Transmissible Ileal Hyperplasia of Hamsters

I. Histogenesis and Immunocytochemistry

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Transmissible ileal hyperplasia (TIH) was experimentally induced in weanling hamsters, and the development of lesions was characterized. Ileal lesions developed in two phases: a hyperplastic phase which was detected by Day 10 and an inflammatory phase which began by Day 20. Hyperplasia began as focal lengthening of villi with expansion of crypt-type epithelium onto villus walls. Diffuse hyperplasia of distal ileum developed; dilated, tortuous crypts penetrated subjacent supporting tissues; but metastases were not seen. Inflammation began in association with focal or segmental necrosis of crypt epithelium, and crypt abscesses developed. Severe pyogranulomatous inflammation of the ileal wall, focal peritonitis, mesenteric lymphadenitis, and portal hepatitis were common in advanced lesions. Development of ileal lesions was closely correlated with accumulation of particulate antigen, detectable by immunofluorescence, in the cytoplasm of mucosal epithelial cells. Antigen was also detected in ileal granulomas, mesenteric lymph nodes, and liver. There was simultaneous development of serum antibody specific for intracytoplasmic antigen. These studies confirm that mucosal hyperplasia is the primary lesion in TIH. (Am J Pathol 91:433–450, 1978)

TRANSMISSIBLE ILEAL HYPERPLASIA (TIH) is a common enzootic disease of weanling hamsters. It is characterized clinically by diarrhea ("wet tail"), dehydration, and high mortality and morphologically by segmental mucosal hyperplasia and pyogranulomatous inflammation of distal ileum.[1-3] The lesion has been called neoplastic ("enzootic intestinal adenocarcinoma") by some workers because columns of proliferating mucosal epithelium penetrate adjacent muscle tunics.[1] Other investigators recognized an early hyperplastic phase but considered the lesion to be primarily inflammatory ("proliferative ileitis").[2] These contrasting interpretations were based exclusively on morphologic studies of naturally occurring lesions, since attempts to induce TIH experimentally were unsuccessful.

We recently described the first serial experimental transmission of TIH and gave preliminary histologic evidence that mucosal hyperplasia preceded ileitis.[3] We also found that hyperplastic epithelium contained an intracytoplasmic antigen(s), detected by immunofluorescence, which was

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morphologically compatible with one or more species of intracellular bacteria. This report is the first detailed description of the morphogenesis of experimentally induced TIH and confirms that the primary lesion is mucosal hyperplasia. The accompanying paper describes the ultrastructural changes of TIH and indicates there is a close association between the development of hyperplasia and the presence of intracellular bacteria.[4]

Materials and Methods

Animals

Male weanling Lak:LVG(syr) hamsters weighing 25 to 30 g (Lakeview Hamster Colony, Newfield, N.J.) were housed in plastic boxes with autoclaved pine chip bedding and filter lids and were fed autoclaved D&G Research Animal Laboratory Diet (Price-Wilhoite Co., Frederick, Md.) and hyperchlorinated (9 ppm) water *ad libitum*.

Animal Inoculation

Pooled, experimentally induced ileal lesions or normal ileums were homogenized to 10% (w/v) in cold sterile saline. Hamsters were infected by gavage with 1.0 ml homogenate. Control hamsters were gavaged with 1.0 ml of homogenized normal ileum.

Necropsy

Hamsters were exsanguinated by cardiac puncture. Transverse sections for immunocytochemistry of duodenum, jejunum, proximal ileum, terminal ileum, cecum, colon, liver, spleen, and mesenteric lymph node were snap-frozen and stored at −60 C. The sections of intestine were taken at standardized distances from the ileocecal junction. Longitudinal and transverse sections of intestine for histology were taken immediately adjacent to sections for immunocytochemistry. Longitudinal sections were opened, spread on paraffin blocks, and immersed in 10% neutral buffered formalin. Pieces of pancreas, liver, thymus, spleen, mesenteric lymph node, brain, heart, trachea, kidney, urinary bladder, esophagus, adrenal gland, salivary gland, and testes were also fixed in formalin. Lungs were inflated with formalin by intratracheal perfusion.

Histopathology

Four- to six-μ paraffin sections were stained with hematoxylin–eosin and examined by light microscopy. Selected sections were stained with periodic acid–Schiff (PAS) stain, Giemsa stain, Kinyon's acid fast stain, Warthin–Starry stain, or Brown and Brenn stain.

The crypt–villus cell column height for each hamster was determined by counting epithelial cells lining at least five randomly selected crypt–villus units from distal ileum of each hamster. A crypt–villus unit was counted only if a continuous ribbon of epithelial cells could be followed from the base of a crypt to the tip of an adjacent villus.

Immunocytochemistry

Six-μ cryostat sections were fixed and stained by the indirect immunofluorescence technique as previously described.[3] Direct staining was performed with immunoglobulin prepared from pooled hamster anti-TIH serum (from hamsters with advanced lesions) by precipitation with 50% ammonium sulfate. Immunoglobulin was conjugated to FITC, and the IgG fraction was recovered by DEAE cellulose ion-exchange chromatography.[5] Sections were examined with a Zeiss fluorescence microscope fitted with a BG-12 UV exciter

filter and No. 53 and No. 44 barrier filters. Reagents were tested for specificity by routine blocking tests and by staining of control sections of antigen-positive or antigen-free ileum with anti-TIH serum or with serum from normal hamsters. Anti-TIH antibody reacted specifically with TIH-associated antigen by direct or indirect immunofluorescence. Anti-TIH antibody did not react with normal ileum.

Anti-TIH antibody titers in serum from experimentally infected hamsters were estimated by indirect immunofluorescence. Replicate sections of an ileal lesion containing large amounts of TIH-associated antigen were incubated with serial dilutions of test serum and then with a 1:20 dilution of a single lot of goat antihamster IgG–FITC. The endpoint was taken as the highest dilution of serum from infected hamsters which produced brighter specific staining than a standard normal hamster serum.

Design of Experiment

One hundred thirty-one hamsters were distributed, 3 to 5 per box, among 27 boxes. They were identified individually by ear notching, and 125 were inoculated with TIH homogenate. Six control hamsters, housed separately, were inoculated with homogenate of normal ileum. They were observed daily for clinical signs. Necropsy was performed on 5 infected hamsters every fifth day for 40 days, beginning with the day of inoculation. Hamsters were selected for necropsy from a table of preassigned numbers so that no more than 1 hamster was taken from a box on a given necropsy day. If a hamster died before its designated necropsy day, the next animal on the list was taken. Forty-five infected hamsters and 6 control hamsters (three necropsies on Day 15 and three on Day 30) were evaluated morphologically. The additional hamsters were included to allow for attrition from TIH-related deaths during the latter half of the experiment.

