EXPERIMENTAL BACILLARY DYSENTERY

An Electron Microscopic Study of the Response of the Intestinal Mucosa to Bacterial Invasion

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Primates are the only animal species naturally susceptible to shigellosis. This fact has greatly retarded our knowledge of the pathogenesis of bacillary dysentery. Although it has been established that the virulence of Shigella organisms can be equated with their power to invade and to multiply in host tissues, there exists as yet no sequential study of the morphologic events attending this invasion. In particular, we lack data at the ultrastructural level on the impact of bacterial invasion on the cells of the intestinal mucosa.

We investigated this problem in the Shigella-infected guinea pig because over the years we have accumulated a considerable body of information on shigellosis in this subject,[1-4] thus facilitating correlation of ultrastructural alterations with light microscopy observations and those obtained with fluorescent antibody staining techniques.

Conventionally raised guinea pigs are naturally resistant to an oral challenge with Shigella organisms. This resistance, however, is not absolute and the animals can be made susceptible to orally administered Shigella by starvation. Shigellosis in guinea pigs so modified, is a rapidly fatal disease with death usually occurring within 48 to 72 hours. The experimental infection resembles fulminating infection in human subjects as is seen in the Ekiri syndrome.[5] The small intestine is involved early, a feature also of certain forms of human shigellosis including the Ekiri syndrome.[6]

In this study we have emphasized the cellular alterations of the gut mucosa incident to enteric infection and related these findings to the tissue response of the mucosa as a whole.

Material and Methods

Culture Preparation. S. flexneri 2a, strain 2457,[1] was used. This virulent strain has been maintained in our laboratory in the lyophilized state. Fresh cultures derived from ampules of lyophilized organisms of this strain were used for each experiment. The organisms were grown on meat extract agar plates for 18 hours, and were sus-

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pended in brain-heart infusion broth in a concentration of approximately 10^8 viable cells per 10 ml of broth.

Technique of Infection. Hartley strain guinea pigs of either sex and weighing 300 to 400 gm were assigned at random to 5 groups of 10 animals each. Prior to oral bacterial challenge, 4 groups were deprived of food for 4 days, but allowed water. The remaining group served as "non-starved" controls. The animals in this group were sacrificed at the same time as the starved controls.

Three groups of starved guinea pigs were infected, each animal receiving 10 ml of infectious broth by stomach tube. One ml tincture of opium was administered intraperitoneally immediately after challenge to inhibit peristaltic movement.[3] These 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; virulence controls were, however, employed.

The remaining group of starved animals served as "starved-controls," each animal receiving sterile broth and opium. These animals were 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. The guinea pigs were killed by cervical dislocation and necropsied in a conventional manner. A segment of ileum was removed and processed as follows:

(1) For fluorescent antibody studies a 5 cm portion was slit 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 *S. flexneri* 2a globulin. The details of the procedure were those used by LaBrec and Formal.[2]

(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), Giemsa, Alcian blue and Feulgen stains.

Thin sections of 1 to 2 μ of plastic embedded material were also stained with azure II and methylene blue or PAS.

(3) For electron microscopy (E.M.) studies (RCA 3G) a third portion of fresh ileum was cut into small fragments, fixed with 1.33 per cent osmium tetroxide in S-collidine buffer [7] for 2 hours, dehydrated in water soluble epoxy resin [8] and embedded in Araldite. Ultrathin sections were stained with lead hydroxide and were examined at an initial magnification of $\times$ 2,000 to $\times$ 20,000.

Critique of Technique. The electron microscopic investigation of a dynamic pathologic process has a built-in sampling problem. In our study the intensity of the inflammatory response varied from animal to animal and even at 24 hours after challenge the inflammatory lesion in the ileum of individual animals was not entirely uniform. We established the range of lesions and a scale of progression of the overall responses of the ileum by first studying fluorescent antibody stains and paraffin-embedded preparations. A corresponding range of lesions was then searched for in 1 to 2 μ sections of plastic embedded material. From these blocks a final selection of tissues was made for electron microscopy.

Bacilli identified by electron microscopy were assumed to be Shigella organisms. We had previously established the fact that under the conditions of the experiment only these organisms penetrated the epithelial lining. The magnitude of the invasion was judged on the basis of their specific fluorescent antibody staining in adjacent sections.

Results

Effect of Starvation. Starvation had no effect on crypt cells in the guinea pig ileum. The absorptive cells on the villi showed a slight dilatation of canaliculi in both the endoplasmic reticulum and the Golgi

apparatus (Figs. 1a and b). Otherwise the fine structure of absorptive cells in the starved guinea pig control specimens was very similar to that reported for the jejunum in fasted rats.[9] Likewise, the ultrastructural organization of crypt cells was comparable to that in the fasting human subject.[10] There was a slight reduction in the cellularity of the tunica propria, principally affecting the number of lymphocytes. The reticular framework was readily discernible (Figs. 2 and 4). The most conspicuous cell type was the macrophage (Figs. 2 and 3), which in specimens from starved control animals occasionally contained complex membrane-bound structures which might have represented phagocytized whole cells.

Effect of Bacterial Challenge. Oral challenge with *Shigella flexneri* elicited an acute enteritis which progressed with time. As early as 8 hours, there was an ileitis, manifested by dilatation of capillaries and venules and by focal infiltrates of neutrophils and macrophages distributed at random. No alterations in the epithelium or an excessive discharge of mucus were noted.

Frozen sections treated with fluorescent antibody (FA) disclosed moderate numbers of specifically fluorescing Shigella organisms in the ileal lumen and rarely an occasional bacillus within epithelial cells. The number was so small, however, and the distribution of organisms so irregular that attempts to demonstrate intra-epithelial localization of bacilli electron microscopically were unsucessful. Similarly, at 12 hours after challenge, the invading organisms could not be found within epithelium, although FA-treated sections revealed increased numbers of intra-epithelial organisms. Also, at this time, fluorescing Shigella organisms were seen in the lamina propria. Large numbers of dysentery bacilli were observed in the intestinal lumen. Light microscopy showed an increase in the inflammatory cellular exudate and in vascular hyperemia; this was present in all 10 animals in this particular group. Neutrophils and some extravasated red cells were observed in the mucosa and in the lumen as well. Separation of epithelial cells and widening of intercellular spaces occurred along the sides of the villus which in contrast to the villus crest is normally free of this phenomenon (Fig. 5). Goblet cells were reduced in number and size. Feulgen-positive inclusions occurred in increased numbers in the epithelial cells at the apex and midportion of the villi (Fig. 6); otherwise the chief cells of the intestinal lining were little affected except for the striated border which in PAS stained preparations demonstrated a slight reduction in height and staining intensity after the 8-hour stage. This was confirmed electron microscopically; the height of the microvilli tended to become more uniform measuring an average of approximately 1.4 μ,

while in the controls, depending on the position of the epithelial cell on the villus, the length of the microvilli varied from 1.46 μ at the base, to 1.9 μ at the mid-villous portion and 1.08 μ at the villus tip.[11]

At the 24-hour stage the conventional bacterial and specific FA stains revealed many bacilli in the sections. By this time the enteritis, which originally had a distinct patchy character, had become nearly confluent. The reaction in the tunica propria (Figs. 7 to 10) was characterized by an exudative response in which both cells and vasculature participated, and which was accompanied by a markedly increased transepithelial migration of inflammatory cells and cellular debris (Figs. 14 to 16). Numerous inflammatory cells were evident in greatly enlarged intercellular spaces adding to the further expansion of these spaces, thus affecting many of the desmosomes. In the intestinal lumen a greatly increased number of bacilli either lay free, singly or in clumps, or within desquamated epithelial and phagocytic cells (Fig. 13).

Leukocytes and macrophages packed the tunica propria. Degranulated leukocytes were readily observed. Macrophages were greatly increased in size and contained numerous and complex inclusions (Figs. 9 and 17). The phagocytes were, in general, well preserved although their acid phosphatase activity was distinctly decreased when compared with controls. (The assistance of Dr. H. Jervis of the Department of Experimental Pathology, Walter Reed Army Institute of Research, in providing the histochemical examinations is gratefully acknowledged.) Degenerative changes in inflammatory cells varied in degree and led to necrosis and secondary phagocytosis by macrophages. These changes also involved cell nuclei. The remaining structures in the tunica, nonmyelinated nerves, smooth muscle cells, fibrocytes and an occasional mast cell showed no significant alteration.

The villous architecture exhibited major changes; the villi were markedly irregular and there was a shift of the villus:crypt ratio with considerable foreshortening of villi. In some areas these changes had advanced to obliteration of the normal villous pattern with a corresponding severe loss of total epithelial surface area. Fusion of villi was frequently observed. Individual epithelial cells became smaller and more cuboidal than normal and in places even became flattened (Figs. 7 and 10). Extrusions from the villi occurred with abnormally high frequency and from unusual sites along the sides of the villi (Fig. 11). Cells were shed individually, in clusters (Fig. 12) and in strips which at times appeared to bridge the intervillous space. In contrast, cells in control specimens were extruded individually from the villus tips only. Micro-ulcers of the mucosa occurred with an irregular, patchy distribution. Emptying of goblet cells was pronounced. The crypt glands

were elongated and in places dilated because of blockage at the crypt orifices.

In the mucosa proper, dysentery bacilli lay both free and intracellularly in epithelial and inflammatory cells. They had a characteristic elongated, truncated shape, were bound by a delicate bacterial cell wall and showed a uniform, dense granular cytoplasm. Bacilli which had penetrated epithelium were either free, causing no noticeable alteration in the cytoplasm (Fig. 14), or were membrane-bound (Figs. 18 and 19). The membranes resembled phagosomes and were at times of a complex nature suggesting an active process of membrane formation, breakdown and re-synthesis. No free lying bacilli were noted with certainty within macrophages (Fig. 17) or leukocytes. Here bacteria always appeared membrane-enclosed (Fig. 20).

While all villus epithelium showed evidence of degenerative change, the intensity of the response varied slightly from area to area seemingly irrespective of the presence or absence of intra-epithelial organisms (compare Figs. 7 and 10). Where cytoplasmic changes were marked there was an overall loss of substance with corresponding loss of cytoplasmic components and particularly severe regressive alterations in the microvilli and mitochondria (Figs. 10 and 14). Changes were less severe in the chief cell nuclei which showed variable loss of chromatin pattern, nuclear swelling and irregular configuration with occasional obliteration of nucleoli. Cellular degenerative changes were accompanied by an increase in the number, size and complexity of membrane-bound cytoplasmic inclusions (Figs. 6, 14 and 16).

The process of epithelial extrusion in Shigella infection was altered in that it was initiated by a separation of a group of cells from the basement membrane and subsequent bulging of the cell mass into the lumen. The rounded shape of the cell aggregate appeared to be associated with two events occurring at the same time. First, the group of cells becoming detached from the basement membrane remained attached to each other at their lateral surfaces and to cells still connected to the basement membrane. The gap created by this detachment was closed by a progressive movement of epithelial cells toward the tip of the villus. The innate tendency of the intestinal epithelium to cover the basement membrane resulted in an extrusion of the group of detached cells. Secondly, the dislodged cells appeared to form new attachments to each other at their newly freed base resulting in a rounded structure which was preserved even when the mass was extruded into the lumen. The final separation occurred at the desmosome. This process must have been a fairly rapid one since the extruded complexes frequently contained well preserved epithelium and inflammatory

cells. The unusual mechanism of cell extrusion might have been responsible for the bridging between adjacent villi noted by light microscopy. Our observations failed to disclose why cell detachments occurred at any particular site or why groups of detached cells remained attached to each other.

