The Histopathology of Acute Intestinal Amebiasis

A Rectal Biopsy Study

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NECROPSY APPEARANCES of late intestinal lesions in amebic colitis have been well described.[1–3] However, descriptions of the pathology of early lesions in man are scarce.[4] While experimental studies in a variety of animals, including kittens, dogs and monkeys,[5–9] have contributed to the understanding of amebiasis, the natural history of the disease and lesions seen in animals often differ from that in man. Tissue autolysis and postmortem migration of amebas make necropsy material unsuitable for the study of early tissue changes.[8,9] Rectal biopsy is now a commonly used procedure and its value in the diagnosis of amebiasis has been referred to in recent years.[4,10,11] These recent studies have been mainly directed at the demonstration of amebic trophozoites, but little has been written about early tissue changes in the gut mucosa. This paper describes the histologic stages in the evolution of early intestinal lesions as seen in rectal biopsies from 53 patients with proven acute intestinal amebiasis.

Materials and Methods

Fifty-one patients were Orang Asli adults (Western Malaysian aborigines) admitted to Gombak Hospital with the complaint of passing blood and mucus in their stools. All of these patients had motile, erythrophagous trophozoites of *Entameba histolytica* in stool specimens or proctoscopic aspirate. In addition, positive biopsies from 2 Chinese patients were available for study. Twelve negative rectal biopsies, taken to exclude amyloidosis in patients without intestinal symptoms, were used as controls.

Rectal biopsies were done using a Welch-Allyn proctoscope with offset rectal biopsy forceps. The specimens were immediately fixed in 10% formalin and subsequently embedded in paraffin. Sections were cut perpendicular to the mucosal surface at 5 μ and stained with hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff (PAS), phosphotungstic acid-hematoxylin (PTAH) and, in selected cases, Gordon and Sweet's reticulin stain.

In addition to qualitative assessment of tissue changes, an attempt was made to

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quantitate intraepithelial and stromal neutrophilic granulocyte (neutrophil) infiltration by using an arbitrary 0 to 4+ grading. In all specimens where the plane of sectioning was considered to be satisfactory, the thickness of the mucosa was measured using a Leitz micrometer eyepiece attachment; in each instance the mean of two readings was recorded.

Results

Normal Rectal Mucosa

The normal rectal mucosa in sections from the control group of biopsies had a smooth surface regularly interrupted by the mouths of the glands (Fig 1). The mucosa ranged in thickness from 300 to 520 μ in 9 specimens (mean, 430 μ). The surface epithelium consisted of a single layer of regular, tall columnar cells and mucus-producing goblet cells (Fig 2); cuboidal cells or intracellullar neutrophils were rarely seen. Straight tubular glands, the crypts of Leiberkuhn, extended from the surface down through the entire thickness of the lamina propria. These were lined almost entirely by a single layer of mucin-filled goblet cells interspersed with occasional simple columnar cells and rare argentaffin cells. Few mitotic figures were seen. The lamina propria contained capillaries, a moderate number of plasma cells and lymphocytes. Neutrophils were rarely seen but eosinophils were relatively frequent. In some sections an occasional lymphoid follicle was present. Sometimes it extended through the muscularis mucosae into the submucosa.

Nonspecific Lesion

The mildest histologic change, seen in biopsies from 22 patients, was thickening of the mucosa. The thickness, measured in 15 specimens, ranged from 550 to 960 μ (mean, 670 μ). This was usually irregular, so that the surface outline was wavy. (Fig 3). The lamina propria was edematous especially in the superficial portions. The capillaries were hyperemic. In addition to the usual plasma cells, eosinophils and mononuclear cells, there was a mild to moderate infiltration by neutrophils (Grade + to 2+). These were prominent within and around capillaries, but there were also small collections beneath the surface epithelium. In some areas they were present within the surface epithelial cells (Fig 4) and occasionally in the surface exudate. Lymphoid aggregates, when present, showed reactive hyperplasia with numerous histiocytic cells. The glands were increased in length and their openings were wider than normal. There were no collections of inflammatory cells in the lumens. Intraepithelial mucin was abundant in most glands, but in some sections there were foci of mucin depletion. Glandular

branching and irregularity were occasionally present, resulting in the appearance of intraluminal epithelial collections. The surface epithelium was intact. In some sections it consisted almost entirely of goblet cells. Elsewhere there were small scattered foci of cuboidal metaplasia associated with loss of mucin and sometimes pyknosis of nuclei. The muscularis mucosae was prominent, showing mild interstitial edema, and was occasionally infiltrated by small numbers of eosinophils and neutrophils. Measurements of its thickness could not be made as it was incomplete in most biopsies.

