
The Process of Microbial Translocation

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The process of microbial translocation was studied using *Candida albicans*, *Escherichia coli*, or endotoxin instilled into Thiry-Vella loops of thermally injured guinea pigs and rats. Translocation of *C. albicans* occurred by direct penetration of enterocytes by a unique process different from classical phagocytosis. Translocation between enterocytes was not observed. Internalization was associated with a disturbance of the plasma membrane and brush border, but most internalized organisms were not surrounded by a plasma membrane. Passage of the candida into the lamina propria appeared to be associated with disruption of the basal membrane with extrusion of cytoplasm of the cell and candida. Organisms in the lamina propria were commonly phagocytized by macrophages but also were found free in lymphatics and blood vessels. Translocation of *E. coli* and endotoxin also occurred directly through enterocytes rather than between them, but translocated endotoxin diffused through the lamina propria and muscular wall of the bowel wall by passing between rather than through the myocytes. These descriptive phenomena provide new insight into the role of the enterocyte and intestinal immune cells in the translocation process.

BERG AND GARLINGTON¹ defined bacterial translocation as 'the passage of viable bacteria through the epithelial mucosa into the lamina propria and then to the mesenteric lymph nodes, and possibly other tissues.' This concept should be refined to include all microbial translocation, defined as the passage of both viable and nonviable microbes and microbial products such as endotoxin across an anatomically intact intestinal barrier.

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Studies in several laboratories have shown that translocation, as measured by recovery of viable organisms from the regional lymph nodes, is increased by hemorrhagic shock,² antibiotic therapy,^{3,4} intestinal obstruction,^{5,6} parenterally administered endotoxin,⁷ hyperpyrexia,⁸ thermal injury,⁹ intravenous feedings,¹⁰ elemental diets,^{11,12} and cytotoxic drugs,¹³ but not by simple protein malnutrition⁷ or T-cell depletion.¹⁴ Translocation of bacteria is decreased by feeding complete enteral diets,¹⁰ glutamine,^{13,15} trophic hormones such as bombesin,¹⁶ and prevention of intestinal ischemia.¹⁷ Based on these observations, it has been postulated that increased rates of translocation increase the hypermetabolic response of injured and septic patients,¹⁸ cause a septic state in surgical patients in the absence of a defined infectious focus,¹⁹ and predispose or contribute to the development of multisystem organ failure.²⁰⁻²²

Few studies have evaluated how microbes pass through the intestinal barrier. Cole et al.²³ demonstrated direct candidal invasion of enterocytes after intragastric inoculation in immunologically immature 5- to 6-day-old mice, and Krause et al.²⁴ demonstrated in a bold clinical experiment that translocation occurred in a normal human following ingesting a large dose of *Candida albicans*. Deitch et al.²⁵ showed invasion of *Escherichia coli* in ulcerations of villus tips following hemorrhagic shock, and Wells et al.²⁶ more recently demonstrated that enterococci have a transmucosal route of translocation. In a previous

study we showed rapid translocation of biotinylated *C. albicans* into the lamina propria through enterocytes.²⁷

To better understand the process of microbial translocation, we have used three translocation 'probes' (viable *C. albicans*, viable *E. coli*, and partially purified endotoxin) to observe and document the anatomic process of translocation by light and electron microscopy. For this purpose a model was selected to achieve relatively high rates of translocation.

Materials and Methods

Animals and Animal Care

Female Hartley guinea pigs (Murphy Breeding Laboratory, Plainville, IL) weighing 350 to 450 g and male Lewis rats (Harlan Sprague Dawley Inc., Indianapolis, IN) weighing 225 to 250 g were used for these experiments. Animals were quarantined for 1 week in standard laboratory conditions so they could adapt to the environment and to exclude animals with pre-existing diseases. The experimental protocols were approved by the University of Cincinnati Medical Center Institutional Animal Care and Use Committee, and the animals were housed in an AAALAC-approved facility. In conducting the described research in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals as set forth by the Committee on the Care and Use of Laboratory Animals, National Research Council, United States Department of Health and Human Services, National Institutes of Health.

Surgical Procedures and Burn Injuries

All operative procedures and potentially painful manipulations were performed under general anesthesia. Guinea pigs were anesthetized with intramuscular injection of 0.2 mg/kg of acetopromazine malate, 0.33 mg/kg, xylazine citrate, and 0.4 mg/kg atropine sulfate. Rats were anesthetized with 65 mg/kg sodium pentothal injected intraperitoneally.

Thiry-Vella loops were created by proximal and distal enterostomy of a 10-cm loop of distal ileum containing at least one Peyer's patch. Bowel continuity was restored by end-to-end anastomosis using a running 6-0 Prolene^R suture, and the animals were allowed to recover for 7 to 10 days before experiments were performed.

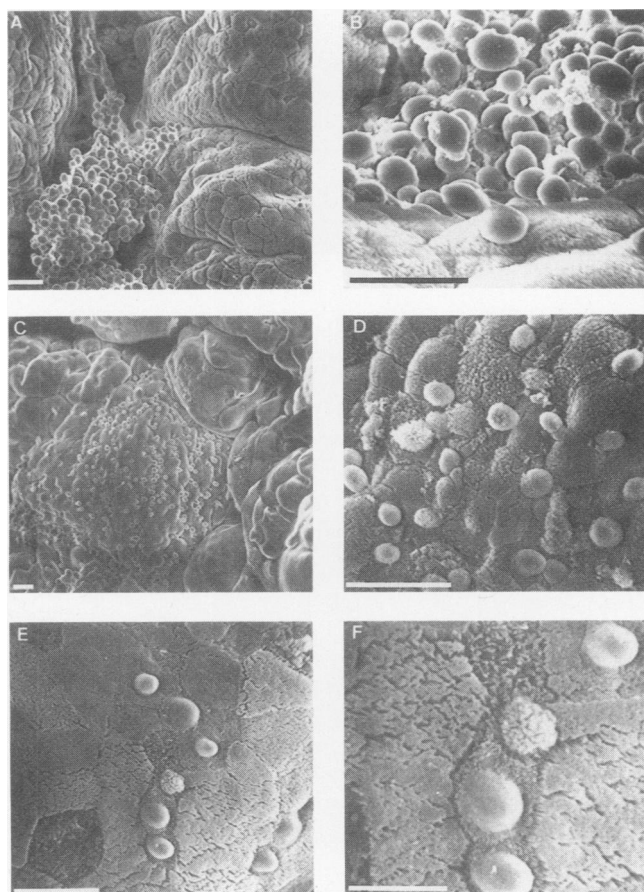
Burn injury of 50% full-thickness total body surface area (TBSA) was inflicted under general anesthesia according to a previously described method.²⁷ After burn injury, intraperitoneal injection of 20 mL lactated Ringer's solution was administered for fluid resuscitation. The surface area of the guinea pig in square centimeters was

calculated using the following formula: $-24.3 + 0.445 \times (\text{weight in grams}) + 10.2 \times (\text{length in centimeters})$.²⁸ The formula of Horst et al.²⁹ was used for calculating the TBSA of the rat, i.e., surface area in square centimeters equals $9.1 \times w^{2/3}$, where w equals body weight in grams.

