# *The Pathogenesis of Experimentally Induced Amebic Liver Abscess in the Gerbil* (Meriones unguiculatus)

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**Sequential development and pathology of experimentally induced amebic liver abscess in the gerbil (*Meriones unguiculatus*) were studied from 1 to 60 days after inoculation. Early lesions were characterized by an acute inflammatory response, which became granulomatous at 5 days. Early granulomas were discrete, with well-defined fibrohistiocytic walls. Trophozoite dissemination as a result of fibrolysis of granuloma walls was confined to the liver parenchyma. The granulomatous cellular infiltrate (<20 days) was characterized by granulocytes and histiocytes; older granulomas (>30 days) were composed of lymphocytic infiltrate, plasma cells, and a few granulocytes, and were characterized by the absence of epithelioid histiocytes. The degree of pathologic change adjacent to liver granulomas followed the sequential development of the amebic liver abscess. Severe changes observed were portal canal lymphocytic infiltration, the presence of foreign body giant cells, periportal fibrosis, proliferation of bile duct epithelium, and hepatocyte anisonucleosis and ballooning degeneration. The pathogenesis of the infection and the usefulness of the gerbil model for the study of human amebiasis are discussed. (Am J Pathol 1984, 117:71–80)**

SUCCESSFUL axenic cultivation of *Entamoeba histolytica*,[1] made possible the studies of experimentally induced bacteria-free amebic abscess in rodents as a model of the human disease. Among laboratory animals, only the rabbit,[2] the hamster,[3–6] and the gerbil[7,8] are known to be highly susceptible to amebic liver infections. With the hamster model much emphasis has been placed on testing the virulence of strains of *E histolytica*, but studies were hindered by the short-lived nature of the amebic liver abscess[3] and amebic metastasis to other sites[9] resulting in high host mortality, thus precluding long-term studies. We have previously reported[8] that the gerbil not only is susceptible to amebic cecal and liver infections but is also tolerant to the infection, and could be a suitable host for long-term studies.

The liver lesions produced by the direct inoculation of *E histolytica* in rodents is characterized by a granulomatous inflammatory response,[3,6,8,10] but its occurrence in nonhuman primates[11–13] and humans is rare. Only in two cases[14,15] was a granulomatous immune response reported in humans with amebic liver abscess. This may be a characteristic of early lesions, because they disappeared later in the infection of experimental animals.[8,10] Since the early lesions of human hepatic amebiasis are not well known, the events that precede the typical fluid-filled cavitary abscess reported in postmortem cases remains unknown. There is even less information regarding the evolution of granuloma formation, growth of the amebic liver abscess, and the histogenesis of the cellular infiltrate. The histopathologic changes of the amebic liver abscess have been reported in human cases[14–21] but represent the events at only one point in time. The amebic liver abscesses in gerbils are localized and free from bacteria and progress to a cavitary abscess, as in human infections. Thus, a study of events leading up to the formation of the cavitary abscess was made. We report here, from a time-course study, the early events of the granulomatous inflammatory response, growth of the amebic liver abscess, characterization of the cellular infiltrate, and histopathologic changes adjacent to the amebic liver abscess.

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## Materials and Methods

### Animals and Parasite

Male Mongolian gerbils (*Meriones unguiculatus*) 50–60 days old and weighing between 55 and 60 g (Tumblebrook Farms) were used in all experiments.

The strain of *Entamoeba histolytica* used was IP:0682:1 (American Type Culture Collection Code) isolated by Dr. E. Meerovitch in June 1982 from the dysenteric stool of a 1-year-old Amerindian child in the amebiasis-endemic area of Saskatchewan, Canada. The initial isolate was established and thereafter grown in Robinson's medium[22] for 20 3-day passages before axenization in TYI-S-33 medium[1] in August 1982. Routine transfers of cultures in TYI-S-33 medium are carried out every 3 days by chilling stock cultures, suspending the amebae and transferring approximately 1 ml of the suspension into 13 ml of fresh medium containing 100 IU/ml penicillin and 100 mg/ml streptomycin in 15–125-mm screw-capped tubes. Cultures prepared for inoculation of gerbils were grown in 30-ml plastic disposable tissue culture flasks (Becton Dickinson). The absence of microbial contamination is monitored every second passage by inoculating approximately 0.3 ml of culture material at the time of transfer into Brewer's thioglycollate medium with cooked meat. Cultures are incubated at 37 C inclined at about 20 degrees from the horizontal.

### Preparation and Inoculation of Amebae

For inoculation of gerbils, 60-hour amebic cultures (mid-log phase) were chilled for 5 minutes in an ice-water bath and centrifuged at 500*g* for 5 minutes, and the amebae in the sediments were pooled and counted with the aid of a Spencer Bright-Line hemocytometer. The concentration of the amebic inoculum was adjusted to $2 \times 10^5$ trophozoites contained in 0.1 m of fresh medium. Each inoculum was loaded into an individual 1-ml disposable tuberculin syringe fitted with a 3/8-inch 26-gauge needle and kept in the vertical, needle-down position prior to use. Liver inoculations were performed after laparotomy in animals anesthetized by an intraperitoneal injection of sodium pentobarbital solution (42 mg/kg). Each gerbil was inoculated in the left lobe of the liver. To minimize injury of the hepatic parenchyma, the inoculum was introduced slowly, producing a subcapsular blister at the inoculum site. After inoculation care was taken to preclude leakage of the inoculum by blotting the area with sterile cotton swabs. The abdominl wall and skin were sutured separately with 3-0 normal catgut (Ethicon, Ontario, Canada). Aseptic precautions were observed throughout, and each operation took about 4 minutes. In this experiment, 56 gerbils were given injections of trophozoites of *E histolytica*, and 10 age-matched controls were similarly given 0.1 ml of medium without trophozoites.

### Experimental Protocol

To follow the development of granuloma formation, abscess growth, the cellular infiltrate, and pathologic changes in the liver, gerbils were killed from 1 to 60 days after inoculation. For the first 2 days, 4 gerbils were killed on each day, and thereafter (Days 3, 5, 10, 20, 30, and 60) 8 animals were killed on each of the above days. To determine the extent of liver injury caused by the injection of the inoculum, 5 control gerbils were killed and examined on Days 10 and 20. In these animals, some hemorrhage at the site of injection was observed on Day 10, and by Day 20 all livers appeared normal, with no evidence of scarring. In histologic sections, liver architecture was restored, and no damage at the injection site was observed.