The cytoplasmic degenerative changes described above were only rarely present and only to a minor degree in the epithelium at the bottom of the crypts; Paneth cells in particular were unaffected by the enteritis. Goblet cells did not accumulate mucus. There was no separation of crypt epithelium, although this was a very common feature in the epithelium abutting on the lumen. In the crypts the active transmigration of inflammatory cells was unaccompanied by the appearance of any intercellular spaces (Fig. 21).

Discussion

In the experimental model employed exudation of cells and fluid into the lamina propria was an early manifestation of enteric challenge with virulent Shigella organisms. Mucosal capillaries and venules were affected at 8 hours, the earliest period following challenge examined, coinciding with the initial demonstration of the organisms in the mucosa. Evidence of stasis and of increased vascular permeability to plasma protein preceded alterations of villous epithelium. A similar sequence of events has been established in human bacillary dysentery by Letterer,[12] who postulated that the mucosal necrosis and ulceration were not caused by direct action of Shigella toxin but were the consequence of a disturbance of circulation.

Our investigations have added to this concept. We noted degenerative changes in the villous epithelium with alteration of microvilli, appearance of dense bodies, sequestration of cytoplasmic components and dilatation of the endoplasmic reticulum and the Golgi apparatus. The changes were nonspecific and could have occurred as the result of tissue hypoxia in the wake of a circulatory disturbance. Similar cytoplasmic alterations were observed by Swift and Hruban[13] in response to a variety of substances specifically inhibiting some phase in the biosynthesis of protein, purine or cholesterol. It may thus be inferred that the same structural changes may be caused by different mechanisms.

An early and important manifestation of epithelial degeneration in our subjects was the loosening of villous epithelial attachment to the basement membrane and an intercellular separation leading to abnormal cell maladjustment. This is a phenomenon not restricted to this form of disorder. Abnormal and accelerated cell extrusions have been

observed in lethal intestinal virus infection in mice (LIVIM).[14] Ashworth and Chears noted increased intercellular separations in nontropical sprue and postulated that this could be a mechanism for excessive loss of intestinal epithelium in this condition.[15] We believe that we are dealing with a general biologic phenomenon, and that premature and abnormal cell separation is one facet of the response of the gut mucosa to injury. In the Shigella-infected guinea pig the extruding cells appeared capable of re-attachment to each other and also of bridging intervillus spaces. It appeared likely that the frequent fusion of villi and obstruction of crypt glands were attributable to this phenomenon.

The rapidly occurring dimunition in height of absorptive cells was due not only to a flattening caused by the tendency of intestinal epithelium to cover the villus surface even in the face of accelerated cell loss but also seemed to be the result of catabolic action and loss of cytoplasm.

Some aspects of the invasion of the intestinal mucosa by virulent micro-organisms appear elucidated. The Shigella bacillus is non-motile and yet within 12 hours it was transported in significant numbers into the tunica propria; no phagocytes were involved in this movement. The organisms were apparently moved by an interaction with cytoplasmic organelles, in particular the endoplasmic reticulum and Golgi apparatus, and with pinocytotic or digestive vesicles. The intensity of the cytoplasmic response was indicated by the frequency with which vesicular aggregates were formed and fragments of cytoplasm were included within membrane-bound vacuoles. The formation of an outer membrane was an active process in the affected epithelium and amounted to a sequestration of the intracellular parasite as well as a mechanism of transporting it. We have no indication that the direction of transport was always towards the tunica propria and to what extent the epithelium was capable of discharging bacilli into the intestinal lumen. In our subjects a sufficient number of organisms reached the tunica and eventually overcame the host. The lysosomal enzymes demonstrated in the lining epithelium of the rat ileum,[16] are presumably also present in the guinea pig. However, there was little recognizable enzyme effect on membrane-bound, intra-epithelial bacilli. Such an effect was unmistakable in organisms engulfed by macrophages and neutrophils.

The presence of bacilli in the tunica elicited a marked macrophage response with the formation or ribosome-packed inclusion bodies suggesting early immunologic stimulation. It is noteworthy that identical bodies were present within epithelial cells and inter-epithelial spaces. Their intracytoplasmic location in otherwise slightly altered cells suggests phagocytosis. It remains undetermined, however, whether these

bodies might also be formed by epithelium itself, and whether they fulfilled any function in epithelial metabolism.

The diversity in the composition of inclusion bodies in the Shigella-infected guinea pig was strikingly similar to Behnke's findings[17] in the fetal rat duodenum. He noted intra-epithelial inclusions with complex organizations during a particularly active phase of epithelial differentiation when the original stratified intestinal lining was undergoing villous transformation. This was accompanied by marked widening of the intestinal lumen. It would thus appear that under the stress of Shigella infection intestinal mucosa is capable of recapitulating alterations which occur during embryonic development. The various intracytoplasmic and inter-epithelial bodies are Feulgen-positive. Similar inclusions may, however, occur in a variety of inflammatory and neoplastic conditions and are occasionally seen in the normal intestinal mucosa.[18–22]

Trier, investigating the effect of methotrexate on the mouse jejunum, suggested the possibility of phagocytosis of lymphocytes by mucosal cells.[23] We have also noted the engulfment of mesenchymal cells by epithelium in Shigella enteritis, a phenomenon suggestive of phagocytosis. This, however, is less frequent than the appearance of inflammatory cells in intercellular spaces. Light microscopy cannot distinguish between the two and may give an exaggerated impression of phagocytosis particularly as the motile inflammatory cells press into adjacent epithelium causing an irregular infolding of the plasma membrane.

During the period of our experiment crypt epithelium was distinctly less affected by bacterial invasion than were cells on the villus surface. It is reasonable to assume that villus epithelium was more exposed to bacterial invasion. Fluroescent antibody procedures, however, demonstrated Shigella organisms in crypt cells. It is possible that in addition to differences in magnitude of bacterial involvement, the anatomic organization of crypt and villus epithelium as well as the relationship of these cells to their respective basement membranes and portions of the tunica propria might account for differences in their susceptibility. For instance, the expansion of the intercellular space so characteristic of mid-villus epithelium in the Shigella-infected animal was absent in the crypt area. The relative paucity of crypt cell changes at 24 hours was in marked contrast to the intensity and variety of responses elicited in villous epithelium by Shigella infection.

Summary

In experimental peroral infection of the preconditioned guinea pig, dysentery bacilli penetrated the intact epithelial lining and reached the tunica propria of the ileum in a matter of a few hours. It appeared that

the non-motile organism was transported passively through the epithelium by organelles related to the endoplasmic reticulum and Golgi apparatus which took the form of membrane-enclosed vesicles resembling phagosomes. The exudative inflammatory reaction in the tunica appeared early and preceded alterations in villus epithelium. The epithelial changes ranged over a wide spectrum from degenerative alterations, with severe loss of cytoplasmic compenents including microvilli, to evidence of heightened cellular activity comparable to that seen in the active fetal phase of intestinal development. The study throws additional light on the mechanism of abnormal sequestration of epithelium from the villus, its dislodgment and its role in the fusion of villi with blockage of crypts and overall architectural alteration.

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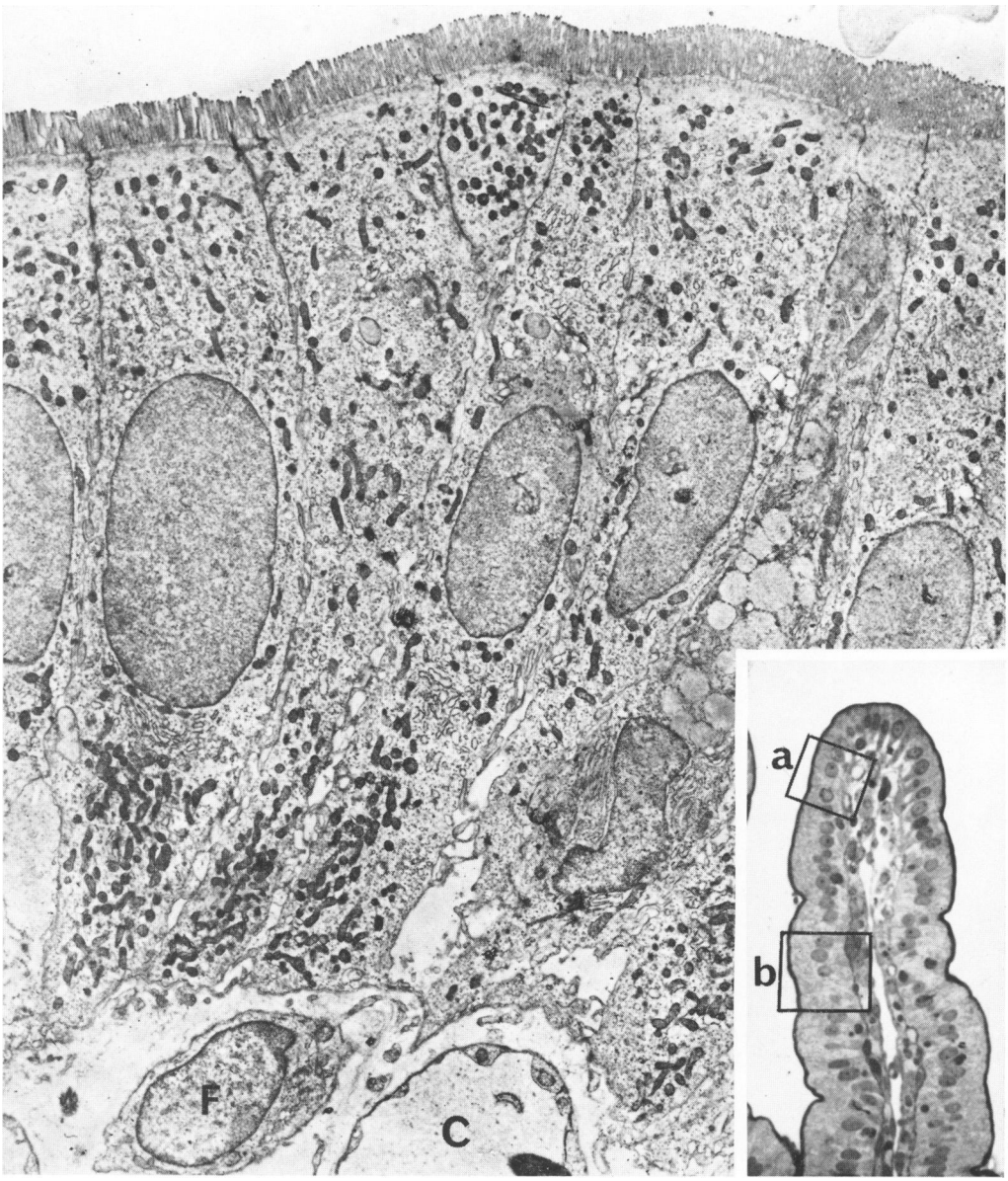
LEGENDS FOR FIGURES

FIG. 1. Ileum, starved, non-infected guinea pig. (a) The apical area of a villus corresponds to the area "a" in the inset which represents the adjacent thick section. × 230. Separations at the base and the formation of intercellular spaces between epithelial cells are apparent. Also shown is dilatation of the Golgi complex and occasional canaliculi of endoplasmic reticulum. There is an electron-lucid character to the interstices of the tunica and the inter-epithelial spaces. Fibroblast (F); capillary (C). × 2,400.



