Pathology of Hepatitis A Infection in the Owl Monkey (Aotus trivirgatus)

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Sequential liver biopsies of owl monkeys that had been experimentally infected with one of two strains of hepatitis A virus (HM-175 or PA-33) were examined for histopathologic alterations. Preinoculation biopsies were normal with only occasional minimal mononuclear cell infiltrates in portal tracts and hepatic lobular parenchyma. Histopathologic features that were present in biopsies taken during the period of elevated serum alanine aminotransferase activity (16–43 days after the intravenous inoculation of virus) included infiltration of predominantly mononuclear inflammatory cells into portal tracts and surrounding parenchyma, degeneration and necrosis of hepatocytes, and hypertrophy of Kupffer cells. Changes were similar in monkeys infected with either HM-175 or PA-33 virus strains. Convalescent biopsies (147–186 days after inoculation) showed resolving lesions with mild portal inflammation and occasional focal collections of inflammatory cells in the parenchyma. These histologic changes are similar to those associated with hepatitis A infection in man, chimpanzees, and several species of marmosets, and support the further use of the owl monkey as a model of human hepatitis A. (Am J Pathol 1984, 115:1–8)

RECENT STUDIES have demonstrated the susceptibility of colony-bred owl monkeys (*Aotus trivirgatus*) to hepatitis A virus (HAV) and have suggested that this species of primate may serve well as a model of human hepatitis A.[1,2] A serologic survey of owl monkeys held at a colony within the United States revealed a high prevalence of antibody to the virus (anti-HAV) among procured animals (60%), compared with a very low prevalence (3%) among colony-bred animals.[1] Furthermore, a sustained outbreak of HAV infection was documented in a colony of owl monkeys in Panama. The virus was recovered from both feces and liver of infected monkeys and was found to be antigenically indistinguishable from human HAV.[1] Almost all newly captured monkeys admitted to this colony developed anti-HAV. In view of these findings, seronegative owl monkeys were experimentally infected with two strains of HAV, including that recovered from owl monkeys in Panama (PA-33) and a strain recovered from an infected human in Australia (HM-175). Details of the biochemical, serologic, and virologic findings as well as a brief summary of the pathologic changes associated with these experimental infections have been presented elsewhere.[2] In the present report we describe the detailed morphologic changes developed in the liver of owl monkeys during HAV infection and compare the infection in the owl monkey with changes characteristically observed in man, the chimpanzee, and the marmoset.

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Materials and Methods

Animals

Twelve owl monkeys that were colony-bred and seronegative for anti-HAV were selected for study.[2] The monkeys were divided into two groups of 6 each. Both groups were inoculated intravenously, the first group with 1 ml of a 0.2% fecal suspension containing PA-33 strain HAV and the second group with 1 ml of a 0.2% human fecal suspension containing HM-175 virus. Details of isolation, housing, diet, and test procedures have been described elsewhere.[2]

Liver Biopsy

Incisional wedge liver biopsies were obtained from each monkey 36–54 days prior to inoculation, 16–43 days after inoculation (generally at the first elevation of serum alanine aminotransferase [ALT]) and in late convalescence, 147–186 days after inoculation. One monkey that did not develop any biochemical or serologic evidence of infection did not have any subsequent biopsies and was not included for analysis. Biopsies were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, periodic acid–Schiff (PAS) with and without diastase digestion, Perl's stain for iron, and Masson's trichrome. All biopsies were read without prior knowledge of animal number, clinical pathology, and time after inoculation by the same observer (C.M.K.).

Virologic Studies

Preinoculation and postinoculation fecal suspensions were tested for HAV antigen by solid-phase radioimmunoassay.[1] Antibody to hepatitis A was detected by radioimmunoassay (HAVAB, Abbott Laboratories, North Chicago, Ill).

