THE KUPFFER CELL REACTION IN MURINE AND HUMAN VIRAL HEPATITIS, WITH PARTICULAR REFERENCE TO THE ORIGIN OF ACIDOPHILIC BODIES

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Parenchymal cell damage in viral liver injury is generally much more striking than damage to the sinusoidal lining cells. This is certainly true of experimental murine hepatitis due to the MHV_3 virus which produces a confluent focal necrosis resembling human acute yellow atrophy.[1] Bang and Warwick[2] have published an electron micrograph showing MHV_3 virus particles between a hepatic parenchymal and a sinusoidal lining cell. The principal object of the present investigation was to decide whether the initial damage in viral hepatitis occurred in endothelial or parenchymal cells. Since it is possible that the endothelial lining consists of only one cell type,[3] we have treated the terms sinusoidal lining cell, endothelial cell and Kupffer cell as synonymous. Hyalinized acidophilic globules resembling the Councilman bodies of yellow fever are frequently seen in mouse hepatitis, and the histogenesis of these bodies was also investigated. Finally, the Kupffer cells and the acidophilic bodies of human viral hepatitis were compared with the corresponding structures in the murine disease.

MATERIAL AND METHODS

Mice

Female mice aged 21 days, of the Swiss-Webster strain, supplied by Budd Mountain Farm, Chester, New Jersey, were used. Repeated blood examinations have shown these mice to be free from *Eperythrozoon coccoides* which enhances the severity of the MHV_3 infection. The animals were fed an unrestricted diet of Purina Chow.

Virus

The MHV_3 strain of mouse hepatitis[4] was used in the form of 10 per cent suspension of liver recently obtained from infected animals. After intravenous injection, the mortality was approximately 100 per cent. Most deaths occurred 4 to 5 days after inoculation. After intraperitoneal injection, deaths occurred somewhat earlier.

Experimental Methods

In the first experiment, 30 mice received injections of 0.1 ml. of virus suspension intravenously. The intravenous route was employed since this is the best method of

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producing a diffuse injury affecting the entire liver.[1] Five mice were killed each day. Sections cut at 1 μ were found to be better suited to study the earliest liver lesions produced by this virus than those of conventional thickness.

In a further experiment, the Kupffer cells were labeled by an intravenous injection of 0.1 ml. of India ink (Günther Wagner C11/1431 a). Three different concentrations of the ink (undiluted, 1:3 and 1:6 in sodium chloride) were injected intravenously into 3 groups of 12 mice. Three days later the animals received 0.1 ml. of the virus suspension. Since the tail vein was no longer available, the virus was injected intraperitoneally. Four mice in each group of 12 were killed daily.

Immediately after necropsy, liver blocks of conventional size were fixed in Carnoy's fluid and embedded in paraffin for sectioning. In addition, small blocks were fixed with Möller's fluid and embedded in a mixture of 85 per cent methyl and 15 per cent ethyl methacrylate. This material was used for sections cut with a Porter-Blum microtome at 1 μ. Conventional and thin sections were stained with hematoxylin and eosin, by the periodic acid-Schiff (PAS) stain with and without diastase digestion, for the Prussian blue reaction for hemosiderin, and with phosphotungstic acid-hematoxylin (PTAH).

Human Tissue

Twenty liver biopsy specimens obtained by needle puncture from patients with viral hepatitis were also examined. These were fixed in buffered formalin, embedded in paraffin and cut at 5 μ. Sections were stained as in the case of the experimental material.

Results

Murine Hepatitis

The Kupffer cells in experimental animals showed striking changes 24 hours after the intravenous injection of MHV3 virus. Affected cells were scattered diffusely throughout the lobules. Mitotic figures were frequent (Fig. 1); their pattern was often irregular, abnormally coarse or fragmented (Fig. 2). Frequently the endothelial lining cells were binucleated, and occasionally foci of hyperplasia were observed. The nuclei in some lining cells were pyknotic or karyorrhectic. Normally the cytoplasm of these cells in mice is scanty and difficult to distinguish from that of adjacent parenchymal cells. Damaged endothelial cells usually had an increased amount of cytoplasm. Occasionally this appeared basophilic and streaked or vacuolated, but more frequently it was eosinophilic, often actually "hyalinized" (Fig. 2). Some sinusoidal cells with pyknotic nuclei and hyalinized cytoplasm appeared swollen, and it seemed possible to trace a transition between these cells and globular acidophilic bodies lying in the sinusoids (Fig. 3). Parenchymal cells adjacent to the great majority of the damaged lining cells were normal. A few of the hepatic cells seemed to be slightly swollen, and in others, cytoplasmic basophilia was increased. Only in the vicinity of extensive lining cell involvement was there actual necrosis of parenchymal elements (Fig. 5). These tiny areas of hepatic cell necrosis centered about sinusoids and were infiltrated by round cells and neutrophils.