1a

FIG. 1. (b) Mid-villus corresponding to rectangle "b" in the inset. High columnar chief cells, slender microvilli and tightly interlocked, interdigitated lateral epithelial plasma membranes may be recognized. Profiles of the Golgi complex and occasional canaliculi of endoplasmic reticulum are dilated. A transmigrating neutrophil (N) shows distinct granules and a lymphocyte (L) appears between epithelial cells. The attachment of these cells to the basement membrane extends broadly. Elements of the tunica propria are loosely arranged although contact between cells is evident. Portion of a goblet cell discharging mucus (G); capillary (C); fibroblast (F); macrophage (M). × 2,400.

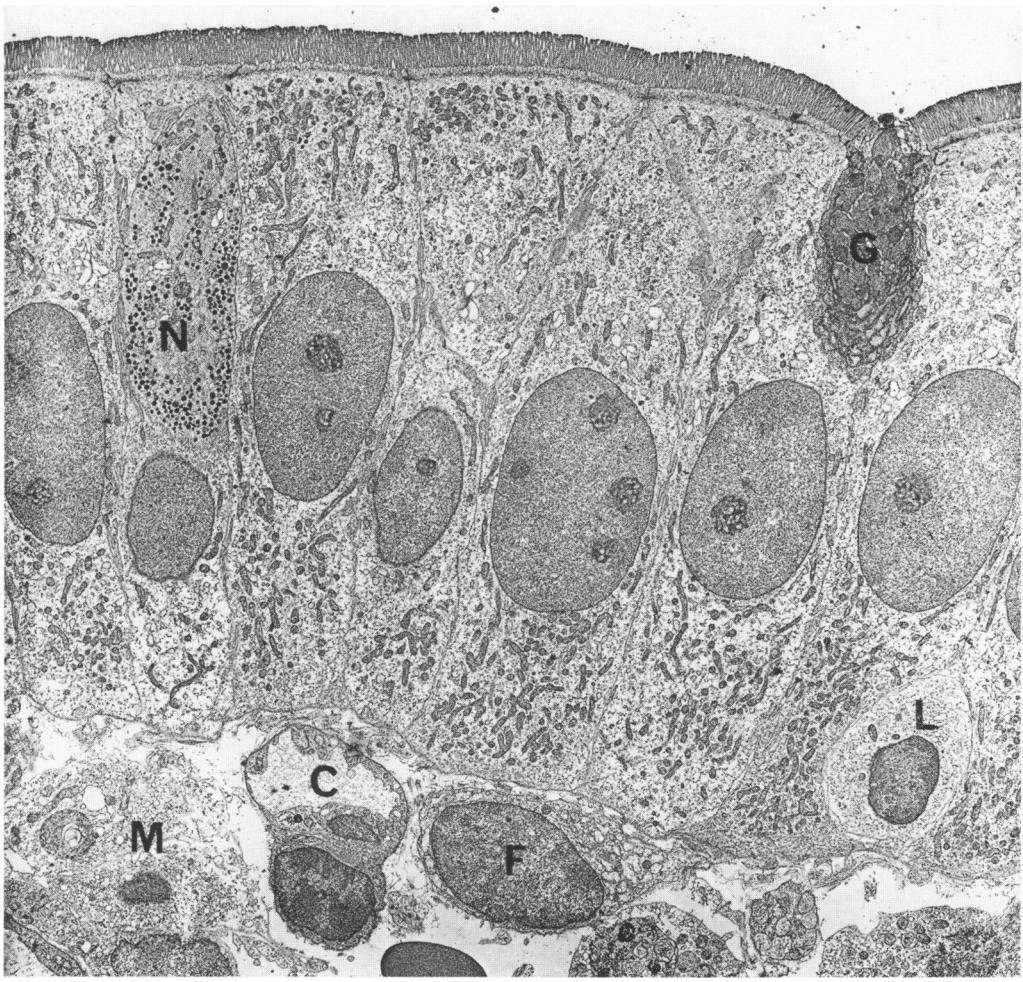


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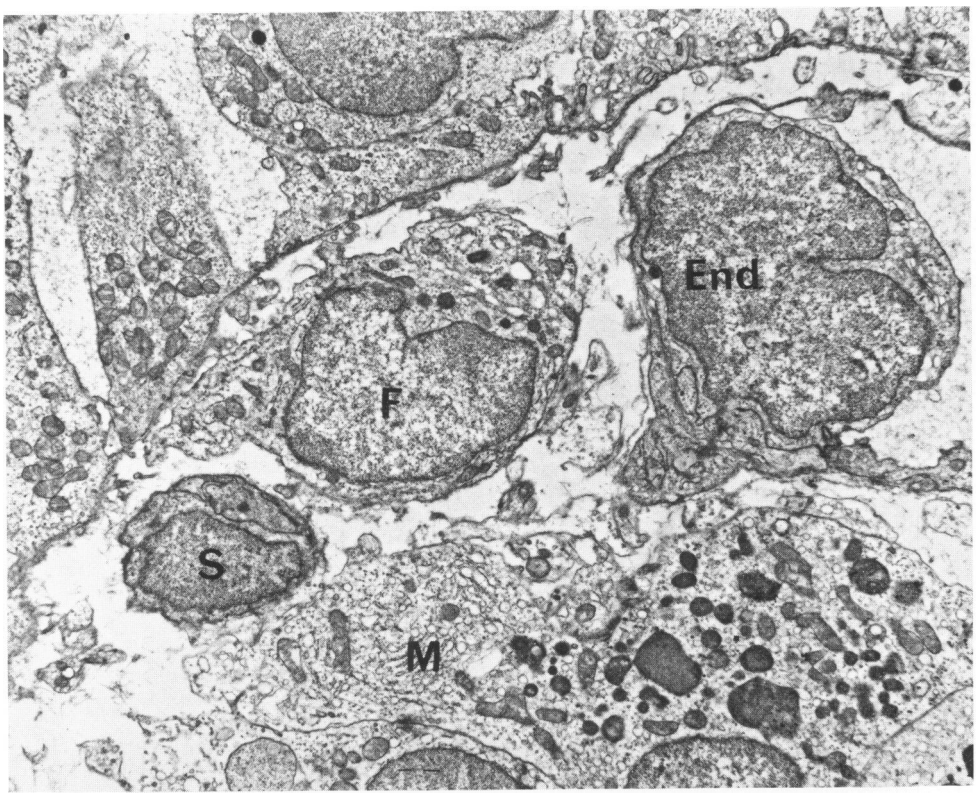
FIG. 2. Basal portion of an epithelial cell and subjacent tunica in a starved, non-infected control. Apical area. The basement membrane is intact and is in close proximity to a smooth muscle cell (S) and a capillary to the basement membrane. A cluster of macrophages (M) abuts upon the capillary. Characteristic inclusions (dense bodies), an absence of extracellular matrix and a delicate, fibrillar framework in the tunica are noteworthy. Endothelial cell (End). × 5,700.

FIG. 3. Tunica propria in the midportion of a villus in a starved, non-infected control. A portion of an enlarged macrophage is included. In the cytoplasm of this cell are several complex inclusions. This finding is uncommon in control specimens in which inclusion bodies often resemble those shown in Figure 2. Nucleus (NU); inclusion body (I). × 6,400.



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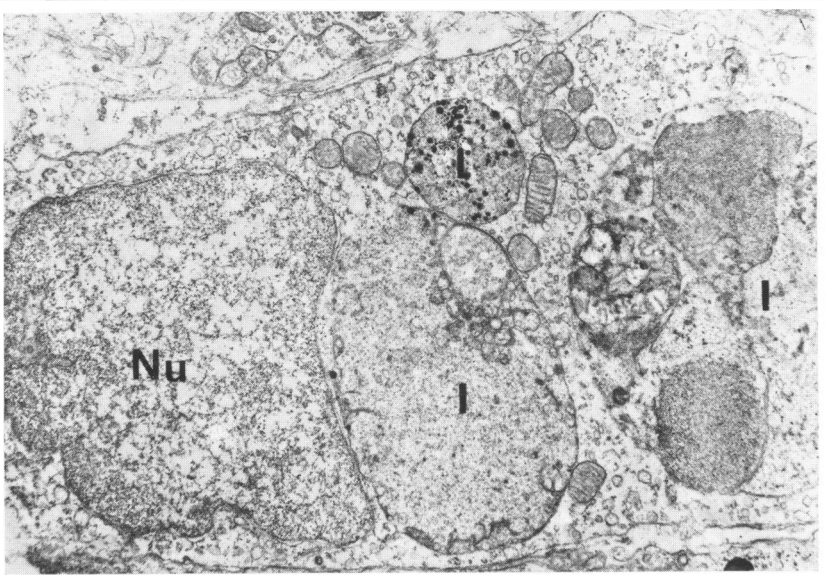

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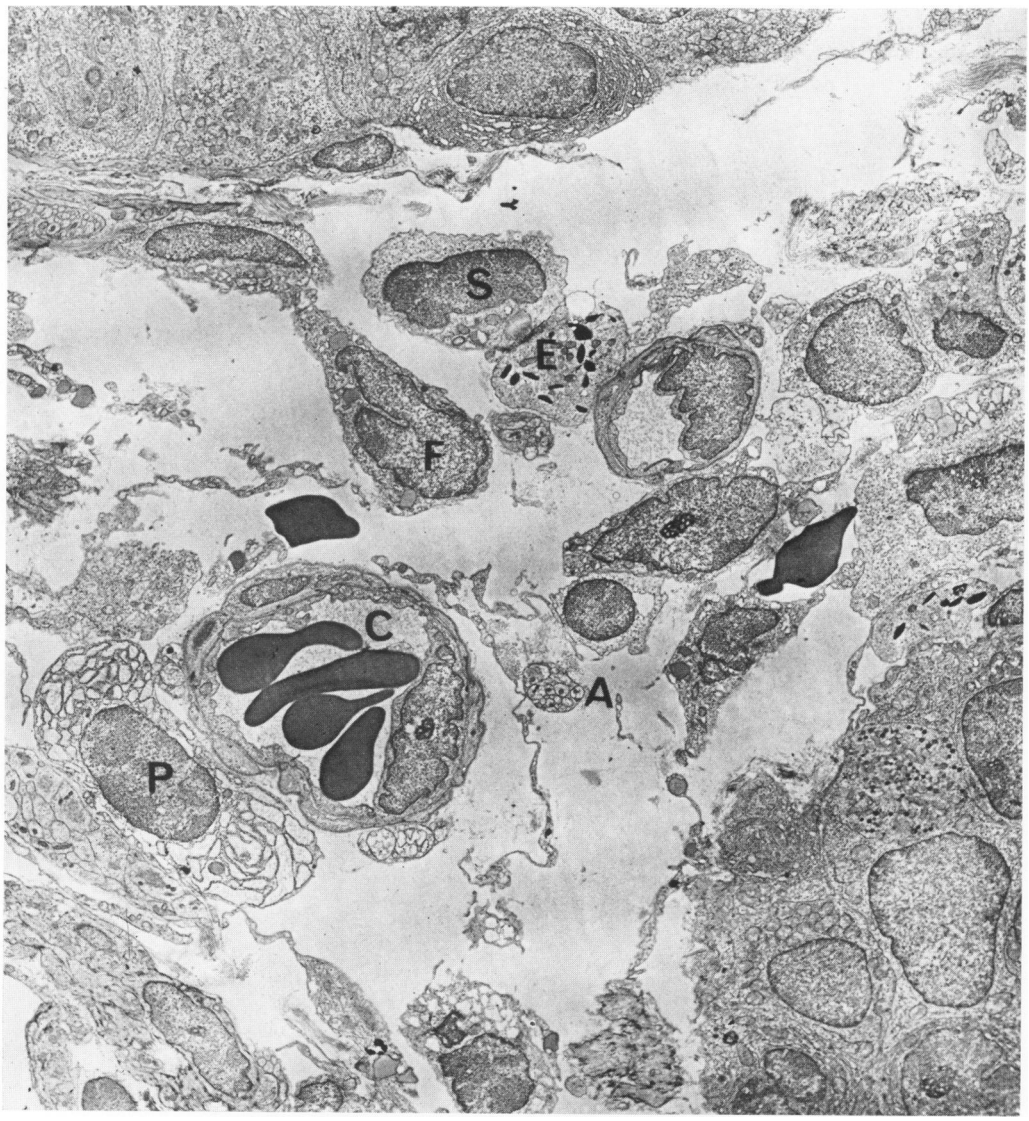

FIG. 4. A cross section through the lamina propria beneath a crypt in a starved, non-infected control. Portions of two crypt glands are seen in the upper left and lower right corner. The epithelium has a compact arrangement in contrast to the rather loosely packed structures in the tunica. Axon of non-myelinated nerve (A); capillary (C); fibroblast (F); plasma cell (P); eosinophil (E); smooth muscle cell (S). × 2,400.

