Colonocyte damage in the rectal mucosa in shigellosis is the result of bacterial invasion and leads to ulceration. Additional factors in colonocyte damage may be the Shigella cytotoxin and, especially in colonic crypt cells, bacterial endotoxin. A vascular lesion was present in the lamina propria of the rectal mucosa, which resembled endothelial damage secondary to bacterial endotoxins. In patients with longer duration of symptoms, relative vascular insufficiency, activated lymphocytes, eosinophil and mast cell degranulation, and antibody-mediated colonocyte damage may all play a role. (Am J Pathol 1986, 123:25-38)

Materials and Methods

Rectal biopsies were available from 10 patients with dysentery from whom shigellae were isolated (Table 1). The age of the patients ranged from 13 to 50 years, and they had lived all their lives in southern India. The first stool sample available after arrival in hospital was cultured on a variety of media as described earlier for identification of enteric bacterial pathogens, including shigellae. Shigella dysenteriae I was isolated from 8, including 4 from a village affected by an epidemic of shigellosis in 1976. The S dysenteriae I was multi-drug-resistant with a transferable plasmid. The sporadic patients were all seen from August to December 1981. The severity of the clinical illness was assessed as described elsewhere, and 4 patients were judged to have severe illness (Table 1).

Rectal mucosal biopsies were obtained with the True-love Salt Suction Biopsy Instrument (Vann Brothers, London, U.K.) 8–10 cm from the anal verge, without"

**Ultrastructural Pathology of the Rectal Mucosa in Shigella Dysentery**

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DIARRHEA with blood and mucus, the result of intestinal infection by the *Shigella* group of organisms, is the prototype of the "dysenteries" caused by invasive bacterial pathogens. *Shigella* infection has considerable public health importance, especially in tropical developing countries. Large epidemics of shigellosis, with a high mortality, were reported during the 1970s from Central and South America and the Indian subcontinent. The association of shigellosis with the hemolytic uremic syndrome, which has a high mortality, and other complications has already been documented in southern India and elsewhere. During 1984 shigellae were again the most frequently isolated organisms in children with acute diarrhea coming to this hospital. A pandemic of shigellosis was reported in northeastern India in 1984. A clearer understanding of the pathogenesis of this major gastrointestinal infection is needed so that better methods of treatment and control can be designed. In addition to tissue invasion by shigellae, enterotoxins and cytotoxins may also contribute to the pathogenesis. The presence of *Shigella* carriers, chronic infections, and reinfection with shigellae may all influence the pathogenesis of dysentery. Valuable information about the pathogenesis of shigellosis was obtained by studies of the ultrastructural changes in the ileal and colonic mucosa in experimentally infected guinea pigs and rhesus monkeys. The ultrastructural changes in the human rectal and colonic mucosa associated with acute infectious diarrhea have been relatively little studied, and there are only two brief reports of single cases of *Shigella* colitis. In this paper we describe the ultrastructural pathologic features of the rectal mucosa in biopsies obtained from 10 patients with dysentery and *Shigella* infection.

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prior preparation, within 24 hours of admission to hospital. The biopsies were immediately fixed in 2.5% glutaraldehyde containing 0.1 M calcium chloride in cacodylate buffer for 3 hours, followed by 1% osmium tetroxide. The tissues were then dehydrated in ethanol and embedded in Araldite. One-micron sections were cut with glass knives and stained with toluidine blue. Selected areas were prepared for thin sectioning with a diamond knife on an LKB UM-4 ultramicrotome. The ultrathin sections were stained with saturated aqueous uranyl acetate and lead citrate and examined in a Philips EM 201C electron microscope. The ultrastructural appearance of rectal biopsies from healthy adults in this population has been reported elsewhere.18