### Histology

At necropsy, the amebic liver abscess was dissected out with about 5 mm of healthy surrounding liver tissue. Tissues from the abscess material and from the abscess/liver interphase from each gerbil were immediately fixed in 10% buffered neutral formalin. After fixation for 48 hours, the tissues were embedded in paraffin and sectioned at 5–7 $\mu$. Sections were stained with either Harris' hematoxylin and eosin (H&E), Harris' hematoxylin–aniline acid eosin–naphthol green B[23] (H&E-NGB), or Wolbach's Giemsa according to the method recommended by Luna.[24]

The differential counts of inflammatory cells were made in the center of the granuloma wall of proliferating superficial granulomas. Coalesced granulomas or those with no trophozoites in their centers were excluded. All counts were made under oil immersion at a magnification of $\times 100$, with the area of the field being 0.017 sq mm. The thickness of the granuloma wall was measured on both coalesced and proliferating granulomas with an eyepiece micrometer disk, with the $\times 10$ objective. All values are presented as means $\pm$ standard deviation (SD) of the means.

## Results

### Gross Pathology

Following the direct inoculation of axenic trophozoites of *E histolytica* into the liver, the lesion appeared as a subscapular blister with minimal hemorrhaging at the injection site. At 2–3 days after

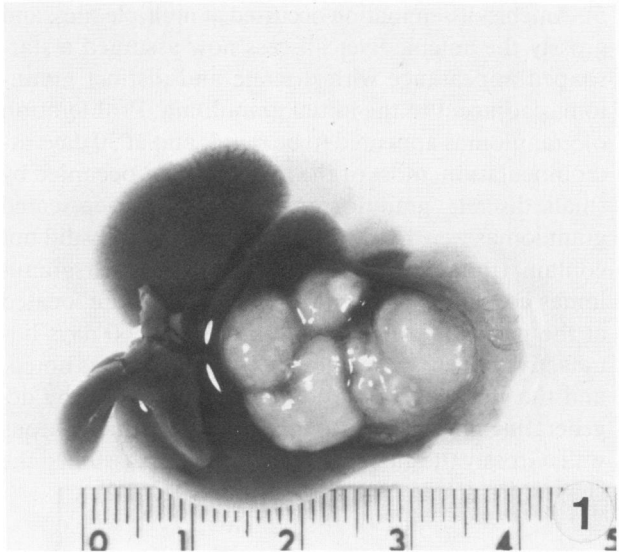
inoculation, a white nodular aggregate formed around the rim of the lesion cavity. Later, at 5 to 10 days, discrete and distinct granulomas were formed as white, clustered nodules; and at 30 days most of the liver lobe was occupied by confluent, granular, white nodular aggregates that appeared as a solid mass (Figure 1). In some cases, the abscess mass appeared as an outgrowth from the lesion site with minimal liver involvement. The peritoneal wall, mesentery, and omentum were usually attached to the abscess surface. The abscess surface was heavily vascularized, and mild pylephlebitis was present in only a few animals. However, at 60 days the abscess appeared as a distinct solid white mass confined to the margin of the liver lobe (Figure 2). Solitary granulomas were occasionally found deep-seated in the liver at a distance from the abscess mass. The centers of lesions at 30 days contained an odorless, creamy-white viscous fluid; and at 60 days most of the necrotic center was occupied by caseous material. Metastases to the other liver lobe or to extrahepatic sites were not observed. Hepatic lymphadenopathy and splenomegaly were common features and correlated with the size of the amebic liver abscess.[8] Host mortality was rare, and only a few gerbils showed obvious illness.

### Granuloma Formation and Growth of the Amebic Liver Abscess

The early events of granuloma formation were observed by histologic examination 1 to 5 days after inoculation. At Day 2, the subscapular lesion site appeared as a hemorrhagic fibrinous neutrophilic mass that contained intact and partially degenerated amebic trophozoites. The underlying liver parenchyma showed disruption in places, and trophozoites were confined to the rim of parenchymal cells (Figure 3). At 2 to 3 days after inoculation, the lesion consisted of a fibrin layer that formed as a corona around the lesion cavity. Neutrophils and a few eosinophils and fibroblasts infiltrated the narrow rim of fibrin peripheral to the lesion, and at 5 days the connective tissue was infiltrated around the lesion site by macrophages. Organized granulomas with epithelioid and vacuolated histiocytes at the inner margin of the lesion cavity and granulocytes in the fibrous zone were most noticeable at 5 days. As the infection progressed, granulomas enlarged, their walls thickened, and at 10 days they assumed the shape of a single cavitary abscess (Figure 4). In the necrotic centers of lesions, trophozoite density increased, and they tended to aggregate at points along the inner margin of the histiocytic layer. Both the histiocytes and the fibrous wall appeared to undergo lysis at these points, enabling the amebae to disseminate to adjacent liver tissue with the formation of new granulomas (Figure 5). Amebic dissemination occurred at multiple sites, and grossly the amebic liver abscess now assumed a star-shaped appearance with discrete and distinct granulomas adjacent to the initial granuloma. Proliferation of granulomas appeared to be rapid, and at 30 days after inoculation, most of the liver lobe was occupied by small discrete granulomas (Figure 6). Deep-seated granulomas were hard and nodular and usually did not contain trophozoites. Superficial thin-walled granulomas contained many trophozoites and were located at the periphery of the abscess mass. At 60 days liquefactive necrosis of coalesced granulomas was noted, and the center of the lesion became occupied by degenerating fibrous partitions enclosed in a thick fibrous wall. Grossly the amebic liver abscess now assumed the shape of a single cavitary abscess (Figure 2).

