The Pathology of Experimentally Induced Cecal Amebiasis in Gerbils (Meriones unguiculatus)

Liver Changes and Amebic Liver Abscess Formation

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The pathogenesis of experimentally induced cecal amebiasis in gerbils (*Meriones unguiculatus*) was studied from 5 to 60 days after inoculation. Ulcertative lesions were noted 10 to 60 days after inoculation. The sequential development of lesions was asynchronous and progressed from destruction of the interglandular epithelium and of glandular crypt elements to loss of mucosa and formation of granulomatous lesions in the submucosa involving the muscularis mucosae. Pathologic changes in the liver correlated with the formation of ulcerative cecal lesions. Subacute hepatic changes showed lymphocytic portal infiltrate, Kupffer cell hyperplasia, multinucleated giant cells, granuloma formation, and sinusoidal mononuclear and granulocytic infiltrates. Metastatic amebic liver abscesses occurred as early as 10 days after inoculation, and small abscesses were found in the portal areas of the right liver lobe. The sequential development and pathologic manifestation of the infection and the usefulness of the gerbil for the study of human intestinal amebiasis are discussed. (Am J Pathol 1985, 119:485–494)

ALTHOUGH the pathology of experimentally induced hepatic amebiasis in laboratory animals has been described,[1–5] little information of this kind is available with respect to intestinal amebiasis. Most studies with guinea pigs,[6–7] gerbils,[8] rats,[9,10] dogs,[11] kittens,[12] and hamsters[13] involved observations over short periods of time or were designed to test the virulence of amebic strains. To date, there have been no reported studies on the progressive development and histopathology of intestinal amebiasis in a laboratory model. There is even less information on pathologic changes in secondary extraintestinal sites, such as early stages in the development of amebic liver abscess.

In the gerbil, the early formation of amebic cecal lesions follows amebic adherence to epithelial cells or may occur in its absence. This is followed by depletion of intraepithelial and goblet cell mucin and cytolysis of the surface epithelium. This process progresses rapidly to sloughing-off of epithelial cells and trophozoite invasion into the lamina propria and crypts.[14] In the present study, the sequential formation and pathologic sequelae of ulcerative cecal lesions in the submucosa and muscularis mucosae of gerbils are reported and compared with similar changes reported in human intestinal amebiasis.[15–18] At the same time, the histologic changes in the liver in the presence of a cecal infection alone or at the early stages of amebic metastasis to the liver were studied.

Materials and Methods

Animals and Parasites

Male Mongolian gerbils (*Meriones unguiculatus*), 45 to 50 days old, weight 50 to 55 g (Tumblebrook Farms, West Brookfield, Mass) were used in these experiments. Prior to experimentation, all animals were pretreated by oral gavage for 3 consecutive days with metronidazole (Flagyl; Rhône-Poulenc, Montreal, Canada) in a

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dose of 200 mg/kg, in order to eliminate naturally occurring *Entamoeba muris* and *Trichomonas* sp.[8] Intracecal inoculation with *Entamoeba histolytica* was done 10 days after treatment.

The strain of *E histolytica* used was IP:1182:2 (American Type Culture Collection Code), originally isolated from a case of acute amebic dysentery contracted in Central America. Amebic cultures were routinely maintained at 37 C, with twice-weekly transfers, in Robinson's medium,[19] supplemented with 500 mg/ml erythromycin, and in some cases additionally with 500 IU/ml penicillin and 500 mg/ml streptomycin. The antibiotics were necessary to prevent overgrowth of *Escherichia coli*, the bacterial associate used in the culture medium.

Preparation and Inoculation of Amebae

Amebae for inoculation were pooled from 48-hour cultures (log phase), centrifuged for 5 minutes at 500*g*, and counted with the aid of a Spencer Bright-Line hemocytometer. The inoculum was adjusted to 5×10^5 trophozoites in 0.2 ml of 0.85% saline. Each inoculum was loaded into an individual 1-ml disposable tuberculin syringe fitted with a 1-inch 21-gauge needle and kept in the vertical, needle-down position prior to use. The gerbils were anesthesized by an intraperitoneal injection of sodium pentabarbitol solution (42 mg/kg) and laparotomized. The amebic inoculum was injected toward the apex of the cecum. The site was blotted with sterile cotton swabs, and the abdominal wall and skin were sutured separately with 3-0 normal catgut (Ethicon, Peterborough, Ontario, Canada). Aseptic precautions were observed throughout the procedure. Fifty-five gerbils were inoculated intracecally with amebae, and 5 age-matched controls were similarly given 48-hour Robinson's medium freed from amebae by repeated centrifugation (conditioned medium).

Experimental Protocol and Grading of Cecal Lesions

Fecal samples from individual gerbils were examined for the presence of amebae throughout the course of the experiment. Randomly selected groups of inoculated gerbils were killed and underwent necropsy 5, 10, 20, 30, and 60 days after inoculation. Control animals were killed and examined on day 10. Positive diagnosis for cecal infections was based on the presence of amebae either in the cecal contents or in cecal wall scrapings. The severity of cecal infection was graded according to the scoring system introduced by Neal.[10] The condition of the cecal wall and contents were graded separately with the use of a number system from 1 to 4. When present, amebic liver abscesses were carefully dissected out and weighed and examined microscopically for the presence of trophozoites.[8] All data are presented as the means ± standard deviation (SD) of the means.