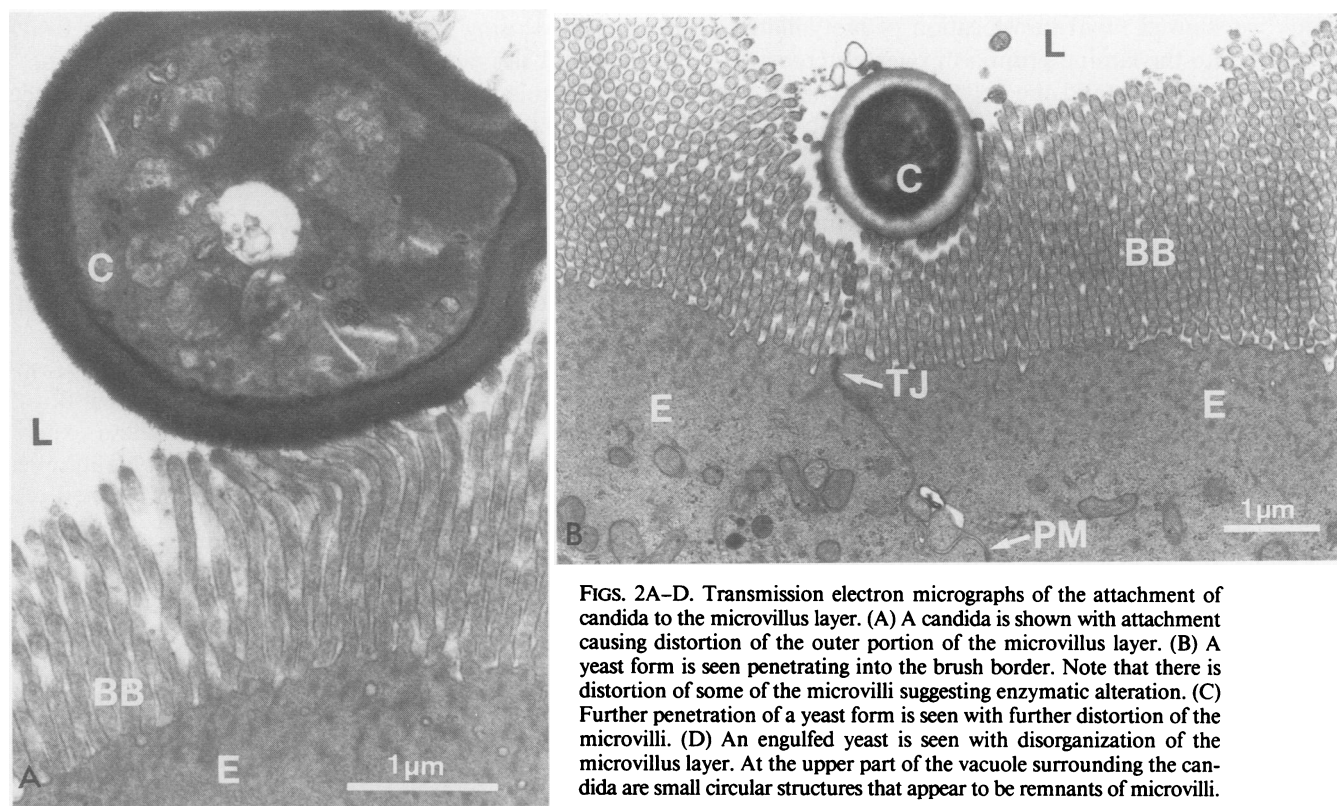
Preparation of the Microbial Probe

To provide a baseline for comparison with other experiments, the *E. coli* and endotoxin were labeled with ¹⁴C glucose.

Preparation of C. albicans. A strain of *C. albicans* isolated from the blood of a burn patient at Shriners Burns Institute, Cincinnati, Ohio, was used for these experiments. This isolate was serotype A and biotype number 177, as characterized by a modified Odds and Abbott sys-



FIGS. 1A-F. Scanning electron micrographs of the mucosal surface of guinea pig Thiry-Vella loops instilled with *C. albicans*. (A and B) Specimens taken 1 hour after burn; (C-F) specimens taken 12 hours after burn. The scale bars on A to E are 10 μ m and the scale for F is 5 μ m. Note that in A and B there is already attachment of the candida to the epithelium, but many of the candida are entrapped by mucous. (C) Diffuse attachment to one of the villi but not to others as was characteristic throughout most of the sections examined. (D, E, and F) Varying degrees of penetration of the candida into the brush border.



FIGS. 2A-D. Transmission electron micrographs of the attachment of candida to the microvillus layer. (A) A candida is shown with attachment causing distortion of the outer portion of the microvillus layer. (B) A yeast form is seen penetrating into the brush border. Note that there is distortion of some of the microvilli suggesting enzymatic alteration. (C) Further penetration of a yeast form is seen with further distortion of the microvilli. (D) An engulfed yeast is seen with disorganization of the microvillus layer. At the upper part of the vacuole surrounding the candida are small circular structures that appear to be remnants of microvilli.

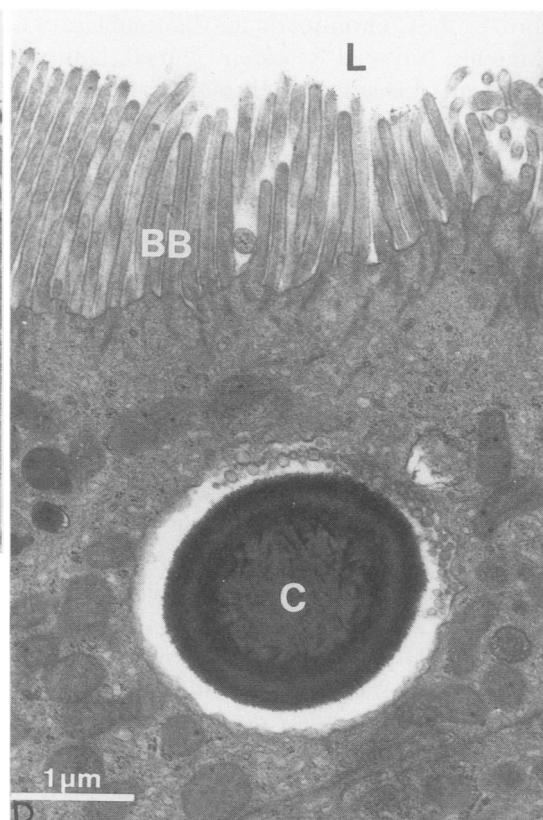
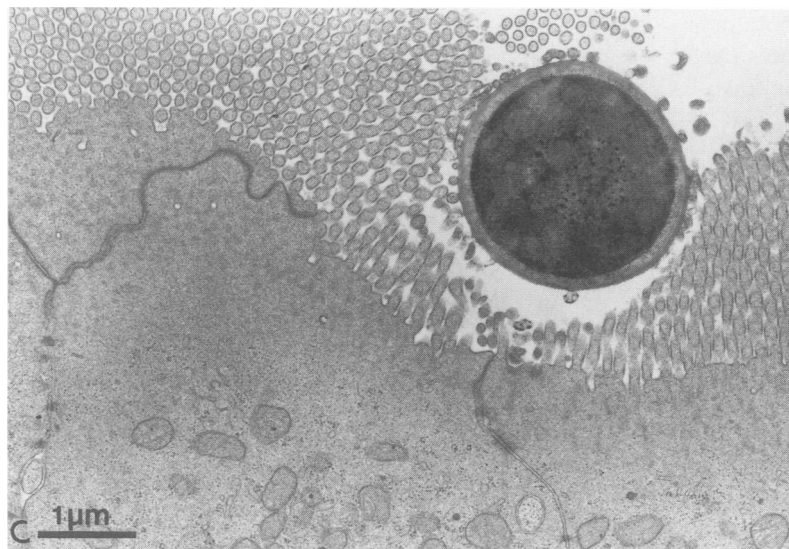


FIG. 2. (Continued)

tem.³⁰ Brain heart infusion broth was inoculated with *C. albicans* stock culture and allowed to grow in a shaking water bath at 37 C for approximately 17 hours. One half milliliter of this culture was plated onto Sabouraud dextrose agar and incubated at 37 C for 48 hours. The plates were maintained at 4 C for 3 to 10 days and scraped to collect the *C. albicans*. The organisms were suspended in phosphate-buffered saline (PBS), pelleted at 2000 rpm, and washed three additional times. The final pellet was suspended in PBS, counted using a hemocytometer, and viability was determined by trypan blue exclusion. Only preparations with more than 90% viability were used with the final concentration adjusted to approximately 1×10^{10} organisms/mL.

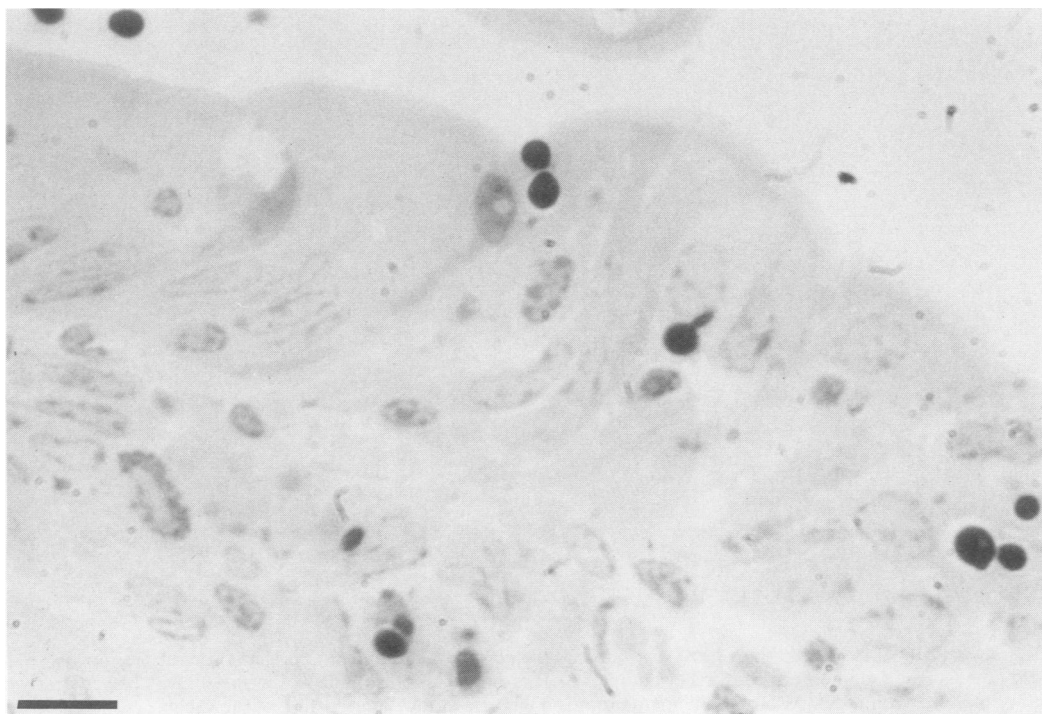
Preparation of ^{14}C -labeled *E. coli*. The *E. coli* strain used, obtained from Richard L. Simmons, M.D., University of Minnesota, Minneapolis, Minnesota, was grown in 75 mL of lipopolysaccharide (LPS) growth media (2.5 g/l peptone, 5 g/l NaCl, 2.5 g/l Na_2HPO_4) in a shaking water bath at 37 C for approximately 17 hours. This culture was used to inoculate 50 mL of fresh LPS media containing 500 μCi of ^{14}C glucose (specific activity 310 mCi/mmol, ICN Biomedicals Inc., Cleveland, OH), and then incubated for an additional 19 hours. The isotope-labeled bacteria were washed, resuspended in sterile saline, and adjusted to a final concentration of 1×10^{10} organisms/mL using a Klett densitometer (Klett Manufacturing Co., Long Island, NY).