Forty-eight hours after intravenous inoculation, many large foci of

necrosis had developed. These, like the damaged endothelial cells, did not have a zonal distribution. The necrotic lesions consisted largely of hyalinized parenchymal cells still continuous with viable liver plates (Fig. 6). Globular eosinophilic bodies were numerous, both in the foci of necrosis and in the sinusoids adjacent to surviving parenchymal cells. A variable number of round cells and neutrophils had infiltrated the necrotic foci. Prior to inoculation of the virus, parenchymal and Kupffer cells contained only a few PAS-positive, diastase-resistant granules. After infection with MHV_3 virus, the parenchymal cells were unchanged, but many Kupffer cells and acidophilic bodies proved to be PAS positive even after diastase digestion. The PAS-positive substance was usually granular in Kupffer cells, but in eosinophilic bodies it was often of diffuse nature. Neither the hepatic nor the endothelial cells contained hemosiderin.

The liver lesions following the intraperitoneal inoculation of the virus in mice prepared with India ink injections resembled those seen after infection by the intravenous route. Many Kupffer cells were crammed with ink. The parenchymal cells, however, rarely contained even a tiny granule of this material. Acidophilic bodies often exhibited small, diffusely scattered particles of ink and occasionally contained more conspicuous coarse aggregations of this substance (Fig. 4). The origin of acidophilic bodies from Kupffer cells was thus suggested.

Human Hepatitis

Liver biopsy specimens from patients with viral hepatitis occasionally showed small areas of necrosis (Fig. 7); these, like those in murine hepatitis (Fig. 5), seemed to center about hepatic sinusoids. Often there was focal proliferation of Kupffer cells (Fig. 8), a few of which were in mitosis. The nuclei in many endothelial cells were abnormally plump, and some of the cells were binucleated. Their cytoplasm was variously scanty to abundant; generally, it was deeply eosinophilic, but occasionally was characterized by a fine dusting of basophilic material. Most lining cells contained yellowish lipochrome granules with a magenta red appearance when stained by the PAS method after digestion with diastase. Occasionally, these cells also harbored bile or hemosiderin. Hyalinized endothelial cells with pyknotic nuclei were occasionally seen. Just as in the murine disease, it seemed possible to trace a transition between swollen (Fig. 9) or hyalinized Kupffer cells (Fig. 10) and eosinophilic bodies in the sinusoids (Fig. 11). Both the Kupffer cells and the eosinophilic bodies were usually PAS positive after diastase digestion, often intensely so. This substance generally stained magenta red and was coarsely granular. However, in eosinophilic bodies it was often of

diffuse nature and occasionally stained with a purple hue. The parenchymal cells, on the other hand, contained only scattered fine diastase-resistant, PAS-stained granules. Swelling and hyalinization of hepatic cells and all the other histologic changes of human viral hepatitis were present in these specimens. Parenchymal cell hyalinization always affected an entire cell in a diffuse manner. Such cells were generally PAS negative after diastase digestion or exhibited only a very faintly positive stain. They were not stained by PTAH.

Discussion

There was definite damage to Kupffer cells 24 hours after the intravenous inoculation of experimental animals with MHV3 virus. Many of these were in mitosis (Fig. 1), and a high proportion of the mitotic figures were abnormal, suggesting degenerative change (Fig. 2). Pyknosis and karyorrhexis were also frequent. At this time the overwhelming majority of the adjacent parenchymal cells were still virtually normal. We therefore concluded that in this infection Kupffer cells are attacked before parenchymal cells. The cytoplasm of damaged Kupffer cells was often hyalinized (Fig. 2). In the sinusoids there were globular acidophilic bodies (Fig. 3), resembling those first described by Councilman [5] in yellow fever. Moreover, it seemed possible to trace a transition between hyalinized Kupffer cells and the acidophilic bodies.