Histology

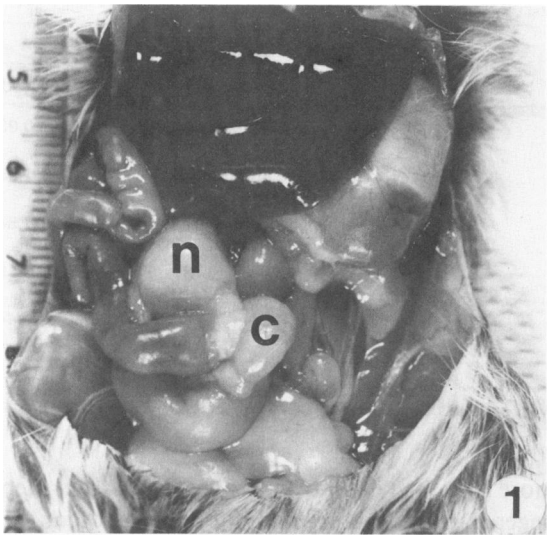
For histopathologic studies, the tissues (entire cecum, liver abscesses, abscess-free right liver lobe) were fixed for 1 week in 10% buffered neutral formalin, embedded in paraffin, and serially sectioned at 4–5 μ. At least two pieces of tissue from the cecum or the liver were examined. Additional liver tissues suspected of having amebic trophozoites but in the absence of gross lesions were completely serially sectioned and examined. Cecal wall sections were oriented perpendicularly to the mucosal surface. Sections were stained with Harris hematoxylin and eosin (H&E), Harris hematoxylin–aniline acid eosin–naphthol green B (H&E-NGB),[20] Giemsa, or periodic acid–Schiff reagent (PAS).

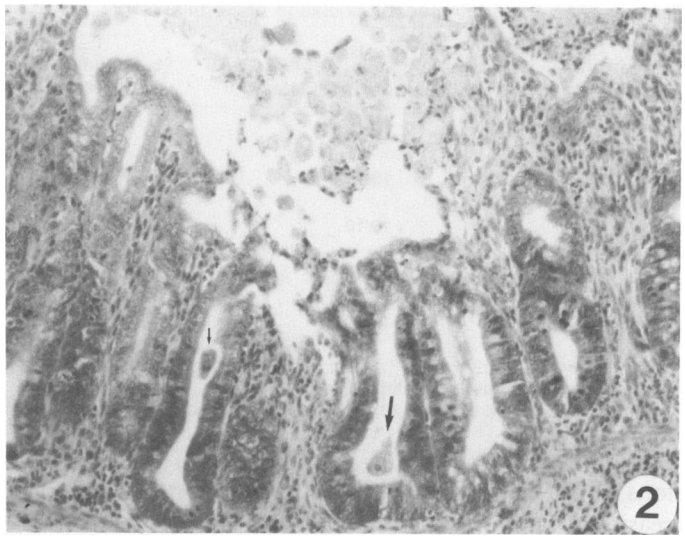
Results

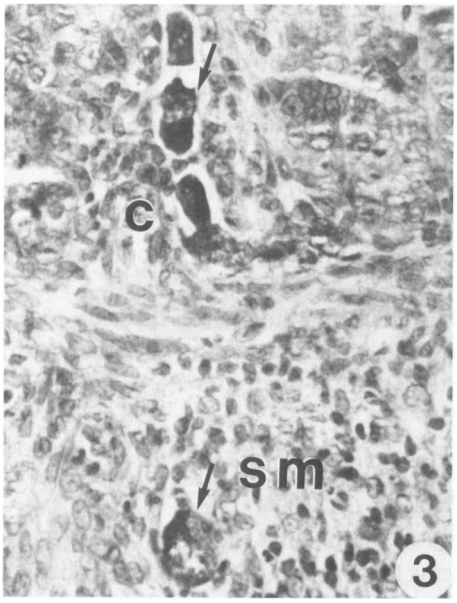
Gross Pathology and Microscopic Examination of Stool and Abscess Material

For about 4 days after inoculation the fecal pellets of gerbils appeared normal (hard, formed), but later they became semi-formed, sticky, and surrounded by a brown glistening mucous coat. Stool consistency after Day 10 remained the same throughout the infection. Motile amebic trophozoites were seen in such feces, as many as 20 per pellet. Frank diarrhea, with or without blood, was not evident, although hematophagous amebae were observed from 10 to 60 days after inoculation. Amebic cysts were not observed. During the course of the infection, most animals refused food, lost a considerable amount of weight (up to 40%, compared with age-matched uninfected controls), and appeared to suffer from acute dehydration, although food and water were provided *ad libitum*. Three animals with severe cecal lesions and liver amebic abscesses died during the course of the experiments. In general, animals with liver abscesses, in addition to cecal lesions, were more severely affected.

At necropsy, as early as 5 days after inoculation, the cecum of most gerbils appeared inflamed, thick-walled, and pale-colored. This was accompanied by inflammation of the ileum and adjacent portions of the large intestine. Mesenteric lymph nodes became progressively enlarged as the infection progressed (Figure 1). In some cases, lesions in the cecum appeared as yellowish white patches protruding from the cecal wall, which had a white creamy exudate. After 10 days, the cecum was usually attached to the abdominal wall and viscera by connective tissue. As the infection progressed, the cecum became contracted and atrophied to about one-third