Preparation of ^{14}C endotoxin. *E. coli* was inoculated into 50 mL of LPS growth media and incubated at 37 C in a shaking water bath for approximately 17 hours. This culture was then added to 2 L of LPS growth media containing 1 mCi of ^{14}C glucose and incubated at 37 C as above for an additional 27 hours. The media were then centrifuged at 6500g for 10 minutes at 4 C, the pellet was resuspended in 40 mL of distilled H_2O and centrifuged at 2000 rpm for 10 minutes. This pellet was washed in the same manner with 40 mL of absolute ethyl alcohol and then 40 mL of acetone. The final pellet was frozen at -70 C for 1 hour and then lyophilized overnight to dehydrate. The LPS was extracted with 1.5 mL of phenol extraction solution (13% phenol, 33% chloroform, and 54% petroleum ether) and sonicated for 15 minutes until the pellet was completely resuspended. The suspension was then centrifuged at 1000g for 15 minutes at 4 C and the supernatant poured through #3 Whatman filter paper into a polypropylene tube, frozen, and lyophilized overnight. Distilled water was added drop by drop to solubilize the phenol and the labeled LPS was collected by centrifugation at 2000g for 10 minutes, frozen at -70 C, and lyophilized for storage at -70 C until use.

Experimental Procedure

Either *C. albicans* 10^{10} in 1 mL PBS, *E. coli* 10^{10} in 1 mL, or endotoxin (1 mg in 1 mL) was instilled into the

FIG. 3. Light micrograph of the guinea pig ileum stained with toluidine blue. Many candidal bodies are seen within mucosal cells, one budding in a subnuclear region. Note especially the penetration of two candida through the brush border in the central portion of the picture (scale bar = 10 μm).



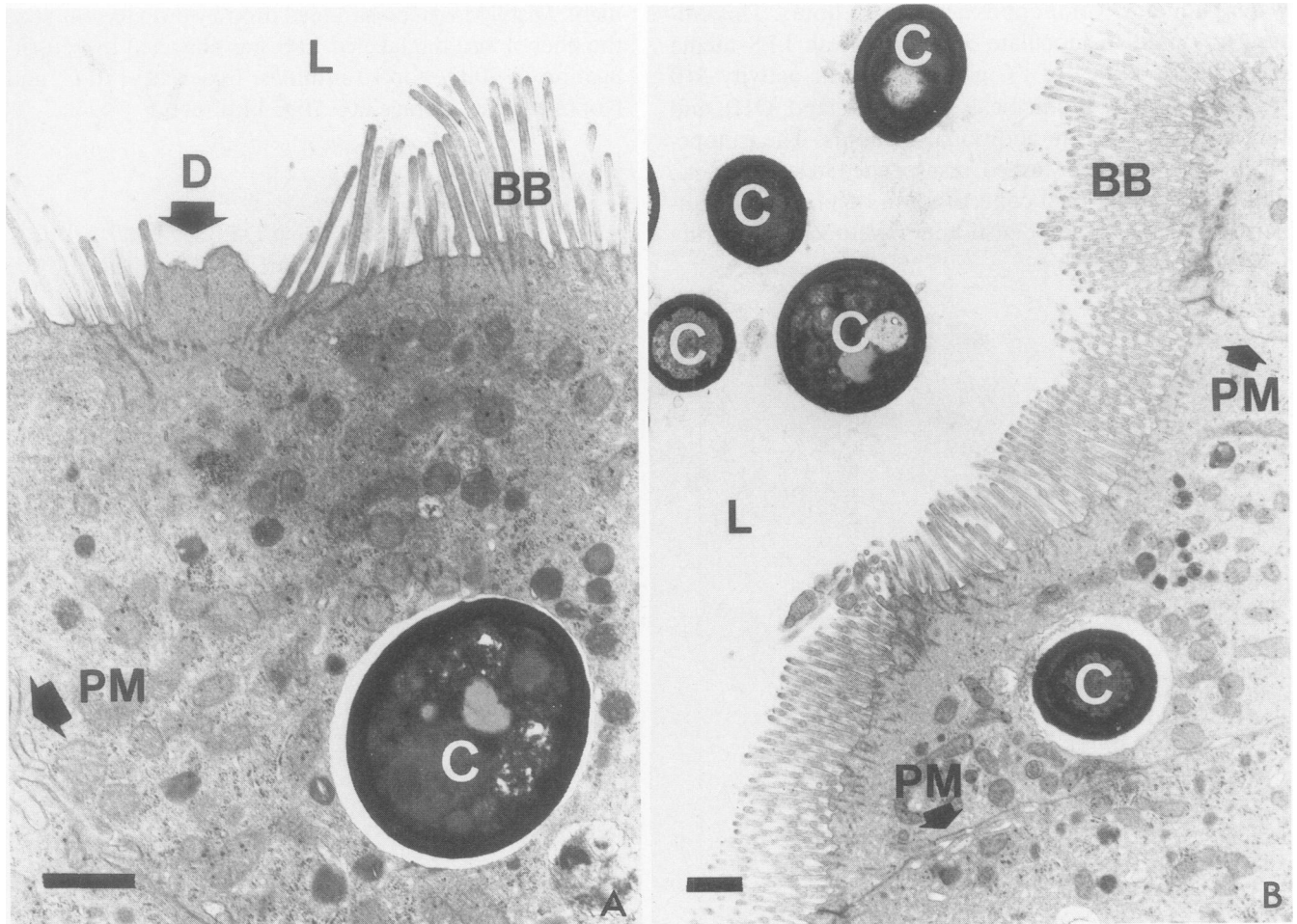
proximal limb of the Thiry-Vella loop after anesthesia but before the time of burn injury. Eight guinea pigs and eight rats were used for each translocation 'probe,' 48 in all. In some animals the Thiry-Vella loop first was irrigated with saline to remove excess mucous within the loop. The distal end was closed with an encircling ligature and the proximal end was similarly closed after instillation of the translocation probe to prevent its discharge. Animals were killed at 1, 4, and 24 hours in most experiments and at 2 and 6 hours in other experiments. When the animals were killed the Thiry-Vella loops were excised and sections taken from the intestine both in areas with and without Peyer's patches. These were frozen with dry ice or placed in a fixative composed of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L (molar) sodium cacodylate buffer with 50 mg/L calcium chloride. In some experiments tissue was taken for frozen sections to be processed

for fluorescent labeling studies, and the remaining loop filled with fixative before taking the sections.

Microscopic Examinations

Preparation of samples for microscopy. Fixed tissue for light microscopy (LM) and transmission electron microscopy (TEM) was washed in 0.1 mol/L sodium cacodylate with 10% sucrose and postfixed with 1% osmium tetroxide in the same buffer. The tissue was washed, dehydrated to 100% ethanol, exchanged into propylene oxide, and embedded in Medcast-Araldite resin (Ted Pella, Inc., Tustin, CA).

Polymerized blocks were sectioned with a Reichert-Jung Ultracut E ultramicrotome (Cambridge Instruments, Inc., Buffalo, NY). For light microscopy, semithin sections ($\sim 1 \mu\text{m}$) were cut and mounted on chromalum gelatin-



FIGS. 4A and B. These TEMs show gross distortion of the plasma membrane and brush border at presumed sites of entry into the enterocyte. (A) Also note lack of a membrane around the candida. (B) This and all other candida within the epithelial layer clearly are seen to be within enterocytes rather than between them. Also note the membranous fragments at the outer portion of the vacuole (scale bars = $1 \mu\text{m}$).

coated slides. Semithin sections subsequently were stained with toluidine blue and covered with cover slips using Permount^R. Thin sections for TEM were mounted on uncoated 200 mesh copper grids and stained with uranyl acetate and lead citrate solutions.