Parenchymal cell damage did not become conspicuous until 24 hours later (48 hours after inoculation with the virus). The most prominent features here were karyorrhexis and hyalinization of entire parenchymal cells (Fig. 6). Expulsion of completely hyalinized parenchymal cells or of hyalinized fragments from the liver plates, as originally postulated by Councilman [5] in yellow fever, was not observed. It was, therefore, considered possible that the globular eosinophilic bodies might develop from Kupffer cells. The evidence in favor of this hypothesis was strengthened by an additional experiment in which the Kupffer cells were labeled with India ink prior to infection. The carbon could be demonstrated not only in a large proportion of sinusoidal lining cells but in numerous acidophilic bodies as well, but was absent from the parenchyma (Fig. 4).

In human viral hepatitis, Kupffer cells often showed nuclear and cytoplasmic enlargement (Fig. 9) with occasional mitosis; there was frequent focal proliferation of sinusoidal lining cells (Fig. 8). These features have been described previously.[6–8] The Kupffer cells contained PAS-positive lipochrome pigment, generally considered an index of parenchymal cell breakdown.[9] More rarely, hemosiderin [10] or bile [11] also appeared in these cells. Some of the mitotic figures observed in Kupffer

cells in human viral hepatitis showed degenerative features, and the nuclei in other lining cells were often pyknotic or karyorrhectic. The cytoplasm in some was swollen, acidophilic and hyalinized (Fig. 10).

The majority of previous authors have considered Kupffer cell alterations to be secondary to parenchymal cell damage. Some investigators,[8,12-14] however, believed that endothelial changes in viral hepatitis might conceivably be primary. Dible, McMichael and Sherlock[15] thought that Kupffer cell proliferation could be associated with necrosis. All of our biopsy specimens were obtained from patients with well-established viral hepatitis; these exhibited alterations in both the parenchymal and the Kupffer cells. It was not possible, therefore, to investigate the sequential development of the liver lesions as could be done in the experimental mice. Whether Kupffer cell changes in human viral hepatitis preceded the parenchymal lesions could not be determined conclusively. However, the Kupffer cell reaction in the human disorder (Figs. 8 and 9) closely resembled that seen in murine hepatitis, and it seemed very possible that in human hepatitis the injury to the littoral cells might also represent a primary feature.

In his original description, Councilman[5] noted that refractile hyaline eosinophilic bodies were not specific for yellow fever and might appear following nonviral hepatic injuries. He considered that they originated from liver cells; later workers have agreed that eosinophilic bodies in infectious hepatitis were formed in liver cells and extruded into sinusoids.[10,14,16] We were unable to observe this phenomenon in human viral hepatitis with any degree of certainty any more than we had been able to do so in experimental hepatitis. There was, on the other hand, suggestive evidence that Kupffer cells might develop into eosinophilic bodies. It has long been recognized that eosinophilic bodies may occur in Kupffer cells.[8] This has generally been considered to be indicative of phagocytosis by the Kupffer cells. It appears to us more likely that the swollen Kupffer cell cytoplasm itself may become hyalinized. This interpretation was supported by the degenerative nuclear alterations in hyalinized Kupffer cells (Fig. 10). Moreover, the eosinophilic bodies resembled Kupffer cell cytoplasm in their content of yellow iron-negative pigment.[17] Kupffer cells and acidophilic bodies were often strongly PAS-positive after diastase digestion (Figs. 9 and 11) while parenchymal cells contained only a few scattered granules with such staining. These findings led us to conclude that injured Kupffer cells in human viral hepatitis might develop into acidophilic bodies. We could not, however, exclude the possibility that some acidophilic bodies could originate from parenchymal cells.

The distinction of acidophilic bodies from "alcoholic hyalin" appear-

ing in nutritional cirrhosis was not difficult. This focal hyalinization of parenchymal cells was never encountered in murine or human viral hepatitis. Conversely, although eosinophilic bodies occasionally appeared in hepatic cells, we could never be certain that they were not actually in a sinusoid and only adjacent to a liver cell. Apart from this difference in location, alcoholic hyalin was also characterized by a more irregular structure. Finally, alcoholic hyalin was PTAH positive and PAS negative[18–20]; acidophilic bodies in viral hepatitis were PTAH negative[21] and PAS positive after diastase digestion.