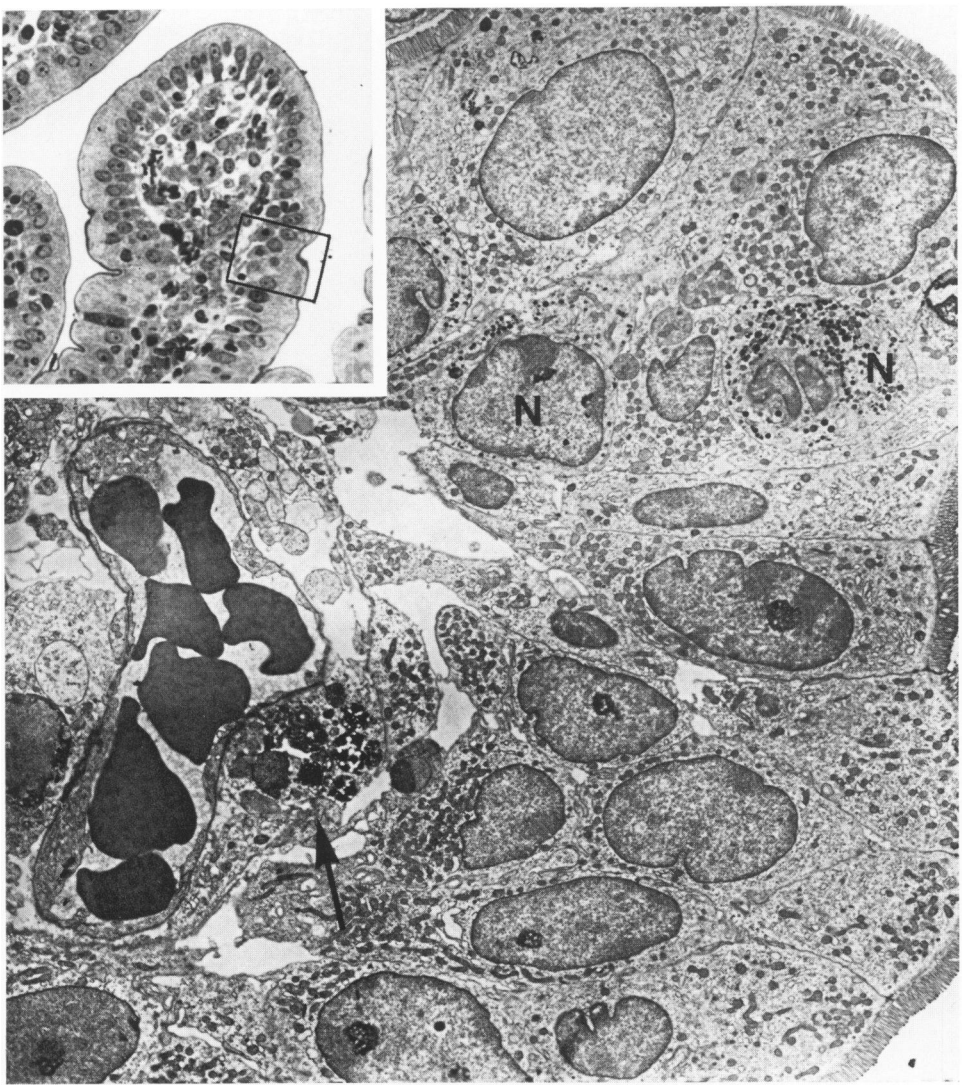

FIG. 5. Mid-villus portion in a Shigella-infected guinea pig at 12 hours post challenge. Inset of an adjacent thick section shows a swollen villus, intact epithelium and increased cellularity in the tunica propria. × 230. The E.M. section is oblique and shows increased separation of epithelial cells at their base with the formation of intercellular spaces between them; (compare with Fig. 1b). The basement membrane is intact. Arrow points to a macrophage in the process of migration through the basement membrane. Transmigrating neutrophils (N). Microvilli are shortened; (compare with Fig. 1a). × 2,200.

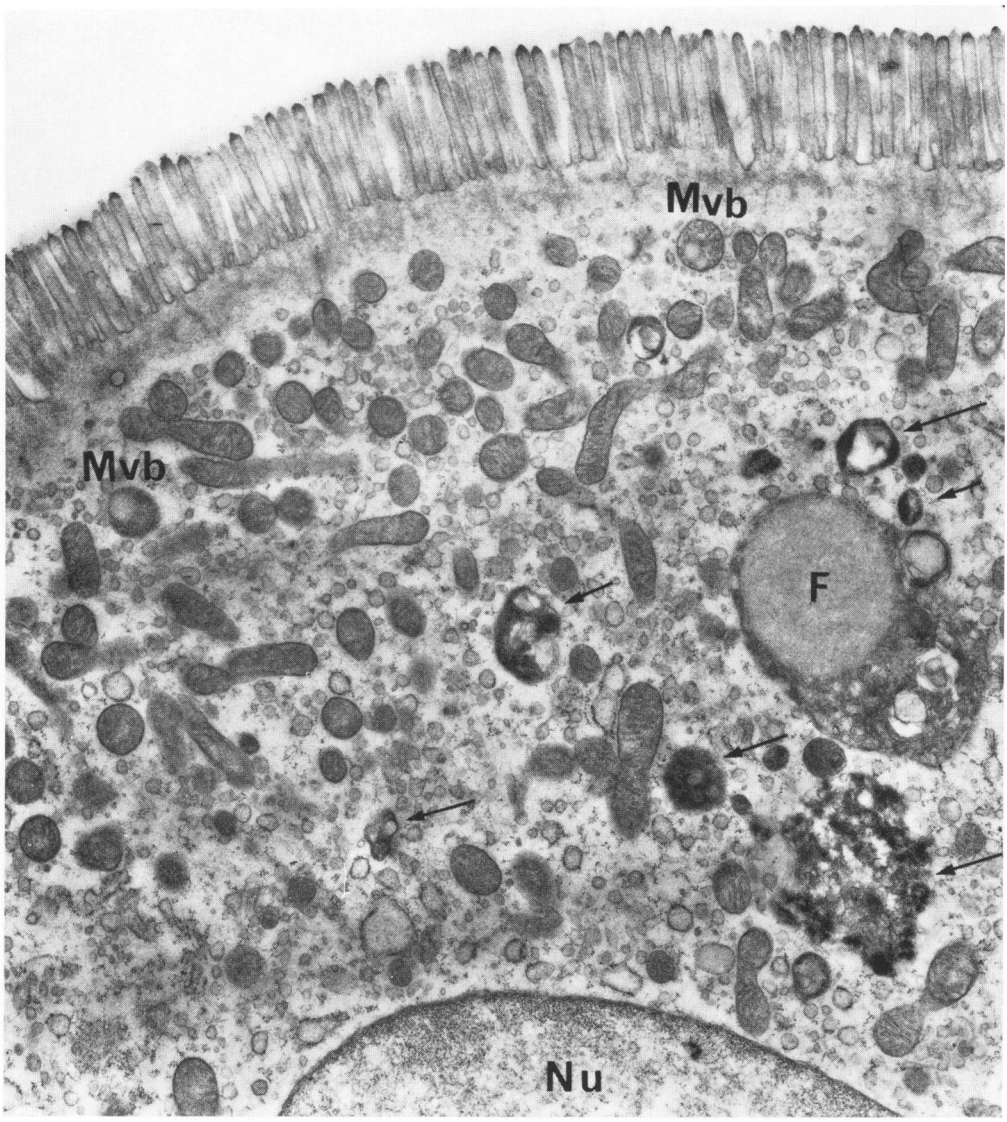

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FIG. 6. Apical portion of a chief cell in the mid-villus area in a Shigella-infected guinea pig at 12 hours. Moderately large, membrane-bound inclusions (arrow) appear in the presence of otherwise well preserved cellular components. The largest inclusion contains a fat droplet (F) and a myeloid figure profile. Several dense bodies and multivesicular bodies (Mvb) are seen. In their aggregate these inclusions correspond to the Feulgen-positive material seen by light microscopy. Nucleus (Nu). × 6,500.