Results

Clinical, Biochemical, and Virologic Findings

All six monkeys inoculated with PA-33 virus developed serologic and virologic evidence of infection, as did 5 of 6 monkeys inoculated with HM-175 virus.[2] Fecal shedding of HAV antigen was first detected 6–27 days after inoculation and persisted for 3–29 days. Somewhat later, each infected monkey developed significant elevations in ALT activity (9–21 days after inoculation of PA-33 virus and 25–39 days after inoculation of HM-175 virus). In 10 of the 11 infected monkeys significant elevations of serum aspartate aminotransferase (AST) levels also developed, and 9 monkeys had significantly elevated serum γ-glutamyltranspeptidase activity. Serum enzyme activities returned to within 3 standard deviations (SD) of base-

Table 1—Histologic Features of Preinoculation, Acute Infection, and Convalescent Liver Biopsy Specimens From Owl Monkeys Infected With the PA-33 Strain of HAV

Monkey	Days after inoculation	Serum ALT on day of biopsy	Portal inflammation	Lobular inflammation	Necrosis	Ballooning degeneration	Acidophilic degeneration	Mitoses	Kupffer cell hypertrophy
WR 68	−54	21	1+	1+	0†	0	0	0	1+
(6-14)*	+17	210	3+	2+	3+	1+	3+	2+	2+
	+147	105	2+	2+	0	1+	0	1+	2+
WR 80	−54	32	1+	0	0	1+	0	0	1+
(10-13)	+16	293	2+	2+	1+	1+	1+	1+	2+
	+147	35	1+	0	0	0	0	0	1+
WR 130	−54	59	1+	1+	0	0	0	0	1+
(8-19)	+16	129	2+	1+	1+	1+	1+	0	2+
	+147	31	2+	0	0	0	0	0	1+
WR 148	−54	34	1+	1+	0	1+	0	1+	1+
(17-22)	+24	176	3+	2+	3+	3+	2+	0	1+
	+147	37	2+	2+	0	1+	0	1+	1+
WB 149	−54	86	1+	1+	0	0	0	0	1+
(7-15)	+17	171	1+	1+	0	1+	0	1+	1+
	+147	49	2+	2+	0	1+	0	0	2+
WR 153	−54	40	1+	0	0	0	0	0	1+
(6-16)	+16	186	3+	2+	2+	2+	2+	1+	2+
	+147	41	2+	0	0	1+	0	0	2+

* The numbers under the animal identification number indicate days after inoculation with positive fecal antigen.
† 0, absent; 1+, minimal; 2+, mild; 3+, moderate.

line values by no later than 49 days after intravenous inoculation of virus in each monkey and were close to preinfection baseline levels by the time of the convalescent liver biopsy in most monkeys.

Anti-HAV was detected by radioimmunoassay as early as 14 days after inoculation of virus and was present in all 11 infected monkeys by Day 56. Seroconversion was also demonstrated in infected monkeys by means of an *in vitro* serum neutralizing antibody assay.[3]

Preinoculation Biopsies

Each of the 11 infected animals served as its own control. Prior to inoculation, all monkeys had histologically normal livers with the exception of only a few minor alterations (Tables 1 and 2). The majority of portal tracts contained a few inflammatory cells including lymphocytes, macrophages, and eosinophils (Figure 1). There were occasional randomly distributed small foci of lymphocytes, macrophages, and polymorphonuclear leukocytes within the hepatic lobule. Minimal individual hepatocellular degeneration was present in some biopsies. Hepatocellular glycogen content was variable but usually most evident around hepatic veins. Kupffer cells were prominent and in most monkeys contained variable amounts of brown Perl's reaction-positive pigment (hemosiderin). Hepatocytes also contained hemosiderin.

Acute Infection Biopsies

There were no differences between the histopathologic changes observed during acute infection in PA-33- and HM-175-infected groups of monkeys (Tables 1 and 2). Nine monkeys had diffuse lesions compatible with acute viral hepatitis. In 5 monkeys (3 infected with PA-33 and 2 with HM-175), these changes were moderate; and in 3 monkeys (2 infected with PA-33 and 1 with HM-175), the changes were mild. One monkey (infected with HM-175) had minimal lesions. In the 2 remaining monkeys, the acute infection biopsy was not distinctly different from the preinoculation biopsy (1 of these monkeys [N2] underwent biopsy 4 days prior to the first elevation of ALT, while the other monkey (WR-149) underwent biopsy 4 days after elevation of ALT).