Amebas were not found in the tissues, or in the crypts, but were present in small numbers in the surface exudate in 3 specimens. They were not found in direct contact with surface epithelium except in one biopsy from a hard mucoid cap adherent to the mucosal surface. Small numbers of trophozoites were present in this mucoid cap and in each section, two or three scattered amebas were seen adjacent to the intact surface epithelium.

Mucopenic Depression with Microulceration

Biopsies from 17 patients showed this lesion. The early lesions in this group consisted of small superficial depressions of the mucosal surface, each of which resembled an ulcer with sloping edges (Fig 5). However, the surface epithelium was only focally eroded and the "pseudoulcer" was due to a decrease in mucosal thickness resulting from a depletion of mucin from the surface and glandular epithelial cells. The depression was accentuated by the presence, in the adjoining mucosa, of all the features of the nonspecific lesion, including increased mucosal thickness.

The surface epithelial cells in the affected area showed a general decrease in height from columnar to low cuboidal and flattened cells. Some contained intracellular neutrophils. Mitotic figures were numerous both in the surface and in the glandular epithelial cells. The lamina propria was hyperemic and infiltrated by neutrophils as well as by plasma cells, eosinophils, macrophages and lymphocytes. Neutrophils were prominent (Grade 2+ to 4+) especially in relation to foci of epithelial lysis. Interstitial edema was less striking than in early nonspecific lesions. The submucosa, when present in biopsies, was hyperemic and edematous with a mild focal perivascular infiltration by a few lymphocytes, plasma cells, eosinophils and neutrophils. The lymphoid follicles showed histiocytic proliferation and occasional plasma cells. There were no follicular abscesses. No bacteria were seen in the tissues, although some were present in the surface exudate. The muscularis

mucosae was prominent, showing interstitial edema and a mild infiltration by eosinophils, neutrophils and plasma cells.

Amebas were identified in 15 specimens. They were confined to the luminal surface, and were particularly abundant adjacent to the sites of epithelial lysis. None were found in the crypts, tissues and vascular channels. They occurred mainly in clusters and contained strongly PAS-positive granules. Ingested erythrocytes were frequently seen. Many showed the characteristic nuclear structure in sections stained with Masson's trichrome and PTAH stains. The amebas were surrounded by a proteinaceous exudate in which were present strands of fibrin, mucin, erythrocytes, occasional neutrophils and mononuclear cells.

Microscopic ulceration was a prominent feature within the mucopenic areas at a later stage of the lesion. Here the histiolysis involved both the interglandular surface epithelium and the subepithelial zone of lamina propria. Engorged blood vessels were often involved with resultant capillary hemorrhage. The epithelium was flattened at the edges of the ulcers and their free ends sometimes projected into the lumen of the gut. A thick proteinaceous surface exudate was often present on the exposed lamina propria at the site of ulceration. The exudate contained fibrin strands, red blood cells, occasional neutrophils and mononuclear cells. Amebic trophozoites were present in large numbers in the exudate but none was seen within the tissues or in the crypts. As in the earlier lesions, hyperemia and cellular infiltration, including a variable number of neutrophils, were seen in the lamina propria and submucosa.

Early Invasive Lesion with Superficial Ulceration

The two smallest lesions in which tissue invasion by amebic trophozoites had occurred showed small interglandular foci of surface epithelial destruction. These foci were sometimes seen only when serial sections are examined. Occasionally, amebas were present directly on the basement membrane, separating it from the damaged surface epithelium (Fig 6). The overlying epithelium had lost some of its mucin and had decreased in height. Elsewhere, there was destruction of both surface epithelium and basement membrane with amebic invasion of the superficial zone of lamina propria. Tissue necrosis and inflammatory cell infiltration at the immediate site of invasion was minimal. Both these lesions were found only in biopsies taken from mucosa adjacent to large necrotic ulcers.

In all other lesions in this group, amebas were present in large numbers. A thin zone of necrosis and neutrophils separated the amebas

from the underlying tissues. In all four specimens with this lesion, destruction of the surface epithelium between the glands preceded tissue invasion. Amebas were not seen in crypts, within normal tissues, in vascular spaces, in lymphoid tissue or in the muscularis mucosae. Involvement of crypts, when present in the more extensive lesions, was confined to the superficial necrotic zone and appeared to occur by extension from the lamina propria rather than from the crypt lumen. The lamina propria beneath and adjacent to the site of invasion showed hyperemia and a mild infiltration by neutrophils. When lymphoid aggregates were present, these showed reactive hyperplasia but there were no organisms, abscesses or follicular ulcers.