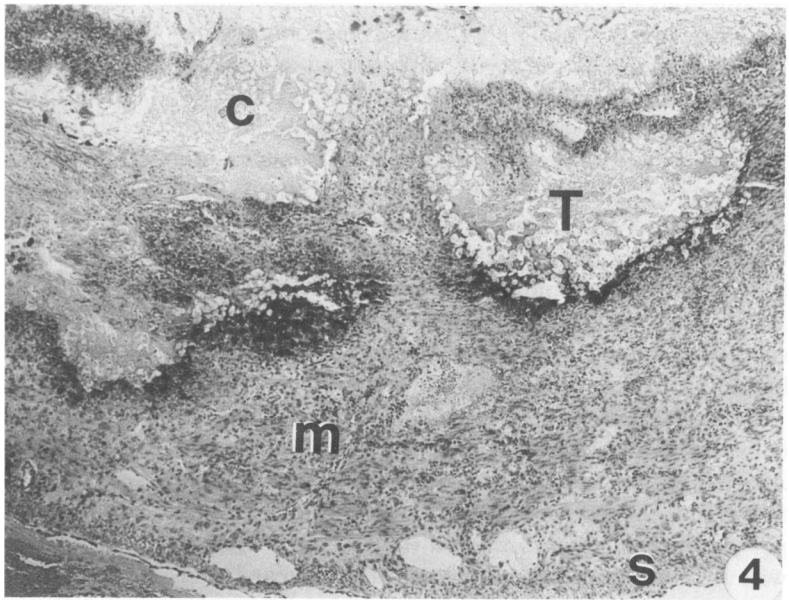


Figure 1—Appearance of the cecum (*c*) and mesenteric lymph node (*n*) in a gerbil inoculated intracecally with *E histolytica*, 20 days after inoculation. The scale bar is in centimeters. **Figures 2–4**—Histopathologic changes in the cecum at various times after inoculation. **Figure 2**—Ten days after inoculation. Destruction of the interglandular epithelium with trophozoites (*arrows*) in the crypts. Note the acute inflammatory infiltrate in the lamina propria. (H&E, ×125) **Figures 3 and 4**—Twenty days after inoculation. **Figure 3**—Cytolysis of the glandular crypts (*c*) and amebic trophozoites (*arrows*) at the base of crypts invading the submucosa (*sm*). (PAS, ×315) **Figure 4**—Deep ulcerative lesions in the submucosa adjacent to the muscularis mucosae (*m*) and serosal layer (*s*). Note the caseous material (*c*) replacing the mucosa and trophozoite aggregates (*T*) in the lesions. (H&E, ×50).

of its normal size. The cecal contents of infected animals appeared as a mucoid, yellowish green exudate, devoid of particulate matter, but containing numerous amebic trophozoites.

Amebic liver abscess became apparent 10 days after inoculation; and, as the infection progressed, abscesses of various sizes were found throughout the liver. Usually a single small abscess was found deep-seated in the liver parenchyma of the right lobe. In other animals, large confluent abscesses occupying the entire liver lobe were noted. Abscess material was either caseous or with a yellowish fluid. Amebic trophozoites were present in all abscesses. Attempts to culture the trophozoites axenically failed, and no bacterial growth occurred in cultures of liver or abscess material in Brewer's thioglycolate medium with cooked meat. Metastases to sites other than the liver were not seen. Splenomegaly was prominent in all animals with amebic liver abscess.

Pathology of the Cecum

Table 1 and Figures 2 to 8 illustrate, respectively, the gross and histologic features of the infected gerbil ceca 5 to 60 days after inoculation.

At 5 days after inoculation, 80% of animals were seen to have cecal lesions; but thereafter, all animals had le-

Table 1—Results of Intracecal Inoculation of Gerbils With 5 × 10^5 Trophozoites of *E histolytica**

Day after inoculation	No. animals infected/ no. animals inoculated	% Animals with cecal lesions	Mean cecal score ± SD (range)		
			Cecal contents	Cecal wall	Combined
5	10/10	80	1.80 ± 0.44 (1–2)	1.40 ± 0.54 (1–2)	3.20 ± 0.83 (2–4)
10	10/10	100	2.70 ± 0.94 (2–4)	2.40 ± 0.96 (1–4)	5.10 ± 1.85 (3–8)
20	10/10	100	3.60 ± 0.69 (3–4)	3.20 ± 0.91 (2–4)	6.80 ± 1.54 (4–8)
30†	15/15	100	3.71 ± 0.48 (3–4)	3.28 ± 0.75 (2–4)	7.00 ± 1.15 (5–8)
60	10/10	100	3.80 ± 0.44 (3–4)	4.00 ± 0.00 (4)	7.80 ± 0.44 (7–8)

* Gerbils were 60 days old at time of infection.
† Three animals died on Days 26, 27, and 29 after inoculation; animals with severe cecal lesions and amebic liver abscess.

sions of different degrees of severity, as shown by increasing cecal score values, depending on time. Early cecal lesions (5 days) were similar to those described by us previously[14]; and only the events accompanying amebic invasion of the crypts, submucosa, and muscularis mucosae will be reported in this paper.

At 10 days after inoculation, the interglandular epithelium, as well as the upper portions of some crypts and the intervening lamina propria, were eroded (Figure 2). There were numerous amebae in the lumen, and some were seen entering the exposed crypts. The cellular infiltrate was predominantly neutrophilic, with a few lymphocytes and eosinophils in the lamina propria. The submucosa was edematous, and lymphoid nodules were hyperplastic with histiocytic infiltrate.

Ulcerative lesions involving the submucosa and muscularis mucosae were seen at 20 days after inoculation. Amebae aggregated in the crypts, causing extensive cytolysis of the glandular epithelium, and invaded the upper portions of the submucosa (Figure 3). The cecal wall was contracted, the submucosa was thickened, and the entire cecum appeared to be atrophied (Figure 1). Trophozoites in the submucosa formed pockets above the muscularis mucosae, and necrosis spread laterally, resulting in the eventual sloughing-off of tissues (Figure 4). The underlying muscularis and serosal layers were edematous, with prominent vascular congestion.

At 30 days after inoculation, the entire mucosa of most animals was destroyed. In some sections, there was granuloma formation in the submucosa, and thrombosed blood vessels were seen extending as far as the muscularis mucosae. In these lesions, an eosinophilic zone of fibrinoid necrosis separated the necrotic exudate from the muscle layer. In other areas, amebae were found deep in the muscularis mucosae, but the cecal wall was never perforated (Figure 5). The cellular infiltrate consisted mainly of neutrophils and some lymphocytes. Proliferating capillaries and granulation tissues were frequently noted. In some animals at 30 days, but more frequently at 60 days after inoculation, there was regeneration of interglandular and glandular epithelial tissues. At this stage, trophozoites were rarely seen in the tissues, and most were restricted to the lumen and upper portions of the mucosal surface (Figure 6). The surface epithelium showed regeneration and migration of epithelial cells and intense neutrophilic infiltration in the lamina propria (Figure 7). Epithelial mitoses were frequently seen, but goblet cells were absent. In other areas, the lamina propria showed fibroblast infiltration, cross-bridging, and mitotic figures in the crypts (Figure 8).