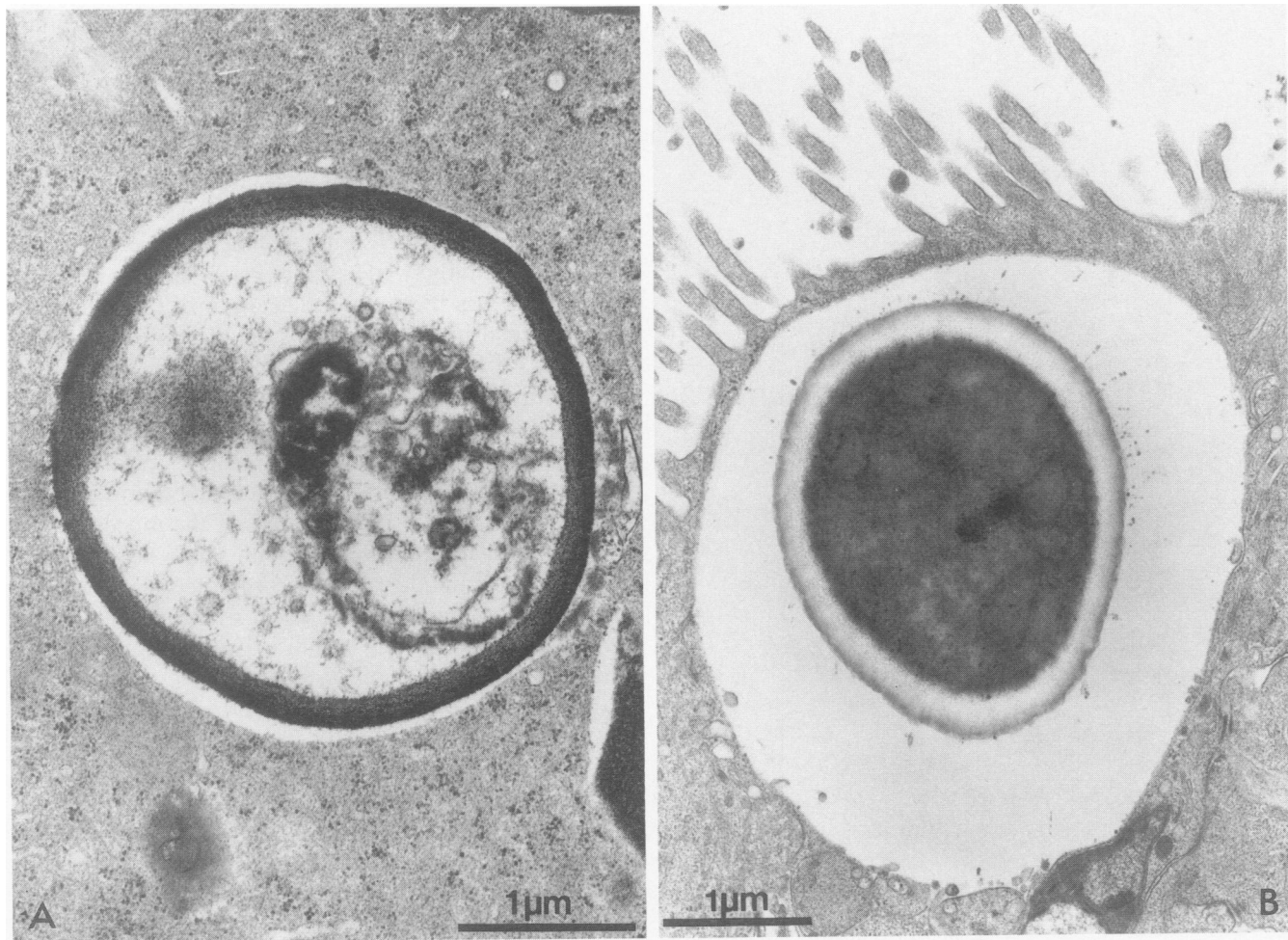
Fixed tissue for scanning electron microscopy (SEM) was washed in osmotically balanced 0.1 mol/L sodium cacodylate, postfixed in 1% osmium tetroxide in the same buffer, rewashed, and dehydrated to absolute ethanol. Exchange to liquid carbon dioxide and critical point drying were accomplished in a DCP-1 Critical Point Dryer (Denton Vacuum, Inc., Cherry Hill, NJ). Dried specimens were mounted on aluminum stubs and coated with gold-palladium in a Fullam Mini-coater (Ernest F. Fullam, Inc., Lathan, NY).

Examination by light microscopy. The semithin sections stained with toluidine blue were examined by standard LM using magnification up to 1000x. Photographs were

made using Kodak Ektachrome 160 Tungsten light film (Kodak, Rochester, NY) at magnifications of 80x and 320x.

TEM and SEM. Transmission electron microscopic observation was accomplished with a JEOL JEM-100C microscope (JEOL Ltd., Boston, MA). Micrography was performed using Kodak electron microscope film 4489. Scanning electron microscopic observation was accomplished with a JEOL JSM-35 microscope and images were recorded onto Polaroid type 55 positive/negative sheet film.

Preparation of monoclonal antibody 2G10. Monoclonal antibody 2G10 was made against the LPS of *E. coli* and *Salmonella minnesota* by the method described by Priest et al.³¹ Monoclonal antibody subisotype determination demonstrated that 2G10 was of the IgG₁ subclass. Monoclonal antibody 2G10 bound strongly by Western immunotransblot analysis to *S. minnesota* Ra, Rb, Rc, Rd,



FIGS. 5A–B. Most of the internalized candida were not surrounded by a membranous structure, suggesting that internalization occurred by a process other than classical phagocytosis.

Re, and wild type, *Salmonella typhimurium*, *E. coli* J5, *E. coli* 0111:B4, and *E. coli* 055:B5. This binding pattern was most compatible with outer/intermediate core region binding and was highly cross-reactive. The enzyme-linked immunoabsorbant assay demonstrated elevated titers against *S. minnesota* Re and *E. coli* J5 LPS and minimal binding against all other types of LPS.

Fluorescence microscopy. Frozen sections on slides were warmed at 70 C for 2 minutes, immersed in cold acetone for 10 minutes, and allowed to air dry. The tissue was then rehydrated in PBS pH 7.4 for 2 minutes, excess PBS was removed, and PBS plus 5% goat serum was added and the slides were incubated for 1 hour at 37 C. After incubation the slides were washed for 5 minutes in PBS and mouse anti-LPS (2G10, 1:40 in PBS + 5% goat serum, described above) was added, and the slides were incubated for 2 hours at 37 C. Following three 10-minute washes in

PBS, goat anti-mouse antibody labeled with fluorescein isothiocyanate (FITC) [1:7 in PBS + 0.1% bovine serum albumin (BSA)] (Kierkegaard and Perry, Gaithersburg, MD) was added and the slides were incubated again at 37 C for 2 hours. Following a rinse in PBS, a solution of propidium iodide (500 ng/mL in PBS) was added for 5 minutes and the slides were washed, allowed to dry, and covered with cover slips.

Results

Studies with *C. Albicans*

Specimens from the rats and guinea pigs provided virtually identical examples and will be discussed together. More than 100 semithin sections stained with toluidine blue were examined by LM, and specimens of special interest were sectioned further for study by TEM. A characteristic appearance of *C. albicans* by LM when stained with toluidine blue as well as a typical appearance by TEM and the presence of pathognomonic budding yeast in many specimens provided easy and sure identification in most specimens.

Translocation directly into the mucosal cells was seen to occur as early as 1 hour after burn. Translocation of candida into the enterocytes was more frequently observed at the villus tips, sometimes associated with submucosal edema. In some specimens there was apparent edema between the epithelial cells but candida were never seen to translocate into these edematous spaces or between cells. Only rarely was there frank ulceration of the villus tips with candidal invasion through the ulcerations. Examination of different specimens from the same animal that showed translocation did not occur uniformly. This variability appeared in some instances to occur because of a lack of access of the organisms to the mucosal surface because of a blanket of mucous. In later experiments attempts were made to remove some of the mucous layer by gentle irrigation of the loop with saline before instillation of the candida, and this promoted a more even distribution of translocation. Even so translocation was seen to occur extensively in villi adjacent to other villi in which no translocation occurred.

Observations of the translocation process. By SEM contact of individual candida with attachment to the brush border was readily observed (Fig. 1). After internalization, the organisms appeared as hemispherical irregularities of the surface. Transmission electron microscopy showed some erosion of the tips of the microvilli, with disorientation of their linear alignment followed by penetration of the organism into the brush border (Fig. 2). Many organisms were seen associated with the brush border and many organisms were seen within the enterocytes im-

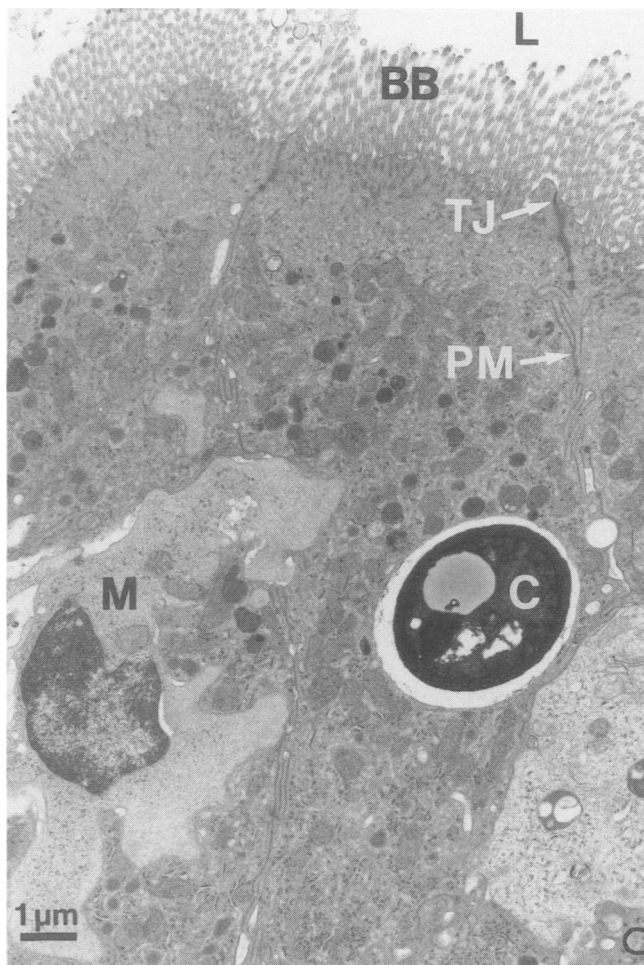


FIG. 5C. An enterocyte containing a candida is bordered on both sides by intraepithelial macrophages migrating through the mucosa via the intercellular junctions.

mediately below the plasma membrane, but the actual process of internalization was observed only once (by LM) (Fig. 3), indicating that the internalization occurred rapidly. Considerable disturbance of the plasma membrane was seen by TEM at sites of entry (Fig. 4), which could represent loss of continuity of the actin filament network within the brush border. Internalized organisms were contained within vacuoles that were rarely lined by membrane with no evidence of lysosomal degranulation (Fig. 5). Sometimes more than one candida were seen within an individual enterocyte, and they were found throughout the apical to basal orientations of the cells. Passage through the basal side of the cell also appeared to be a rapid process and was infrequently observed. However, in one specimen, candida appeared to be extruded into the lamina propria through a disruption of the basement membrane carrying along with them portions of the cytoplasm and intracellular organelles (Fig. 6). In this example the disruption may represent local perforation of the plasma membrane allowing release of candida. Morphologically enterocytes appeared otherwise uninjured by this process of transport.