Mucus depletion was prominent and mitotic figures were frequent in the glandular epithelium. No crypt abscesses were seen. As in the other lesions, the muscularis mucosae was intact and showed mild fluid and cellular exudation.

Late Invasive Lesion with Deep Ulceration

This is the classical "flask ulcer," with undermined edges. Biopsies from 10 patients showed this lesion with ulceration extending through the mucosa and muscularis mucosae into the submucosa. A thick exudate was present in the floor of the ulcer. This contained acellular proteinaceous material, red blood cells and strands of fibrin. Groups of amebic trophozoites, sometimes surrounded by fibrin strands were present, especially in the deeper layers of the exudate. Apart from one specimen (in which there was a heavy neutrophil infiltration), inflammatory cells were infrequently seen in the exudate. The few cells present were usually mononuclear in type.

A deeply eosinophilic zone of fibrinoid necrosis separated the exudate from underlying viable submucosa. The latter was edematous, hyperemic and heavily infiltrated by plasma cells. Some lymphocytes and macrophages were present. Eosinophils were rare. Neutrophils were abundant in the submucosa of two specimens but were less frequent in the others. Blood vessels showed swelling of endothelial cells and sometimes occlusive thrombosis. Proliferating capillaries and undifferentiated mesenchymal cells were also present.

Amebas were usually confined to the necrotic tissue and surface exudate. In some sections however, there were foci where small numbers of amebas were present in the tissue immediately beyond the zone of necrosis. Occasionally they were present within a ruptured blood vessel, but none was seen in blood vessels in deeper tissues.

In contrast to the ulcerated area, the intact mucosa at the ulcer

edge showed hyperemia and a predominantly neutrophil infiltration. Epithelial mitoses were frequent and there was loss of mucin from the goblet cells. The surface epithelium sometimes showed cuboidal metaplasia. In one specimen there was metaplasia to stratified squamous epithelium at the free edge.

Granulating Ulcer

There were three specimens in which loss of mucosa and muscularis mucosae had been followed by formation of granulation tissue. In two specimens, the surface exudate had presumably sloughed off to expose a base consisting of granulation tissue lined by a thin zone of necrotic debris and neutrophils; no amebas were identified. The edge of the ulcer did not show undermining. There was regeneration and early migration of surface epithelium at the edge of one of the lesions.

In the third specimen, there was excessive granulation tissue formation in addition to the features described above. This resulted in a "microameboma" projecting as a mass above the mucosal surface. The surface was devoid of epithelium and only a few amebas were present in the scanty surface exudate.

Discussion

The two mild mucosal changes (the nonspecific lesion and the mucopenic depression) seen most frequently in this study do not appear to have been described before. The mildest lesion obtained from areas of rectal mucosa showing no macroscopic ulceration was histologically nonspecific as it did not contain amebic trophozoites. It was, however, a definite lesion, easily recognized on light microscopy. Glandular hyperplasia, transmigration of neutrophils, stromal edema, hyperemia and infiltration by neutrophils have also been described in ulcerative colitis.[12] However, there were no crypt abscesses and little acute inflammatory exudate on the surface. The increase in mucosal thickness may be explained on the basis of the edema, hyperemia and the interstitial cellular infiltration. Possible factors which may explain the waviness of the surface outline are irregular proliferation of glandular epithelium, variation in epithelial mucin content and spasm of the muscularis mucosae. We have not been able to study the submucosal changes that may also have contributed to this feature. The mechanism causing this early mucosal lesion is uncertain, but most likely it is due to the presence of toxic factors in the lumen, arising either from amebas or from products of tissue necrosis elsewhere in the gut.

We think that this early lesion, although nonspecific, is part of the

picture of amebic colitis in these patients because (1) it was found in patients with proven acute amebiasis who did not have other concomitant intestinal disease, and (2) it was found in rectal mucosa adjacent to mucopenic and invasive lesions. Although the nonspecific lesion has not been referred to specifically in the literature, it is of interest that Figure 3 of Juniper *et al*[4] shows waviness of the mucosal surface and variation in its thickness consistent with this lesion. Flick *et al*[13] have referred to the wavy appearance, glandular branching and increased mucosal thickness in patients with other conditions, including cardiac edema, acute colitis and chronic ulcerative colitis.

Histologically unequivocal trophozoites of *E histolytica* were seen in the mucus in only two specimens with the nonspecific lesion, although typical trophozoites were present on both stool and proctoscopic aspirates in all these patients. This was probably because no attempt was made to remove mucus exudate with the tissue biopsies. However, we agree with Juniper *et al*[4] that the removal of mucus plugs with the tissue is desirable when biopsy is done primarily for diagnostic purposes.