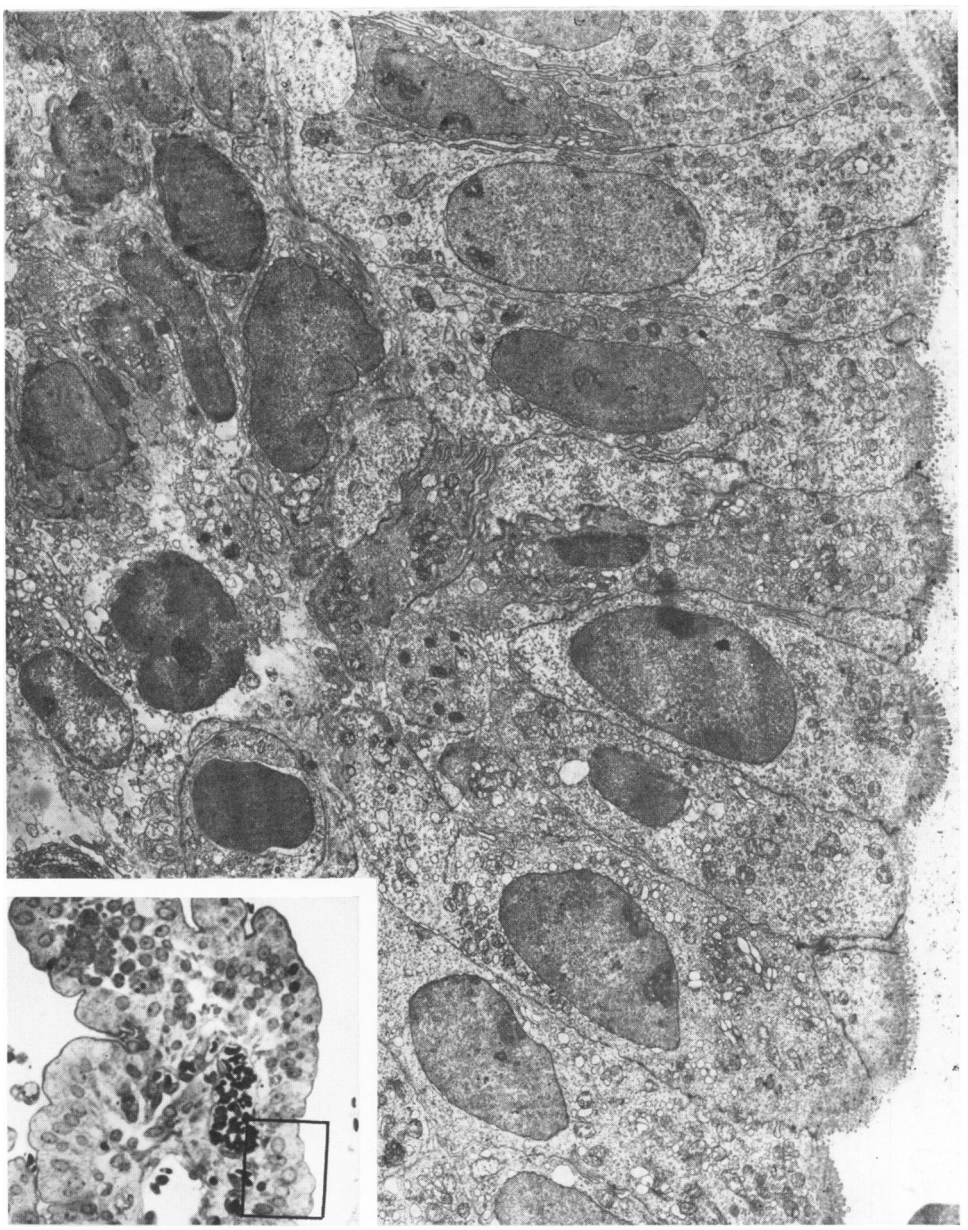

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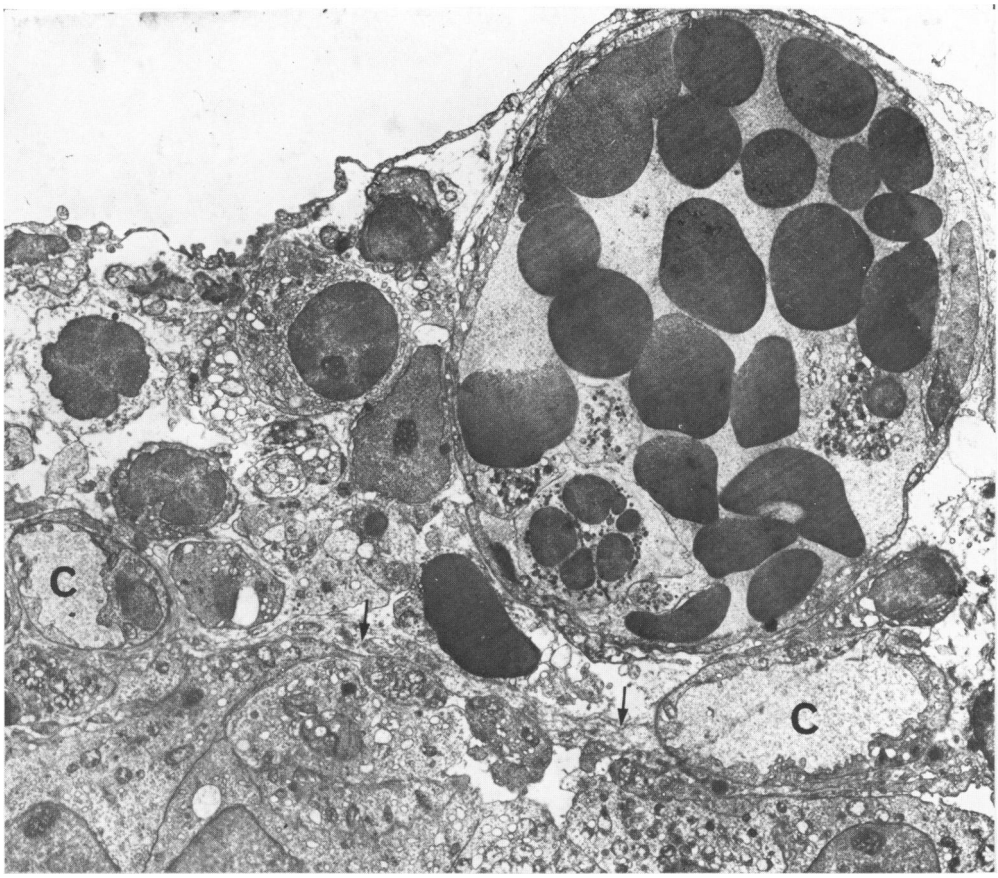
FIG. 7. Mid-villus portion, 24 hours after Shigella infection. The continuity of the epithelial lining is preserved. There is scalloping at the lumen surface. Microvilli are reduced in height and number. Endoplasmic reticulum and mitochondria are swollen. The basement membrane is intact. Elements of the tunica propria are more compactly arranged. × 2,400. Inset, × 200.

FIG. 8. Tunica propria at the mid-villus level, 24 hours following Shigella infection. The clear space on top is a lacteal. Congestion of cellular elements (red cells, neutrophils and thrombocytes) distends a venule. The blood plasma in both venule and capillaries (C) has increased electron density, suggesting fluid loss from the vascular lumen. The interstices in the tunica are filled with cells and cellular debris. The basement membrane is intact (arrow). × 2,400.

FIG. 9. Tunica propria, 24 hours after Shigella infection. The interstices are packed in part by membrane-bound, complex structures originally cytoplasmic inclusions in macrophages. Some are now free in an extracellular location and correspond to the cellular debris observed by light microscopy. Some of the extracellular bodies are packed with ribosomes, contain rough endoplasmic reticulum and resemble functioning albeit atypical organelles rather than cellular breakdown products. × 5,900.



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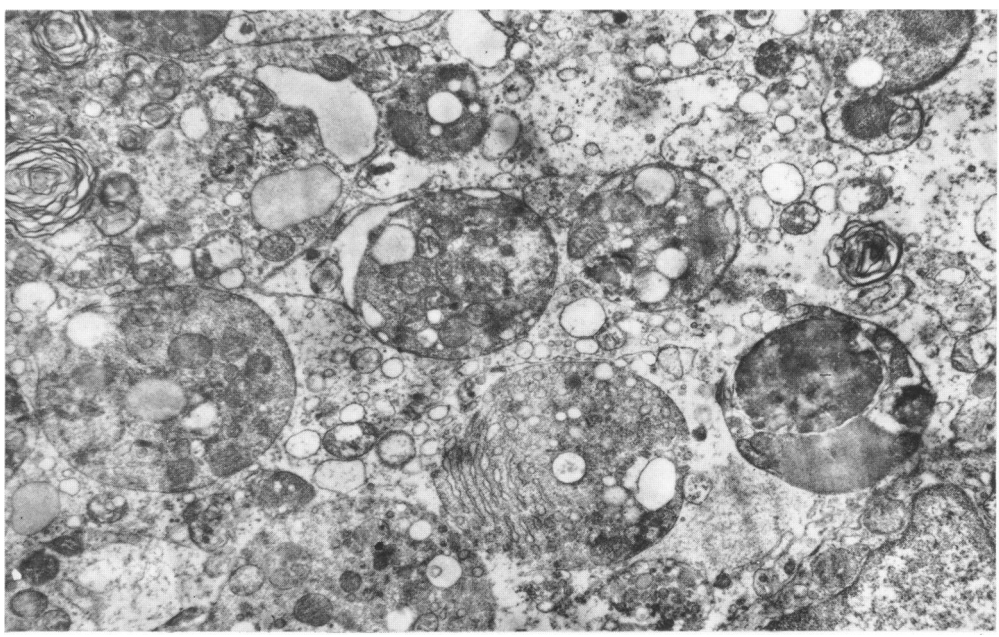

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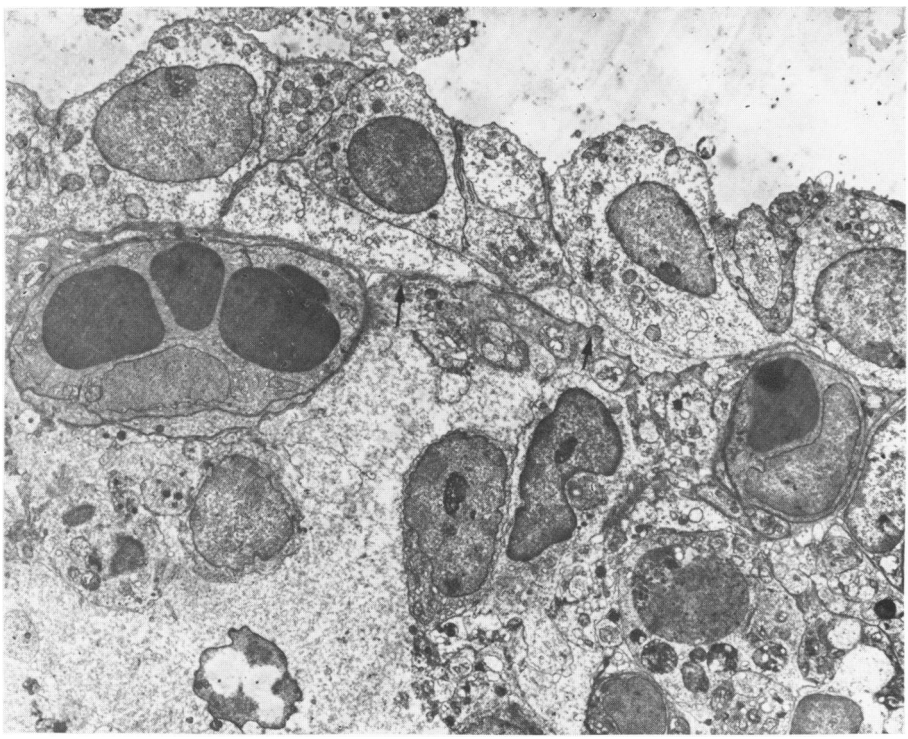
FIG. 10. Midportion of villus, 24 hours after Shigella infection. The lesion is in a more advanced stage than illustrated in Figure 7. Although the continuity of the epithelium is still preserved, the individual epithelial cells show marked regressive changes with great reduction in cell height, severe loss of cytoplasmic content, including microvilli. Most of the remaining mitochondria are swollen. The basement membrane is intact (arrow). Capillary lumens show evidence of blood plasma concentration. The amorphous precipitate in the interstices of the tunica is presumably edema fluid. × 2,000.

FIG. 11. Cell extrusion from an abnormal site along the midportion of a villus. The epithelial cell (note degenerated microvilli) lies free in the lumen except for a remnant of attachment at the surface desmosome (arrow), the last structure to separate. Regressive changes in the nucleus and cytoplasm of an extruded cell are similar to those in the cells still attached. × 6,100.