The histopathologic changes associated with acute HAV infection (Tables 1 and 2) were characterized by inflammation of the portal tracts, with involvement of parenchyma surrounding the portal tracts (Zone 1 of the acinus of Rappaport with some extension into Zone 2) and occasional involvement of the area immediately surrounding the hepatic vein. Portal tracts were enlarged with an infiltrate of predominantly mononuclear inflammatory cells (Figure 2). Lymphocytes were the most numerous cell type, with a few macrophages, eosinophils, neutrophils, and a rare plasma cell. The inflammation was usually uniformly distributed, involving all portal tracts to a similar extent. Although the inflammatory infiltrate sur-

Table 2—Histologic Features of Preinoculation, Acute Infection, and Convalescent Liver Biopsy Specimens From Owl Monkeys Infected With the HM-175 Strain of HAV

Monkey	Days after inoculation	Serum ALT on day of biopsy	Portal inflammation	Lobular inflammation	Necrosis	Ballooning degeneration	Acidophilic degeneration	Mitoses	Kupffer cell hypertrophy
N2	−36	43	1+	1+	0†	0	0	0	1+
(27-34)*	+28	52	1+	0	0	0	0	0	1+
	+186	38	1+	1+	0	1+	0	0	2+
N3	−36	53	1+	0	0	0	0	0	1+
(8-32)	+35	102	3+	3+	3+	3+	3+	3+	2+
	+186	73	1+	0	0	0	0	0	1+
E4	−36	33	1+	0	0	1+	0	0	1+
(9-21)	+28	165	3+	3+	3+	3+	3+	2+	2+
	+186	52	1+	0	0	0	0	0	1+
WR 154	−36	48	1+	0	0	0	0	0	1+
(8-37)	+43	303	2+	1+	1+	0	1+	0	1+
	+186	42	1+	1+	0	0	0	0	1+
WR 155	−36	39	0	0	0	0	0	0	1+
(8-24)	+35	227	1+	1+	1+	1+	0	0	1+
	+186	98	1+	1+	0	0	0	0	1+

* The numbers under the animal identification number indicate days after inoculation with positive fecal antigen.
† 0, absent; 1+, minimal; 2+, mild; 3+, moderate.

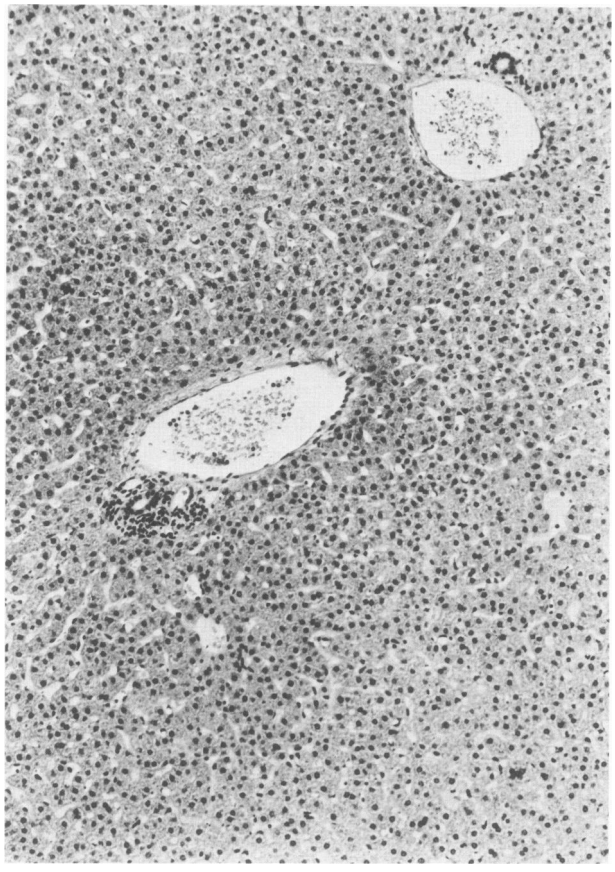

Figure 1 – Preinoculation liver with only a few inflammatory cells in the portal area. (H&E × 100)

