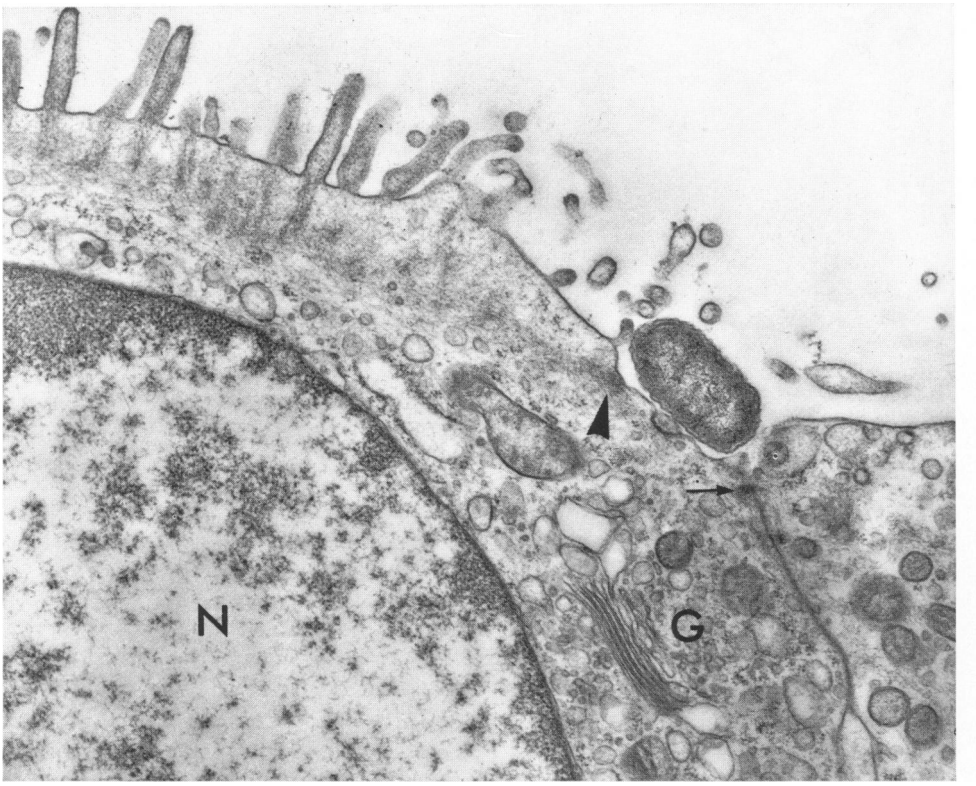
FIG. 8. A bacterium lies close to a junctional complex in which a desmosome (thin arrow) is present. The terminal web (thick arrow) of the cell at the left ends abruptly at the lumen surface. The corresponding terminal web of the cell at the right is absent. The degeneration of microvilli and apical cytoplasm is more severe than that seen in Figure 3. Golgi (G), nucleus (N). (Text-fig. 1.3B). × 15,000.

FIG. 9. A bacterium appears in a cavity formed by two lateral plasmalemmas of two adjacent cells. The terminal web as well as the terminal bar (arrows) are interrupted by the cavity containing a bacterium. (Text-fig. 1.4B). × 33,000.