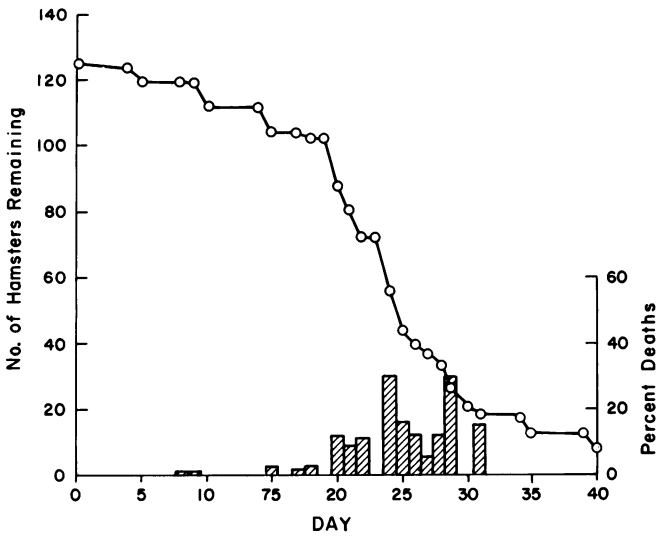
Results

Clinical Disease

Clinical signs occurred only in infected hamsters and began approximately 3 weeks after inoculation. Typical signs included weight loss, soiled rear quarters (diarrhea), progressive dehydration, anorexia, hunched posture, and reluctance to move. Ropelike abdominal lesions were palpable in most hamsters 5 to 10 days before clinical disease appeared. Death usually occurred 3 to 4 days after the onset of clinical signs, although several hamsters died without premonitory signs. Mortality was low for 3 weeks after inoculation, although several deaths from acute enteritis occurred by Day 10. Mortality increased between Days 10 and 30, and all dead hamsters had TIH (Text-figure 1).

Macroscopic Lesions

Gross visceral lesions were restricted to the alimentary tract and mesenteric lymph nodes. Mild diffuse enlargement of ileum extending proximally from the ileocecal orifice for 2 to 8 cm was seen by Day 15. The thickness of the ileal wall increased to 1.0 mm, and the mucosa was slightly roughened and hyperemic. The serosal surface also had a red hue. The transition from thickened to normal ileum was usually abrupt, but



TEXT-FIGURE 1—Spontaneous mortality among hamsters with experimental TIH. The histogram shows the percent spontaneous mortality of hamsters remaining alive at various days after inoculation. The total number of hamsters (*open circles*) decreased progressively due to spontaneous mortality and tissue collections made at 5-day intervals.

occasionally the transition zone was tapered. The cecum was flaccid and its contents were fetid and watery.

By Day 20 distal ileum was rigid, with walls 1.5 to 2.0 mm thick. The mucosa was raised, rough, and brown-red and the lumen occasionally contained blood. The serosal surface was smooth and red to gray; the transition to normal proximal ileum was sharp. Lesions were usually in distal ileum, but several extended proximally from the ileocecal junction for more than 10 cm. Cecal dilatation had increased and the contents were watery or mucoid. Peyer's patches and mesenteric lymph nodes were slightly enlarged. The colon contained yellow-green liquid feces or normal fecal pellets.

By Day 30 ileal walls were up to 4.0 mm thick, the mucosa was partially covered with gray-yellow plaques, and the lumen was either stenotic or dilated. Multiple 1- to 2-mm gray-white nodules (granulomas and abscesses) studded the serosa. The small intestine proximal to ileal lesions was frequently dilated and filled with yellow-brown liquid which was occasionally tinged with blood. The cecum remained fluid-filled and flaccid. Peyer's patches were enlarged, and mesenteric lymph nodes were twice normal size.

By Days 35 to 40 large sections of distal ileal serosa were effaced by clusters of gray-white nodules and there were focal adhesions between ileum, adjacent mesentery, and parietal peritoneum.

Although ileal lesions usually developed adjacent to the ileocecal junction, there were several exceptions. Segmental hyperplasia of distal ileum occurred 1 to 2 cm proximal to the ileocecal junction in a few hamsters,

and 1 hamster had severe thickening of proximal colon with normal ileum. Intussusception of thickened terminal ileum into cecum occurred in most moribund animals by Day 20. Control hamsters had no macroscopic lesions. Typical gross lesions at several stages of development are shown in Figures 1 through 3.

Histopathology

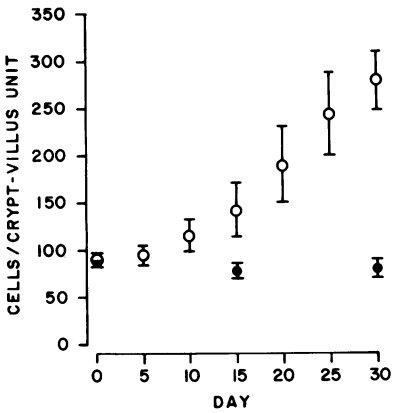
Lesions associated with TIH occurred in the intestinal tract, mesenteric lymph nodes, and liver of infected hamsters. Several infected and control hamsters had mild bronchopneumonia compatible with Sendai virus infection.

Intestine

A brief description of ileum from control hamsters is given for comparison with lesions of TIH. The mucosa consisted of crypt–villus units supported by a thin lamina propria (Figure 4). Crypts were lined by columnar epithelium with deeply basophilic, basally oriented nuclei and amphophilic cytoplasm. Segments of crypt epithelium were pseudostratified. Paneth cells with large eosinophilic cytoplasmic granules were found at the base of many crypts. Mitotic figures were common among crypt cells. The crypt–villus junction was marked by a genu where epithelial cells changed from the "crowded" crypt to typical columnar absorptive cells. Nuclei of differentiated villus cells were round to oval and vesicular and were basally oriented. The cytoplasm was eosinophilic and free of inclusions. There was no pseudostratification or mitotic activity. Some goblet cells were present. Dying epithelial cells with pyknotic nuclei were extruded from village tips. The muscularis mucosa was several myofibers thick and the submucosa was narrow. The muscle tunics consisted of three to five layers of myofibers. The lumen contained some trichomonads.

Ileal lesions developed in two phases: a hyperplastic phase which began by Day 10 and an inflammatory phase which began by Day 20. Cell counts of crypt–villus units increased progressively after Day 5, and by Day 30 the average number of cells per column had increased threefold (Text-figure 2). Counts were not made after Day 30 because of mucosal distortion from inflammation.

Hyperplasia began by focal elongation of villi with expansion of crypt-type epithelium onto the lower one third to one half of villus walls (Figures 5 and 6). Mitotic figures were plentiful in crypts, and some crypt cells contained intracytoplasmic inclusions resembling necrotic cells or clumps of nuclear material. Mucosal hyperplasia stopped at the ileocecal junction, and the proximal edge of most lesions was marked by an abrupt reduction in villus height.



TEXT-FIGURE 2—Average number of mucosal epithelial cells per crypt–villus unit among hamsters with TIH (*open circles*) compared with control hamsters (*closed circles*). Bars indicate ±SE.