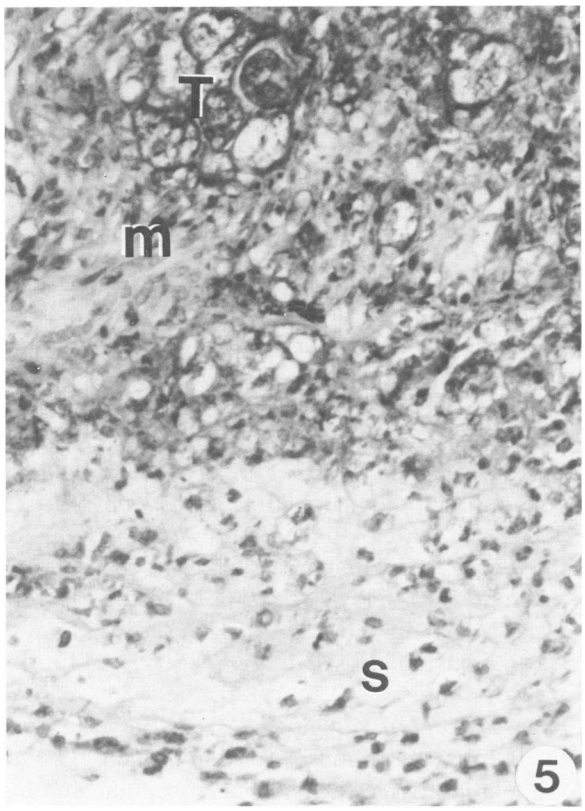
No pathologic changes were seen in the ceca of the control animals 10 days after injection with the conditioned Robinson's culture medium.

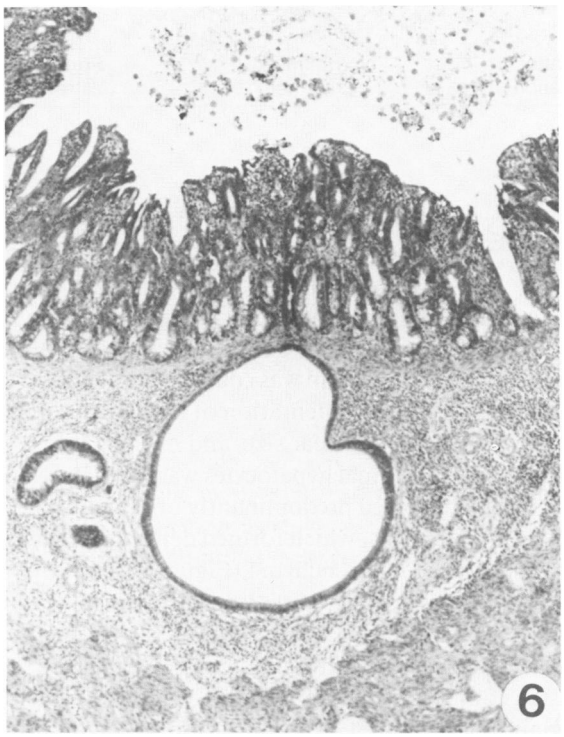
Pathology of the Liver in Animals With Cecal Amebiasis

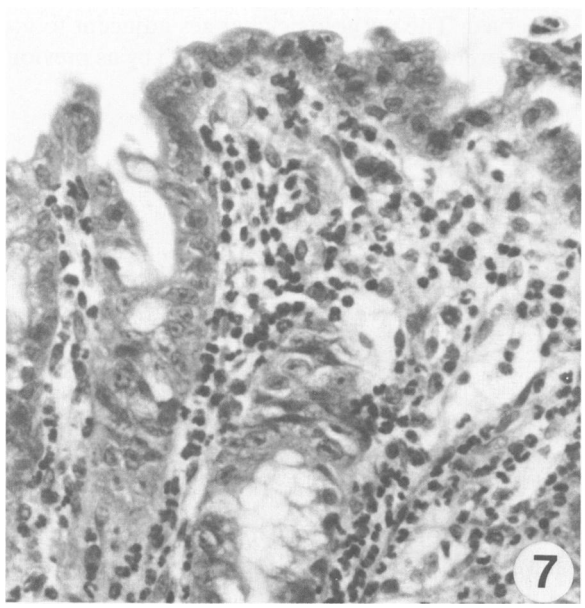
In an attempt to understand the early pathogenesis of amebic abscess formation, the morphologic changes and the cellular infiltrate in the right liver lobes of animals with cecal amebiasis, but with no evidence of amebic liver abscess, were studied from 5 to 60 days after inoculation. The major pathologic features found are shown in Table 2 and illustrated in Figures 9 to 14.

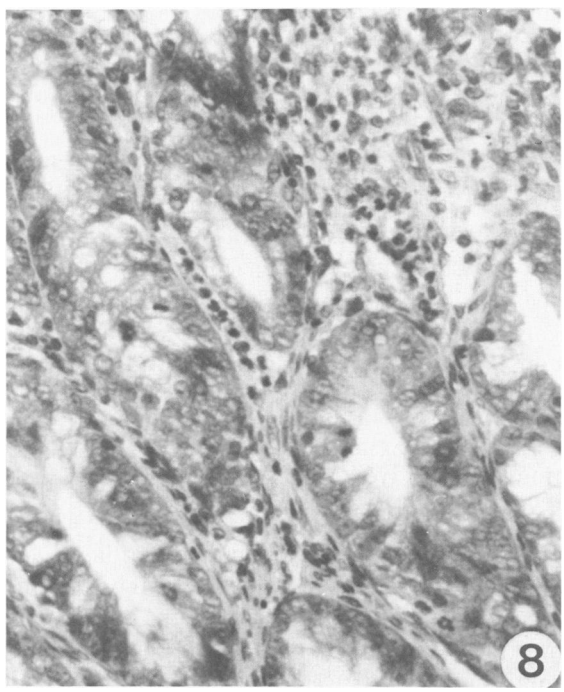
At 5 days after inoculation, there was no evidence of any pathologic change in the liver parenchyma. However, at 10 to 60 days after inoculation, the most prominent changes were lymphocytic portal infiltrate, Kupffer cell hyperplasia, multinucleated giant cell formation, and sinusoidal granulocytic infiltrate (Table 2).

The earliest evidence of parenchymal change was Kupffer-cell hyperplasia and anisonucleosis 10 days after inoculation. Hepatocytes were often seen to be anuclear; and, in other areas, hepatocyte regenerative activity was evidenced by increased numbers of bi- or trinucleated cells (Figure 9). There was minimal disorganization of hepatic cell plates.