Results

Light-Microscopic Appearance

Epithelium

In half the biopsies (Table 2) the surface epithelium was ulcerated and covered with an inflammatory exudate containing numerous neutrophil polymorphs, desquamated epithelial cells, red blood cells, bacteria and many fibrin strands. The severity of the ulceration paralleled the numbers of bacteria in surface colonocytes (Figure 1A). The colonocytes were shorter and flattened in areas adjacent to ulceration. There was variation in staining density of colonocytes, with a few dark pyknotic cells and many pale-staining swollen cells with indistinct luminal borders. The microvilli were poorly formed, and the cells appeared immature, with large nuclei containing prominent nucleoli. The crypt lumens were dilated, and there were occasional crypt abscesses containing neutrophil polymorphs. Mucus depletion of the crypt epithelium was a feature, especially in patients with shorter duration (Table 2). The cytoplasm of the crypt cells, down to the base of the crypts, appeared vacuolated, but bacteria were not present in these cells (Figure 1B). Mitotic activity was increased, and the epithelial layer was infiltrated by lymphocytes, neutrophil polymorphs, macrophages, mast cells, and eosinophils. Blast transformation and mitotic activity was seen in epithelial lymphocytes.

Lamina Propria

There was edema, congestion of blood vessels, and focal or more diffuse hemorrhage at all levels of the

Table 1—Clinical Features of 10 Patients With Shigellosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Duration (days)</th>
<th>Severity</th>
<th>B &amp; M*</th>
<th>Edema</th>
<th>Congestion</th>
<th>Ulcers</th>
<th>Organism†</th>
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<td>10</td>
<td>50</td>
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<td>6</td>
<td>Mild</td>
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<td>+</td>
<td>+ S flexneri-3</td>
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* B & M, I, Blood and mucus initially; B & M, A, blood and mucus at admission; +, present; −, absent.
† S dys. 1, Shigella dysenteriae I. Cases 5–8 were from a village affected by epidemic dysentery in 1976. Other patients were sporadic cases seen at the hospital. Grading of clinical severity is as given in Choudari et al.17

Table 2—Changes in Surface Epithelium and in Colonocytes in Rectal Mucosa in Acute Shigellosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration (days)</th>
<th>Ulceration</th>
<th>Bacteria in colonocyte</th>
<th>Surface damage</th>
<th>Crypt damage</th>
<th>Goblet cell depletion</th>
<th>Mitochondrial damage</th>
<th>Increased IEL</th>
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IEL, Epithelial lymphocytes; severity of extent of abnormalities graded as follows: absent, −; mild, +; moderate, ++; severe, +++.
Figure 1—Light micrographs of rectal mucosal biopsies.  
A—Case 2. _S. dysenteriae_ of 6 days' duration. Damage surface colonocytes adjacent to an ulcer. Note intracytoplasmic bacilli. (x 1080)  
B—Crypt, lower third of Case 2, with mucus depletion and cytoplasmic vacuolization. (x 1270)  
C—Case 3. _S. dysenteriae_ 1. Three days. Blood vessel near the lower third of the crypt with denudation of endothelial cells and a mitotic figure in the remaining cell. (x 825)  
D—Case 1. _S. dysenteriae_ 1. Six days. Capillary in the subluminal lamina propria with many fibrin strands (F), stagnation of red blood cells in the lumen of the vessel, and extravasation into the lamina propria. (x 900)

lamina propria, with increased neutrophil polymorphs and mononuclear cells. Edema was more in the upper half of the lamina propria, and hemorrhage mainly in the deeper layers. In many areas the endothelial lining of capillaries and venules was denuded, and remaining cells were hyperplastic, with several cells in mitosis (Figure 1C). In scattered blood vessels throughout the lamina propria there were fibrin strands suggesting intravascular coagulation and evidence of thrombus formation (Figure 1D).