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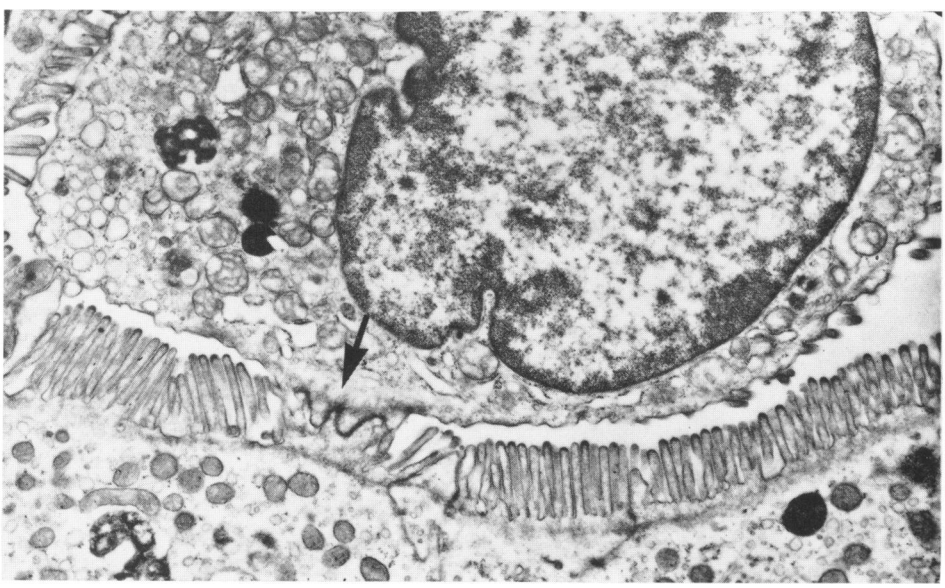

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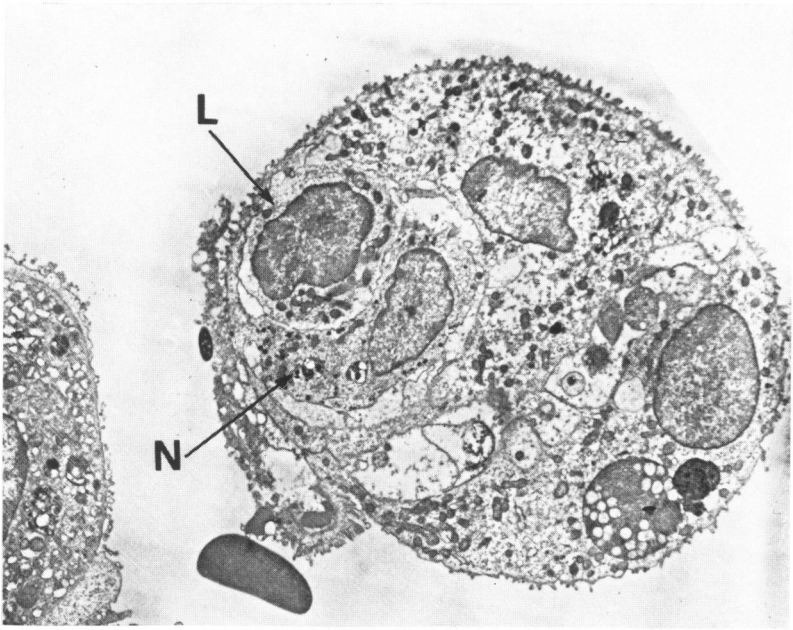
FIG. 12. An extruded cell complex lies free in the lumen and consists of a rounded-off aggregate of altered epithelium and a migrating lymphocyte (L) and neutrophil (N). × 2,200.

FIG. 13. A degenerated cell in the intestinal lumen has a preserved cell membrane containing multiple Shigella organisms. Aggregates of osmiophilic material resemble nuclear substance. × 3,200.



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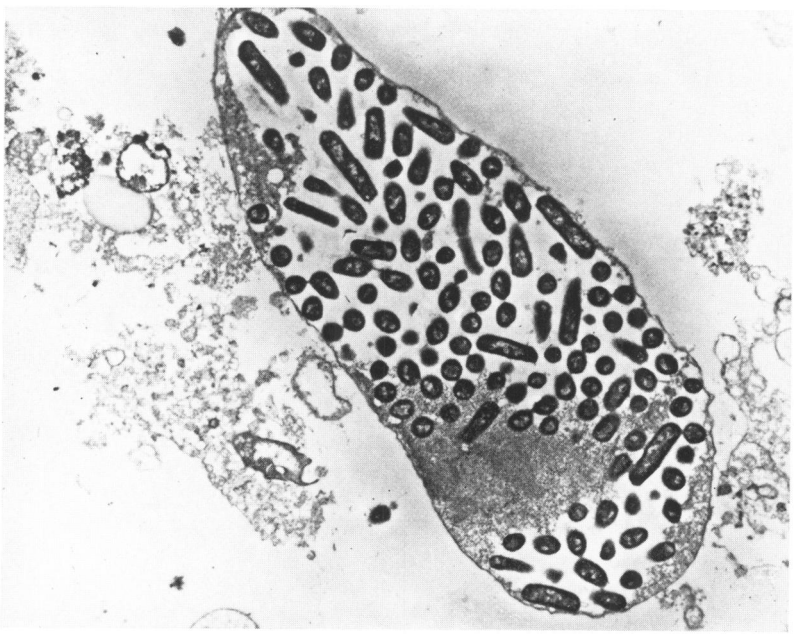

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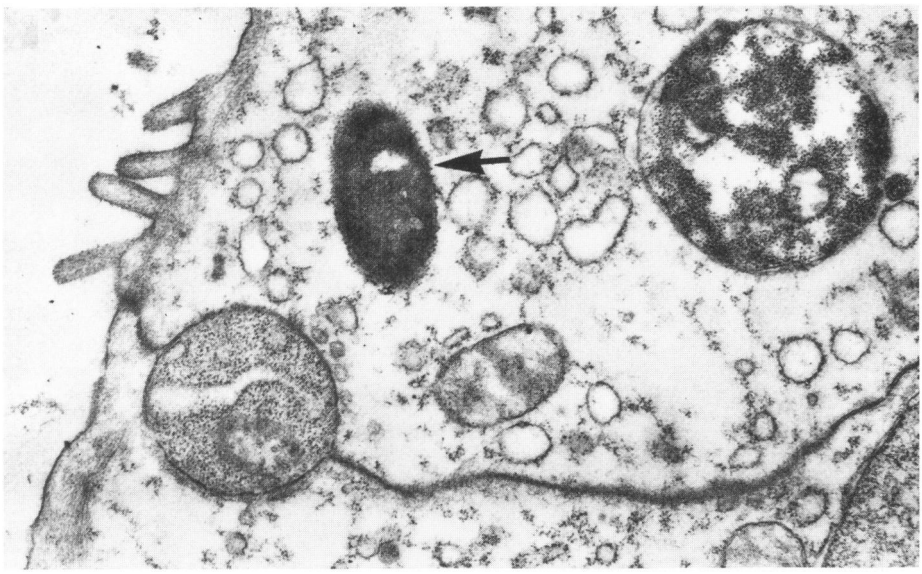
FIG. 14. Apical portion of intestinal epithelium, 24 hours after Shigella infection. A Shigella organism (arrow) lies free in the cytoplasm near the lumen surface. The microvilli and terminal web have undergone regressive changes. The endoplasmic reticulum is swollen. Membrane-bound intracytoplasmic inclusions contain osmiophilic granular material resembling ribosomes and a mitochondrium. A similar structure is present in the intercellular space. × 8,000.

FIG. 15. Present in the intercellular space between two chief cells is a complexly arranged membrane-bound inclusion body containing ribosomes, ergastoplasm, mitochondria and vacuoles. Arrow in tunica propria points to basement membrane. × 7,100.



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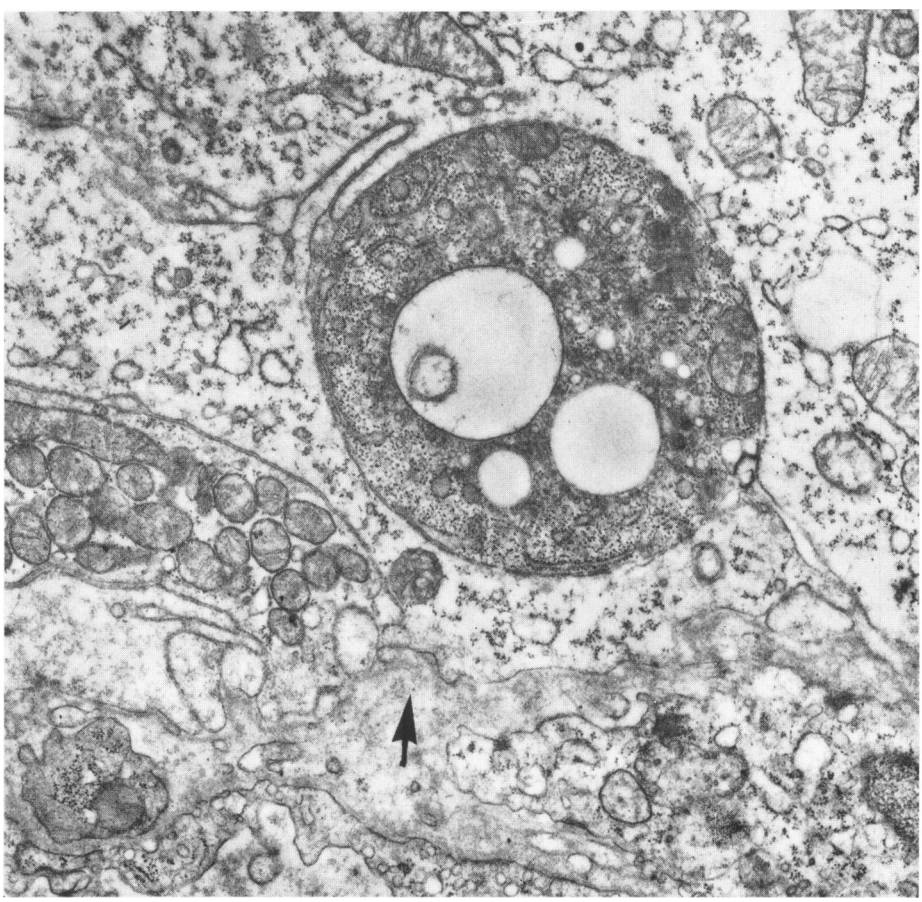

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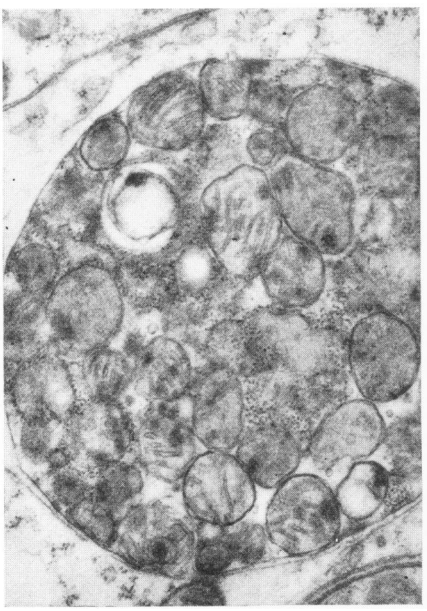
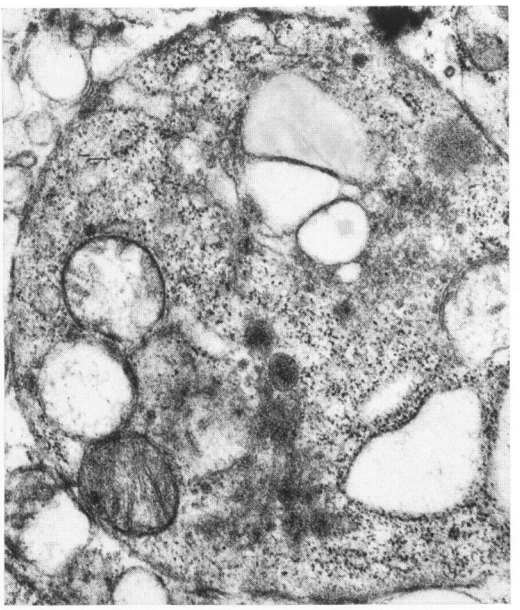
FIG. 16 a and b. Variants of membrane-bound, intra-epithelial inclusion bodies containing packed mitochondria, ribosomes, dense bodies and vacuoles. × 7,400.