### Cellular Infiltrate

The histogenesis of the cellular infiltrate in and around the amebic liver abscess was followed from 5 to 60 days after inoculation. A qualitative analysis of the inflammatory cells is presented in Table 1, and the distribution of the cells in the granuloma wall is shown in Figures 7 to 12. Early in the infection, from 5 to 10 days after inoculation, the most prominent cell types were polymorphonuclear leukocytes, which were diffusely distributed throughout the granuloma wall. Among these cells, neutrophils were present in high numbers and outnumbered the eosinophils (Table 1). The inner margin of the granuloma wall at Day 10, had a distinct zone of histiocytes (Figure 7), and as they merged toward the necrotic center, lysis of cells was observed. Trophozoites were in close contact with histiocytes, but granulocytes were absent from the histiocyte zone and from the centers of granulomas. The necrotic center consisted of a granular eosinophilic mass. Outside the histiocytic zone, granulocytes were the predominant cell type, and scattered throughout the granuloma wall were fibroblasts and collagen fibers (Figure 8). Mast and plasma cells were present at the periphery of the granuloma wall. At 20 days after inoculation, the granuloma wall thickened and the predominant cell type was the lymphocyte, and there was a 65% reduction of granulocytes (Figure 9, Table 2). Epithelioid histiocytes were restricted to the inner margin of the granuloma wall, and granulation tissue was observed throughout. Plasma cells infiltrated the outer fibrous layer of the granuloma wall and appeared as a rim of cells encircling the wall. By 30 days the granuloma wall had receded in size (Table 1), and many granulomas were coalesced. Lymphocytes were tightly packed around the margin of the fibrous zone (Figure 10). The histiocytic infiltrate was now absent from the inner margin, and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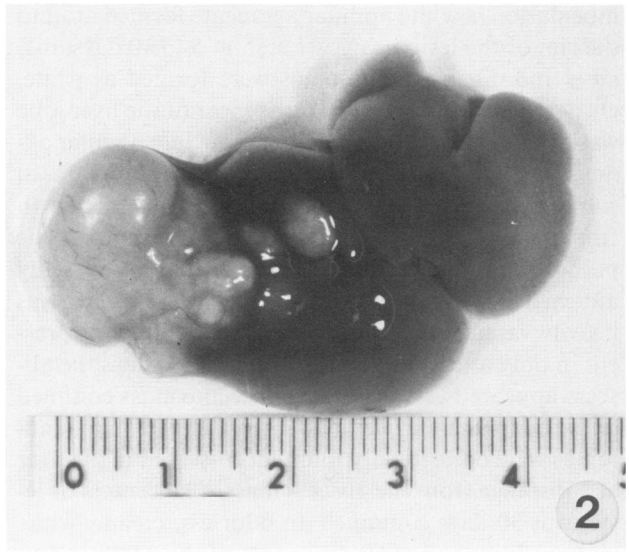
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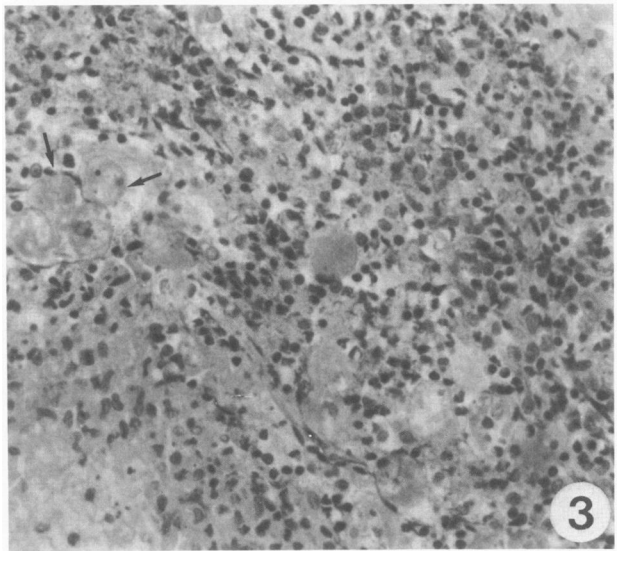
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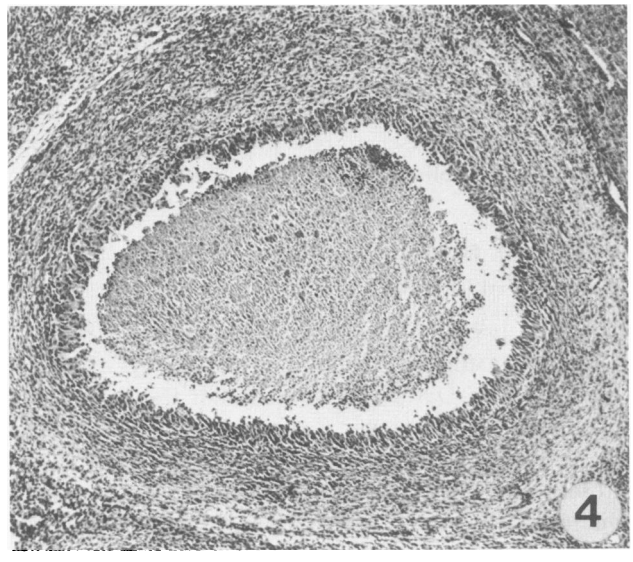
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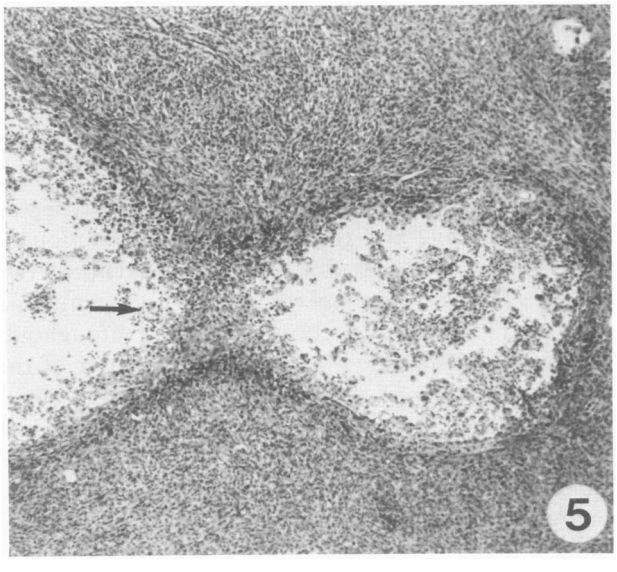
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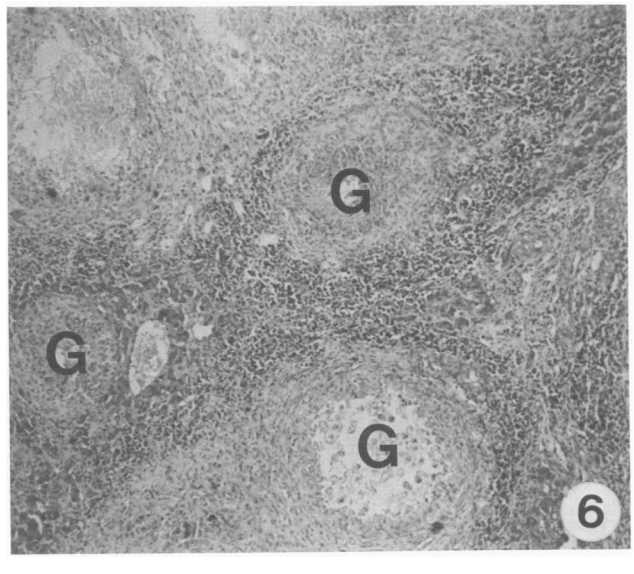
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Table 1—Number of Granulocytes in Amebic Liver Granulomas of Gerbils at Various Times After Inoculation With 2 × $10^5$ Axenic Trophozoites of *Entamoeba histolytica*