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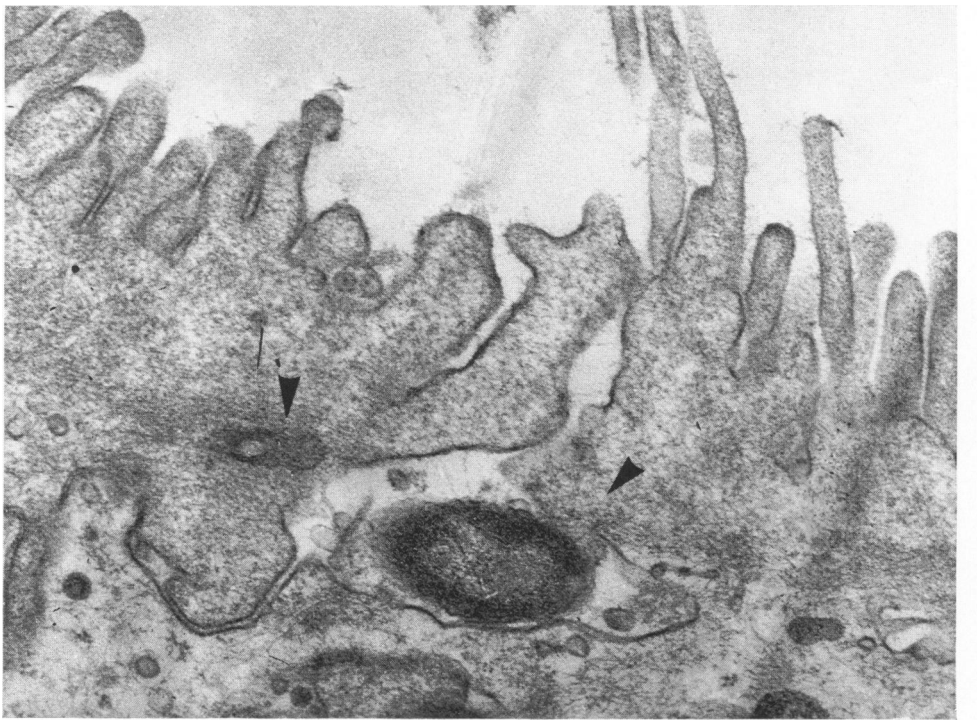

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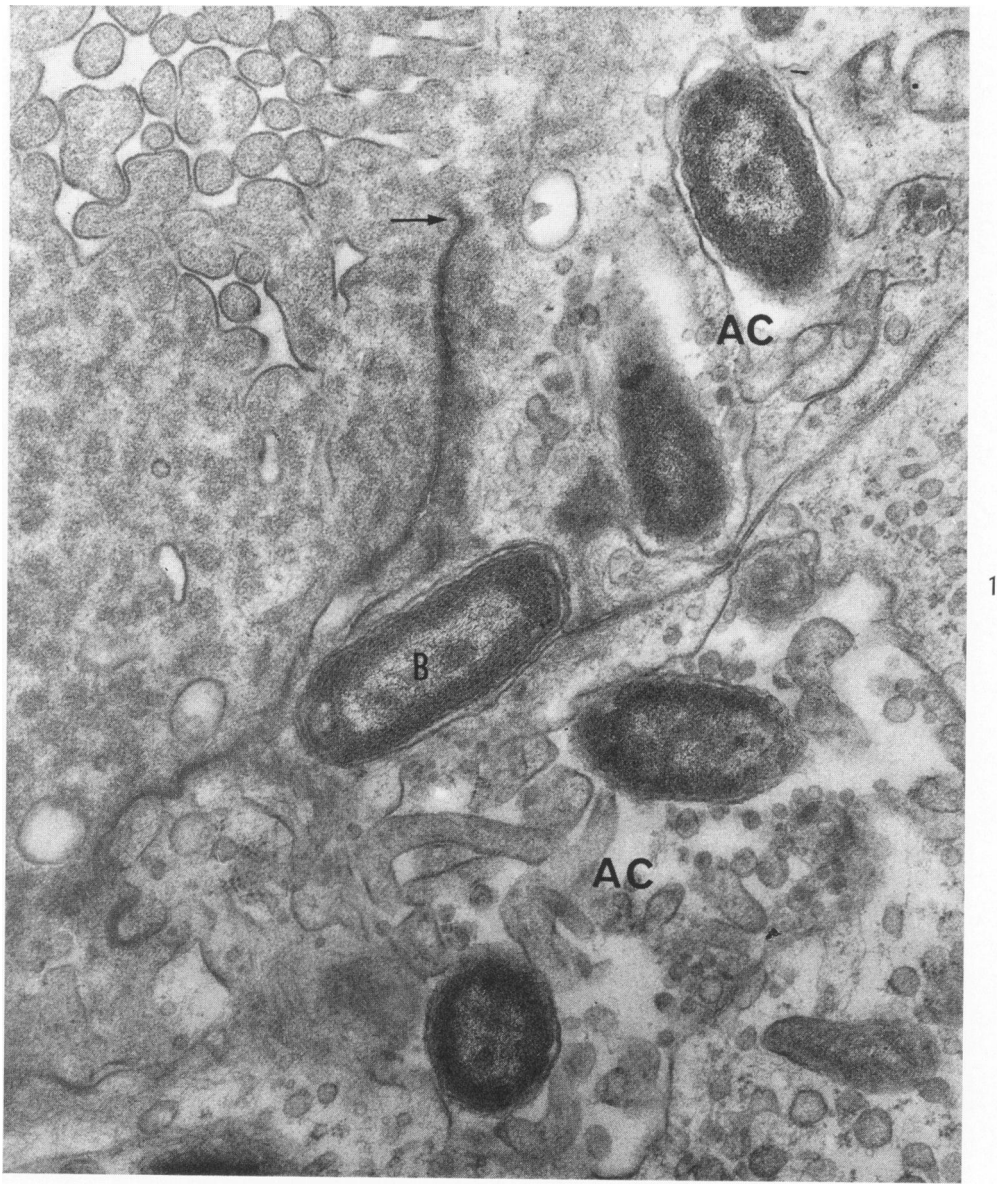
FIG. 10. An oblique section through the apical cytoplasm shows a bacterium (B) tightly enclosed by the lateral plasma membrane of two neighboring epithelial cells. The adjacent cavity (AC), enclosed by a single membrane, contains bacteria, remnants of microvilli, blebs and vesicles. In the left upper corner, the central fibrillar cores of microvilli and intercellular tight junction (arrow) are seen in a cross section. The cytoplasm contains more vesicles than are shown in Figure 1. (Text-fig. 1.5B). × 32,000.