Ileal hyperplasia was diffuse by Day 15, but distal ileum was more severely affected. Crowded crypt-type epithelium with numerous mitotic figures and cytoplasmic inclusions covered the lower one half to three quarters of each villus. Villi were longer and more tortuous, with prominent extrusion zones. Villi were also wider than normal because hyperplastic epithelial cells were elongated and pseudostratified. Some crypts were mildly dilated and crypt lumens contained cell debris. Paneth cells were not evident. There was a trace of inflammation in 1 hamster, characterized by focal necrosis of crypt epithelium and infiltration of adjacent submucosa by a few neutrophils and macrophages. The macrophages had abundant pale eosinophilic cytoplasm containing some PAS-positive granules. There was also mild inflammatory edema of cecal lamina propria.

By Day 20 villi in distal ileum were two to three times longer than normal and were covered by hyperplastic epithelium; cells in mitosis were seen commonly near villus tips (Figure 7). Villus tips were blunted, necrotic, or hemorrhagic. Adjacent villi were frequently fused. Intrusion of some Peyer's patches by hyperplastic mucosa occurred so that crypts nearly abutted on muscle tunics (Figure 8). Some crypts had also extended laterally under neighboring crypts; dilated, distorted crypts with intralumenal debris were common. Ileitis had developed in 1 hamster. The submucosa was diffusely infiltrated by neutrophils and macrophages with abundant PAS-positive, non-acid–fast cytoplasmic granules (Figure 9).

Some crypts were packed with neutrophils, and cryptal and subcryptal abscesses communicated through rents in crypt walls. Mucosal hyperplasia stopped at the ileocecal junction (Figure 10), but cecal lamina propria was often infiltrated with lymphoid cells and neutrophils.

By Day 25, fronds of hyperplastic epithelium had either dissected

laterally beneath adjacent crypts or had penetrated the muscularis mucosa and portions of the muscle tunics (Figure 11). Penetration occurred in both inflamed and noninflamed areas (Figure 12) and pyogranulomatous foci in the submucosa were more frequently associated with necrosis of crypt epithelium and crypt abscesses than with mucosal encroachment on supportive tissues. However, hyperplastic epithelium did not breach its basement membrane and metastases were not detected in any hamsters. Laminar necrosis and hemorrhage of perilumenal hyperplastic mucosa were common, but necrosis of epithelium on segments of individual village walls and panmucosal necrosis also occurred.

By Day 30, many crypts were abscessed, and pyrogranulomatous infiltrates filled the lamina propria and submucosa. Granulation tissue and immature fibrous connective tissue developed at the margins of granulomas.

By Day 35, thick collars of pyogranulomatous tissue encircled the hyperplastic mucosa. Tapered columns of epithelium or large crypt diverticula penetrated the inflammatory tissue, and muscle tunics were either disrupted or effaced (Figure 13). Granulomas were often multiloculated with central liquifaction necrosis. Focal peritonitis was common, with adhesions of mesentery and cecum to ileal lesions.

Although mucosal hyperplasia was primarily restricted to ileum, severe mucosal hyperplasia occurred in the proximal colon of 1 hamster from Day 20 (Figure 14). Segmental hyperplasia of cecal mucosa was also found in 1 hamster on Day 25.

Mesenteric Lymph Nodes

Germinal centers were prominent by Day 20 and medullary cords were filled with plasma cells. Cortical and medullary sinuses usually contained some neutrophils. There were multiple foci of PAS-positive macrophages in cortical sinuses and paracortical regions.

Liver

There was spotty nonsuppurative portal hepatitis by Day 15. In hamsters with advanced ileal lesions, portal hepatitis was diffuse and some intrasinusoidal granulomas were seen.

Immunofluorescence

In the intestine, TIH-associated antigen was confined to hyperplastic segments of mucosa, including the hyperplastic colon previously described. Clumps of brightly fluorescing antigen typically were in the apical cytoplasm of mucosal epithelial cells (Figure 15). Fluorescing rod-

shaped bodies were observed in some cells and were seen occasionally in intervillus spaces and in crypts.

Antigen was detected in ileum by Day 10, and by Day 15 continuous or segmental ribbons of antigen outlined individual villi (Figure 16). Antigen accumulated in the cytoplasm of macrophages in the lamina propria and submucosa as pyogranulomatous inflammation developed (Figures 17 and 18). By Day 15 clumps of antigen were detected in large mononuclear cells in Peyer's patches and in the subcapsular sinuses and cortices of mesenteric lymph nodes. The distribution of fluorescing cells corresponded to the histologic distribution of macrophage aggregates. The number of fluorescing foci in lymph node gradually increased through Day 30. Antigen was detected in hepatic portal triads on Day 35, but deposits were small and sparse.

Bacterial stains of ileums revealed intracellular gram-negative rod-shaped bodies in the apical cytoplasm of mucosal epithelium by the time TIH antigen was first detected (Day 10) (Figures 19 and 20). Neither intracytoplasmic bodies nor TIH-associated antigen were seen in intestines from control hamsters nor in normal portions of intestine from infected hamsters.

Antibody Response

Anti-TIH antibody was first detected on Day 10 and titers ranged from 0 to 1:80. After Day 15, most serums had titers of 1:320 or greater (Table 1). Serums from control hamsters were negative. All serums which reacted with TIH-associated antigen in homologous ileum also reacted with antigen in autologous ileum.

Table 1—Titers of Serum Anibody to TIH-Associated Antigen in Hamsters With TIH as Determined by Indirect Immunofluorescence

	No. of days after infection								
Titer	0	5	10	15	20	25	30	35	40
2560									
1280						1	1		
640					4			1	1
320				1	1	4	3	3	2
160				3			1	1	1
80			1						1
40			1						
20			2						
10				1					
<10	5	5	1						

Numbers under each day represent numbers of individual serums tested.

Table 2—Incidence of Mucosal Hyperplasia, TIH-Associated Antigen in Hyperplastic Lesions, and Anti-TIH Antibody in Hamsters Infected With TIH

	No. of days after inoculation								
	0	5	10	15	20	25	30	35	40
No. of hamsters	5	5	5	5	5	5	5	5	5
Hyperplasia	0	0	4	5	5	5	5	3	4
Antigen	0	0	4	5	5	5	5	3	4
Antibody	0	0	4	5	5	5	5	5	5

Correlations Between Lesions, Antigen, and Antibody

All inoculated hamsters with ileal lesions had TIH-associated antigen and anti-TIH antibody. Four inoculated hamsters killed on Day 10 or after did not have lesions or antigen. However, 3 of them developed anti-TIH antibody (Table 2).

Discussion

These experiments confirm previous impressions that TIH begins as hyperplasia [2,3] of ileal mucosal epithelium and that the pyogranulomatous phase occurs after severe hyperplasia has developed.[3] Focal crypt hyperplasia was detected 10 days after infection of weanling hamsters. By 20 days, villi over long segments of ileum were elongated, intermittently fused, and covered by mitotically active crypt-type epithelium. Cell counts of crypt–villus units also increased by 10 days after inoculation and, in severe lesions, average units contained approximately three times as many cells as normal units. Elongation of villi was followed by penetration of crypts into supporting tissues of ileal wall. Mild focal inflammation of basal lamina propria or submucosa preceded penetration in some sections, but this was not common.