rounded bile ducts, it usually did not disrupt them. In some ducts, epithelial cells were swollen and there was an occasional lymphocyte present between cells. In many of the portal tracts the limiting plate was focally disrupted by an inflammatory cellular infiltrate which extended out into the surrounding parenchyma (Figure 3). Hepatocytes in the periportal zone were often swollen with granular cytoplasm, and individual hepatocytes adjacent to the limiting plate were occasionally surrounded by inflammatory cells. Foci of hepatocellular degeneration and necrosis radiated out from the portal tracts but were also randomly distributed throughout the periportal and intermediate zones, with occasional extension into the acinar zones 3 (central zones). Hepatic veins were uniformly spared inflammatory changes. Degeneration of hepatocytes was characterized by swollen cells with thin wisps of cytoplasm (ballooning degeneration) or shrunken eosinophilic cells (acidophilic degeneration) (Figure 4). Necrotic hepatocytes, either singly or in small clusters, were often surrounded by lymphocytes, macrophages and a few polymorphonuclear leukocytes (Figure 5). Clusters of these inflammatory cells very often accumulated in sinusoidal spaces or replaced necrotic hepatocytes. In addition, a moderate number of hepatocellular mitoses were present immediately adjacent to areas of necrosis and inflammation (Figure 6). Cholestasis was not evident in any acute infection biopsy. Hepatocellular glycogen content was either unchanged or slightly increased over that seen in preinoculation biopsies.

In 6 of the 11 monkeys, there was a mild to moderate increase in the size and apparent number of Kupffer cells. In 5 monkeys there was a mild increase in the amount of hemosiderin in Kupffer cells and hepatocytes. Kupffer cells and portal macrophages occasionally contained a small amount of PAS-positive, diastase-resistent granular material.

Convalescent Biopsies

At the time of the convalescent biopsies, most monkeys had serum ALT levels that were within normal ranges (Tables 1 and 2). Minimal to mild portal and lobular inflammation was present in all monkeys. Most of the portal tracts contained some lymphocytes and macrophages and a few eosinophils and plasma cells. Small foci of similar inflammatory cells were occasionally present in the lobular parenchyma (Figure 7). An occasional degenerating hepatocyte was observed within the cluster of inflammatory cells. There was no evidence of active hepatocellular necrosis. Kupffer cells were still prominent, and both these cells and hepatocytes contained variable amounts of hemosiderin.

Discussion

The characteristic lesions of acute viral hepatitis in man include hepatocellular degeneration and necrosis, mononuclear cell infiltration in portal and parenchymal locations, and activation of sinusoidal lining cells.[4,5] Histopathologic changes observed in the owl monkey closely resemble those reported for man and correlated well with biochemical alterations, particularly elevations of serum ALT. There was prominent portal inflammation, which consisted primarily of lymphocytes with a few macrophages and polymorphonuclear leukocytes. Hepatocellular degeneration, necrosis, and associated mononuclear inflammatory cell accumulation occurred predominantly in a periportal location with extension into the intermediate zone. As in man, the disruption of the limiting plate, extension of inflammatory cells into the periportal parenchyma, and periportal hepatocellular necrosis observed during the acute phase could be difficult to distinguish from chronic active

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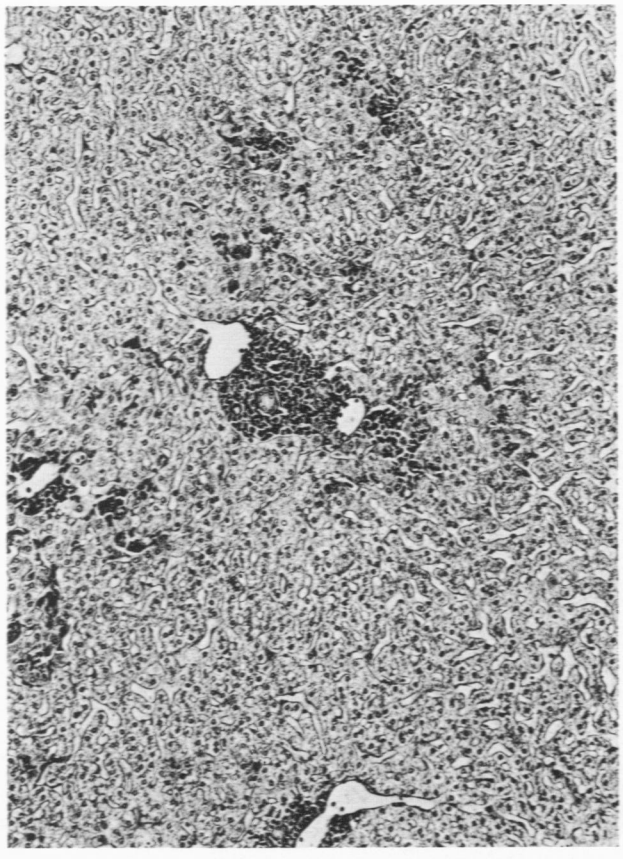