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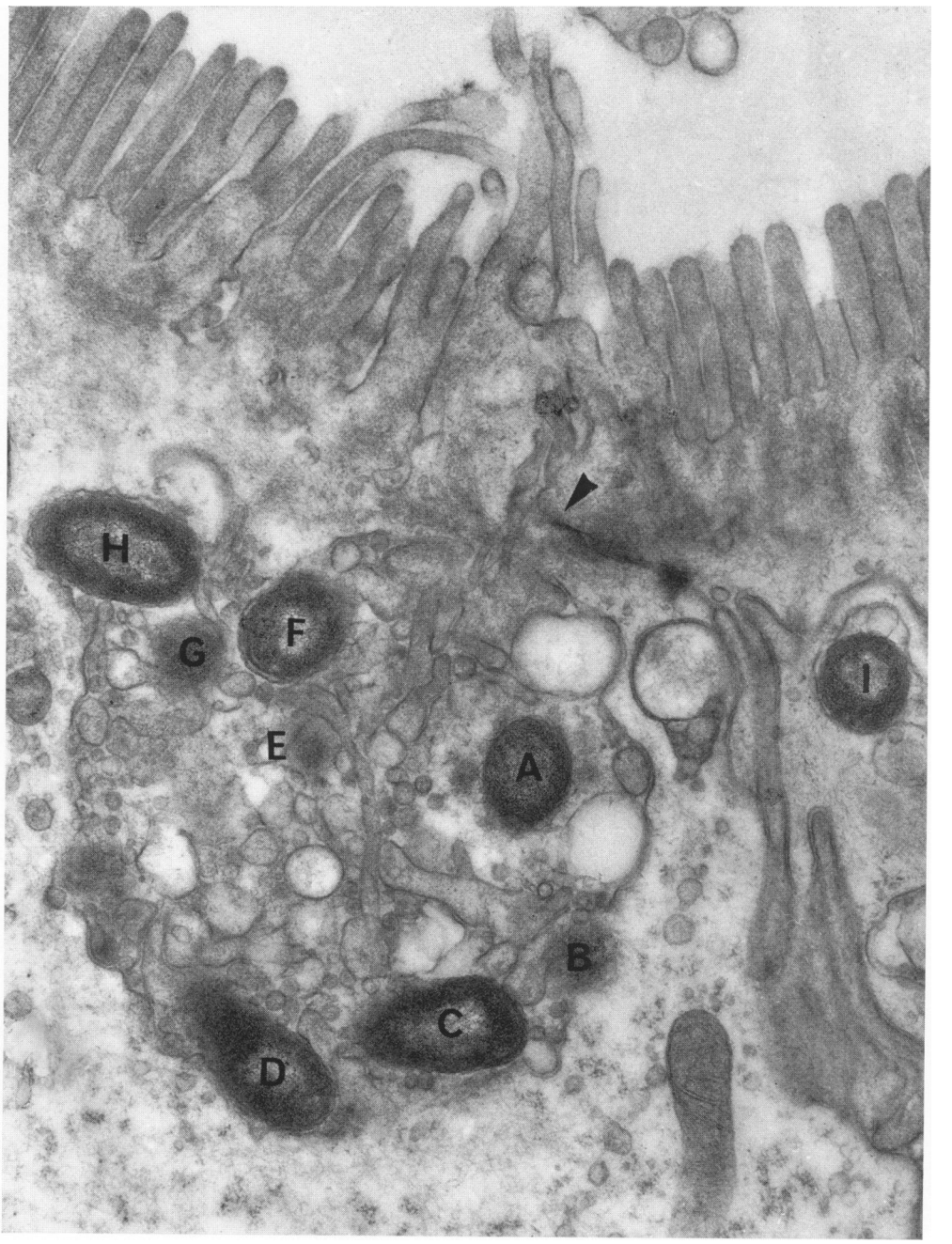