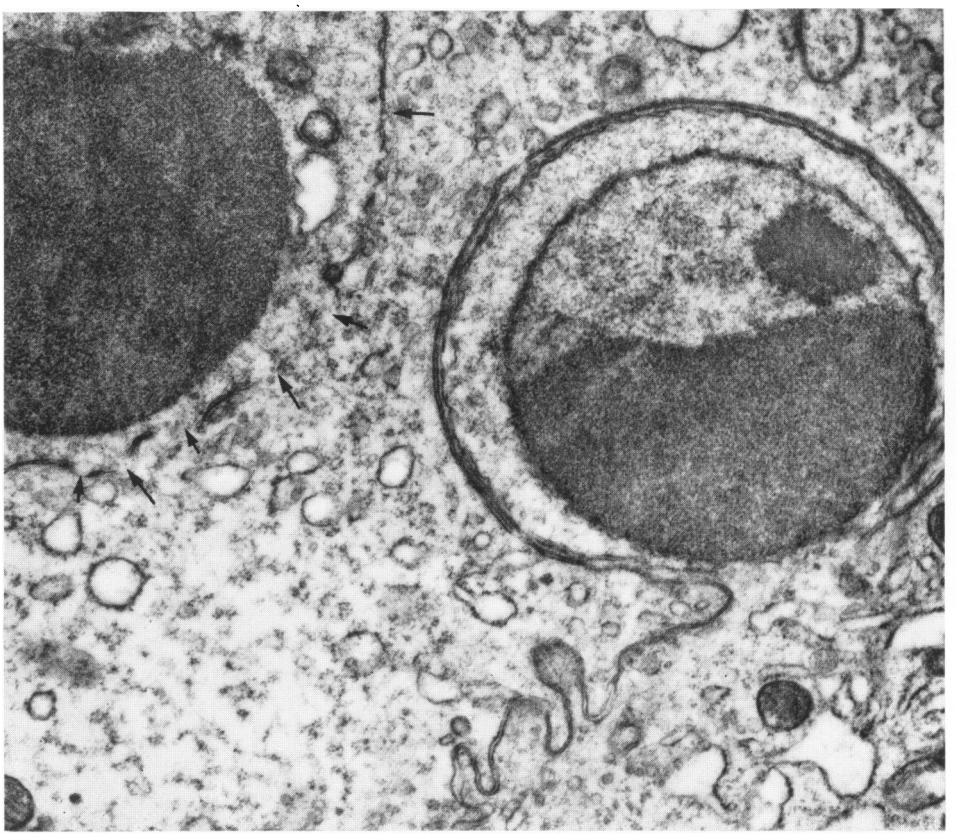
FIG. 16c. Another type of large, membrane-bound inclusion within absorptive cells is represented by lymphocytes in different stages of preservation. A projection of an intracellular lymphocyte appears on the right. The lymphocyte on the left shows disruption of its cell membrane (arrows), a remnant of which persists. × 7,500.

16a





b



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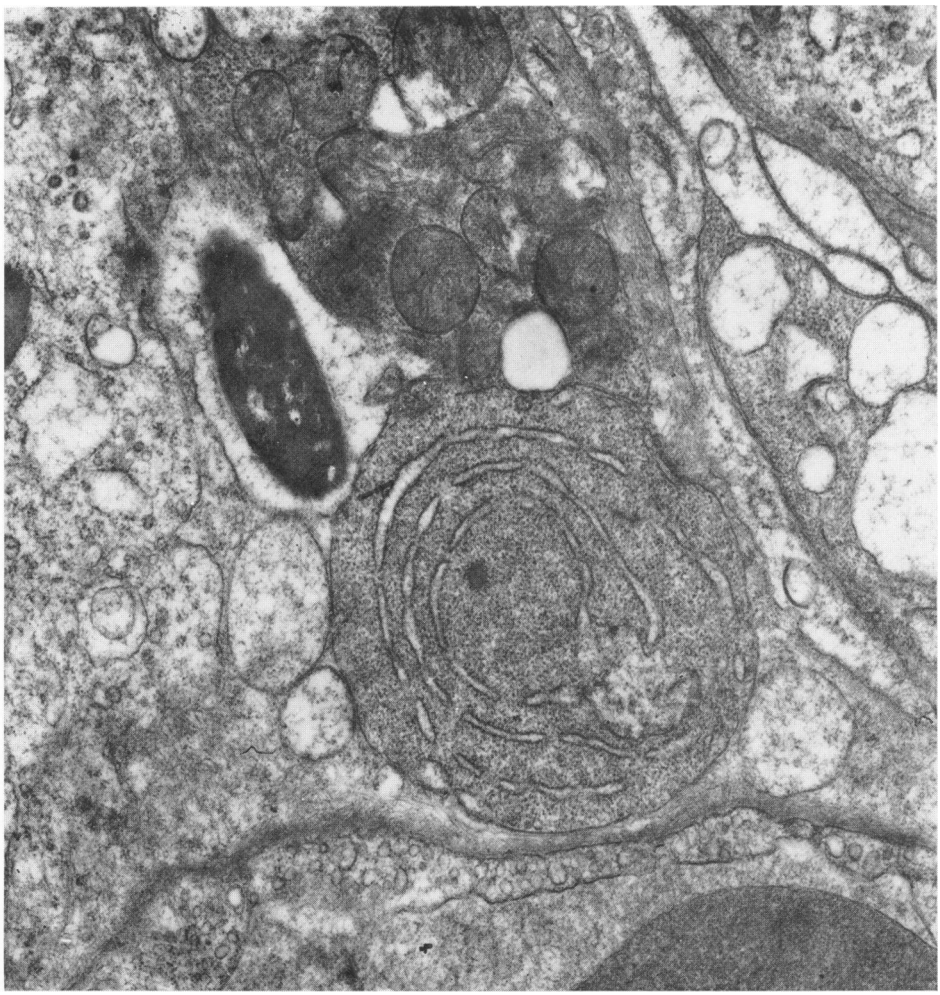


FIG. 17. Tunica propria. Above the capillary wall a membrane-bound portion of a macrophage contains osmiophilic granular material suggestive of packed ribosomes subdivided by concentric rings of slightly dilated vesicles of endoplasmic reticulum. There is a resemblance of this structure to the inclusion bodies seen in Figures 9, 14, 15, 16 and 18c. A Shigella organism lies in close proximity to this structure. × 7,800.

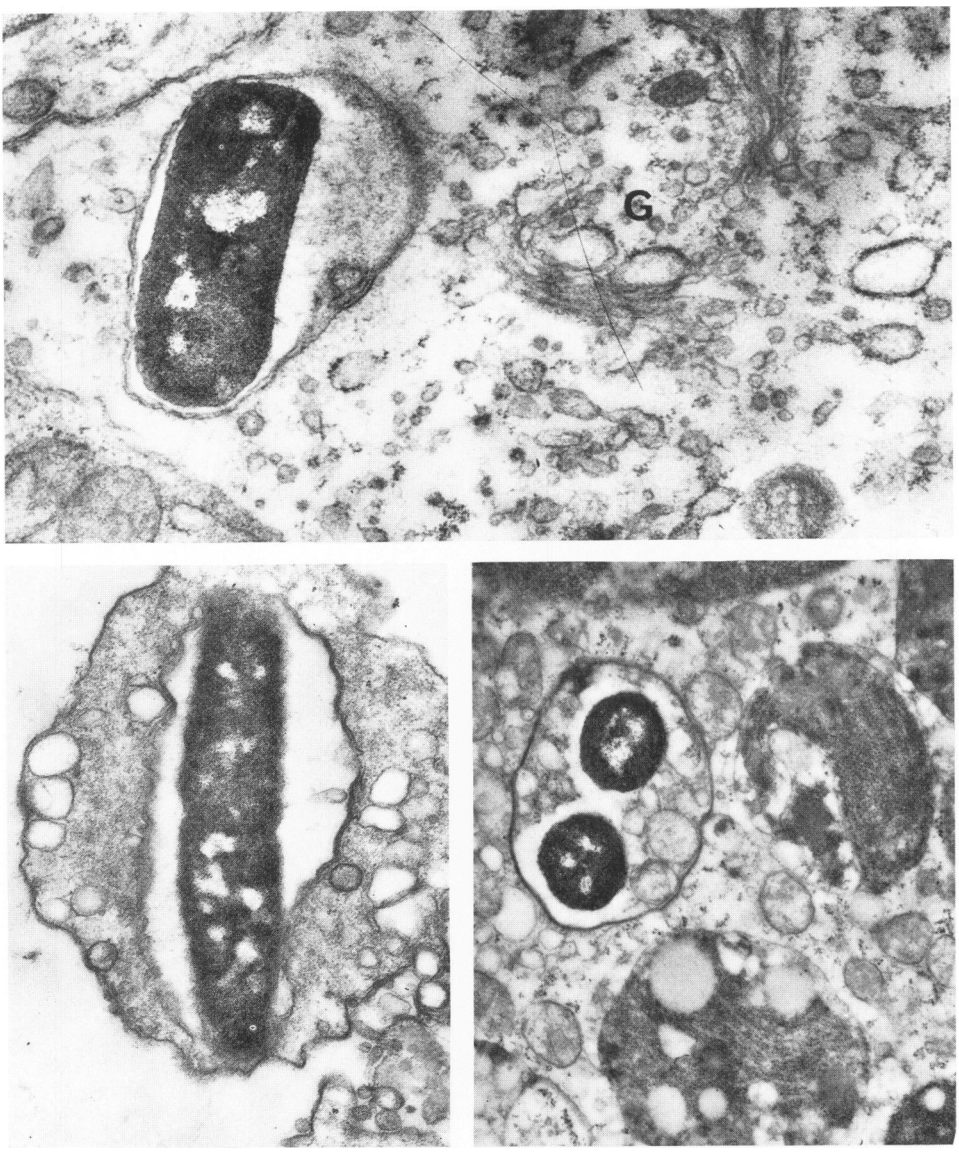


FIG. 18. A composite of intra-epithelial, membrane-enclosed Shigella organisms. Golgi apparatus (G). The membranes frequently enclose cytoplasmic components. a, ×10,000; b, × 7,500; c, × 6,500.