It is of interest that amebas were not found in direct contact with surface epithelium in the nonspecific lesions except in one specimen. This was obtained from a hard mucoid nodule adherent to the intact mucosal surface. The absence of superficial epithelial erosion and mucus depletion in this lesion is unusual because, in all other lesions where amebas were in contact with the mucosal surface, mucopenic depressions had resulted. The difference in this lesion may be due to the fact that amebas were present only in very small numbers and the hard mucoid material in which they were embedded may in some way have protected the mucosa from more severe damage.

The mucopenic depression, the earliest specific histologic lesion seen, is characterized by a localized area of mucin loss. It has been shown experimentally that mucus release from colonic goblet cells can be induced either by faradic stimulation of the nervi erigentes or by local irritation in the absence of nervous stimulation.[14] The latter is the likely explanation for the localized mucopenic depressions in amebiasis as these lesions were related to the presence of amebas in the immediate vicinity.

The local irritation may be due either to enzymes released by the amebas or to products of tissue destruction or to their combined presence. The hypertrophy and hyperplasia of the mucosal glands and the abundant intracellular mucus seen in the early nonspecific lesion probably resulted from relatively mild stimulation where the rate of mucus production is as rapid as its release. It seems likely that the mucopenic

depression may represent a stage of "exhaustion" of mucus-producing cells due to excessive local irritations. It is of significance that large numbers of amebic trophozoites were present in the adjacent exudate in 15 of the 17 cases. The only two biopsies in which amebas were not found were incomplete, consisting only of the edges of the lesions.

It is generally felt, on the basis of necropsy studies, that neutrophils are not a feature of intestinal amebiasis [15] and their presence in some of the late ulcers has been ascribed to secondary bacterial infection.[16] However, in monkeys experimentally infected with *E histolytica,* neutrophils were prominent even in early lesions.[8,9] Neutrophils have also been noted in human rectal biopsies from mucosa showing only mild changes.[17] In contrast, Juniper *et al* [4] commented on the noticeable absence of neutrophils in their biopsies, except in the more extensive ulcers. In our material, neutrophils were the predominant inflammatory cells in the nonspecific and mucopenic lesions but were less frequently seen in invasive lesions. They were rare in normal rectal biopsies taken by an identical technic by the same observer. Enteropathogenic bacteria including *Salmonella edwardsellia* were isolated from some of the patients, but the number of neutrophils present was independent of their presence. The neutrophil infiltration is probably an early response to local irritation and/or tissue damage, although it can also occur in some deep ulcers with secondary infection.

The presence of "crypt abscesses" has been referred to by several authors.[4,10,17] We have not seen collections of neutrophils at the base of crypts in any of our specimens, although neutrophils were often present within the epithelial layer, especially toward the mucosal surface.

Eosinophils are usually described as being only occasionally present in the normal rectal mucosa.[12] However, in our biopsies of normal rectal mucosa, they were relatively frequently seen. This may be because intestinal helminthic infestation is common among the Orang Asli. There was no noticeable increase in eosinophils in any of the biopsies from patients with amebiasis, but they were notably infrequent in the late ulcers.

The mucosal glands have been regarded as the site of initial amebic invasion by various authors.[1,15] Their observations were based on necropsy material in which postmortem migration may well have occurred. However, in monkeys experimentally infected with *E histolytica* and subsequently sacrificed, amebas were found between the glands but not within the crypts.[8,9] It has been suggested that secretion from the glands may prevent the entrance of amebas into the crypts.

Our observations confirm the interglandular surface epithelium as

the site of initial penetration in man. We have not seen invasion of the glands except in the late invasive lesions with extensive tissue necrosis.

The exact method by which amebas invade tissues is uncertain. Some authors [9,15] have referred to the mechanical action of pseudopodal migration. Others regard cytolysis to be of major importance.[8,18] In our material, cytolysis and tissue destruction, due to enzymes liberated by amebas, appeared to be the major factor involved in invasion for the following reasons: (1) In biopsies from 17 patients showing mucopenic depressions, there was focal superficial epithelial erosion adjacent to collections of amebas. The epithelial lysis preceded invasion as amebas were not found within the tissues at this stage. (2) Amebas appeared intact and did not show detectable degenerative changes on light microscopy; the characteristic nuclear structure was well seen in many amebas. (3) In invasive lesions, a zone of tissue necrosis usually separated amebas from viable tissue.