Ultrastructural Appearance

_Epithelium_

Bacterial bodies with signs of degeneration were found in the neutrophil polymorphs of the luminal exudate (Figure 2). Bacterial bodies in colonocytes of the surface and upper third of the crypt epithelium were either free in the cytoplasm (Figure 3A), in membrane-

Figure 2—Case 4. _S. dysenteriae_ 1. Four days. Part of the cytoplasm of a neutrophil polymorph from luminal exudate with intact (arrow) and disintegrating (D) bacilli. (x 8000)
bound vesicles (Figure 3B), or in the intercellular spaces (Figure 3C) and were usually intact. The severity of the colonocyte cellular organelle damage did not correlate with the presence of bacteria. Some surface colonocytes with bacteria had only minimal damage (Figure 3D), and other surface and most crypt colonocytes with damaged organelles contained no bacteria.

Surface colonocytes with minimal changes had only shortened microvilli, with poorly formed glycocalyx and microvillus rootlets (Figure 4). Swollen pale nuclei with chromatin margination, luminal surface almost devoid of microvilli, and cytoplasm with areas of rarefaction and increase in glycogen granules characterized colonocytes with more severe damage. In such cells mitochondria were swollen, with loss of cristae, rough endoplasmic reticulum was dilated, with degranulation, and there was disaggregation of polyriboosomes with reduction in ribosomes in the cellular matrix. The Golgi apparatus

Figure 3A—Case 3. *S. dysenteriae*. Three days. Luminal colonocyte with absent microvilli. Mitochondria are swollen, with loss of cristae and myelin figures. Rough endoplasmic reticulum is dilated. A bacterial body is seen free in the cytoplasm (arrow). (× 11,400)  
B—Case 2. *S. dysenteriae*. Six days. Luminal colonocyte containing bacterial body (arrow) in a membrane-bound vesicle. A portion of an adjacent neutrophil polymorph (N) can be seen. (× 7900)  
C—Case 10. *Shigella flexneri*. Six days. Widened intercellular space in surface epithelium containing bacilli. Adjacent cells have mitochondrial damage and increased lysosomes. (× 4700)  
D—Case 3. *S. dysenteriae*. Three days. Luminal cells with many bacteria (arrows) in the cytoplasm. Cellular organelles are intact. (× 7200)
was intact in some cells, and in others it was dilated. There was increase of lysosomes and infranuclear fat droplets in the cytoplasm (Figure 5). Scattered colonocytes were dark-staining and pyknotic, with condensation of the damaged cytoplasm. In patients from the epidemic of shigellosis several adjacent colonocytes or the entire surface colonocytes had poor nuclear and cytoplasmic staining, with ruptured apical membranes and paucity of cellular organelles. The mitochondria and endoplasmic reticulum was damaged in such cells, but no bacteria were present. In the patients with duration of illness longer than 10 days, there was evidence of colonocyte regeneration, many colonocytes contained large autophagosomes and the epithelium was infiltrated by macrophages containing phagolysosomes. 

Mitochondrial swelling was a prominent feature in the middle and lower third of the crypts. Other cellular organelles appeared intact in most crypt cells except for
scattered cells with dilatation of the rough endoplasmic reticulum. Mucus depletion, with goblet cells containing numerous arrays of vertically placed rough endoplasmic reticulum, was a feature in all biopsies. Mitochondria were less affected in these goblet cells (Figure 6). Electron-dense bodies were increased in all epithelial cells. Damaged colonocytes were extruded into the lumen at all levels of the rectal crypt tubules.

In patients with longer duration, where the surface colonocytes were already showing signs of recovery, the crypt cell damage was more prominent. There was nuclear swelling with chromatin margination, loss of mitochondrial cristae, and dilatation of the rough endoplasmic reticulum; other cells were dark and pyknotic.