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FIGS. 11 and 12. The same membrane-enclosed cavity is shown cut at different levels. The cavity is formed by the lateral plasmalemmas between two adjacent host cells and is continuous with a displaced intercellular junctional complex (arrow). The complexity of the interdigitated and invaginated lateral plasmalemmas is noteworthy. There are degenerated microvilli, blebs and vesicles. A–a, B–b, C–c, D–d, E–e, F–f, G–g, H–h, and I–i represent identical organisms. × 31,000.

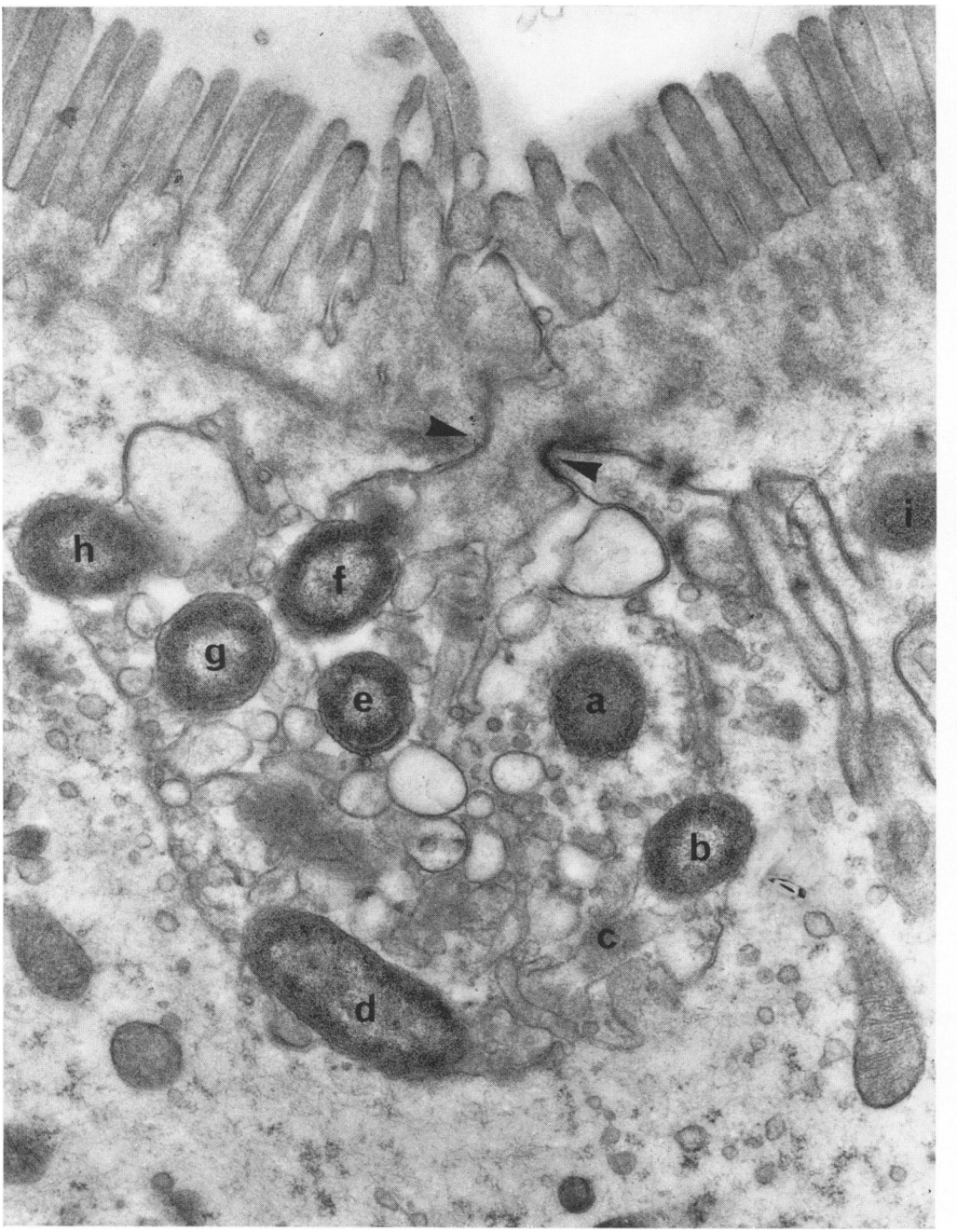