Downward expansion of dilated or tortuous hyperplastic crypts had been interpreted by some investigators as a neoplastic change.[1] We could not, however, demonstrate violations of epithelial basement membrane by proliferating cells, and cellular atypia was minimal. Vascular or lymphatic invasion or metastases were not detected. Thus, it is more likely that penetration resulted from the pressures of proliferating crypt epithelium against ileal walls rendered turgid by preceding villus expansion than from invasion by neoplastic epithelium.

The inflammatory phase was characterized by severe pyogranulomatous infiltration of ileal wall. It was usually preceded by dilatation of crypts with crypt abscess formation and necrosis of flattened crypt epithelium. Inflammation was probably caused in some areas by preexisting tissue necrosis, but the role of TIH-associated antigen must be considered.

Inflammation began after antigen appeared in mucosal epithelial cells. Macrophages in inflammatory foci in ileum, lymph node, and liver also contained large quantities of antigen. Ultrastructural studies [4] indicate that TIH-associated antigen is probably a bacterium (or bacteria) and thus could provoke inflammation as a foreign body or through release of chemotactic substances. Tissue invasion by enteric organisms through rents in crypt abscesses undoubtedly exacerbated the development of inflammation.

Serum antibody to TIH-associated antigen was detected by 10 days after inoculation and was found in all infected animals after Day 15. The effect of anti-TIH antibody on the progression of disease has not been studied, but its appearance indicates that immune recognition of TIH-associated antigen is an early event. The antigen-eliciting anti-TIH antibody has not been identified. Current evidence suggests it is the intracellular bacterium (or bacteria) associated with TIH [6] despite the fact that anti-TIH antibody cannot be absorbed from serum by anaerobic or aerobic bacteria isolated from normal or affected hamsters by conventional techniques.[10] In any case, detection of anti-TIH antibody in infected animals offers promise as a serodiagnostic test for TIH, since antibody has been detected only in hamsters exposed to disease and it does not react with normal intestinal mucosa.

The etiology of TIH remains unknown. Previous filtration studies indicated that the causative agent(s) is compatible with a bacterium.[3] In the current study, gram-negative rod-shaped bodies were detected in apical cytoplasm of hyperplastic cells. There is ultrastructural evidence that bacteria colonize ileal epithelium in both naturally occurring and experimental TIH.[4,6] Several attempts to isolate an etiologic agent have proved unsuccessful.[2,3,6-9] However, we have recently been able to transmit disease with ileums from neomycin-treated hamsters whose gastrointestinal tracts were free of detectable aerobic but not anerobic organisms. The aerobically sterile ileums used for transmission contained large amounts of TIH-associated antigen.[10]

Although lesions developed primarily in distal ileum, they sometimes extended proximally to jejunum; in 1 animal severe colonic hyperplasia occurred. Other workers have also reported some variation in location of lesions.[1] In the present study, TIH antigen was found in all cases in which extra-ileal lesions occurred. The cecum was usually spared, although focal hyperplasia was detected in 1 hamster. We found recently that experimentally infected hamsters given 10 mg of neomycin daily developed severe cecal mucosal hyperplasia, and their mucosal epithelium contained

abundant TIH-associated antigen.[10] Therefore, the predilection of lesions for different levels of intestinal tract may depend as much on the local microbial flora as on variations in mucosal sensitivity to proliferative stimuli.

Proliferative lesions of the small intestine have been reported in several species.[11,12] Of particular interest is a syndrome in swine called "intestinal adenomatosis,"[13] which resembles TIH morphologically. Rowland and Lawson [14] found that hyperplastic segments of intestinal mucosal epithelium contained intracytoplasmic bacteria which they identified as *Campylobacter sputorum mucosalis* by immunofluorescence techniques. *Campylobacter* was isolated from ileums of infected swine but not from normal swine.[15] There have been no reports of experimental induction of lesions in swine with either ileal homogenates or pure cultures of the organism. *Campylobacter* has not been detected in hamsters with TIH.

TIH does not mimic any disease of humans. Proliferative lesions of small intestine are rare.[16] TIH may be an excellent model to study regulation of mucosal proliferation. Granulomatous lesions are characteristic of Crohn's disease [17,18] and Whipple's disease.[19] PAS-positive macrophages with intracellular bacteria are found in both Whipple's disease and TIH. However, in Whipple's disease, mucosal epithelium is not hyperplastic and intracellular bacteria are found primarily in inflammatory cells of the lamina propria.[19] Bacteria have, however, long been suspected of playing a role in the etiology of Whipple's disease.[19] Clancy and coworkers have recently isolated a cell-wall–deficient *Streptococcus* from a patient with Whipple's disease and have demonstrated organisms in granulomatous ileal lesions by immunofluorescence.[20]

TIH has been given various names, most of which reflect morphologic interpretations of spontaneous lesions.[1,2] Many observations were made on fully developed lesions and thus stressed the inflammatory phase. We had proposed, from results of a preliminary pathogenesis study, that the name "atypical ileal hyperplasia" be adopted [3] as a more accurate reflection of the primary pathologic process. "Atypical" was included to account for the penetrative behavior of the lesion. Based on the current study, we propose that "atypical" be dropped and that "transmissible ileal hyperlasia" be adopted as a working designation. Since the primary lesion in TIH is mucosal hyperplasia, kinetic studies of ileal epithelium are underway to characterize alterations in the rates of cell replication and migration. Experiments to determine if TIH increases the incidence of intestinal neoplasms in hamsters given 1,2-dimethylhydrazine [21,22] are also in progress.

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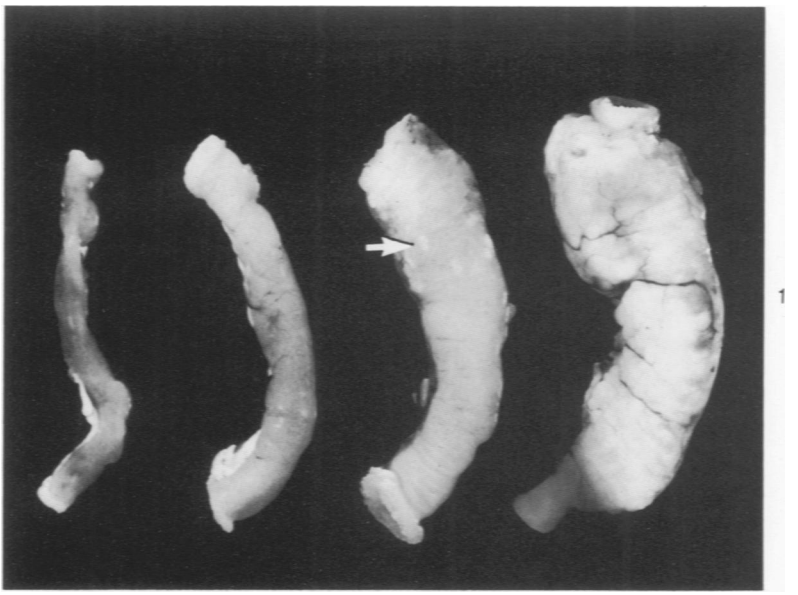