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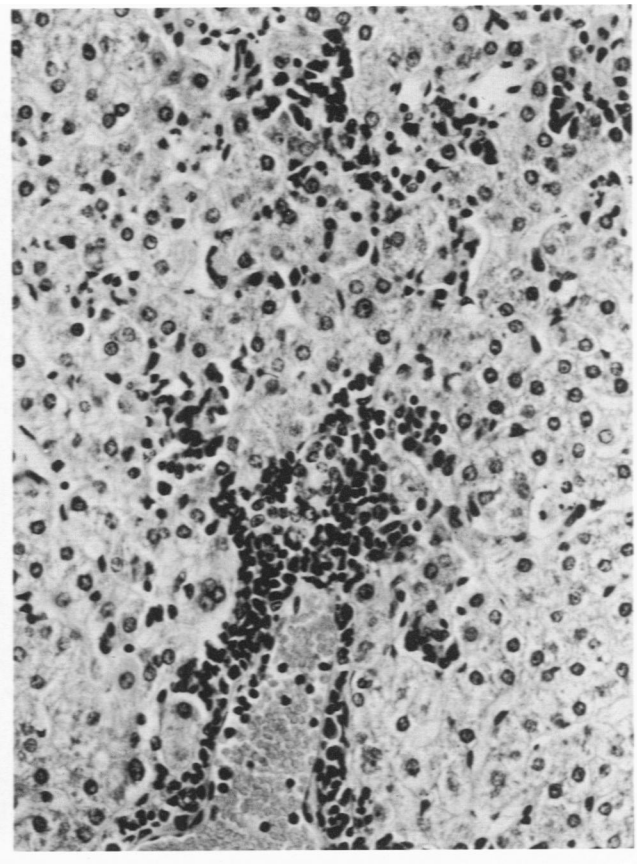