However, in two debilitated patients with severe necrotic ulcers, biopsies of apparently intact mucosa showed early invasion without a preceding zone of tissue lysis. In these specimens, very small numbers of amebas had succeeded in passing through the interglandular surface epithelium into the lamina propria. The surrounding tissue showed very little damage or inflammatory reaction. In the adjacent interglandular zones, where larger numbers of amebas had invaded, both cytolysis and inflammatory reaction had occurred. It would appear that local tissue resistance in these two patients is defective in some way, enabling even small numbers of amebas to invade the tissues. Also, invasion by a few amebas is associated with only minimal tissue damage whereas large numbers are necessary for significant cytolysis to occur. The factors influencing the differences in tissue response to infection by *E histolytica* have been discussed by Shaffer *et al.*[15]

Doxiades and Yiotsas [11] in a study of rectal biopsies from 1878 patients whom they considered to have chronic amebiasis found "cellular formations" which they regarded as "atypical forms of entameba histolytica with deformed nuclei" in 24.4% of the biopsies. These structures were found in otherwise normal mucosa and were often located between the intestinal glands and the muscularis mucosae in the absence of damage to the overlying tissues. Two of the 5 illustrative cases they reported had blood and mucus in their stools and other features which fulfill our criteria for acute amebiasis. Likewise, Tandon *et al* [17] refer to one case in which the diagnosis of amebiasis was made by the demonstration of an ameba in the depths of an otherwise normal mucosa. The single structure in their illustration which had been interpreted as an

ameba lacked the nuclear characteristics of *E histolytica.* We have not been able to find isolated groups of normal or degenerate amebas near the muscularis in the absence of destruction of superficial tissues. It has been shown that PAS-positive macrophages tend to concentrate in the lamina propria near the subepithelial reticular layer and near the muscularis mucosae.[12] We agree with Juniper *et al*[4] that the demonstration of the characteristic nuclear structure is essential for accurate identification of trophozoites of *E histolytica* in biopsy material.

Summary

Rectal biopsies from 53 patients with acute amebic colitis showed five types of histologic changes: nonspecific lesion, mucopenic depression, early invasion, late invasion and granulating ulcer. The nonspecific lesion and mucopenic depression are preinvasive stages which do not appear to have been recognized before. In both these stages, mucosal changes included the presence of significant numbers of neutrophils although "crypt abscesses" were not seen.

Presence of amebas within tissues was accompanied by destruction of superficial tissues in every instance. The interglandular surface epithelium was the site of initial penetration. Glandular invasion occurred only in the late stages.

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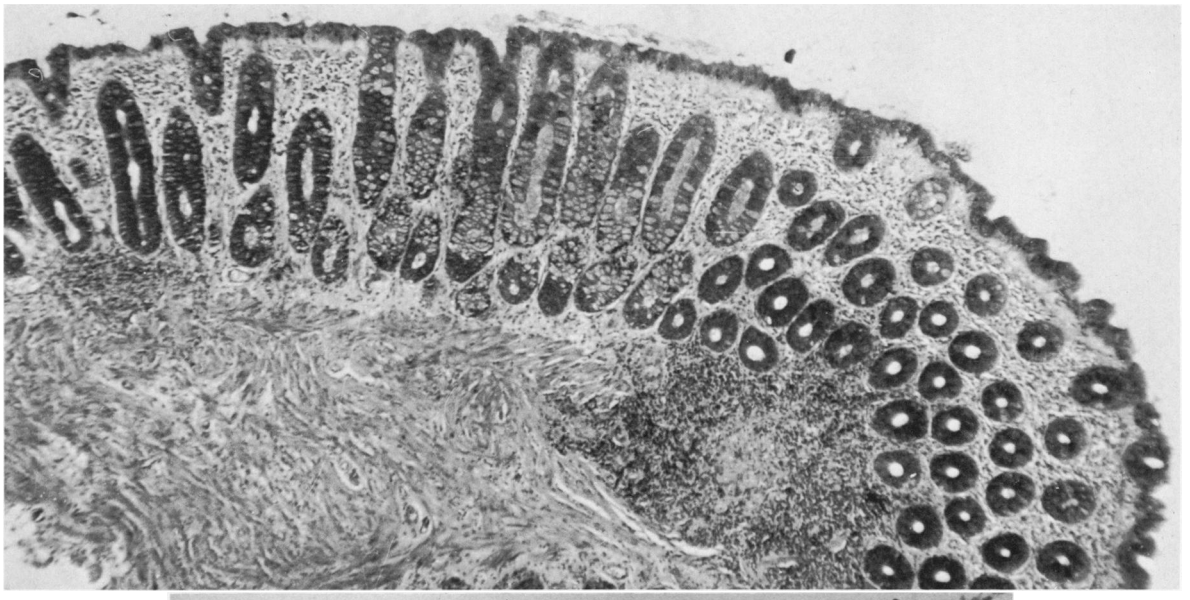
[Illustrations follow]

Fig 1. (*Top*) Low-power photomicrograph of normal rectal mucosa showing smooth surface interrupted by mouths of glands. Periodic acid-Schiff (PAS) technic. × 94.