Figures 5–8—Histologic changes in the cecum 30–60 days after inoculation. **Figure 5**—Thirty days after inoculation. Trophozoite (*T*) invasion of the muscularis mucosae (*m*). The serosal layer (*s*) shows moderate infiltration with granulocytes (PAS, ×312) **Figures 6–8**—Sixty days after inoculation. **Figure 6**—Diffuse cellular infiltrate, edema, and regeneration of epithelial elements in the submucosa. (H&E, ×50) **Figure 7**—Neutrophilic infiltrate in the lamina propria. The surface epithelium shows regeneration and migration of epithelial cells. Note the absence of trophozoites in the tissues. (Giemsa, ×312) **Figure 8**—Regeneration of glandular epithelium, cross-bridging, and fibroblastic infiltrate in the lamina propria. (Giemsa, ×312)

Table 2—Histologic Changes in the Livers of Gerbils at Various Times After Intracecal Inoculation With *E histolytica* Trophozoites

Days after inoculation	No. animals* examined	Percentage of animals with			
		Portal infiltrate	Kupffer-cell hyperplasia	Giant cells	Granulocytic infiltrate
5	10	0	0	0	0
10	8	13	13	25	38
20	7	43	29	72	72
30	6	50	17	84	84
60	8	50	25	75	100

* Animals with metastatic amebic liver abscesses were not examined.

Liver changes were more prominent at 20 to 60 days after inoculation (Table 2). Hepatocytes were often swollen, and their cytoplasm was coarsely granular. Occasionally, there was fragmentation of the cytoplasmic contents, variation in nuclear size, and nuclear pyknosis. Necrosis of individual hepatocytes was rare. The cellular infiltrate consisted predominantly of granulocytes and mononuclear cells, which formed dense aggregates throughout the liver parenchyma (Figure 10). In other areas, granulomas and giant cells were also present (Figure 11). In most cases, prominent changes occurred in the portal areas and the surrounding parenchyma. These consisted commonly of pylephlebitis of the portal vein with giant cells (Figure 12) or infiltration with lymphocytes and neutrophils. Central veins were not affected. There was no fibrosis of the portal or interlobular areas. However, the hepatocytes around the infiltrated portal areas showed marked glycogen depletion and some indication of fibrous tissue deposition (Figure 13). Proliferation of bile duct epithelium was noted only in a few cases. Liver necrosis with microabscess formation was observed around the portal areas in 5 animals, but in no cases were amebae seen (Figure 14). Pigmentation, fatty degeneration of hepatocytes, and hepatomegaly were not observed. Bacteria were not seen in the histologic sections nor in the culture from tissues taken at different times during the infection.

Histology of the Liver Abscesses

As shown in Table 3, amebic liver abscesses were present in 13 of the 55 intracecally inoculated gerbils. Liver abscesses were first observed 10 days after inoculation, but their prevalence was not related to the duration of the infection. They always occurred in the right liver lobe, and their weights were variable.

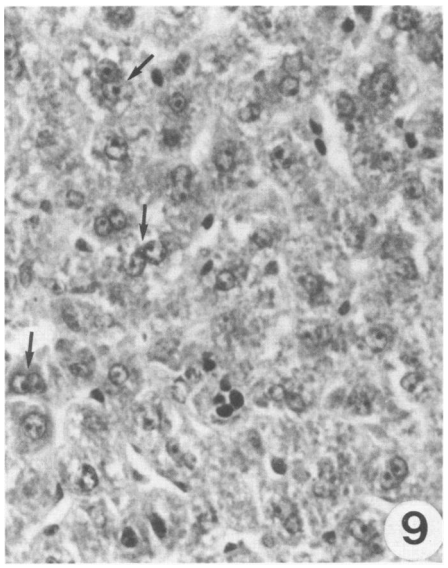
In histologic sections, the earliest amebic liver abscess (20 days after inoculation) was seen lying adjacent to the portal areas. Abscess material contained numerous granulomas and necrosis in the adjacent areas (Figure 15). Granulomas had no wall or a very thin layer of fibrous tissue. There was marked compression and congestion of the surrounding hepatocytes (Figure 16). Trophozoites were present in the centers of all lesions; they were associated with pyknotic cells and acute inflammatory infiltrate (Figure 17). In larger abscesses, usually involving a large portion of the liver lobe, there was periportal fibrosis and lymphocytic infiltration in the portal areas. The centers of the lesions contained caseous material with amebae in close association with the fibrous wall (Figure 18). Granuloma walls showed granulation tissues as well as neutrophilic and histiocytic infiltration. Fluid-filled cavitary abscesses were not observed. The pathologic changes adjacent to granulomas were similar to those described by us previously.[5]

Discussion

Infections with *E histolytica* may result in invasion of the colonic mucosa and subsequent spread to extraintestinal sites. In the majority of cases, however, the parasite remains as a harmless commensal in the lumen of the large intestine. Although the pathophysiology of initial mucosal damage remains obscure,[14,16] the histopathology of intestinal lesions has been described.[15-18] In acute intestinal amebiasis of man, lesions vary from superficial microerosions to late invasive lesions with deep ulceration or to granulating ulcers.[15-16]

In the Mongolian gerbil model used in this study, we have previously reported that the earliest type of mucosal damage showed foci of interglandular epithe-