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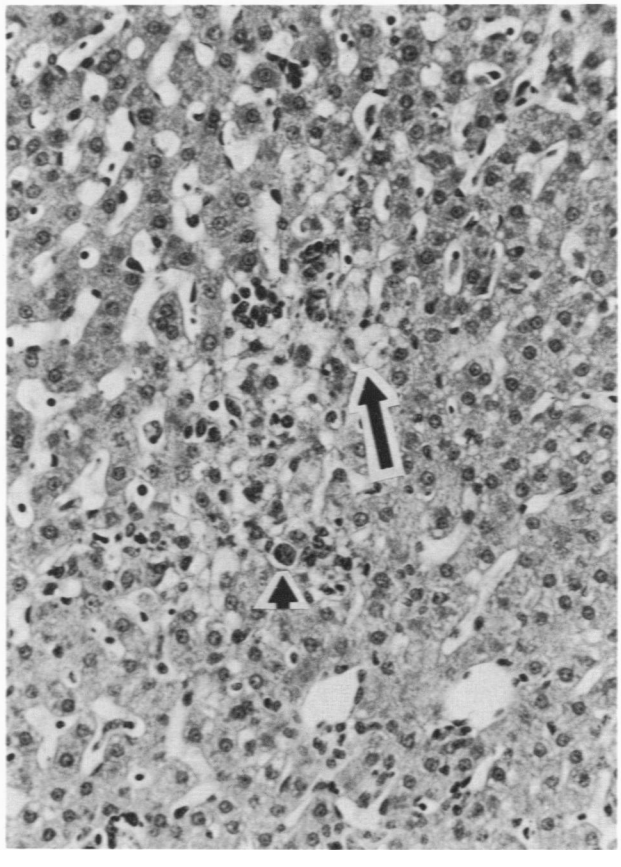
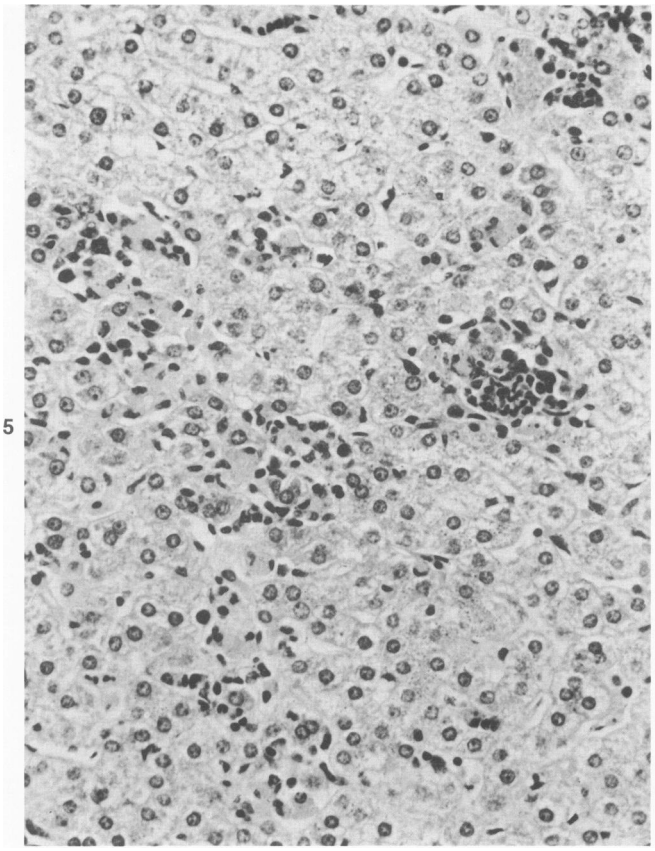
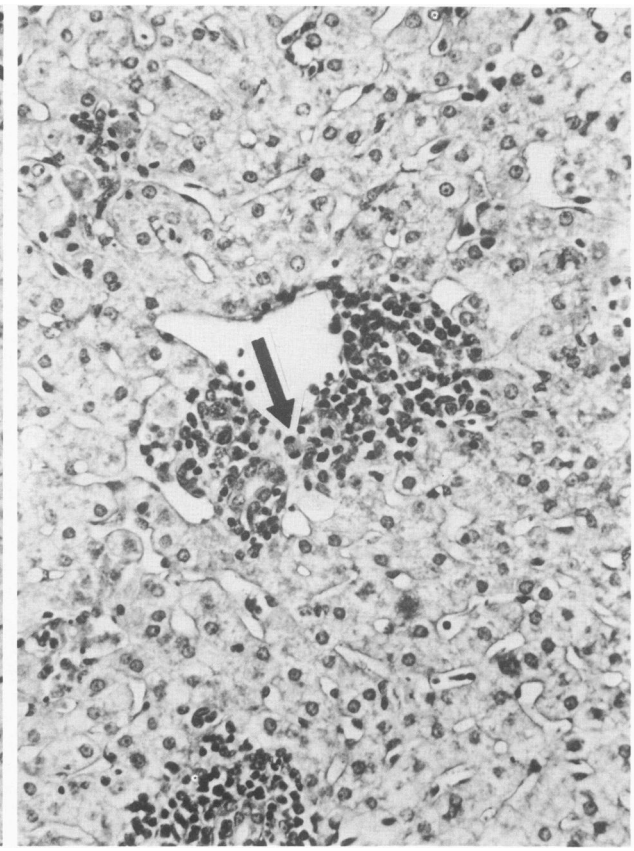


Figures 2-4—Livers during acute infection with hepatitis A virus. **Figure 2**—Enlargement of portal tracts with predominantly mononuclear inflammatory cells and foci of necrosis and inflammatory cell accumulation in the surrounding parenchyma. (PAS with diastase digestion, × 100) **Figure 3**—Erosion of the limiting plate and extension of the inflammation into the parenchyma. (H&E × 250) **Figure 4**—Focus of hepatocellular degeneration showing ballooning degeneration (*arrow*) and acidophilic degeneration (*arrowhead*). (PAS, × 250)