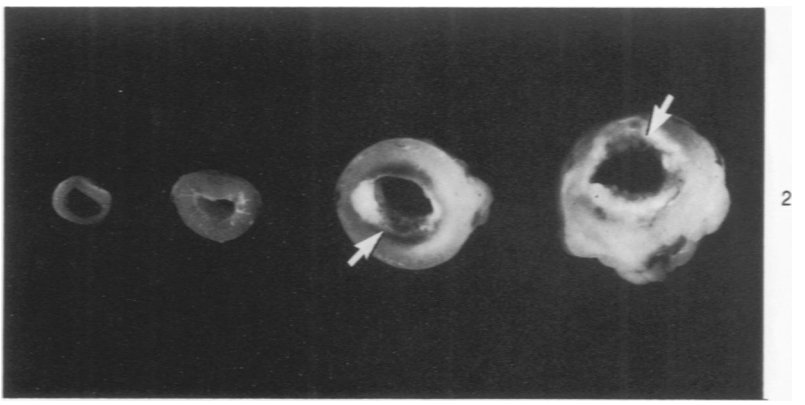

Figure 1—Distal ileums from hamsters with experimental TIH. Specimens were taken just proximal to the ileocecal junction (*top*). From left to right: ileums collected on Days 0, 15, 25, and 35. The ileum at the left is normal. The other three specimens demonstrate progressive enlargement. The 25-day specimen has small granulomas visible through the serosal surface (*arrow*). The 35-day specimen has extensive granulomatous inflammation and a sharp demarcation between affected and normal ileum. **Figure 2**—Transverse sections of the ileums shown in Figure 1. There is progressive thickening of walls due to mucosal hyperplasia. The specimens taken at 25 and 35 days have gray-white mottling of mucosa from necrosis and hemorrhage (*arrows*) and the 35-day ileum has extensive granulomatous thickening of ileal wall.

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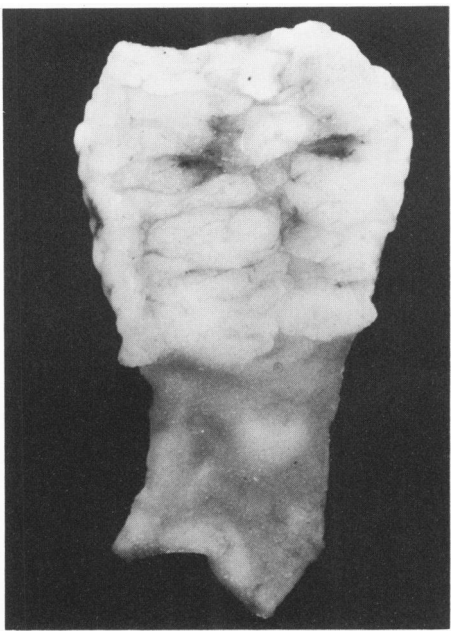

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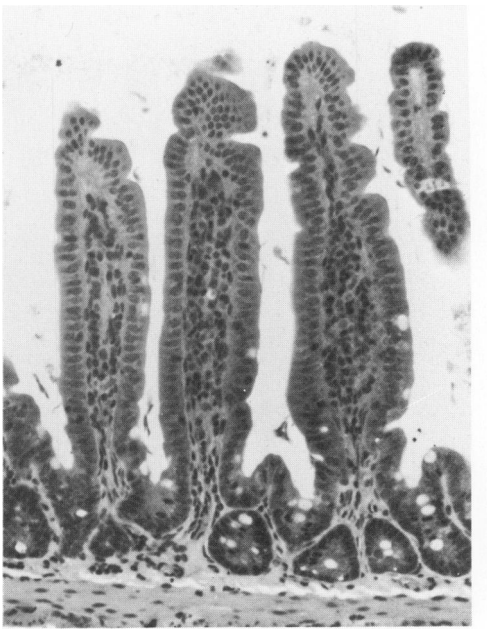

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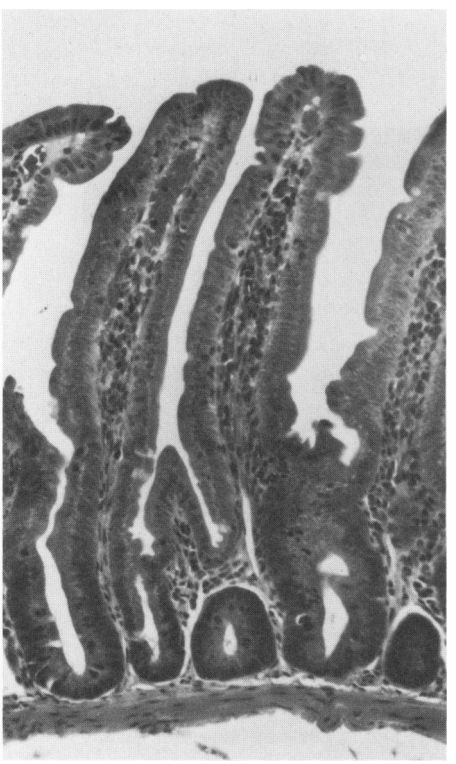