| Days after inoculation | Mean thickness* of granuloma wall (μ) ± SD (range) | Mean number of neutrophils ± SD (range) | Mean number of eosinophils ± SD (range) | Mean number of granulocytes† ± SD (range) |
|---|---|---|---|---|
| 5 | 9.40 ± 3.14 (7.18-16.59) | 56.60 ± 6.73 (45-62) | 32.60 ± 5.85 (25-40) | 89.20 ± 11.86 (70-102) |
| 10 | 33.45 ± 4.93 (27.65-43.45) | 52.60 ± 13.08 (42-75) | 28.20 ± 8.25 (20-40) | 80.80 ± 9.03 (74-95) |
| 20 | 52.93 ± 14.19 (35.55-71.15) | 18.14 ± 6.30 (9-25) | 10.42 ± 2.63 (8-15) | 28.57 ± 4.75 (22-36) |
| 30 | 16.45 ± 3.64 (11.84-24.49) | 14.16 ± 3.76 (10-17) | 8.83 ± 2.31 (6-12) | 23.00 ± 5.25 (16-29) |
| 60 | 68.05 ± 12.00 (55.30-89.27) | 18.00 ± 5.04 (12-25) | 9.80 ± 3.63 (8-16) | 28.00 ± 3.39 (24-33) |

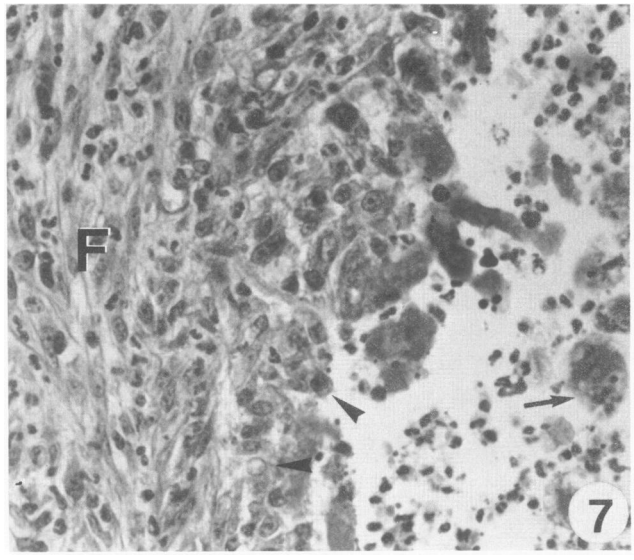
* Mean of 20 granulomas.
† Mean of 10 counts; area of field, 0.017 sq mm.

trophozoites were in direct contact with the fibrous wall. Granulocytes were not numerous and were diffused among the lymphocytes. At 60 days, granulomas had coalesced and necrosed, and their remnants were seen as partitions enclosed by a thick fibrous wall. There was heavy infiltration of lymphocytes in the fibrous wall (Figure 11) and lymphoid aggregates were found at the periphery of the abscess mass (Figure 12).

### Changes in the Liver Parenchyma Around the Amebic Liver Abscess

The pathologic changes in the uninvolved liver parenchyma adjacent to the granulomas were followed from 5 to 60 days after inoculation. The major pathologic findings are shown in Table 2 and Figures 13-18. Early in the infection (3 to 5 days) only minimal changes were noted, but by Day 10, the liver parenchyma adjacent to the granuloma wall showed flattening and congestion of cells. Portal areas were moderately infiltrated with lymphocytes (Figure 13). Perivascular spaces showed considerable neutrophilic cellular infiltrate, and foreign body giant cells were often seen around the portal areas. During proliferation of the amebic liver abscess at 20 days, multinucleated giant cells, Kupffer cell hyperplasia, and anisonucleosis were common (Figure 14). Often, nuclei were absent from hepatocytes, and in other cells regenerative activity was evidenced by increased numbers of bi- or trinucleated cells. Hepatocytes were often swollen, and the cytoplasm was coarsely granular. Hyperchromatism of nuclei was frequently observed, as nuclei contained large nucleoli. Further away from the granuloma wall hepatocyte changes were only equivocal. Between 20 and 30 days, during maximum proliferation of the amebic liver abscess, hepatic changes around the abscess were most pronounced. Portal areas were heavily infiltrated with lymphocytes, and those between granulomas showed fibrosis, with an increased amount of proliferating bile duct epithelium (Figure 15). In other areas, thrombosis of the portal vein was frequently encountered. In some cases perivascular infiltration of lymphocytes and plasma cells could be detected away from the lesion. Large numbers of foreign body giant cells were readily observed both in granuloma wall (Figure 16) and in adjacent liver parenchyma. In most animals hepatocyte degeneration, anisonucleosis, Kupffer cell hyperplasia, and periportal fibrosis were common features (Table 2). In other areas close to the granuloma wall, hepatocytes were often swollen, with increased amounts of bile or iron pigments. The interlobular areas showed congestion with erythrocytes, but few inflammatory cells (Figure 17). At some distance away from the lesion, hepatocytes showed ballooning degeneration with or without nuclei and loss of cytoplasmic material. The surrounding cells showed marked multivacuolar fatty change (Figure 18). At 60 days, when granulomas were coalesced, changes in the hepatocytes adjacent to abscess mass were minimal.