Among the cells infiltrating the crypt epithelium, the increase in epithelial lymphocytes was most striking. These cells were activated, with mitosis (Figure 7A) and prominent pseudopods (Figure 7B). Often such lymphocytes were adjacent to degenerated colonocytes (FIGU
Figure 7A—Case 7. *S. dysenteriae*. Twenty days. Crypt cell base infiltrated by an interepithelial lymphocyte in mitosis. (×4600)  
B—Case 8. *S. dysenteriae*. Twenty days. Lower third of the crypt. Interepithelial lymphocyte (L) with many pseudopodia. Adjacent cells are damaged. (×4600)  
C—Case 1. *S. dysenteriae*. Six days. Lower third of crypt showing an interepithelial lymphocyte (L) adjacent to damaged colonocytes. (×7000)
Figure 8—Case 3. S. dysenteriae 1. Three days. Juxtaluminal lamina propria with disintegrating neutrophil polymorph (N) and plasma cells (P) with prominent endoplasmic reticulum. Intercellular space contains amorphous material with many fibrin strands (F) and red blood cells (R). (×4500)

Figure 9—Case 3. S. dysenteriae 1. Three days. Lamina propria near the crypt base with plasma cells containing dilated rough endoplasmic reticulum and plasmacytosis. (×4500)
Table 3—Changes in Lamina Propria (LP) in Acute Shigellosis

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration (days)</th>
<th>LP hemorrhage</th>
<th>Endothelial damage</th>
<th>Platelet thrombi</th>
<th>Fibrin and intravascular coagulation</th>
<th>Neutrophils</th>
<th>Plasma cells</th>
<th>Eosinophils</th>
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</tbody>
</table>

Severity of abnormalities graded as follows: absent, −; mild, +; moderate, + +; severe, + + +.

* Organized thrombi.

ure 7C). Degranulated eosinophils and mucosal mast cells were also present in the epithelial layer.

**Lamina Propria**

The abundant edema fluid was rich in protein and contained an amorphous material with many fibrin strands and red blood cells (Figure 8). Lymphatics at the base of the crypts were markedly dilated and contained a similar amorphous material. The cellular infiltrate in the luminal half of the lamina propria consisted of neutrophil polymorphs in various stages of disintegration, macrophages with heterogenous phagolysosomes, and plasma cells, some with dilated rough endoplasmic reticulum (Figure 8). In the lower half macrophages and neutrophil polymorphs were less abundant, but plasma cells were strikingly increased in number and often showed plasmacytosis (Figure 9). In patients with longer duration of symptoms, in the deeper layers of the lamina propria, especially pericryptally, there were many eosinophils and mast cells with degranulation. The increase in plasma cells, macrophages, and eosinophils was maximal in the patients.

*Figure 10—Case 4. S. dysenteriae. Four days. A capillary with markedly swollen endothelial cells (E), part of transmigrating neutrophil (N), adhesion of platelets (P) at the site of denudation, and fibrin strands within the lumen (arrow). (x 5500)*
Figure 11—Case 1. *S. dysenteriae* 1. Six days. A capillary with endothelial cell (E) undergoing mitosis. (x 4500)

Figure 12—Case 10. *S. flexneri*. Six days. Blood vessels near the crypt base containing distorted (arrow) and dehemoglobinized (R) red blood cells in the lumen. (x 4250)
who were victims of the village epidemic of shigellosis and who had longer duration of symptoms (Table 3).

Vascular congestion was a feature in virtually all the blood vessels in the lamina propria. The subluminal capillaries were dilated, with intact fenestrae and contained many fibrin strands. The lining endothelium of these capillaries was swollen with mitochondrial damage, dilatation of the rough endoplasmic reticulum and marked reduction in pinocytotic vesicles (Figure 10). Other endothelial cells were pyknotic. In many capillaries, there was focal or extensive denudation of endothelial cells with accumulation of platelets and fibrin strands at the site of denudation. Some of the endothelial cells, especially in vessels with extensive denudation, had mitotic activity and cellular hyperplasia (Figure 11). Stagnation of blood with distortion and dehemoglobinization of the red blood cells was a striking feature in the larger vessels (Figure 12). Many of these larger vessels contained platelet thrombi or cellular thrombi with large strands of fibrin enmeshing red blood cells, platelets, neutrophil polymorphs, and eosinophils. These thrombi often occluded the lumen, and blood vessel rupture led to hemorrhage into the surrounding lamina propria. In patients with longer duration of symptoms, organizing thrombi were often seen in vessels near the crypt base (Figure 13). Reduplication of the basal lamina occurred in all the damaged and thrombosed blood vessels. The arterioles in the deeper part of the lamina propria were serpiginous, and their lumen was occluded because of contraction of the smooth muscles and swelling of endothelial cells (Figure 14). Endothelial and smooth muscle mitochondria were damaged in the arterioles.