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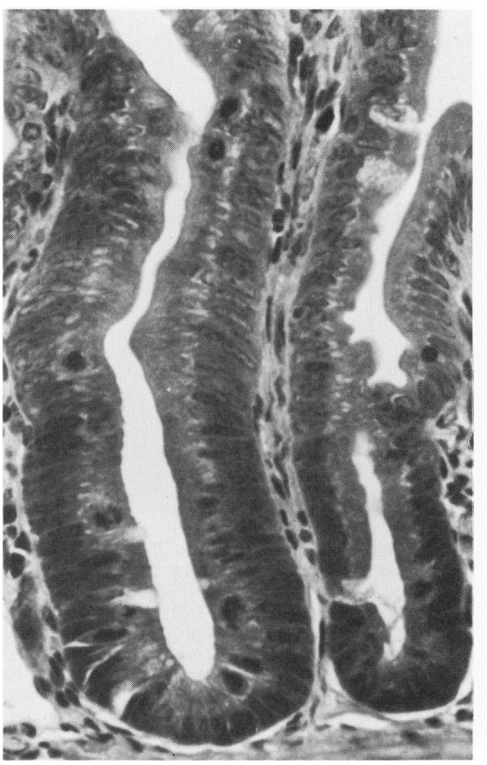
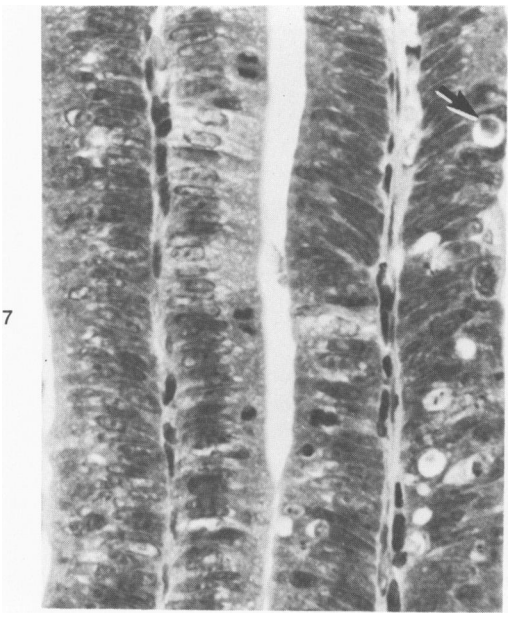
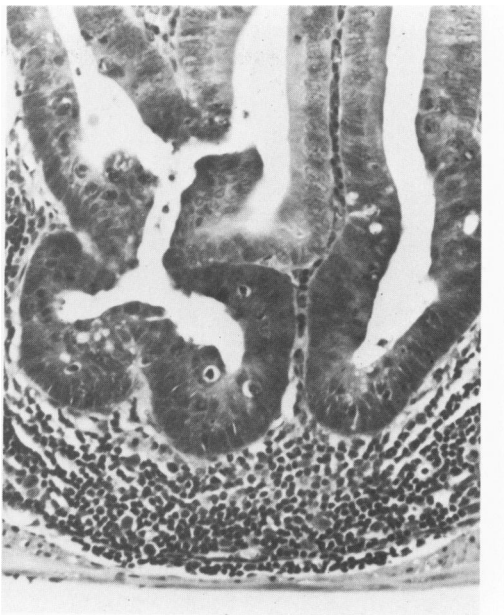
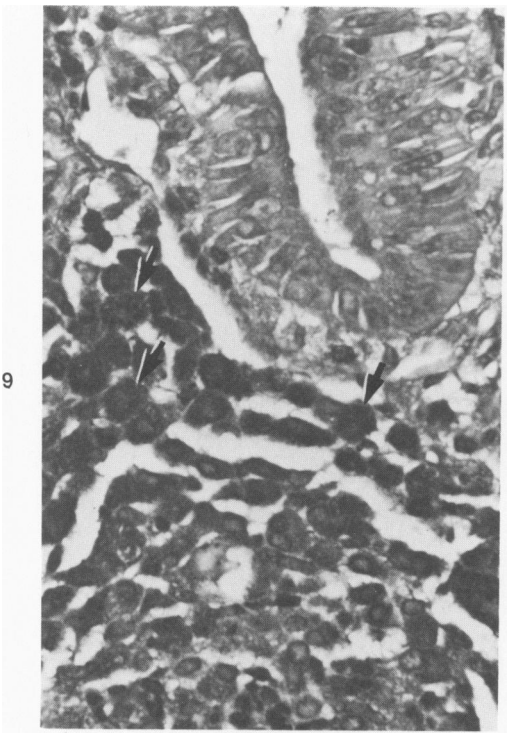


Figure 3—Mucosa of distal ileum from a hamster with experimental TIH 30 days after infection. The mucosal surface is raised, there is distortion of surface texture due to fusion of hyperplastic villi, and there are foci of mucosal necrosis and hemorrage. **Figure 4**—Ileum from a control hamster showing normal crypts with crowded, mitotically active cells and normal villi covered by mature columnar epithelium. The lamina propria contains some mononuclear cells. (H & E, × 175) **Figure 5**—Distal ileum from a hamster with TIH. Day 10 after inoculation. Crypt compartments have expanded and villi are longer than normal. Crypt-type epithelium extends onto lower portions of villus walls. (H & E, × 175) **Figure 6**—Higher magnification of villus in Figure 5. Hyperplastic crypt epithelium extends onto villus walls. Cells are pseudostratified and mitotic figures are common. (H & E, × 440)







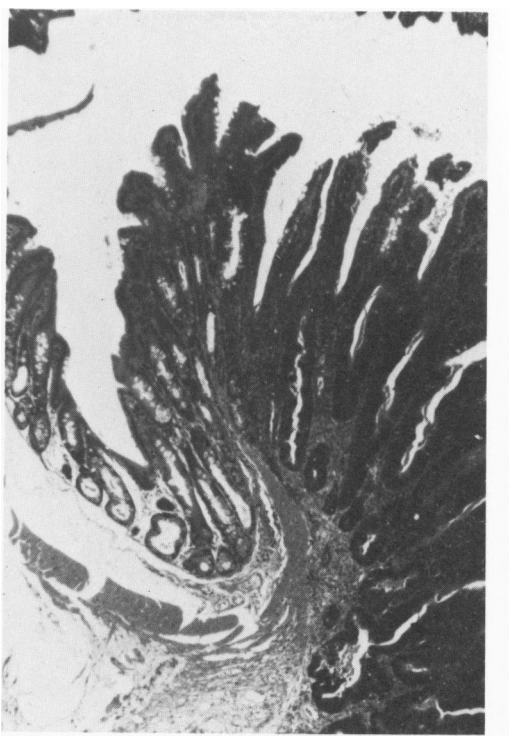


Figure 7—Upper third of two villi showing typical changes of TIH. Day 20 after inoculation. Mature villus epithelium has been replaced by hyperplastic crypt-type epithelium with numerous dividing cells. Many cells contain tinctorially heterogenous intracytoplasmic inclusions (*arrow*). The lamina propria is compressed so that no mononuclear cells remain. (H & E × 440) **Figure 8**—Ileal Peyer's patch compressed by downward expansion of tortuous hyperplastic crypts. The subjacent segment of muscle tunics is thin. Day 20 after inoculation. (H & E, × 175) **Figure 9**—Macrophages infiltrating the lamina propria and submucosa adjacent to a hyperplastic crypt. The macrophages are filled with intracytoplasmic PAS-positive granules which appear dark gray (*arrows*). Day 20 after inoculation. (PAS, × 440) **Figure 10**—Ileocecal junction demonstrating the typical abrupt transition from severely hyperplastic ileal mucosa to normal cecal mucosa. Day 20 after inoculation. (H & E, × 70)

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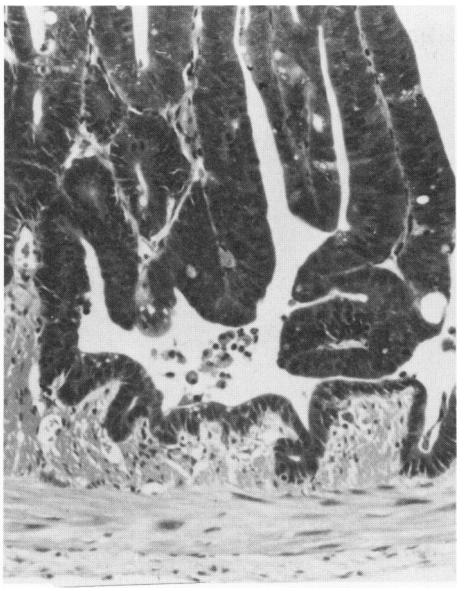