**Figure 1**—Amebic liver abscess in the gerbil 30 days after inoculation. **Figure 2**—Cavitary liver abscess localized at the left lobe of the liver, 60 days after inoculation. Notice the granulomas away from the abscess mass. The scale bar is in centimeters. **Figure 3**—Inflammatory infiltrate and pyknotic cells in a lesion 2 days after inoculation; trophozoites (*arrows*) can be seen at the rim of the lesion cavity. (H&E, ×312) **Figure 4**—Organized granuloma with thick fibrohistiocytic wall and central area of necrosis 10 days after inoculation. (H&E, ×50) **Figure 5**—Budding of granulomas and trophozoite movement (*arrow*) in the direction of a bud at 10 days after inoculation. (H&E-NGB, ×50) **Figure 6**—Coalescing granulomas (*G*) with or without trophozoites at 30 days. The center of a lesion is undergoing liquefactive necrosis (*top left*) at places, and others are surrounded by bands of fibrous tissue. (H&E, ×50)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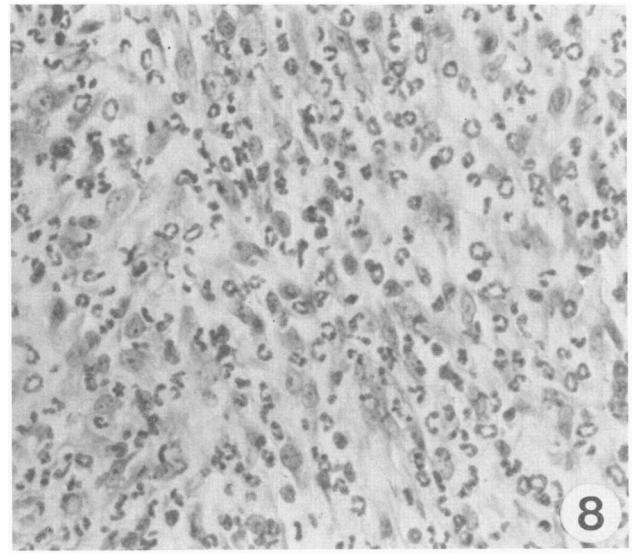
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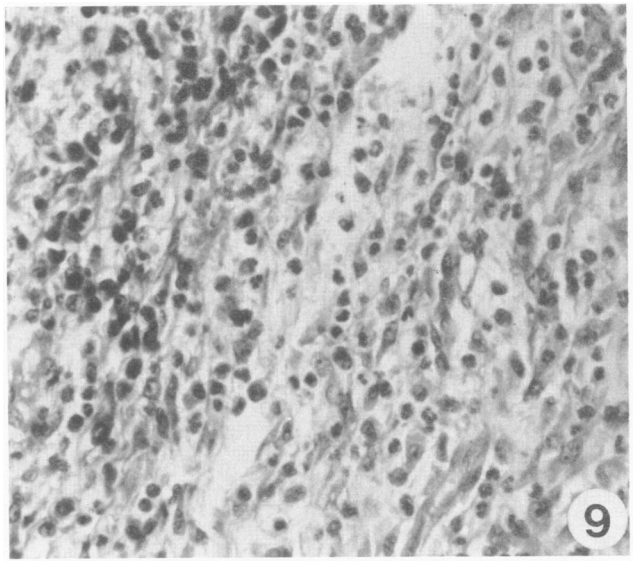
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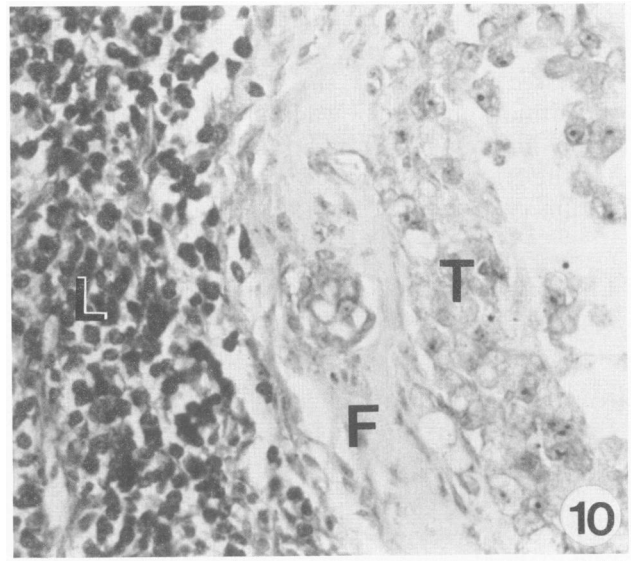
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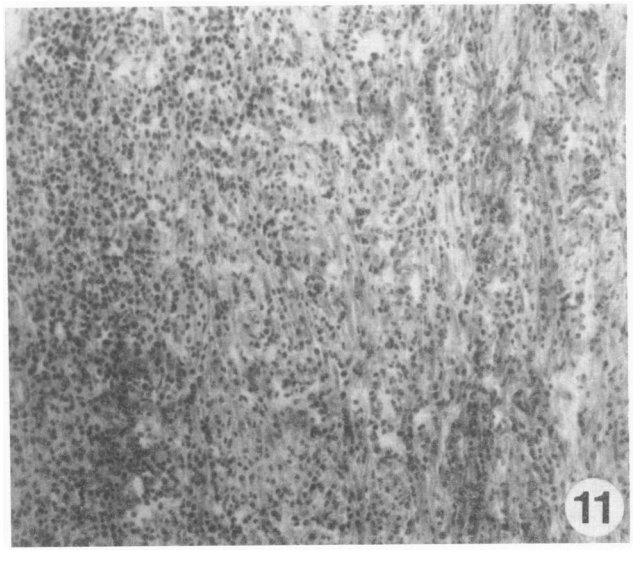
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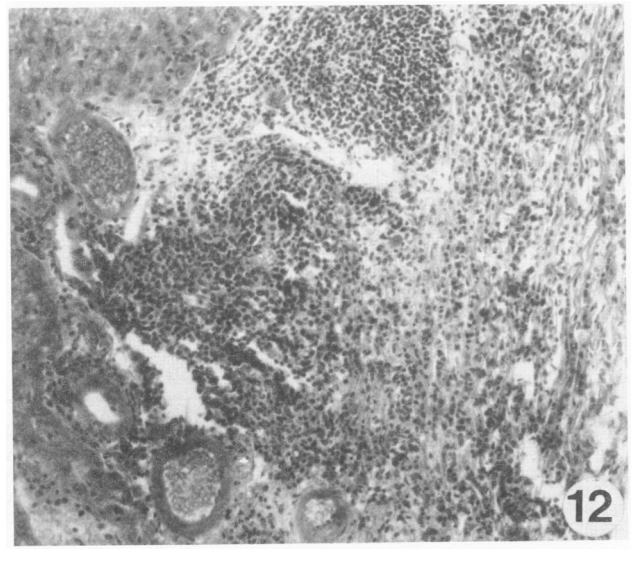
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Table 2—Histopathologic Changes in the Liver Tissue of Gerbils at Various Times After the Induction of Amebic Liver Abscess