Figures 5 and 6 – Livers during acute infection with hepatitis A virus. **Figure 5** – Small foci of necrosis of hepatocytes with accumulation of mononuclear inflammatory cells. Notice the prominence of the Kupffer cells. (H&E, ×250) **Figure 6** – Two mitotic figures (*arrow*) are present amid the inflammatory cells in a portal tract. (PAS with diastase digestion, ×250)

hepatitis.[6,7] However, convalescent biopsies showed diminution of the portal inflammation and no active necrosis following recovery from HAV infection. Acinar zones 3 remained relatively free of inflammation, and there was usually no inflammation of the hepatic vein with HAV infection. These histopathologic findings were consistent with what has been reported in the chimpanzee,[8,9] the marmoset,[4,10,11] and man[5,7,12] during acute hepatitis A infection. However, in man there may be some variability in the changes affecting zones 3 with accentuation of hepatocellular damage, inflammation, and cholestasis in those zones.[6] An increase in the size and possibly number of sinusoidal lining cells or Kupffer cells occurred in the infected owl monkeys. This has also been observed in man, the chimpanzee, and the marmoset.[4,5,8] The significance of this change is uncertain. HAV antigen has been demonstrated in Kupffer cells by immunofluorescence[13] and by peroxidase-conjugated antibody,[14] but it is not known whether its presence represents phagocytosis of virus or viral replication. Like the chimpanzee, the owl monkey exhibited minimal nonspecific changes before inoculation which were readily distinguishable from the changes associated with acute infection. In many of the owl monkeys residual portal inflammation and small foci of lobular inflammation were present after the acute infection had subsided and the enzymes had returned to normal. This has also been reported in the chimpanzee.[8]

The degree of hepatitis (in terms of both serum chemical and histopathologic changes) seen in the owl monkey, chimpanzee, and marmoset is generally milder than that observed in infected human adults. It should be remembered, however, that most HAV infections in children, especially those under 3 years of age, are not associated with jaundice and usually are totally unrecognized.[15] A recent report on fulminant hepatitis A in a chimpanzee would indicate that nonhuman primates are capable of developing severe disease.[16]

In order for an animal model of hepatitis A infection to be useful for pathogenesis studies or new vaccine testing, the infection in the animal should have virologic, serologic, and pathologic features with temporal patterns which closely parallel the natural

disease in man. In man, hepatitis A has an incubation period of approximately 14–28 days. Clinical symptoms may be nonspecific. Although jaundice is present in most adult infections, it occurs less frequently in children.[15,17] Fecal shedding of viral antigen usually begins before the appearance of clinical illness and becomes maximal several days before the appearance of enzyme elevations and histologic lesions.[18–20] The onset of illness is marked by elevations in ALT and AST and may be followed by elevation of serum bilirubin levels and occasionally alkaline phosphatase levels. Serum antibody to HAV appears early in the disease, usually coincident with the onset of acute hepatitis.[18,19] The antibody which appears initially during acute infection is comprised of both IgM and IgG, and IgM anti-HAV is a useful diagnostic marker of acute infection.[21] The characteristic morphologic lesions of acute viral hepatitis occur during the period of enzyme elevation.

The temporal course of experimental infection in the owl monkey is very similar to that described in man. The incubation period following intravenous inoculation ranged from 9 to 39 days.[2] During this time there was little change in clinical appearance, although 2 monkeys had palpably enlarged livers, and 3 monkeys had transient diarrhea. Virus excretion was detected 6–9 days after infection, and peak excretion occurred several days to a week before elevation of enzymes. Serum ALT was significantly elevated, and in most monkeys AST also became elevated. Serum bilirubin levels remained normal. Peak elevation of serum ALT coincided with the development of anti-HAV antibody detectable by both radioimmunoassay and a serum neutralization assay.[2,3] In a study of naturally occurring infection in an owl monkey colony, the first measurable serologic response to HAV consisted partly of IgM.[1] Biopsy at the time of enzyme elevations in experimentally infected owl monkeys revealed lesions characteristic of acute viral hepatitis. Thus, the course of HAV infection in the owl monkey is highly analogous to that of the infection in man.

Infection in the chimpanzee has been well characterized and resembles what we observed in the owl monkey. The incubation period is approximately 15–30 days, and the animals are usually without signs of disease, although some may exhibit lethargy and loss of appetite. Shedding of virus in the stool reaches a peak before elevation of serum ALT, and detection of antibody to HAV occurs prior to or coincident with the rise in enzymes.[4] Lesions characteristic of acute viral hepatitis occur during elevation of ALT. However, the chimpanzee is an endangered species and not readily available for further studies.