Figure 9–14—Histologic changes in the liver of animals with cecal amebiasis. **Figure 9**—Ten days after inoculation. Regenerative activity of parenchymal cells showing large numbers of binucleated cells (*arrows*). (H&E, ×312) **Figure 10–11**—Twenty days after inoculation. **Figure 10**—Mononuclear and granulocytic infiltrate in the liver parenchyma. Kupffer cells are hyperplastic. (H&E, ×312) **Figure 11**—Granulomas (*G*) and giant-cell formation throughout the liver parenchyma (H&E, ×312) **Figures 12–14**—Thirty days after inoculation. **Figure 12**—Pylephlebitis of a portal vein with mononuclear and granulocytic cells. Giant cells (*arrowheads*) surrounding the periphery of the cellular infiltrate. (H&E-NGB, ×312) **Figure 13**—Portal area showing moderate infiltration with inflammatory cells. The adjacent areas show mild fibrous tissue deposition (*f*) and glycogen depletion from hepatocytes. (PAS, ×125) **Figure 14**—Microabscess (*Ab*) adjacent to the portal areas (*pa*). Notice the absence of an abscess wall and prominent necrotic center of the lesion. No amebic trophozoites were noted. (PAS, ×125)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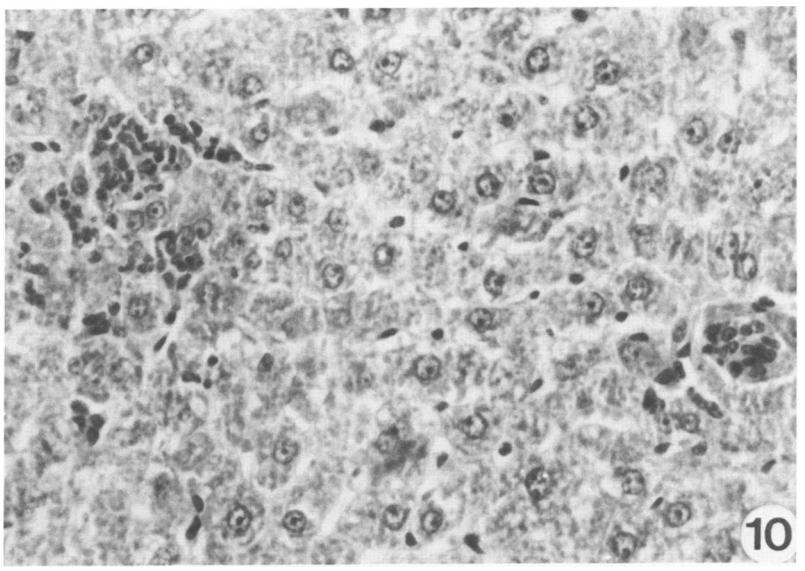
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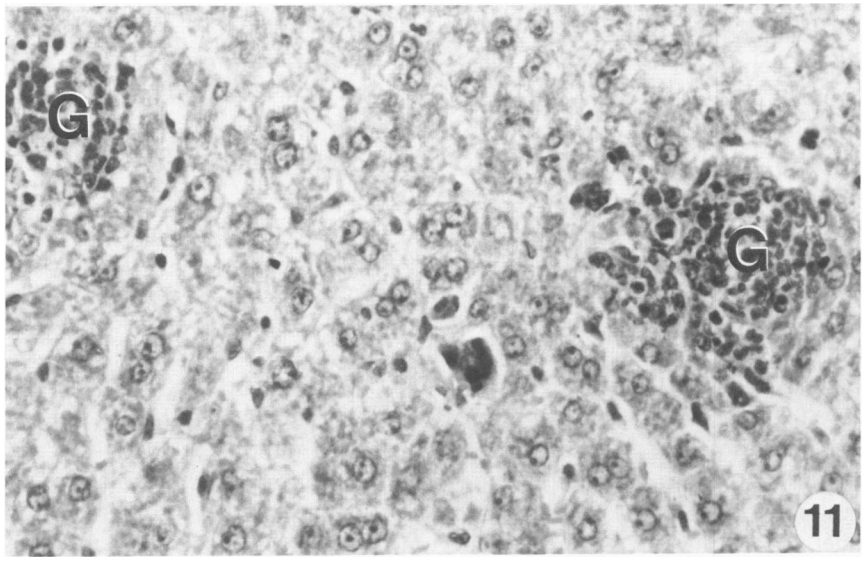
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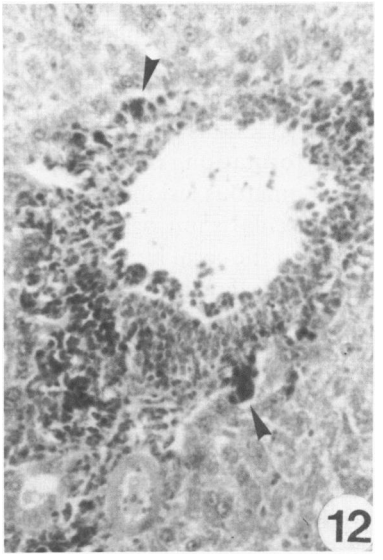
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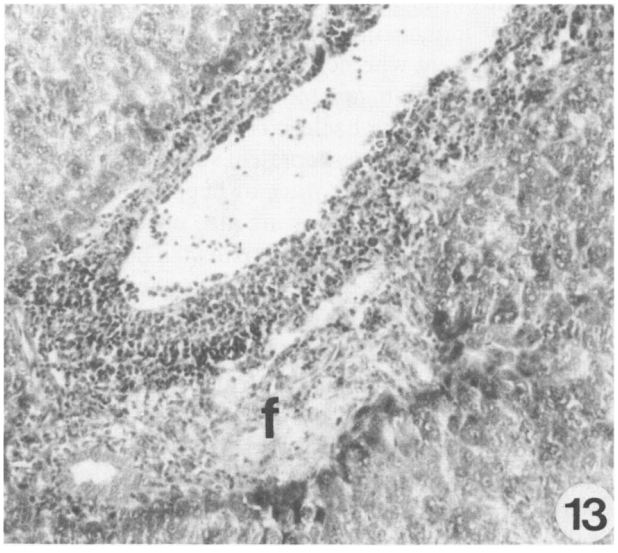
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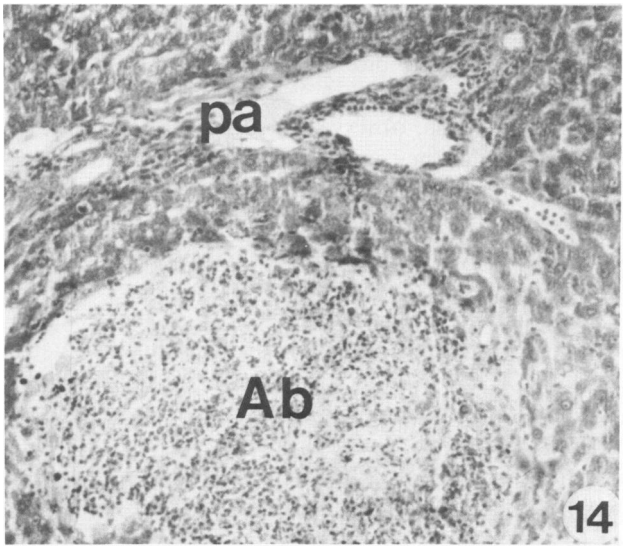
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Table 3—Location and Weight of Amebic Liver Abscesses in Animals Inoculated Intracecally With 5 × 10^5 Trophozoites of *E histolytica*