| Days after inoculation | Number of animals | Percentage of animals with | | | |
|---|---|---|---|---|---|
| | | Portal infiltrate | Periportal fibrosis | Hepatocyte degeneration | Fatty change |
| 3 | 8 | 25 | 0 | 0 | 0 |
| 5 | 8 | 25 | 0 | 0 | 0 |
| 10 | 8 | 50 | 25 | 13 | 0 |
| 20 | 8 | 100 | 75 | 50 | 13 |
| 30 | 8 | 100 | 100 | 88 | 50 |
| 60 | 8 | 75 | 75 | 50 | 38 |

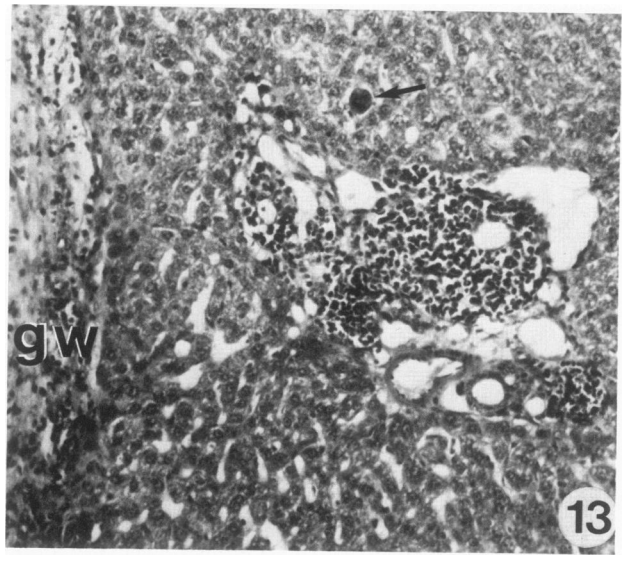
Only the portal areas were hyperplastic. In no instance was hepatomegaly noted. The hepatic changes in the other liver lobes were not examined.

## Discussion

In humans, amebic liver abscess formation begins when hematophagous amebae from the intestine reach the liver via the portal circulation and become lodged in portal radicles and liver sinusoids.[17,19] Hepatocytes are rapidly destroyed, and this destruction leads to wedge-shaped areas of coagulative necrosis. The formation of cavitary lesions results from the coalescence of small necrotic lesions,[20] and these are the type most frequently reported from patients with chronic disease.[25,26] Liver abscess formation in the gerbil following direct inoculation of trophozoites in the liver, also progresses to a cavitary abscess[8] and mimics the events occurring in man. However, very little is known about the acute stages of the disease in humans; and the few histopathologic studies done have reported a spectrum of findings.[15,17,19,21]

In the gerbil model, the early stages of the amebic liver lesions were characterized by an acute inflammatory response which progressed rapidly to become granulomatous in nature within 5 days after inoculation. Polymorphonuclear leukocytes (PMNs) were the predominant cell type early in the infection, and later macrophages and histiocytes surrounded the lesion cavity in a fibrinous corona. The latter events have been reported in the hamster,[10,27] but the sequence of events leading up to the granulomatous response has not been reported. In humans the early lesions are also characterized by a necrotic area, the absence of fibrosis, and a PMN infiltrate.[19] In the gerbil, necrosis of liver cells, combined with the lytic secretory products of inflammatory cells, probably aggravates the necrotic process until fibrosis occurs. The granulomatous response probably occurs as a result of the inefficiency of the acute inflammatory response[28] in sequestering and destroying the trophozoites. Trophozoites of *E histolytica* are known to kill human PMNs[29,30] and, together with the cytotoxic effect of the amebae, could contribute to the initial necrotic process. Both the inflammatory and granulomatous response to the amebic liver infection occurred simultaneously; and when histiocytes and lymphocytes infiltrated the granuloma walls, PMN numbers decreased. The persistence of neutrophils and eosinophils in granulomas was probably in response to the presence of antigen-antibody complexes in the pus of the amebic liver abscess. Because abscess material contains antiamebic antibodies,[31-32] amebic antigens,[33-34] and complement,[31] immune complexes may form locally and give rise to an Arthus-like reaction.[35] PMNs have no direct effect on virulent strains of *E histolytica*; in fact, they are phagocytized and destroyed rapidly.[29] This may explain the apparent absence of PMNs in the necrotic center of lesions. The cytopathic effect of trophozoites was not limited to PMNs; histiocytes and the fibrous wall of granulomas were readily destroyed. This could result in the "budding" of granulomas. It is not known whether trophozoites destroy histiocytes directly; cytolysis of these cells probably occurs in response to the lysosomal enzymes of destroyed PMNs. Of interest is the disappearance of histiocytes (30 days) and the subsequent infiltration of lympho-