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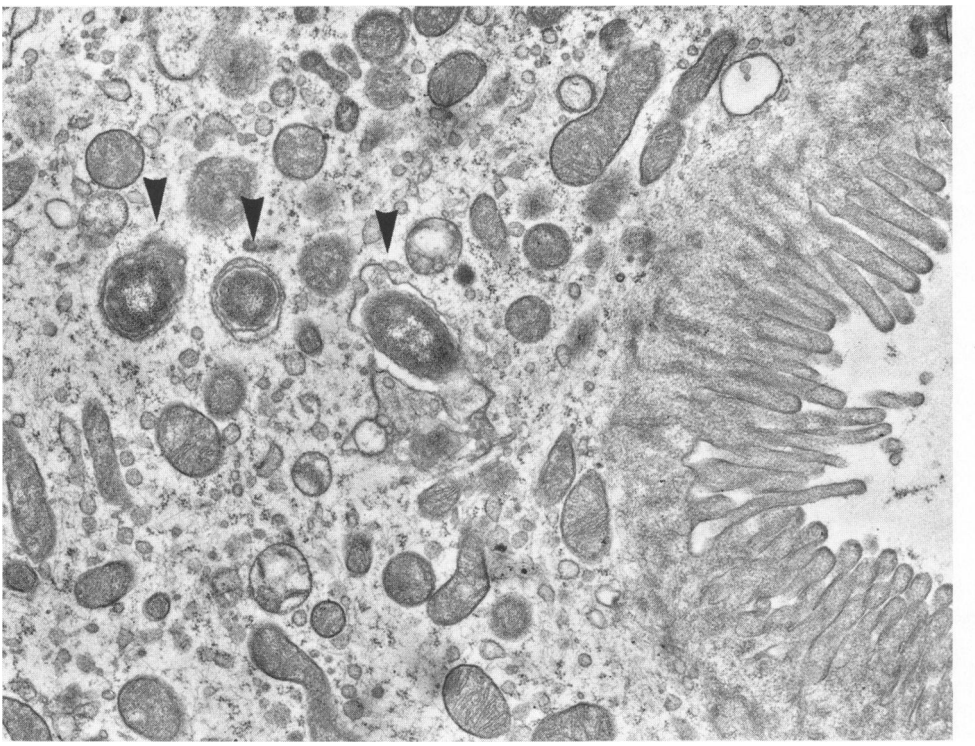
FIG. 13. In the apical cytoplasm, bacteria lie within single membrane-bound enclosures (arrows). The bacterium-containing vacuole at the right contains osmiophilic material and few vesicles; it corresponds to Text-figure 1.5A. In contrast the other two bacterium-containing vacuoles are reduced in size and contain only osmiophilic material. The overlying microvilli and terminal web are not yet completely reconstituted. (Text-fig. 1.6A). × 11,000.