Many candida were found in the lamina propria, indicating that residence there was relatively prolonged. Most of the organisms were found near the villus tips or

in the middle of the villi, with only a few at the base, suggesting an orderly egress from this anatomic position. By both LM and TEM, most organisms were engulfed by phagocytic cells, predominately macrophages. Macrophages were encountered that had ingested several candida, and candida were observed in different states of degradation. However in many specimens candida were found to be budding both within macrophages and in the extracellular spaces. Perhaps 20% to 30% were within extracellular spaces, many of these free within lymphatics (Fig. 7a) or less frequently in veinules (Fig. 7b). In the submucosal lymphatics, the organisms were found both free and within macrophages, sometimes associated with leukocyte aggregates.

Examples were seen in which a high frequency of translocation occurred through the mucosal epithelium covering Peyer's patches. Organisms were seen throughout the structure of the Peyer's patch and appeared to move into the germinal centers through the pericortical tissue. Macrophages within the germinal centers had many inclusions with the microscopic appearance of degrading candida. However similar structures also were seen in germinal center macrophages in animals not injected with candida. Nevertheless the presence of budding yeast cells within germinal center macrophages, found in several

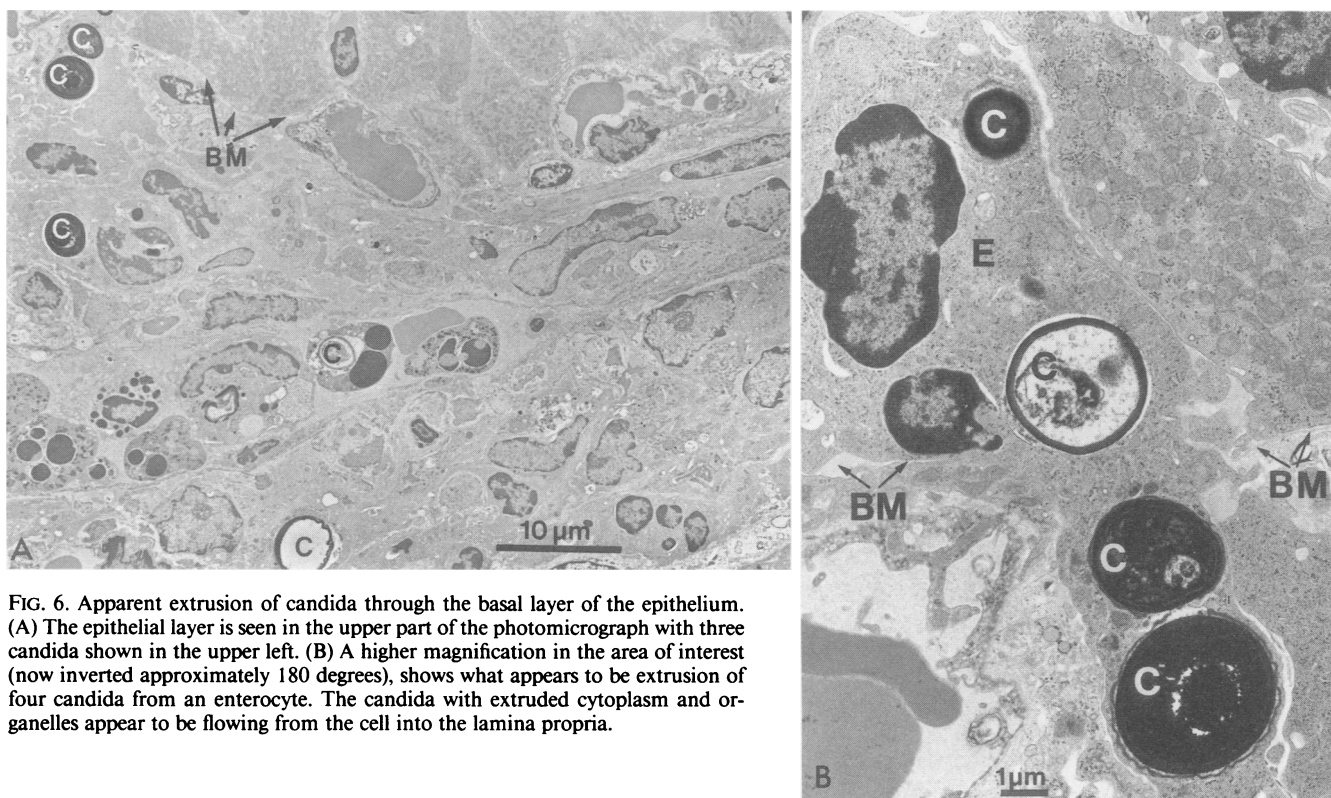
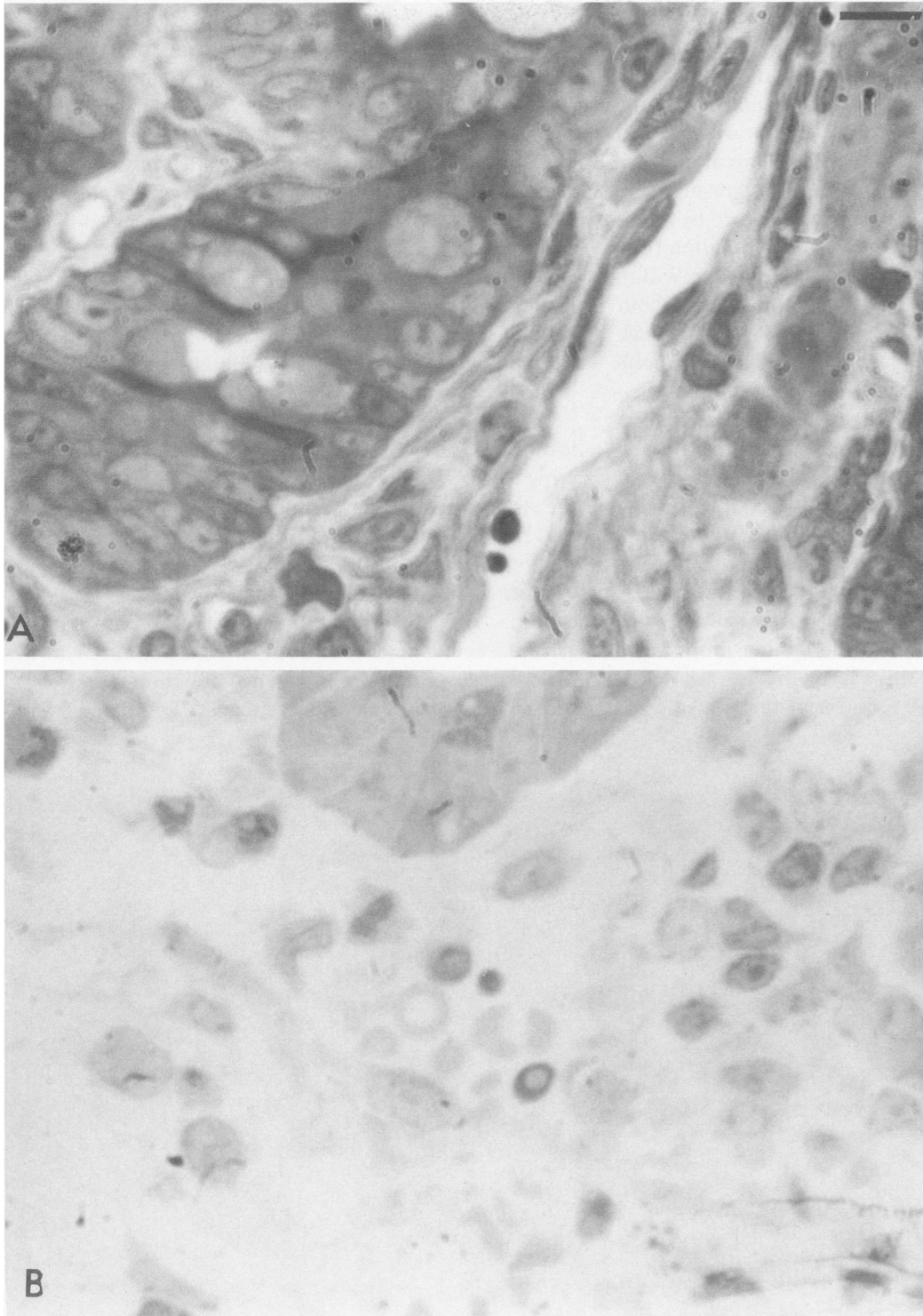


FIG. 6. Apparent extrusion of candida through the basal layer of the epithelium. (A) The epithelial layer is seen in the upper part of the photomicrograph with three candida shown in the upper left. (B) A higher magnification in the area of interest (now inverted approximately 180 degrees), shows what appears to be extrusion of four candida from an enterocyte. The candida with extruded cytoplasm and organelles appear to be flowing from the cell into the lamina propria.