**Figures 7-12**—The cellular infiltrate of amebic granulomas at various times after inoculation. **Figure 7**—Rim of a lesion cavity showing a central area of necrosis with trophozoites (*arrow*) at 10 days. Histiocytes (*arrowheads*) are confined to the area of necrosis enclosed by a fibrous (*F*) wall. (H&E-NGB, ×312) **Figure 8**—Center of granuloma wall at 10 days, with granulocytes, mainly neutrophils and eosinophils. Lymphocytes are rarely present. (Giemsa, ×312) **Figure 9**—Granuloma at 20 days, showing lymphocytic infiltrate at the periphery of the granuloma wall. Granulocyte numbers are low, and a few plasma cells are noted. (H&E, ×312) **Figure 10**—Coalesced granuloma at 30 days, with abundant lymphocyte infiltrate (*L*) adjacent to the narrow rim of fibrous tissue (*F*). Notice the trophozoites (*T*) in close contact with the fibrous wall. Histiocytes are absent, and granulocyte numbers are low. (Giemsa, ×312) **Figure 11**—Diffuse lymphocytic infiltrate in the fibrous wall encircling the cavitary liver abscess at 60 days. Collagen fibers and granulation tissue are prominent. (H&E, ×125) **Figure 12**—Periphery of cavitary liver abscess at 60 days, showing lymphoid aggregates in the portal areas. (H&E, ×125)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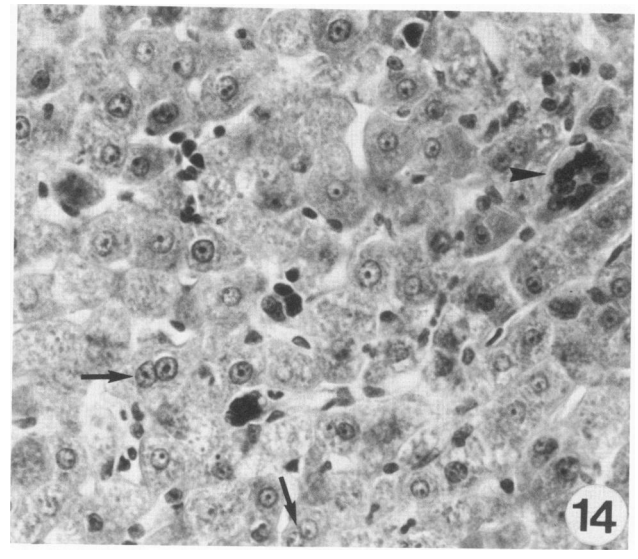
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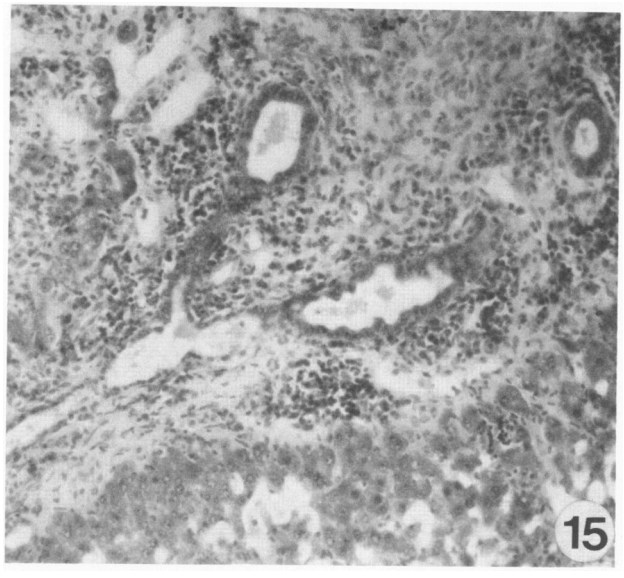
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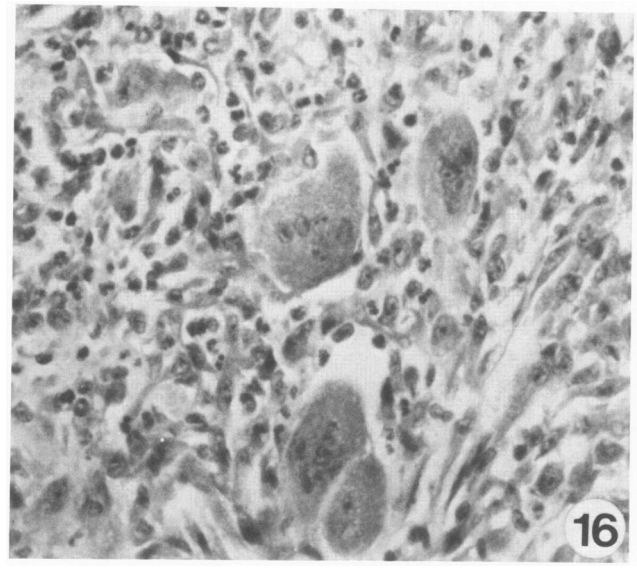
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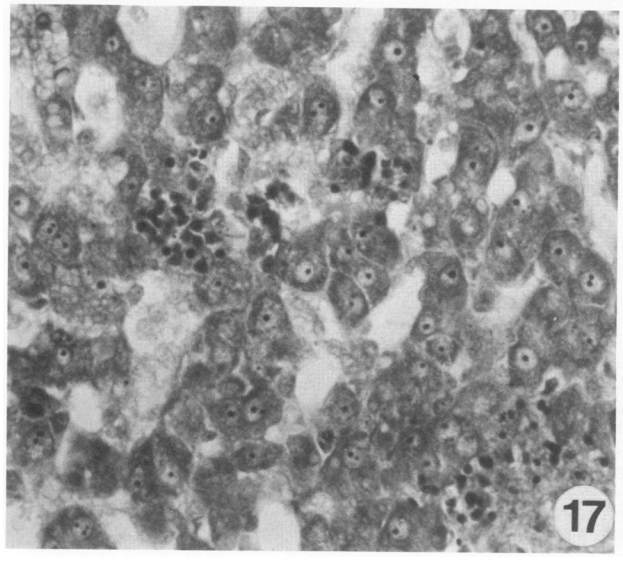
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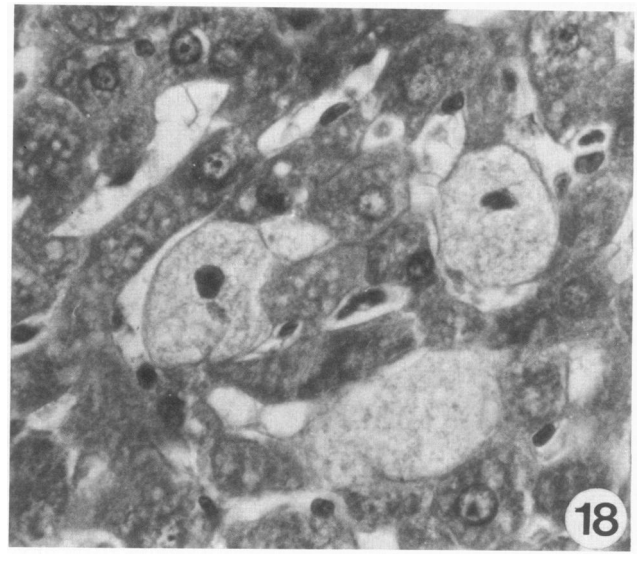
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cytes which precedes the coalescence of granulomas. During maximum proliferation of the amebic liver abscess at 10–30 days after inoculation, T-cell depletion occurs in both lymph nodes and spleen of infected animals (unpublished results). The depletion of lymphocytes in the lymphoreticular tissues is probably in response to the accumulation of these cells around the amebic liver abscess. Lymphocytic infiltrate and the apparent inhibition of proliferation of granulomas strongly suggest that T cells control the growth of the amebic liver abscess. This effect may be partly responsible for both coalescence of granulomas and the formation of cavitary liver abscesses. T-lymphocytes derived from experimental animals[36] and humans[37] with amebic liver abscess are cytotoxic to trophozoites of *E histolytica in vitro*, and their presence around the abscess may serve as an effective barrier preventing the dissemination of trophozoites.