Fig 2. (*Bottom*) Normal rectal mucosa. Surface epithelium consists of tall columnar cells and goblet cells. Neutrophils are rare. H&E. × 268.



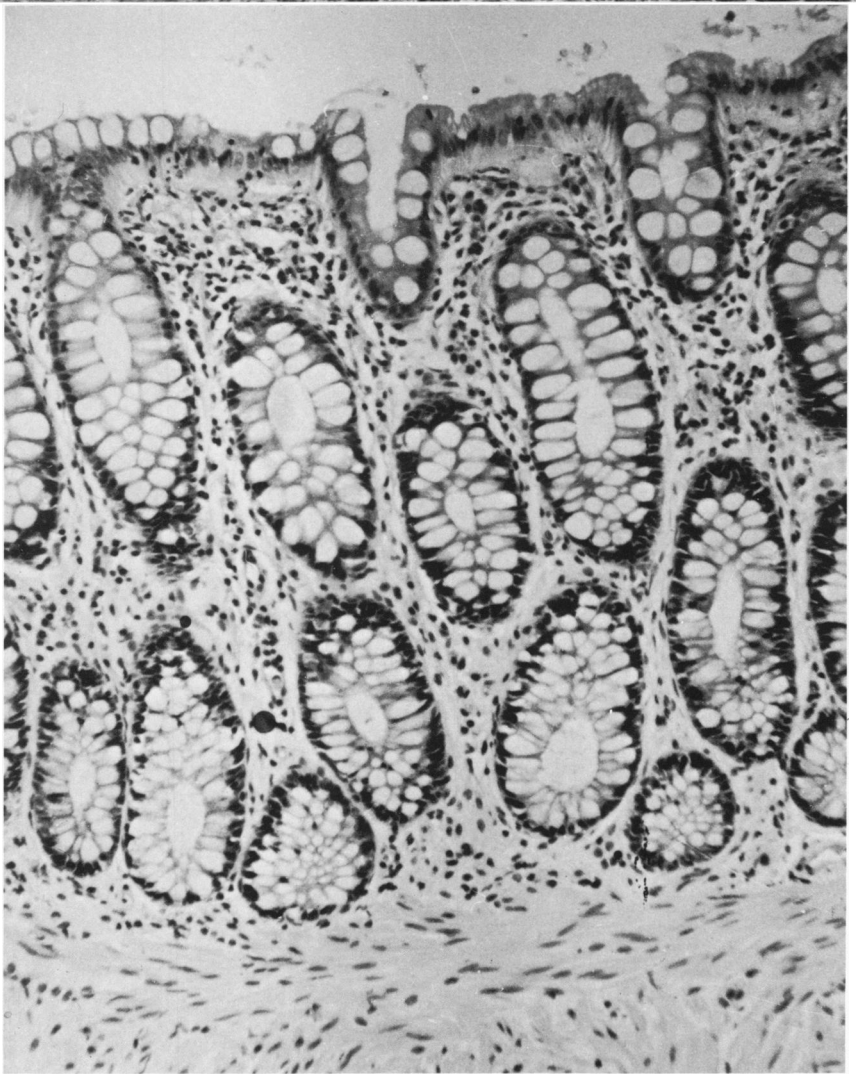


Fig 3. (*Top*) Nonspecific mucosal lesion showing waviness of surface outline, goblet cell hyperplasia and prominence of gland openings. PAS technic. × 268.

Fig 4. (*Bottom*) Nonspecific lesion. Neutrophils are present within surface epithelial cells and in edematous lamina propria. H&E. × 1080.