Day after inoculation	No. animals with abscess/ No. animals inoculated (%)	Location of amebic liver abscess	Mean weight of abscess (g) ± SD (range)
10	2/10 (20)	Right lobe	0.48 ± 0.23 (0.32–0.65)
20	3/10 (30)	Right lobe	0.35 ± 0.24 (0.12–0.61)
30	6/15* (40)	Right and median lobes	0.97 ± 0.68 (0.20–2.10)
60	2/10 (20)	Right lobe	1.10 ± 0.14 (1.0–1.2)

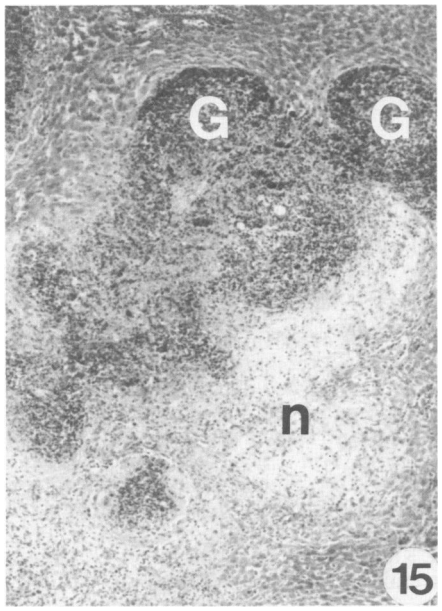
* Three animals died on Days 26, 27, and 29 after inoculation.

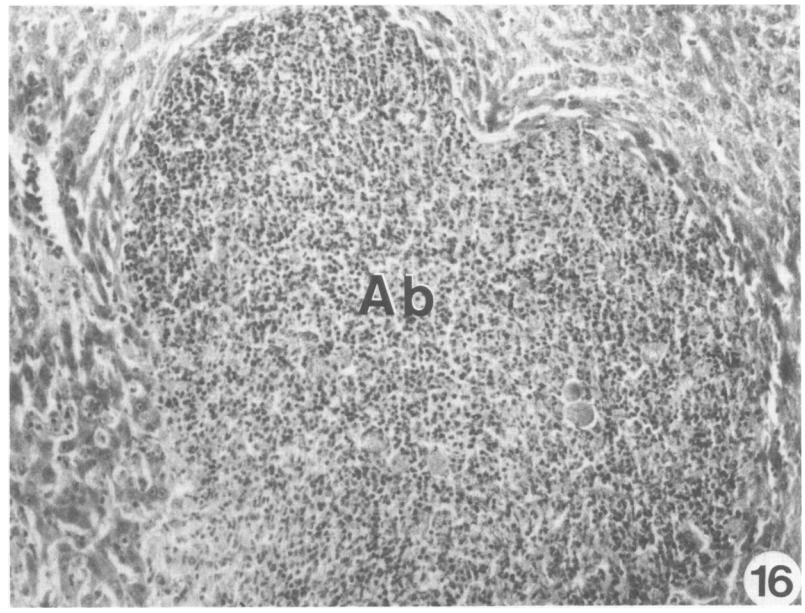
lial cell disruption or histolysis of the surface epithelium.[14] However, the sequence of events leading up to ulcerated lesions has not been reported. In this study the progressive development of lesions with undermined edges, was of three types: 1) lesions with ulceration of the submucosa and destruction of glandular crypt elements; 2) lesions involving the loss of the mucosa and with formation of granulation tissues; and 3) granulomatous lesions in the submucosa extending to the muscularis mucosae. The development and aggravation of the cecal lesions were asynchronous in different animals, and all three types of lesions were observed from 10 to 60 days after inoculation. The most prominent gross pathologic feature was atrophy of the entire cecum, and the most prominent histopathologic features were the loss of mucosa and the formation of granulation tissues. The submucosal invasion of trophozoites with lateral spread along the submucosa resulting from the sloughing-off of the mucosa have never been reported in an animal model. Neither have the deep ulcerative lesions associated with regeneration of epithelial and crypt elements in adjacent areas. Similar types of lesions, however, have been observed in human intestinal amebiasis.[15-16] Granulomatous lesions in the submucosa, observed in some animals, appear to be rare, since they were reported only once in an experimental animal model.[21] Granulomas were diffuse throughout the cecum, and there were only minimal amounts of fibrous tissue. In this respect, such granulomas are not considered to be amebomas.[22]

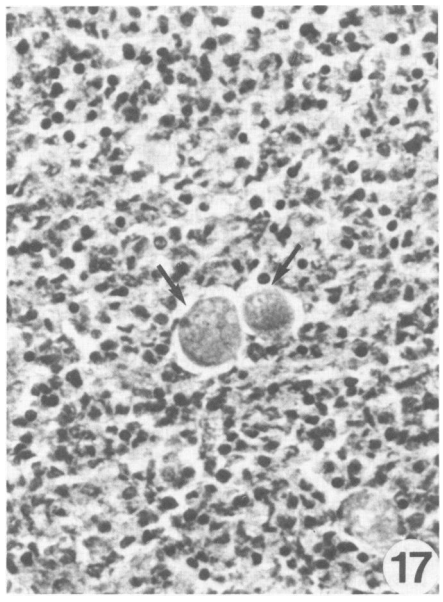
Regardless of the type and severity of the cecal lesions, the cellular infiltrate was predominantly neutrophilic. This is in contrast to a mixed lymphocytic-neutrophilic response observed in the gerbil at the very early stage of mucosal damage, prior to the destruction of the interglandular epithelium.[14] The neutrophilic response could be a direct one to the amebae, or to a secondary bacterial invasion. Similar cellular infiltrates have been observed in monkey[23-24] and human[15] infections. Because pathogenic *E histolytica* trophozoites are known to be able to phagocytose neutrophils or to exert their cytotoxic effect upon them,[25-26] and in this study were frequently observed contacting and ingesting neutrophils, the neutrophilic response is probably nonprotective. The ameba-mediated lysis of neutrophils may result in the release of large amounts of lysosomal enzymes, which, in turn, may aggravate the pathologic process. Products released by live or dead amebae may also contribute to the acute inflammatory response or to tissue damage. The early appearance of neutrophils in response to tissue damage in intestinal amebiasis has been reported by previous workers.[15]