FIG. 14. An aggregate of several organisms, vesicles and dense osmiophilic material is enclosed within a single membrane-bound vacuole which is indistinct at the right margin (arrow). Reconstitution of the overlying brush border is almost complete. (Text-fig. 1.6B). × 10,000.



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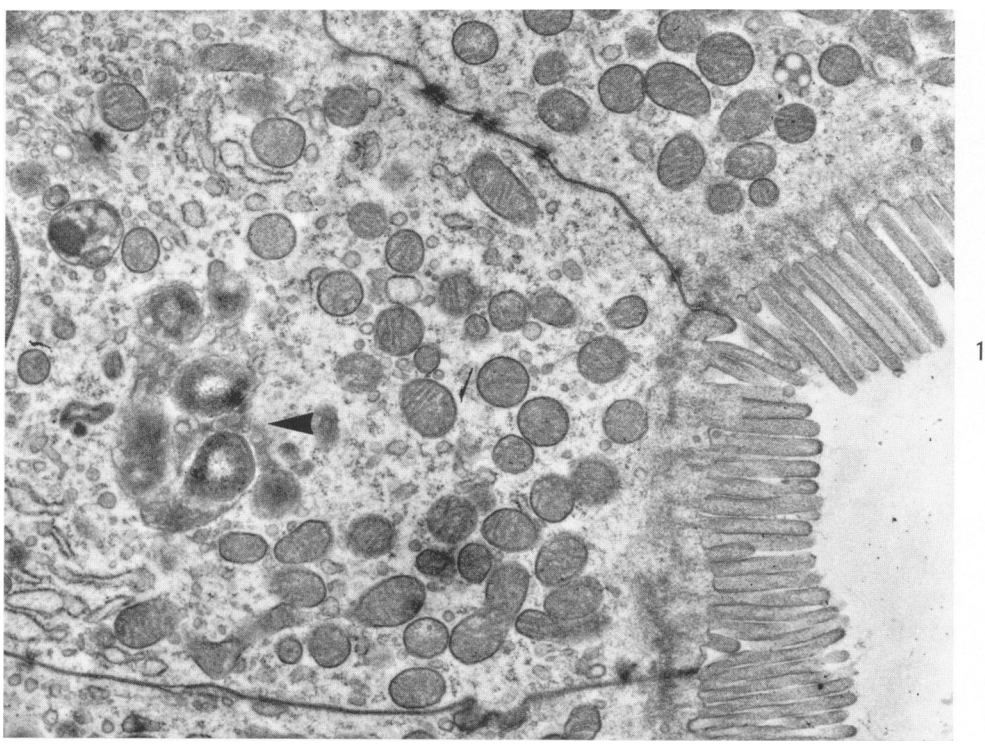

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FIG. 15. A membrane-bound body, at the supranuclear region contains bacteria (arrows) and various cyto-components including mitochondria, endoplasmic reticulum, ribosomes, small vesicles and fragments of membranes. Golgi (G). (Text-fig. 1.6C). × 15,000.

FIG. 16. A membrane-bound vacuole at the supranuclear region contains bacteria and osmiophilic material of increased density. (Text-fig. 1.6A). × 27,000.

FIG. 17. Bacteria at the subnuclear region are tightly enclosed by a single membrane. (Text-fig. 1.7A). × 15,000.

FIG. 18. Bacterial disposition is shown at the subnuclear region. Several organisms are surrounded by dense osmiophilic material. (Text-fig. 1.7B). × 30,000.