The extent of the vascular lesion was maximal in the patients with a duration of less than 8 days (Table 3). Intravascular coagulation, endothelial damage and platelet thrombi were least prevalent in the patients from the village with an epidemic of shigellosis in whom the duration of symptoms was longer than 10 days. However, organized thrombi were present in the areas near crypt bases in all three of these patients (Table 3). Scattered in the lamina propria, there were myofibroblasts with prominent rough endoplasmic reticulum. The smooth muscles of the muscularis mucosa also had mitochondrial damage and marked vacuolization of the cytoplasm. A few bacteria were detected in the superficial lamina propria in 2 of the 10 patients.

Discussion

The pathogenesis of Shigella dysentery is recognized to be a complex process, since the organism invades and multiplies in colonocytes, produces an enterotoxin which promotes fluid secretion in the small intestine, and also has a cytotoxin with neurotoxic properties. In fact, Shigella dysentery has been characterized as “part
cholera, part carcinoid, and part colitis.8 The mechanism of bacterial invasion of the colonocytes is still not understood, although it is likely that the first step in this is digestion of the glycocalyx either by shigellae or by other colonic organisms.9 Subsequent to this, shigellae are found in the colonocytes in membrane-bound vesicles and then digest the membranes to appear free in the cytoplasm. The colonocyte organelle damage is likely to be the result of the cytotoxin which is known to inhibit cellular protein synthesis. The Shiga toxin has been shown to damage intact epithelial cells without invasion.10 Damaged colonocytes without bacterial invasion suggest that this mechanism is operative in vivo also, although these could be cells through which organisms have already transmigrated. Colonocytes with bacterial bodies and no cell damage may just have been penetrated, but histochemical identification is necessary to show that these are shigellae and not other colonic bacteria which have invaded through a compromised epithelial luminal barrier.

Colonocytes in the middle and lower third of the crypts did not contain bacteria, which confirms observations in experimentally infected monkeys.10 However, mitochondrial damage was widely prevalent in these cells. The cytotoxin of shigellae is not known to damage mitochondria, but it inhibits protein synthesis by damaging polysomes and ribosomes. Bacterial endotoxins specifically damage mitochondria.21,22 Release of endotoxin from invading shigellae and enhancement of endotoxin absorption from the lumen through the damaged epithelium with absent glycocalyx13 could be the basis of this mitochondrial damage.

The outpouring of neutrophil polymorphs which attempt to engulf and destroy bacteria in the luminal exudate suggests that this is the first line of defense against invasion by shigellae. The predominant inflammatory cells in the epithelium, especially adjacent to degenerated colonocytes, were lymphocytes. This suggests that cell-mediated damage to colonocytes may also play a role in the morphogenesis of the epithelial lesion in acute bacillary dysentery. The invasion of the epithelial layer by macrophages in patients with longer duration is part of the process of cleaning up the debris for replacement by regenerating colonocytes. The primary role of neutrophil polymorphs in the defense against shigellae is further confirmed by their predominance in the luminal area of the lamina propria. The rarity of bacteria in the lamina propria despite their abundance in the epithelium suggests that the neutrophil polymorphs and their attendant macrophages with

Figure 14—Case 10. Shigella flexneri. Six days. An arteriole near the muscularis showing a serpiginous course. The lumen (arrow) is occluded by swollen endothelial cells. There is marked mitochondrial swelling with loss of cristae in smooth muscles (M) of the arteriole and muscularis mucosa. (×5650)
abundant phagolysosomes are an efficient defense. An alternative explanation for the paucity of bacterial bodies in the lamina propria may be that shigellae invade and damage primarily colonocytes, which are then extruded into the lumen. Further studies are needed to resolve this.