FIGS. 7A and B. Light micrographs stained with toluidine blue. (A) Three candidal organisms are seen within lymphatics of the lamina propria (2) and submucosa (1) without evidence of phagocytosis. (B) Two candida are found within a small venule at the base of the lamina propria without evidence of phagocytosis (scale bars = 10 μ m).

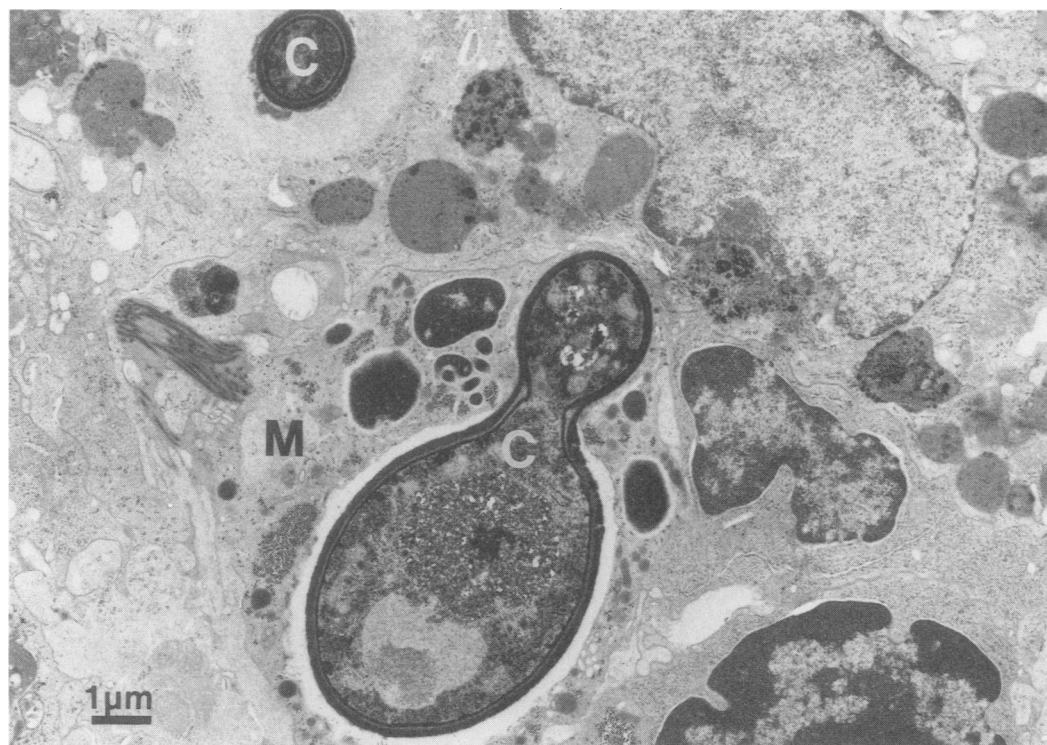
specimens (Fig. 8), indicated that the germinal centers might be an active site of degradation for candida along with other potential pathogens and effete host cells.

The regional lymph nodes showed candida less frequently than the mucosal cells and lamina propria. When

yeast bodies were found, they were both free in lymphatics and engulfed by macrophages throughout the nodes (Fig. 9).

In specimens taken 24 hours after intraluminal infusion of the yeast, there were fewer numbers within the entero-

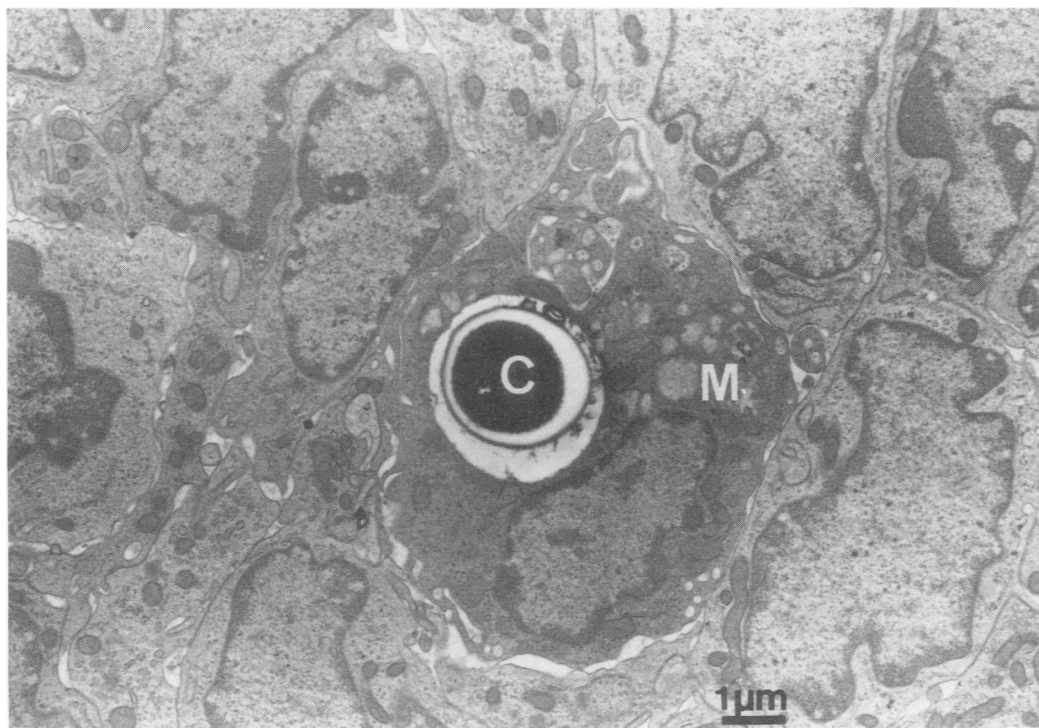
FIG. 8. Budding yeast within a macrophage in a germinal center of a Peyer's patch.



cytes and the lamina propria. However microabscesses with budding yeast were found in the lamina propria (Fig. 10), and vascular occlusion by budding yeast was found in some of the large vessels. Of interest was the presence

of yeast on the serosal surface of the intestine with invasion into the muscular layer. Rarely, in specimens examined from animals killed closer to the time of the burn, were yeast forms found within the muscular wall.

FIG. 9. Candida within a macrophage in the pericortical area of a mesenteric lymph node.



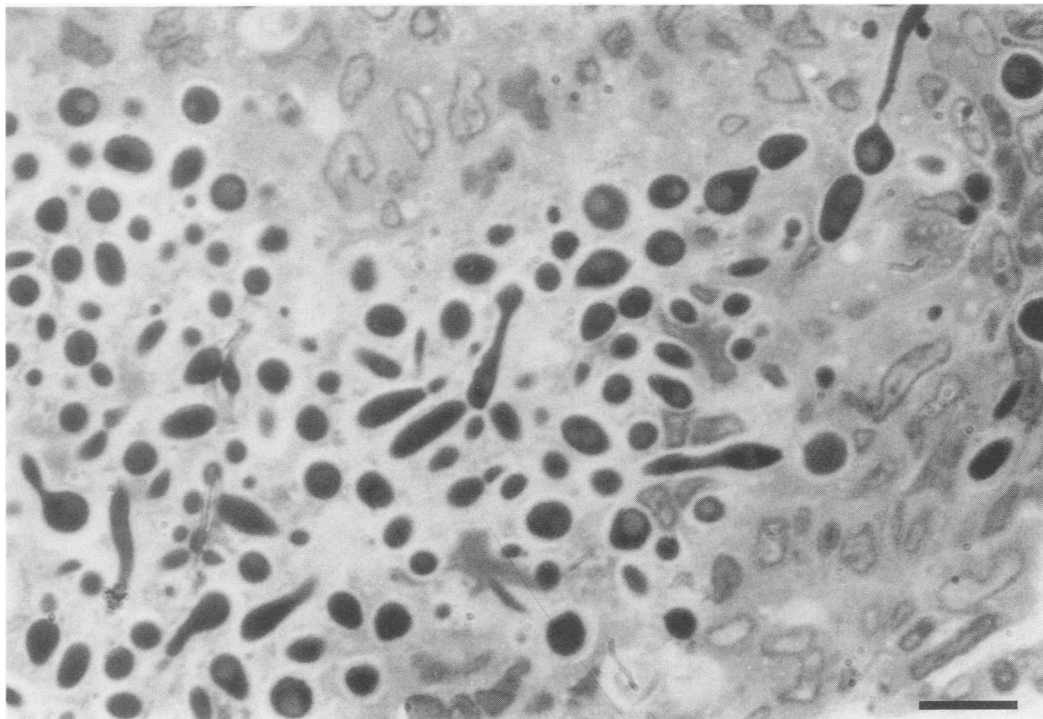


FIG. 10. Light micrograph of a small microabscess with budding candida in the lamina propria of a rat 24 hours after instillation of candida into the loop. The mucosa was intact over the microabscess (scale bar = 10 μ m).