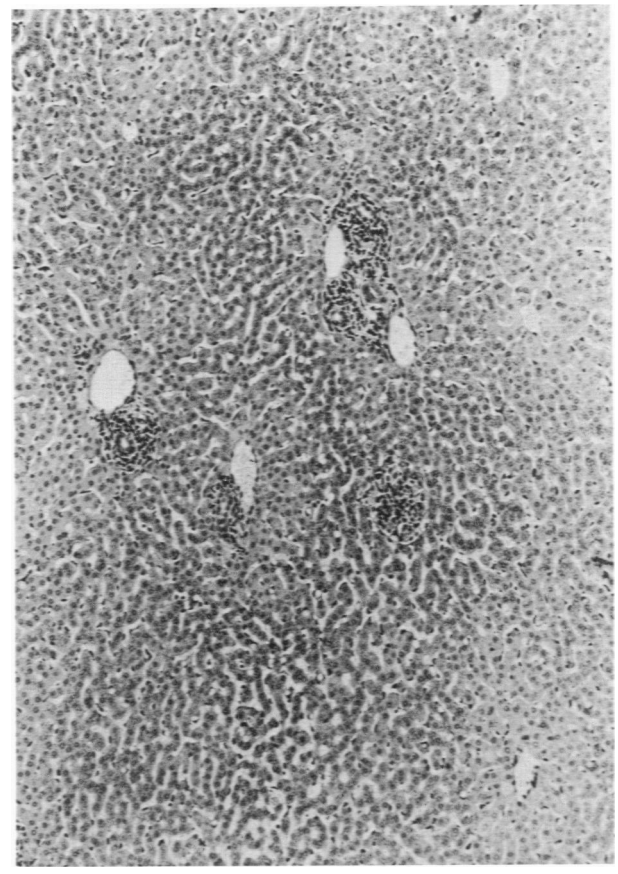


Figure 7—Convalescent liver with minimal numbers of mononuclear inflammatory cells in the portal tracts. Kupffer cells are still very prominent. (H&E, ×100)

Several species of South American marmosets have been proposed as experimental models of hepatitis A.[22] However, there are some differences from the course of infection observed in man, the owl monkey, and the chimpanzee. Excretion of virus in the stool often coincides with the elevation of liver enzymes, in contrast to the situation in man, the chimpanzee, and the owl monkey, in which it usually precedes enzyme elevation.[10] The most sensitive biochemical indicator of hepatic disease is serum isocitrate dehydrogenase, an enzyme that is not routinely measured in man. The marmoset is also difficult to obtain and less easily manipulated than the owl monkey. For these reasons the owl monkey appears to be a better model of HAV infection.

The pathogenesis of hepatitis A has not been resolved, although different mechanisms have been proposed based on morphologic and immunologic observations. One hypothesis is that HAV replication results in a direct cytopathic effect on the hepatocyte.[4] However, the morphologic appearance of type A hepatitis is markedly different from lesions produced by viruses with known cytopathic effect such as

herpes simplex virus. Herpes infection in primates is characterized by large randomly distributed areas of acute coagulative necrosis with little or no inflammatory cell accumulation. Also, if HAV were cytopathic, peak viral excretion in the feces might be expected to occur concurrently with enzyme elevation and liver damage. This is not the case in man, the owl monkey, and the chimpanzee, in which viral excretion is generally on the decline before onset of enzyme elevation and histopathologic evidence of liver damage.[2,4,18] In addition, although HAV has been propagated in cell cultures, the virus is noncytopathic in every type of cell culture tested to date.[23,24] A more attractive hypothesis is that the mechanism of injury involves immune-mediated damage. In support of this hypothesis is the observation that the onset of demonstrable liver injury often occurs with the appearance of antibody.[18-20] Furthermore, lymphocytes in contact with degenerating or necrotic hepatocytes suggest a possible role for cell-mediated immunity.[8]

Much work remains to be done to clarify the mechanism by which cellular injury occurs in hepatitis A, and accurate animal models will play an essential role in this work. In addition to the previously defined primate models (chimpanzee and marmoset) of the disease, in the owl monkey the disease has been shown to be similar in many respects to that in man. The owl monkey is easier to obtain and offers several significant advantages over the chimpanzee and marmoset. It should be an excellent model for further studies.

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