Plasma-cell activation was more marked in the deeper layers of the lamina propria. The striking plasma cell response even in patients with short duration of symptoms may be an index of prior exposure to these enteric pathogens. It has been shown in the immunized rat intestine that specific IgA antibody-containing cells accumulate around the crypt. An early IgM response to *Shigella* infection has been documented in man. The type of immunoglobulin produced by the activated plasma cells in the present study was not investigated.

In patients with ulcerative colitis it has been suggested that plasma cells in the deeper layers of the lamina propria may produce IgG, which may play a role in antibody-mediated colonocyte damage. The striking increase of plasma cells in the deeper regions of the lamina propria, especially in patients with longer duration raises the possibility that this may be an additional mechanism in prolonging colonocyte damage.

The most striking changes in the lamina propria were found in the blood vessels. We have earlier described a local Shwartzman-like reaction in rectal mucosal lamina propria blood vessels in adults with acute diarrhea characterized by endothelial damage, platelet adhesion and clumping, leading to intravascular coagulation and vascular dehiscence with hemorrhage. This lesion was found even in patients with diarrhea associated with noninvasive bacterial pathogens such as *Vibrio cholerae* and in patients in whom no bacterial pathogen could be demonstrated. The prevalence of this lesion correlated well with the severity of clinical illness when it was assessed objectively. This vascular lesion, whose pathogenesis was postulated to be endothelial cell damage mediated by bacterial endotoxin, was present in many of the capillaries and venules of the lamina propria in the present group of patients with invasive dysentery due to *Shigella* infection. The vascular lesion was maximal in patients with duration of less than 10 days, although blood vessels with organized thrombi as residual lesions were present in cases with longer duration (Table 3). The role of endotoxin in the pathogenesis of the vascular lesion is suggested not only by its morphologic resemblance to the local Shwartzman reaction but also by the extensive damage to mitochondria in endothelial cells, smooth muscle cells, and in colonocytes in the deeper layers of the rectal crypt tubules where there was no bacterial invasion. Endotoxin could reach the lamina propria blood vessels from lysis of shigellae and other bacteria which may invade the damaged epithelium and also by enhancement of the normal absorption of endotoxin from the colon through a failure in the mucosal barrier. Further studies are needed to determine the role of bacterial endotoxins in the pathogenesis of the mucosal lesion in diarrhea.

In patients with longer duration of illness, cellular organelle damage was more in the deeper layers of the crypt. The vascular abnormalities in the lamina propria leading to a relative perfusion defect is likely to be a factor contributing to persistent epithelial lesions. Degranulation of eosinophils and mast cells and activated lymphocytes found in such patients could also perpetuate the crypt colonocyte damage by release of cytotoxic factors.

These results suggest the following sequence in the morphogenesis of the rectal mucosal lesion in shigellosis. Colonocyte glycocalyx is damaged and digested with invasion of the mature cells on the upper third of the crypts and the surface by shigellae in membrane-bound vesicles. This elicits mucus secretion, with depletion of goblet cells and transfection of neutrophils to the lumen. The invading shigellae digest the vesicles, migrate laterally into the adjacent cells, and also may invade the lamina propria directly or through paracellular pathways. *Shigella* cytotoxin rapidly reduces colonocyte protein synthesis, which could be compounded by mitochondrial damage by endotoxin and leads to destruction of cellular organelles, with cell death, extrusion, and the formation of microcervices in the surface epithelium. Shigellae that enter the lamina propria are destroyed rapidly, with release of bacterial endotoxin, which, along with possible enhanced endotoxin absorption from the lumen, leads to thrombosis and hemorrhage, causing vascular insufficiency, mitochondrial damage, and persistent damage to crypt cells, especially in patients with prolonged symptoms. Accelerated cell turnover and the scavenging effects of macrophages gradually replace the damaged epithelium. While polymorphs appear to be the first line of defense, epithelial lymphocytes and eosinophils are playing an as yet not understood role in the production of colonocyte damage.

**References**