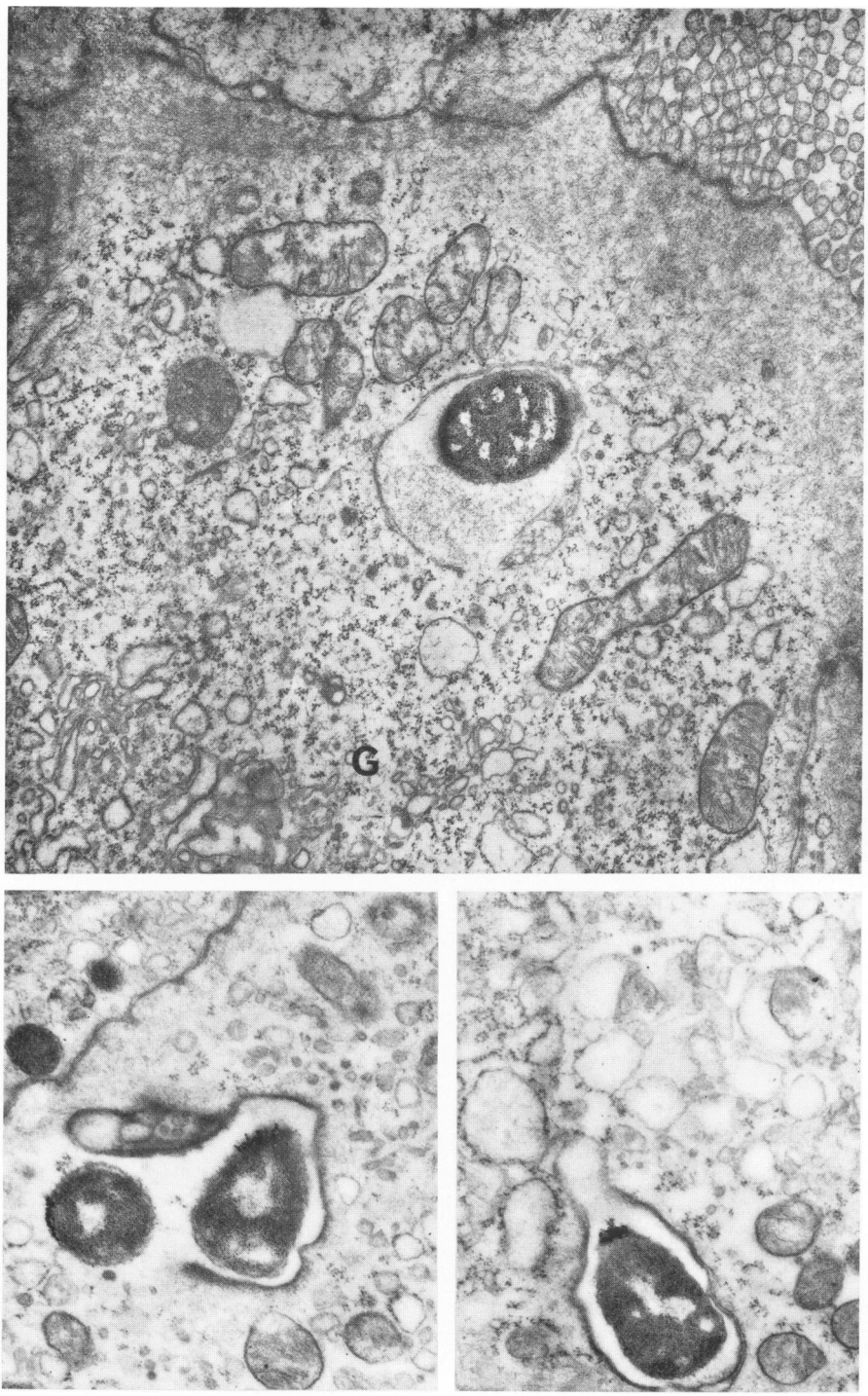
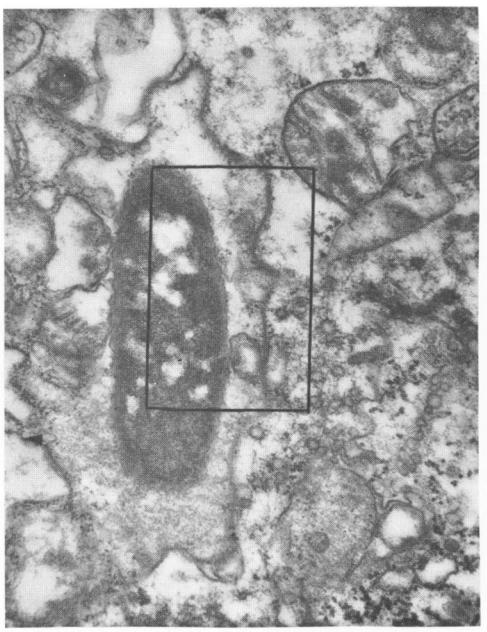
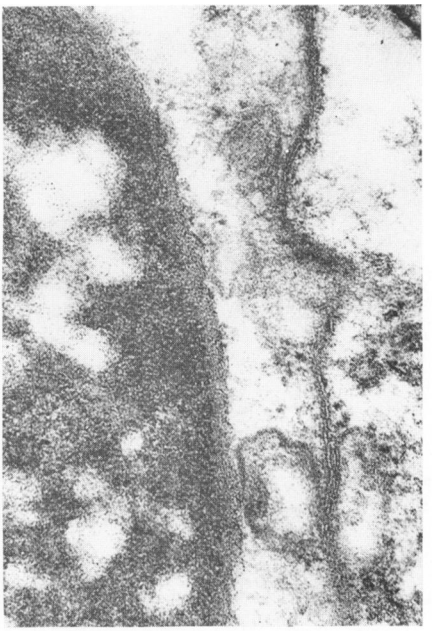


FIG. 19. A composite of intra-epithelial Shigella organisms illustrates exceptional types of membrane enclosure. Golgi (G). In "a" and "b" the membrane envelope is incomplete and in places has undergone complex coiling and reduplication. In "c" the membrane envelope connects with a cytoplasmic component indistinguishable from swollen endoplasmic reticulum. × 7,400.



20a



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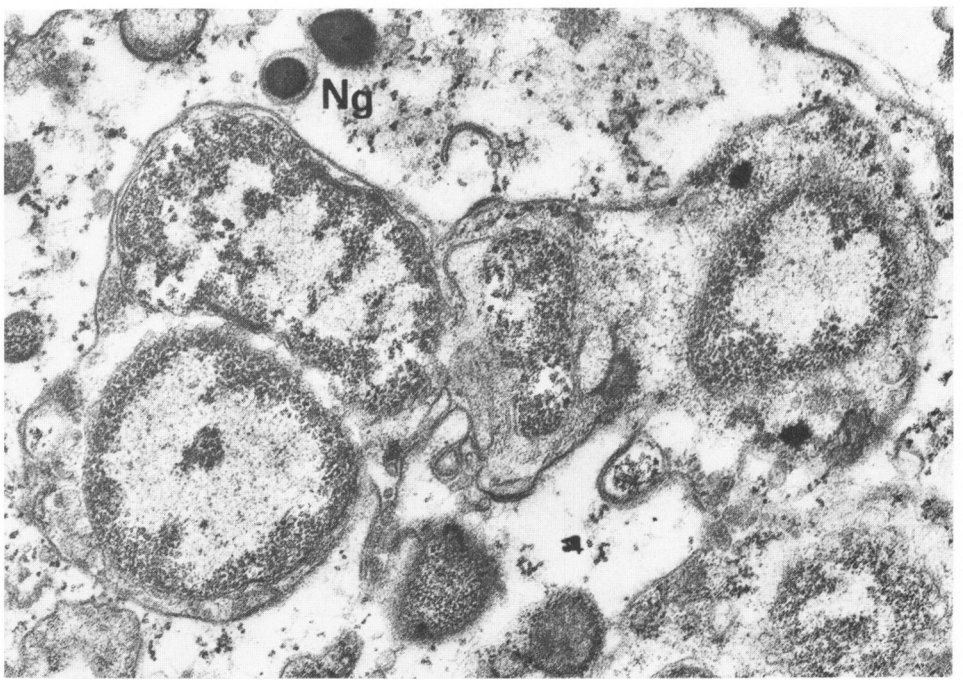

c

FIG. 20 a, b and c. A neutrophil with seemingly intact Shigella organisms within a lysosome evidenced by a distinct unit membrane. Disintegrating Shigella organisms also appear within a neutrophil. Neutrophil granules (NG). a, × 7,500; b, × 30,000; c, × 15,000.

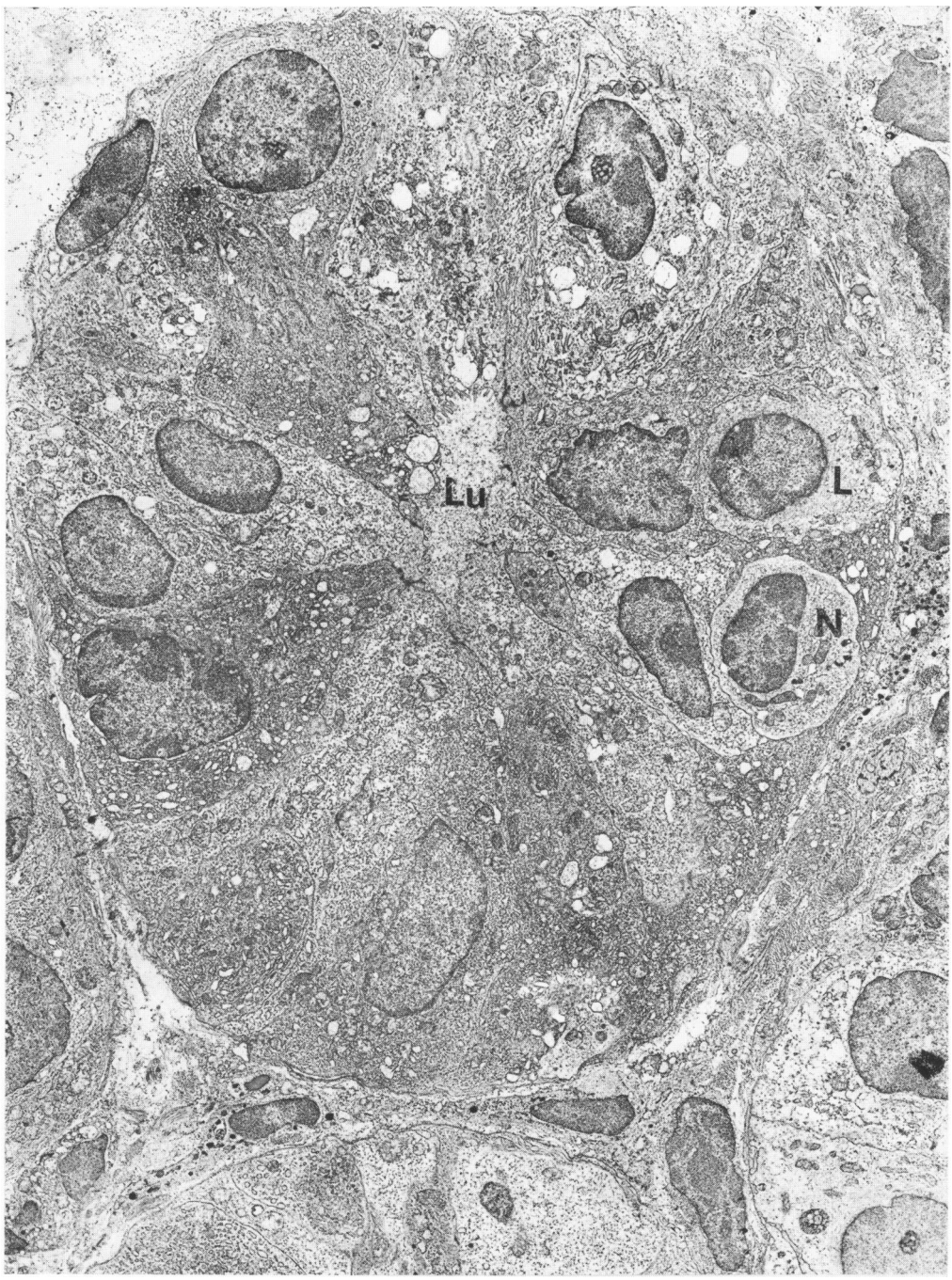

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FIG. 21. Survey illustrations of an upper crypt 24 hours after Shigella infection. There is striking lack of involvement when compared with the villus portion of the mucosa. Lumen (Lu); lymphocyte (L); neutrophil (N). × 2,400.