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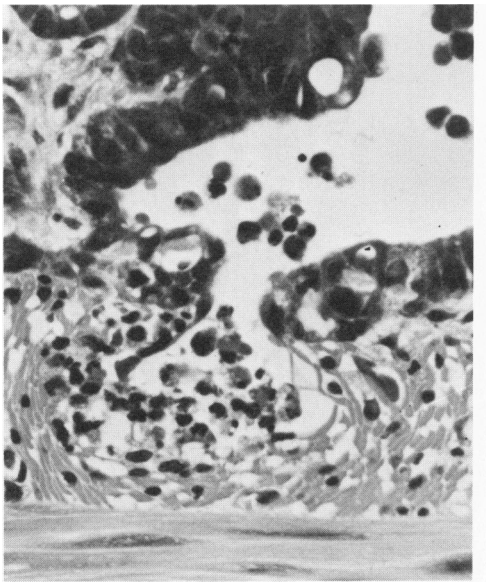

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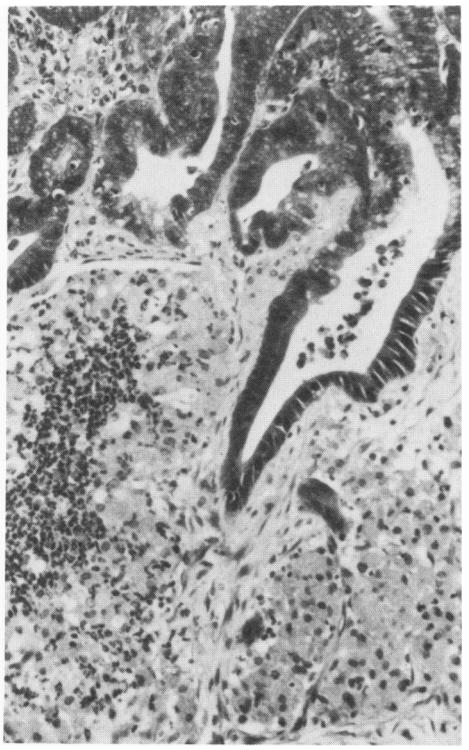

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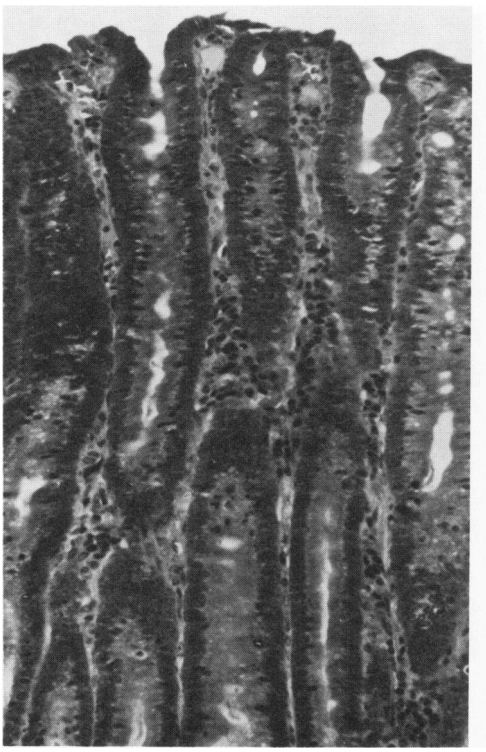
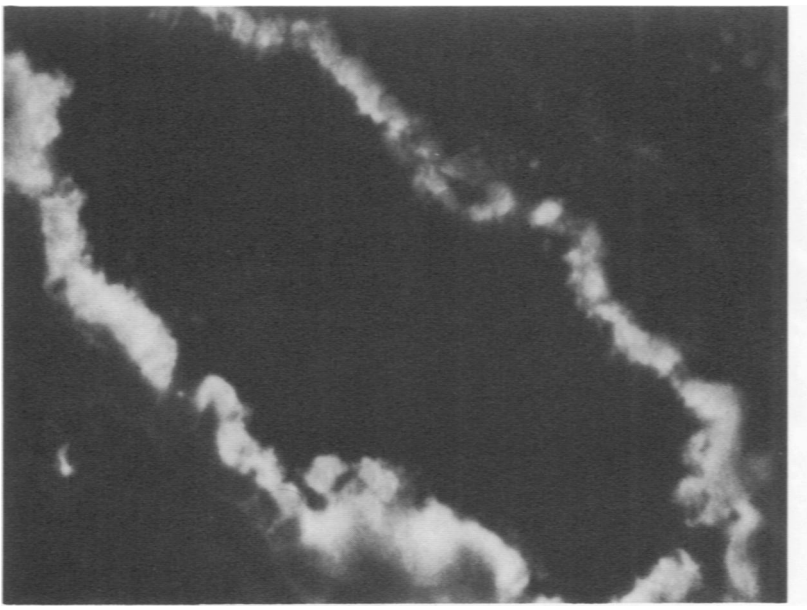


Figure 11—Tortuous dilated crypts have penetrated subjacent hypertrophied muscle tunics and extended laterally beneath neighboring crypts. There are some necrotic cells in the lumen of the crypt. There is no inflammation associated with the penetrating fronds. Day 25 after inoculation (H&E, × 175) **Figure 12**—Focal necrosis of dilated crypt which has penetrated the submucosa and inner muscle tunic. Muscle tunics are hypertrophied. There is an abscess developing beneath the rent in crypt epithelium. Day 25 after inoculation. (H&E, × 440) **Figure 13**—Typical advanced lesion of TIH. Day 35 after inoculation. Fronds of hyperplastic epithelium are penetrating underlying supporting tissues distorted by severe pyogranulomatous inflammation. (H&E, × 175) **Figure 14**—Severe colonic mucosal hyperplasia in hamster with TIH. Day 20 after inoculation. Crypts are three to four times normal height and are lined by mitotically active epithelium. (H&E, × 175)