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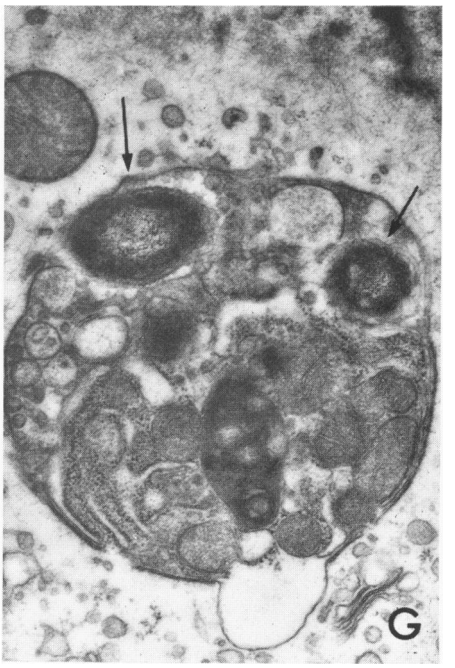

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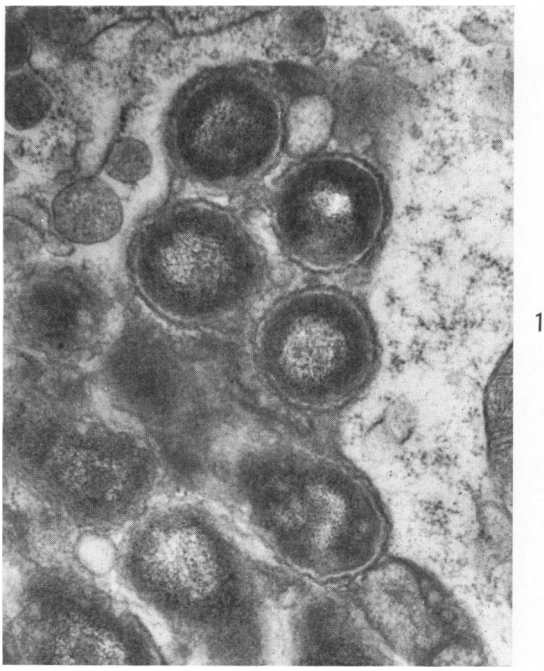

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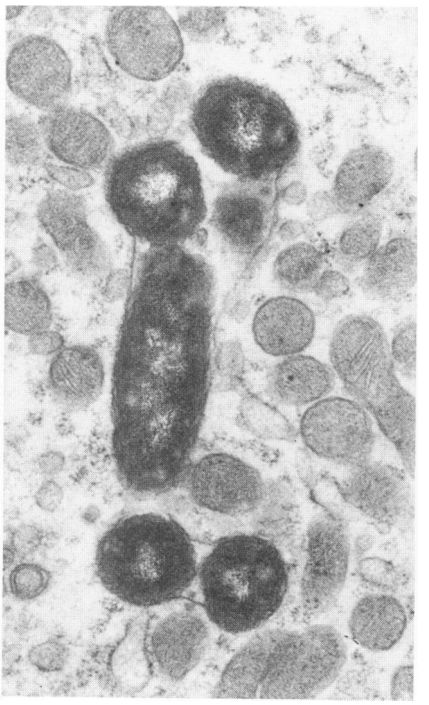

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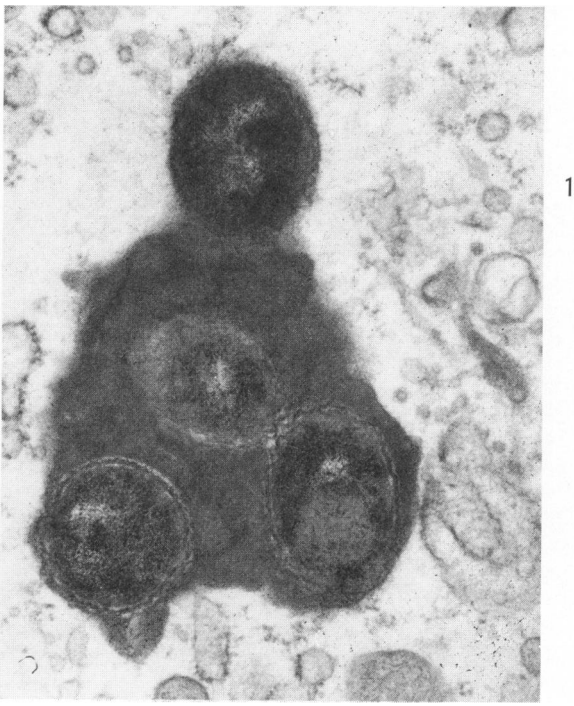