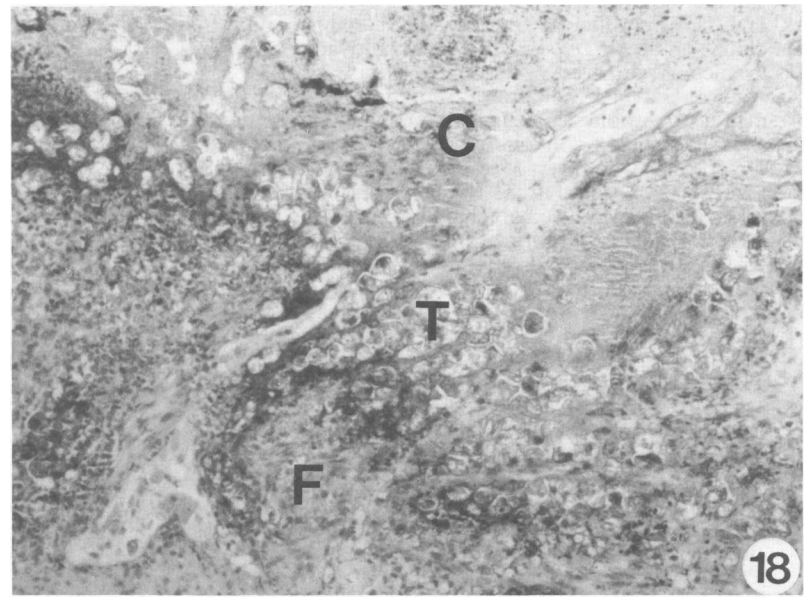
Regardless of the type or severity of the cecal lesions, the majority of the amebae were observed within the cecal lumen, but they were also found in all lesions. Amebic metastasis to the liver was first noted at 10 days after inoculation, corresponding to the destruction of the interglandular epithelium and trophozoite invasion of the crypts and lamina propria. Dissemination of amebae probably occurred as a result of their entry into the capillaries of the lamina propria. Since amebae were never seen in the capillaries of the cecum or in the hepatic portal vein, it appears that dissemination is a very rapid process.

The histologic changes of the liver parenchyma in animals with cecal amebiasis were found to be diverse. The earliest changes seen at 10 days after inoculation were Kupffer cell hyperplasia, anisonucleosis, and granulocyte infiltration in some areas. Liver tissue changes became more pronounced with the aggravation of the cecal lesions. They consisted of granuloma formation, appearance of multinucleated giant cells, and lymphocytic portal infiltration. At this stage of the process, amebic trophozoites were never seen in the liver parenchyma, even in the presence of microabscesses around the portal areas. Similar findings were reported in cases of human intestinal amebiasis.[27-28] It is not known at present whether these changes are due to actual amebic invasion, amebic enterotoxins/cytotoxins[29] released in the gut, or bacterial toxins. It has been suggested that these changes represent subacute hepatic involvement at the earliest stage of hepatic amebiasis.[28] Similar changes in the liver are also characteristic of those caused by bacterial endotoxins.[30] The only proof of amebic metastasis to the liver was the demonstration of early liver abscesses around the portal areas containing amebic trophozoites. Abscesses were found in the right lobe of the liver and probably were the result of dissemination of amebae via the portal circulation. Similar observations have also been noted in human infections.[31-32] Early abscess formation was characterized by the absence of a well-developed fibrogranuloma wall and the presence of a cellular infiltrate composed of granulocytes and mononuclear cells.[5]









Figures 15–18—Histopathologic features of amebic liver abscesses resulting from a cecal infection. **Figure 15–17**—Early amebic liver abscess 30 days after inoculation. **Figure 15**—Note numerous granulomas (*G*) and adjacent necrotic (*n*) areas within an abscess in the portal area. (PAS, ×50) **Figure 16**—Higher magnification of a portion of the abscess (*Ab*) showing the absence of a fibrous wall and congestion of the surrounding hepatocytes. (H&E, ×125) **Figure 17**—Necrotic center of a lesion with amebic trophozoites (*arrows*) and pyknotic cells. (H&E, ×312) **Figure 18**—Confluent abscess 20 days after inoculation. Fibrogranuloma wall (*F*), trophozoites (*T*), and caseous material (*C*) in the center of a lesion. (PAS, ×125)

It is not known whether amebae were able to establish and multiply in previously necrosed areas of the liver, or whether they were restricted to the portal areas. Nor is it clear whether previous or concomitant liver injury during a cecal infection is a prerequisite for the establishment of amebic trophozoites and eventual abscess formation. The minimum number of amebae *in situ* necessary to produce an abscess is also not known. It is suggested that amebic dissemination to the liver is a continuous process during the course of invasive intestinal amebiasis. When fixed macrophages (Kupffer cells) are damaged, or when necrotic areas develop, impairment of liver function takes place,[27] enabling the amebae to establish and multiply. It was shown that hamsters with previously induced liver injury were more susceptible to amebic liver abscess formation.[33] A state of transient immunodepression may also be necessary for the entry of amebae into the systemic circulation and their survival. Mice,[34] guinea pigs[35–36] and humans[37] with intestinal amebiasis, when subjected to immunosuppressive treatment, became more prone to develop amebic liver abscess. Local and systemic immune

responses in gerbils with cecal amebiasis are probably suppressed, as is the case with experimental hepatic amebiasis.[38]

This study has shown the progressive development of disease and host responses during the formation of ulcerative cecal lesions in the gerbil. This is the first time that the pathologic changes in the liver as a result of a cecal infection and the early formation of metastatic amebic liver abscess in an animal model have been described. These events correlate with similar ones recorded in human amebiasis.[15-17]

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