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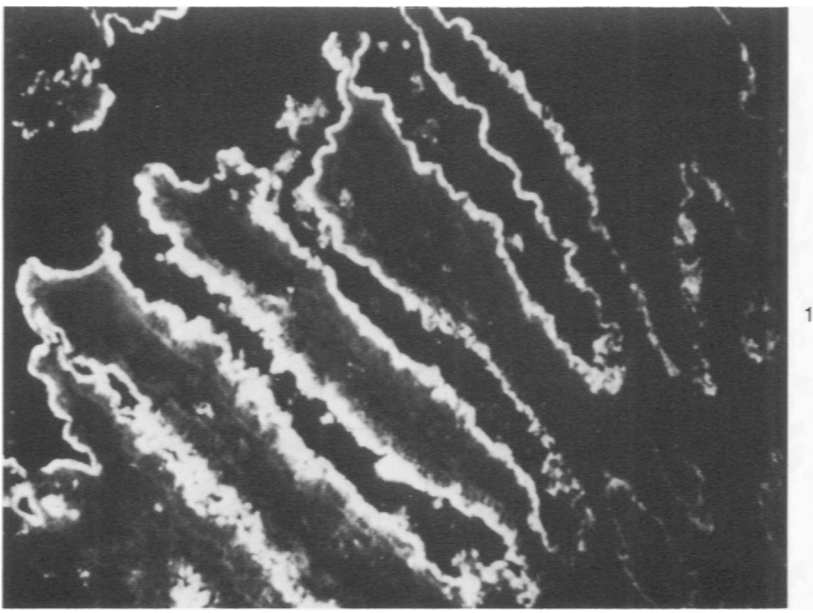

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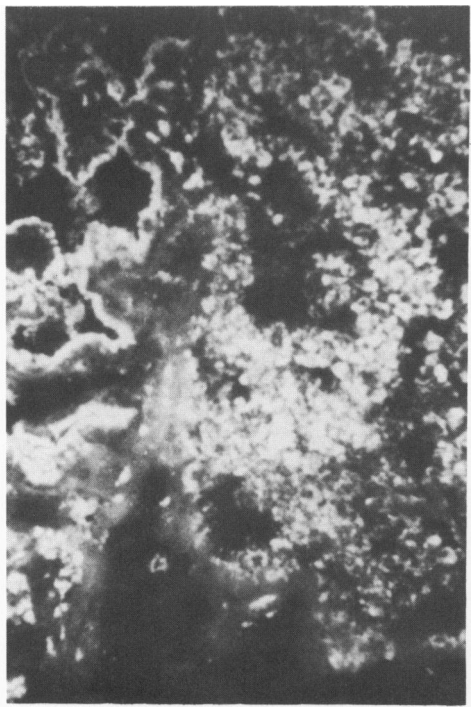
Figure 15—Particles and clumps of TIH-associated antigen detected by indirect immunofluorescence in the apical cytoplasm of epithelial cells covering two villi. Day 15 after inoculation. Cell nuclei are black, and antigen-free cytoplasm is gray. (× 700) **Figure 16**—Typical ribbon-like pattern of TIH-associated antigen demonstrated by indirect immunofluorescence staining of hyperplastic ileal villi. Antigen has accumulated in the apical cytoplasm of epithelial cells. A few fluorescing exfoliated cells occupy intervillus spaces. Day 15 after inoculation. (× 110)



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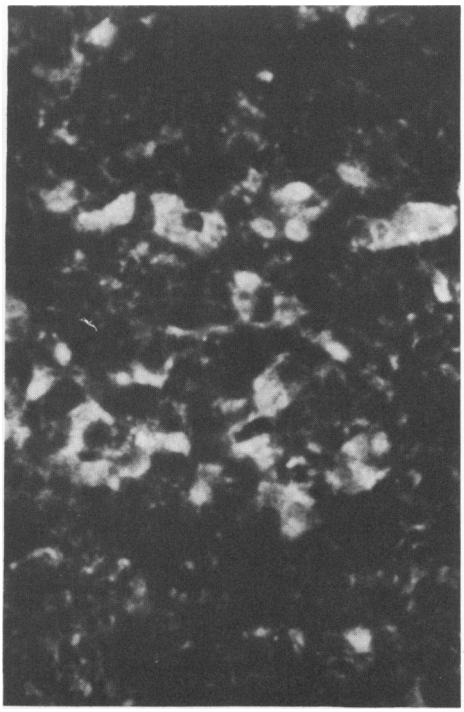

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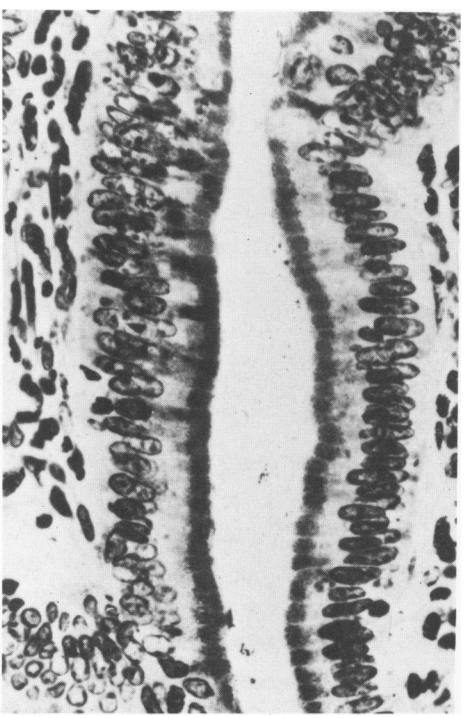

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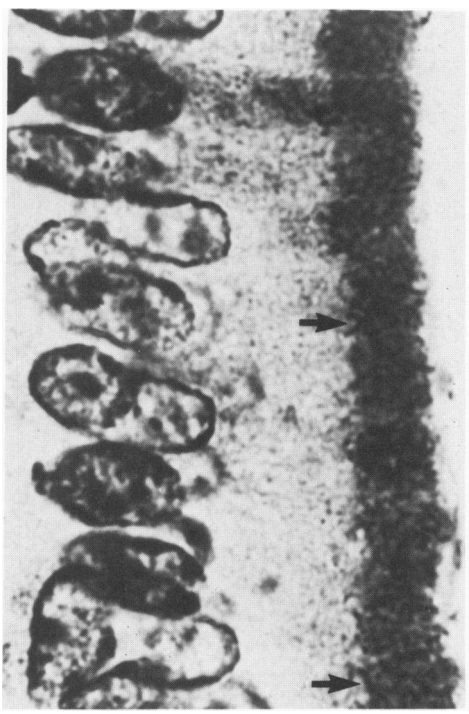

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Figure 17—TIH-associated antigen in an advanced ileal lesion. Antigen has accumulated in macrophages infiltrating supporting tissue tunics (*lower half of field*) and typical ribbon-like distribution of antigen is apparent in villus mucosa (*upper half of field*). Day 35 after inoculation. (× 110) **Figure 18**—TIH-associated antigen in macrophages and in interstitial tissues of ileal wall on Day 35 after inoculation. Macrophage nuclei appear black, and cytoplasmic fluorescence is granular. These cells were PAS-positive, as shown in Figure 9. (× 700) **Figure 19**—Hyperplastic mucosal epithelium on adjacent villi with accumulation of particulate material resembling bacteria in the apical cytoplasm. Day 10 after inoculation. Note that the distribution of material is identical to the distribution of TIH-associated antigen. It is gram-negative by Brown and Brenn stain. (Warthin-Starry, × 700) **Figure 20**—Higher magnification of cells in Figure 24, demonstrating particles in apical cytoplasm. Many resemble rod-shaped bacteria (*arrows*). (Warthin-Starry, × 1750)