E. coli

Study of translocation of *E. coli* in Thiry-Vella loops by light microscopy failed to provide sufficient resolution to accurately trace the process. Because of this it was difficult to accurately select areas for thin-section processing. Nevertheless, using TEM, several organisms were seen embedded in the brush border as well as within the cytoplasm (not shown). Like candida the *E. coli* was always within the enterocytes and never was seen passing between them.

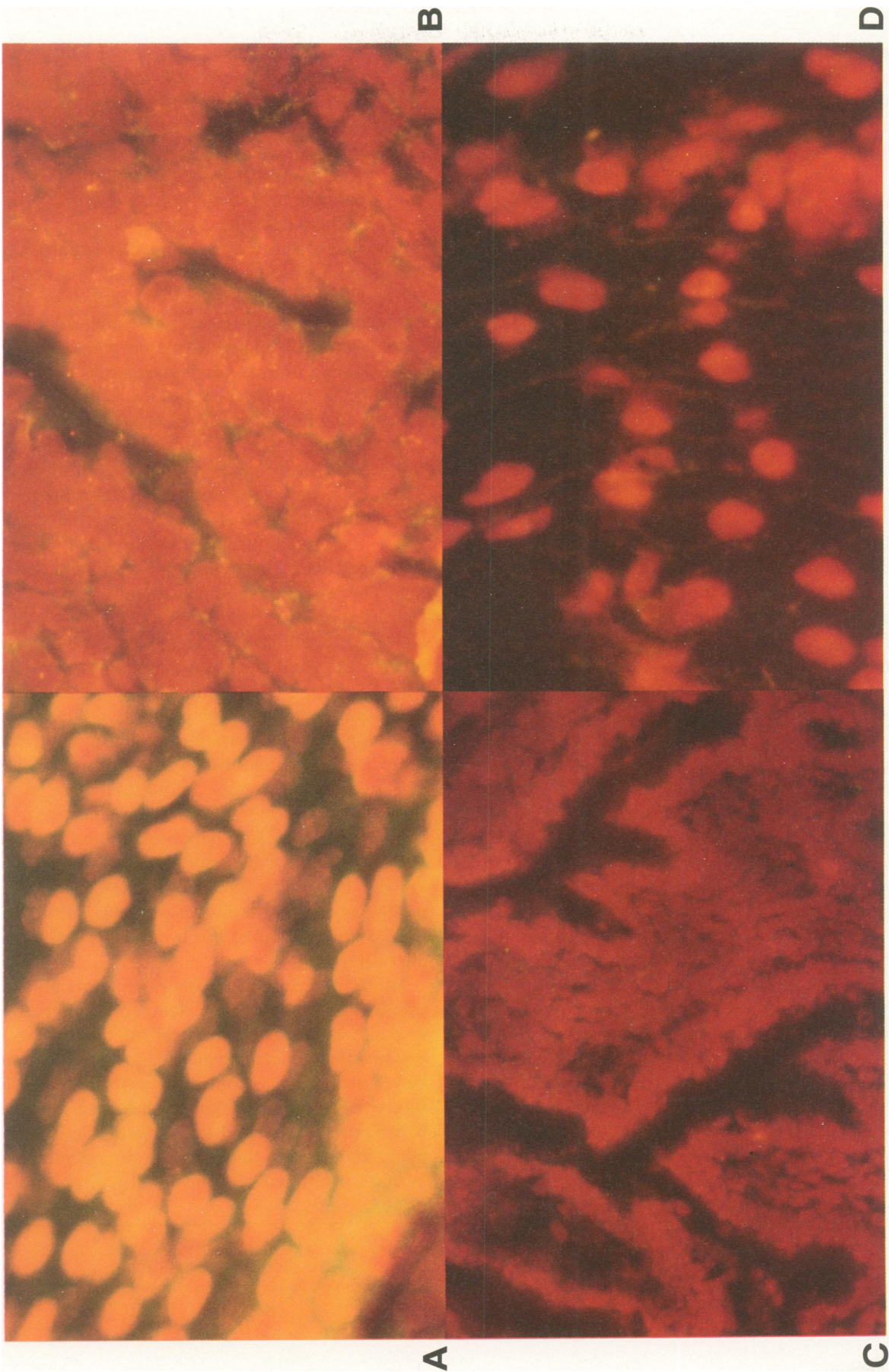
Examination of frozen sections of tissue stained by a fluorescent antibody technique using a lipopolysaccharide-specific monoclonal antibody revealed that the translocation process for *E. coli* was both similar to and different from that of *C. albicans*. This difference arose because fluorescent staining occurred not only of the infused, intact live organisms but also of live resident *E. coli*, infused and resident dead *E. coli*, and endotoxin that was free from bacterial cells.

Within 1 hour granular staining was prominent within the enterocytes, the granules being both quite small and

the size of the *E. coli* organism itself (Fig. 11). Clumping and aggregation of these particles became more prominent in the lamina propria with beginning concentration within cells, presumably macrophages. The relative numbers of stained cells and their density increased as the bottom of the lamina propria was approached. Stained cells as well as free fluorescent particles were readily demonstrable within the submucosa but insufficient resolution was not possible on frozen sections to determine whether the stained particles were largely within vascular structures or outside them. A remarkable feature was that the specific granular stain was seen to extend throughout the muscular layers to the serosa, concentrating between rather than in cells.

The above observations were interpreted to show that both *E. coli* and its endotoxin product, which were identified by the lipopolysaccharide-specific monoclonal antibody, pass into the lamina propria directly through enterocytes in a manner similar to the larger *C. albicans* and not through the intercellular spaces. Once within the lamina propria, the *E. coli* and endotoxin appeared to be both free and cell bound, presumably to phagocytic mac-

FIGS. 11A-E. (A) *Top left*. Immunofluorescent stain of a villus 1 hour after burn injury and instillation of *E. coli*. Note both diffuse and granular stains within both the mucosal layer and lamina propria. Some of the granular stain is located within macrophages. (B) *Top right*. Negative control, not treated with the anti-lipopolysaccharide monoclonal antibody, of a villus from the same animal. (C) *Bottom left*. There is increased localization of stain within phagocytic cells in the lamina propria. (D) *Bottom right*. The muscular layer of the intestine 2 hours after burn shows heavy specific staining for endotoxin in a granular pattern with apparent localization between the cells. (E) Page 509, *top left*. Negative control not stained with the anti-LPS antibody.



rophages. As the *E. coli* and endotoxin passed through the lamina propria, there was increasing concentration in cells, but there also was a considerable diffusion directly through the intercellular spaces of the muscular wall to reach the serosal surface.

Endotoxin

Samples to examine the translocation of endotoxin by fluorescence microscopy were processed in exactly the same manner as were samples for *E. coli*, and the findings were similar, with the exception that the stain within the enterocytes was more diffuse than granular (Fig. 12). Once inside the lamina propria, the endotoxin was concentrated within cells, presumably macrophages, and also spread diffusely throughout the intercellular spaces into and through the muscular wall, passing between the myocytes.

Discussion

Several factors are known to lead to increased translocation of microbes across the intestinal barrier.³² These include (1) direct injury to the enterocytes, as may occur with gamma irradiation or toxins; (2) reduced blood flow to the intestine, as may occur with hemorrhagic shock or the systemic administration of endotoxin or inflammatory agents; (3) an increase in the microbial load, as may occur with resistant organisms following the use of antibiotics or antacids; and (4) altered nutrition, as may occur with the administration of defined elemental enteral diets or intravenous hyperalimentation. Particle size also may influence the rate of translocation, with smaller particles translocating more easily.