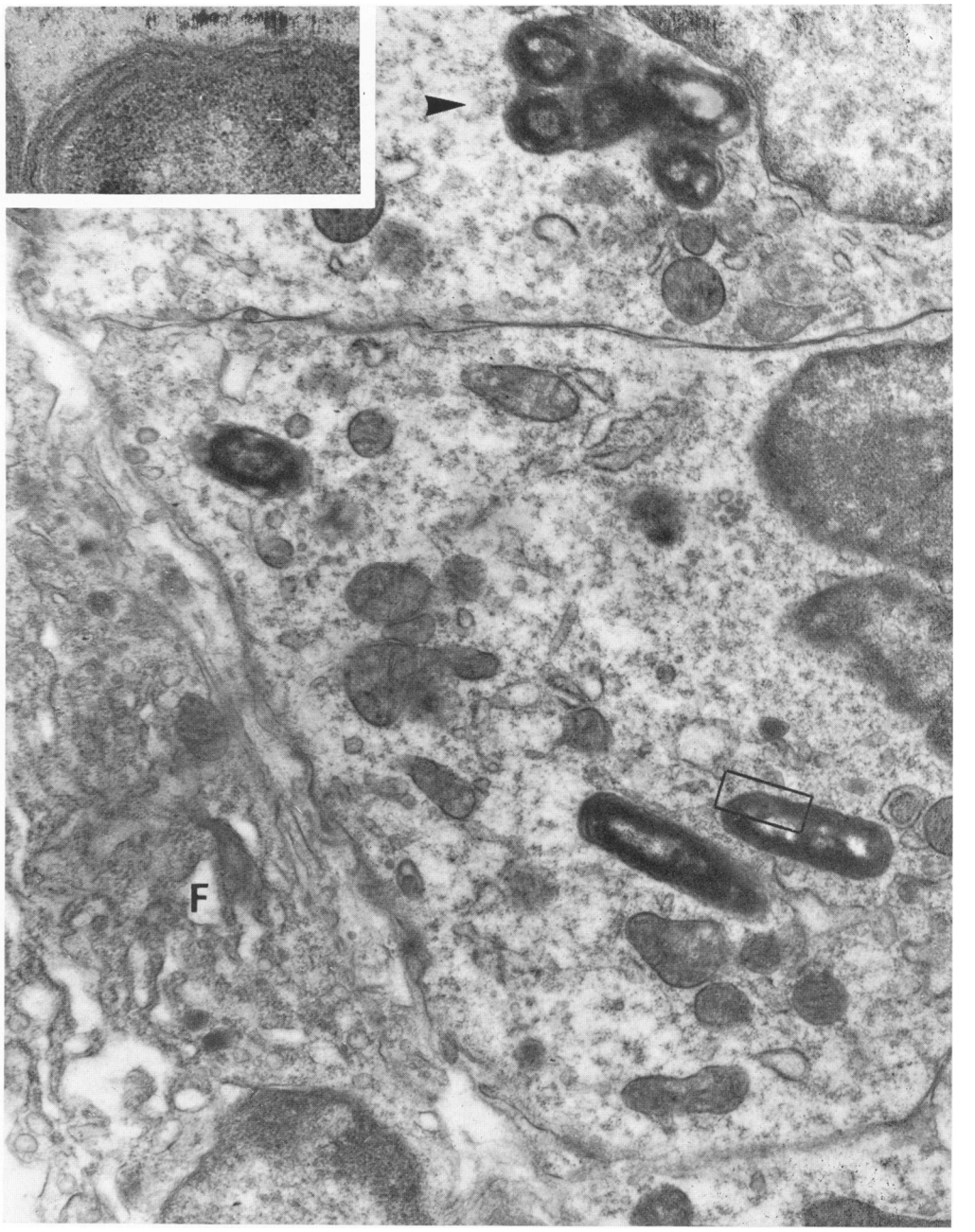

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FIG. 19. At the basal portion, groups of bacteria (arrow) are surrounded by osmiophilic material and tightly enclosed by a single membrane. × 12,000.
Inset. The relationship between the enclosing single membrane and a bacterial cell wall is demonstrated. A fibroblast (F) is apparent below the delicate basement membrane. × 75,000.