The histopathologic changes of the uninvolved liver parenchyma adjacent to the liver granulomas produced a spectrum of findings. The changes were correlated with growth and proliferation of the amebic liver abscess. Prior to granuloma formation and fibrosis there was only mild inflammatory cellular infiltrate in the portal canals. With granuloma formation and subsequent fibrosis at 10 days after inoculation, the parenchyma adjacent to granulomas showed flattening of cells, hepatocyte degeneration, and Kupffer cell hyperplasia. Severe parenchymal changes occurred at 20–30 days after inoculation, as is also most frequently reported in cases of amebic liver abscess in humans. Portal infiltrate, portal fibrosis, and proliferation of bile duct epithelium were most pronounced; and similar changes have also been reported in humans[19] and in experimental animals.[10] In a few animals, portal and hepatic venous thrombosis was observed but was not a common feature. Disturbances of the portal circulation in human infections has been attributed to portal hypertension, splenomegaly, and congestion of the liver[20,38]; but only splenomegaly has been indicated in this study. Splenomegaly in gerbils with amebic liver abscess appeared not to be due to disturbances of the portal circulation, because it occurred in the absence of hepatic venous thrombosis. Diffuse hepatocellular degeneration ("amebic hepatitis")[14,15,21] and spotty necrosis[20] have also been reported in humans but were not found in the present study. Reactive changes such as anisonucleosis and pigmentation of hepatocytes were found consistently at 30 days, and similar changes have also been reported in humans.[39] The appearance of ballooning hepatocytes and multivacuolar fatty changes were also indicative of the specific changes caused by the amebic liver abscess. Degenerative changes of the hepatocytes did not proceed to necrosis as reported for humans.[19]At 60 days after inoculation the histopathologic picture changed, and only few animals showed marked liver pathology adjacent to the abscess mass. The reversal of pathologic changes in the gerbil is probably indicative of control of the infection, because abscess growth and proliferation ceased at this time. This stage of the infection may represent the chronic state of the disease in humans, because cavitary liver abscesses were fully formed but rarely progressed to the fluid-filled stage, as in human infections.

This study has clearly shown that an acute inflammatory response precedes a granulomatous response when trophozoites are injected directly into the liver of gerbils. The histopathologic changes of uninvolved liver parenchyma were correlated with the proliferation of granulomas and showed changes typical of the acute stage of the disease in humans. The coalescence of granulomas results in the formation of a cavitary liver abscess, closely resembling abscesses commonly found in humans. The wide spectrum of findings and discrepancies reported from human infections are probably due to the fact that other disease conditions may be superimposed upon the specific changes induced by the amebic liver abscess. Similarly, it is difficult to interpret the events reported at one point in time, because the pathologic changes in acute and chronic stages of the disease may be different. The present study clearly shows the distinct progression of events leading up to the cavitary liver abscess. Since in the gerbil the abscess is free of bacterial contamination, is localized in the liver lobe, and can be observed for long periods of time, the gerbil seems to be an excellent model host for the study of the development of hepatic amebiasis in man.

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**Figures 13–18** – Histopathologic features of liver parenchyma adjacent to amebic granulomas at various times after inoculation. **Figure 13** – Ten days after inoculation. Portal area showing moderate infiltration with inflammatory cells. Foreign body giant cell (*arrow*) and flattening of hepatocytes adjacent to granuloma wall (*gw*) was common. (H&E, ×125) **Figure 14** – Regenerative activity of parenchymal cells at 20 days, showing large numbers of binucleated cells (*arrows*), anisonucleosis, and multinucleated giant cells (*arrowhead*). (H&E-NGB, ×312) **Figures 15–18** – Thirty days after inoculation. **Figure 15** – Periportal fibrosis, proliferating bile duct epithelium, and hepatocellular unrest. (H&E, ×125) **Figure 16** – Large numbers of foreign body giant cells in the granuloma wall and adjacent liver parenchyma. Liver cells are absent in granulomas. (H&E, ×312) **Figure 17** – Hepatocellular unrest, anisonucleosis, and pigmentation of hepatocytes. Inflammatory infiltrate is absent, and a few areas show congestion (*top left*). (Giemsa, ×312) **Figure 18** – Ballooning degeneration, pyknotic nuclei, and marked multivacuolar fatty change of hepatocytes. (H&E-NGB, ×500)

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