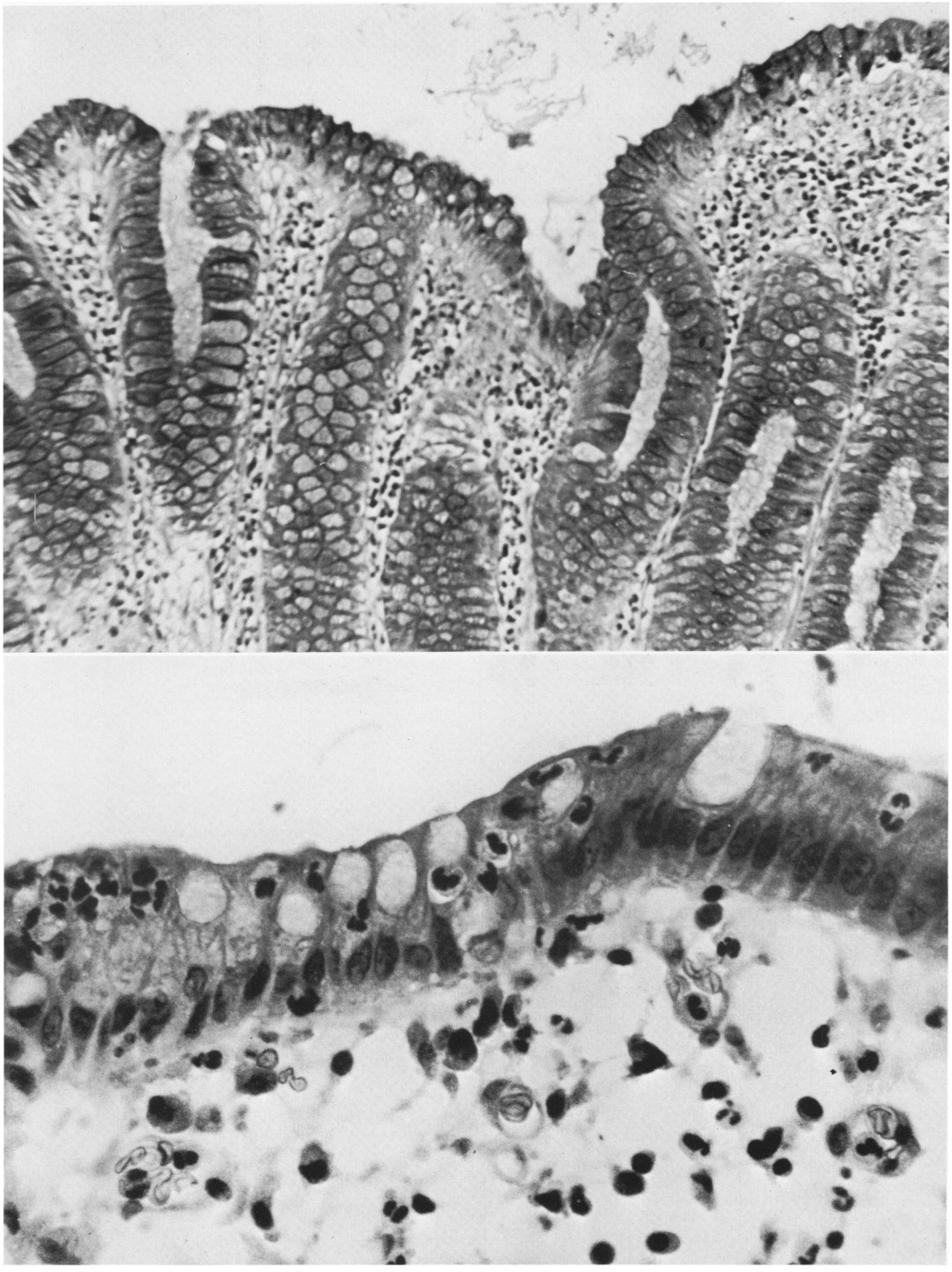
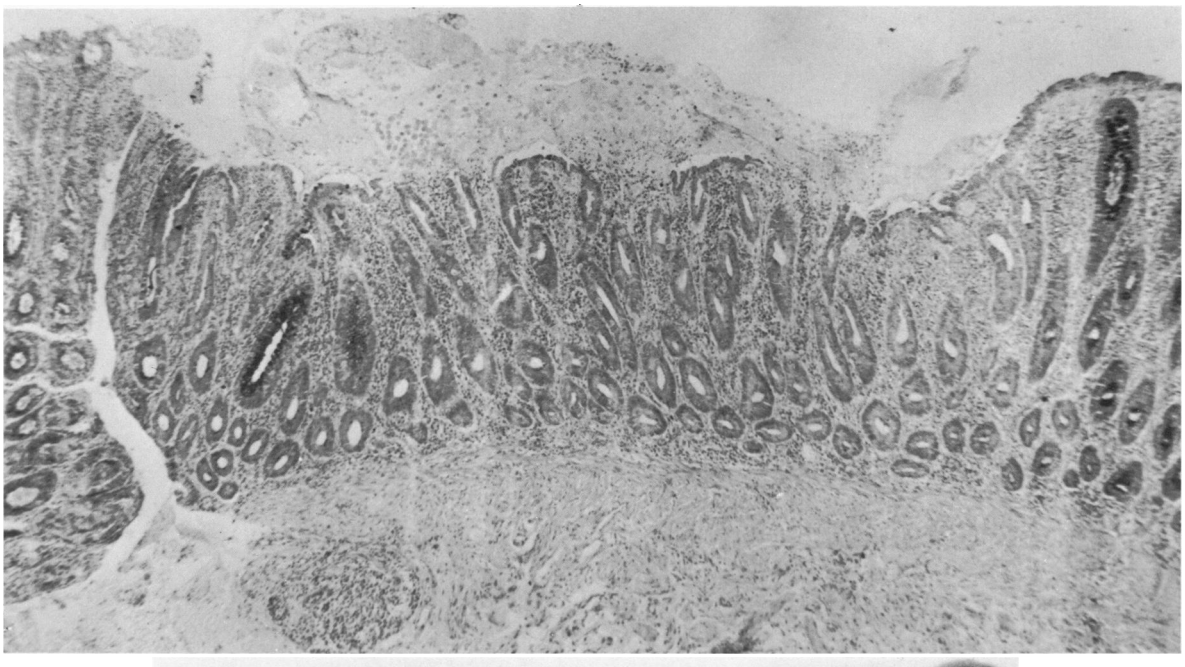
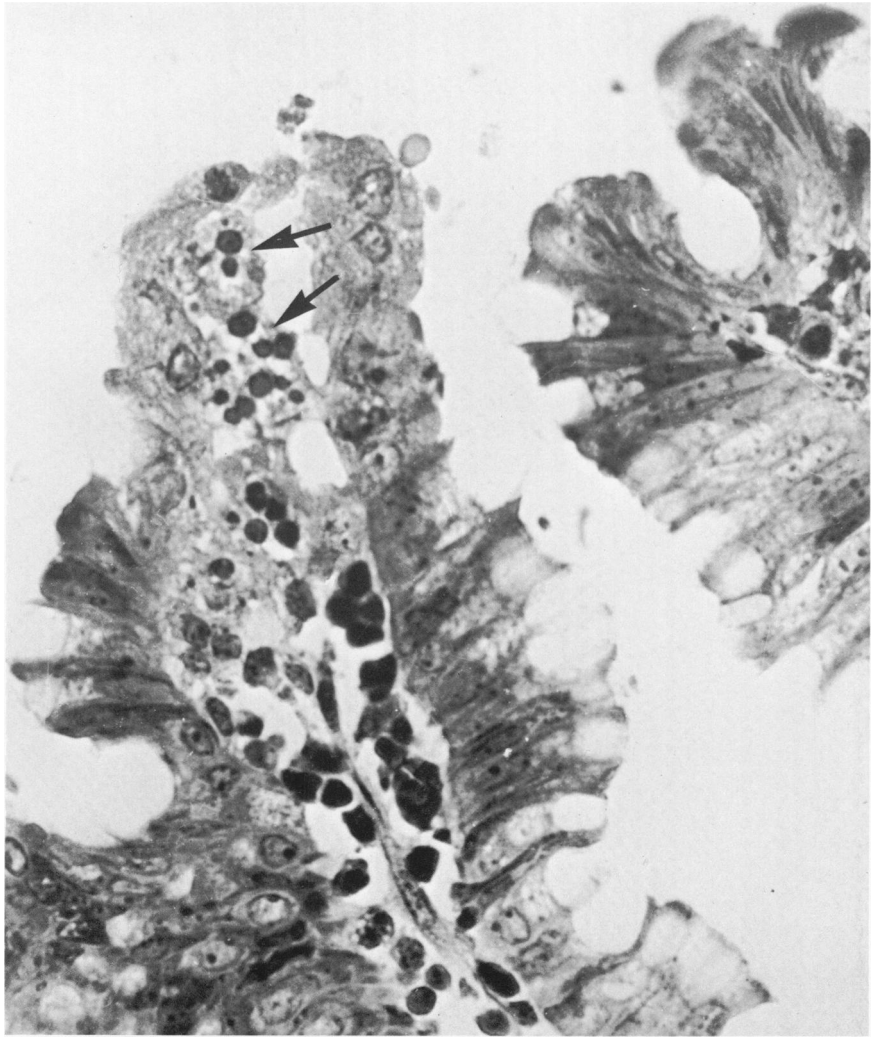


Fig 5. (*Top*) Mucopenic depression with microulceration showing localized decrease in mucosal thickness due to loss of mucin. Surface epithelium is only focally eroded. Amebas are present in surface exudate. H&E. × 70.

Fig 6. (*Bottom*) Early invasive lesion showing two amebic trophozoites (*arrows*) beneath damaged surface epithelium. Masson's trichrome. × 1080.





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