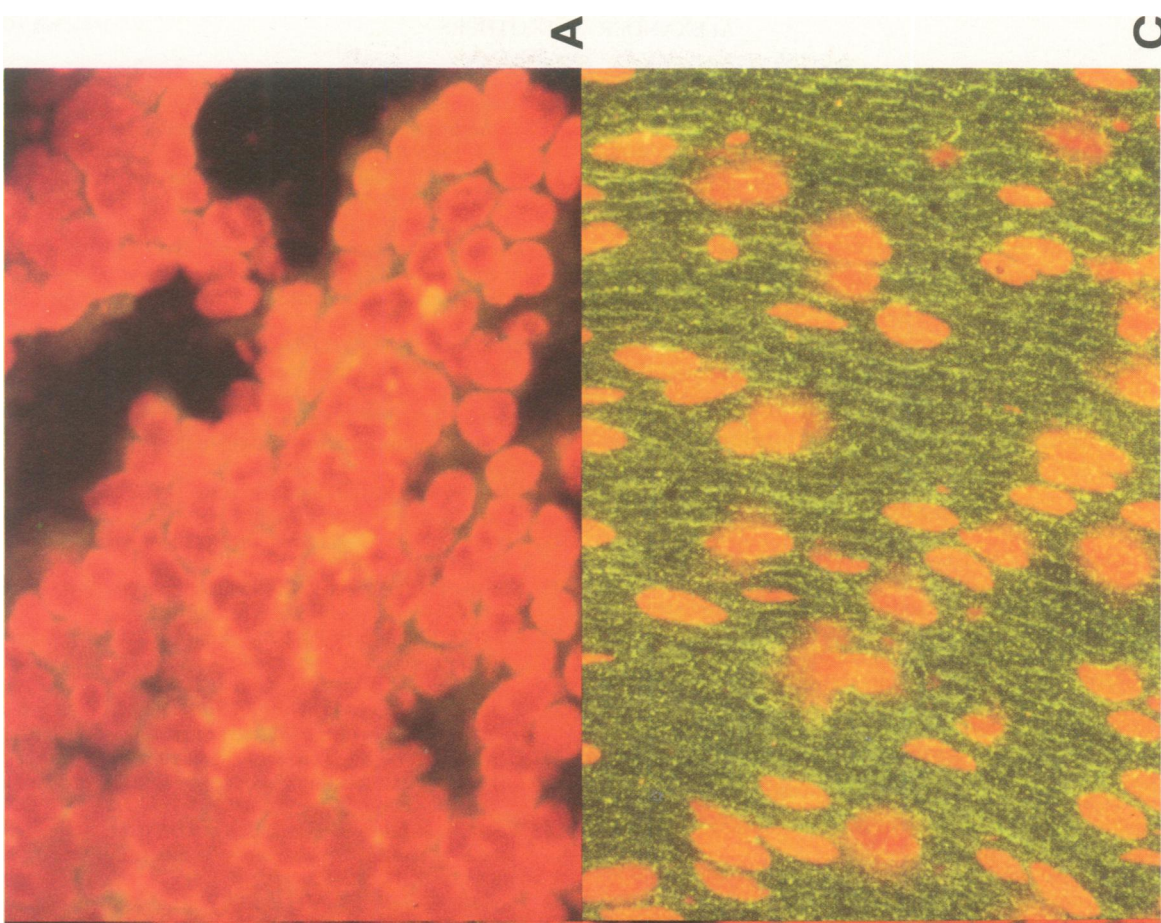
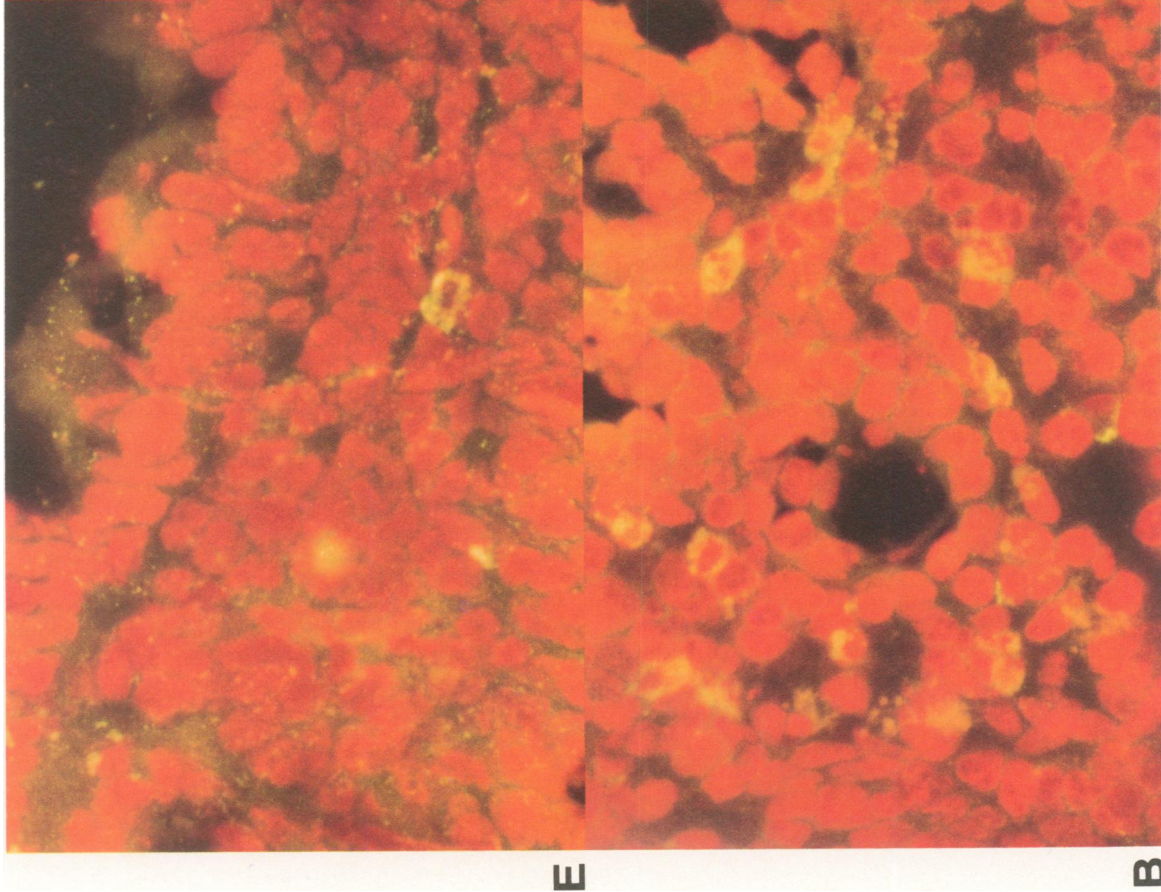
In this study we used a model that was associated with a relatively high rate of translocation. In the isolated Thiry-Vella loop, lack of luminal nutrients is associated with atrophy of the mucosa, even in well-nourished animals. However Deitch et al.⁷ have shown that malnutrition with mucosal atrophy in itself will not necessarily result in an increase in translocation of bacteria. Use of a 50% burn coupled with a high concentration of microbes within the lumen of the Thiry-Vella loop was associated with a high rate of translocation, which, at least in the case of *C. albicans*, occurred by precisely the same mechanisms as were observed in intact gastrointestinal tracts of animals receiving thermal injury with or without antibiotics (unpublished observations).

The most important finding of our study is that both large (*C. albicans*) and small (*E. coli*) intact microbes and endotoxin translocated directly through morphologically intact enterocytes through a similar mechanism, which is different from classical phagocytosis and exocytosis. Translocation through rather than between enterocytes is consistent with the previous observations of Cole et al.,²³ who demonstrated direct internalization of candida by enterocytes after intragastric inoculation into 5- to 6-day-old mice and also the recent study of Wells et al.,²⁶ who showed that enterococci translocated through rather than between enterocytes in mice. These studies are remarkable in that endotoxin and perhaps some intact organisms once in the lamina propria pass between cells and through the muscular layer of the bowel to the serosa. This finding is consistent with the observations of Wells et al.,³³ who showed translocation of enteric bacteria through the intestinal wall into experimental intraperitoneal abscesses, and Papa et al.,³⁴ who demonstrated transmural migration of endotoxin through ischemic colons in dogs.

The observation that large amounts of endotoxin translocated across the mucosa is also an important finding. The usual method for quantitation of microbial translocation in experimental models is by enumerating culturable organisms in the mesenteric lymph nodes or other organs, but clearly this does not accurately reflect what has passed the intestinal barrier. The use of monoclonal antibodies as a semiquantitative marker for this process provides a more meaningful assessment for endotoxin. In an unpublished study using ¹⁴C-labeled *E. coli*, infused into the gastrointestinal tract of mice, we showed that less than 0.1% of the radioactive label localizing in the mesenteric lymph nodes and the spleen was associated with viable bacteria when the animals were killed 1 to 4 hours after a 30% thermal injury. In this model, as well as the model with the Thiry-Vella loop, translocation was found to occur early, less than 1 hour after injury.

We showed that early feeding after burn injury decreases translocation in animals,³⁵ dampens the hypermetabolic response, and preserves intestinal morphology.¹⁸ This suggests that abnormally high rates of translocation may be relatively easy to control by nutritional therapy after injury. Endotoxin from the gut has been shown to cause hepatic dysfunction and activation of Kupffer cells,³⁶ and many investigators think that continued microbial translocation in injured or critically ill patients provides the

FIGS. 12A–C. Specific stain for endotoxin using anti-LPS antibody in the ileum 1 hour after burn of an animal that received endotoxin in the Thiry-Vella loop. (A) *Top right*. There is diffuse staining of the cytoplasm of the enterocytes as well as the lamina propria. (B) *Bottom left*. Negative control without LPS antibody. (C) *Bottom right*. Specific anti-LPS immunofluorescent staining in the muscular wall is localized between rather than within the myocytes.



stimulus for a 'septic state,' even in the absence of a demonstrable focus of infection or bacteremia, and that this ultimately leads to the development of multiple-system organ failure.¹⁹⁻²² While the current studies do not directly address the persistent question of clinical relevance of translocation, they provide clear evidence that translocation of viable microbes and endotoxin occurs with great frequency through morphologically intact enterocytes. Further investigation of the mechanisms regulating enterocyte function should lead to a better understanding of how to control the translocation process and better assess its role in the pathophysiology of human disease.

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