Experimental infection with MHV3 virus is not the only form of viral hepatitis in which Kupffer cells appear to be attacked before parenchymal cells. A similar sequence has been observed in ectromelia[22] and in experimental yellow fever.[23] Through the kindness of Dr. H. Smetana we have been able to examine liver sections from monkeys inoculated intraperitoneally with yellow fever virus. We have confirmed his observation that the Kupffer cell changes precede any parenchymal cell alterations. In addition, acidophilic bodies were found in some specimens before significant hepatic cell lesions had developed. Here, too, as in human and murine hepatitis, many Kupffer cells and some eosinophilic bodies were PAS positive after diastase treatment. Our observations in murine and human hepatitis as well as those of others in yellow fever and ectromelia suggest that virus uptake by the injury to sinusoidal lining cells may be the primary event in hepatic viral disorders. It appears to us that at least some of the hyaline globular eosinophilic bodies evident in these infections originate from damaged Kupffer cells.

Summary

The earliest liver lesions induced by murine hepatitis virus have been investigated. Kupffer cells often exhibited mitotic figures, nuclear pyknosis and karyorrhexis 24 hours after intravenous inoculation with the virus. Some Kupffer cells were fragmented but others were diffusely hyalinized; it was possible to trace transitions between the hyaline cells and globular "acidophilic bodies." Parenchymal cell alterations at this stage were minimal but developed later in the infection.

In a second experiment, India ink was introduced intravenously in order to label Kupffer cells before inoculation with murine hepatitis virus. Particles of India ink were found in Kupffer cells and in occasional acidophilic bodies but not in parenchymal cells. Damage to Kupffer cells thus preceded alterations in parenchymal cells. Moreover, injured Kupffer cells were apparently transformed into acidophilic bodies.

Examination of biopsy specimens from human subjects with viral

hepatitis showed a Kupffer cell reaction closely resembling that seen in the murine disease. It seemed possible to trace transitions between swollen Kupffer cells, hyalinized Kupffer cells and eosinophilic bodies. All these structures were usually diffusely PAS positive after diastase digestion; parenchymal cells contained only a few granules giving this reaction. It is concluded that in human viral hepatitis, as in the murine disease, injury of Kupffer cells may be a primary event and acidophilic bodies ("Councilman bodies") may subsequently develop from these cells.

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LEGENDS FOR FIGURES

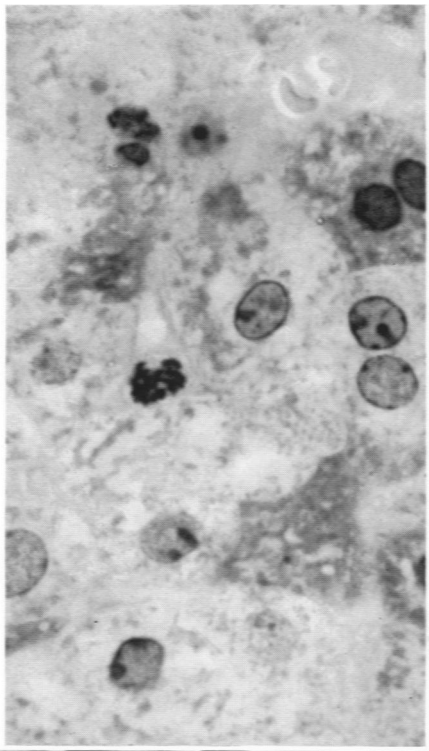
Unless otherwise noted, sections were cut at 1 μ and stained with hematoxylin and eosin.

FIG. 1. A Kupffer cell in mitosis in a mouse liver, 24 hours after intravenous inoculation with murine hepatitis virus (MHV3). × 1,300.

FIG. 2. An abnormal mitotic figure appears in a hyalinized swollen Kupffer cell. Same animal shown in Figure 1. × 1,300.

FIG. 3. Acidophilic bodies are evident in a sinusoid; one has a pyknotic nucleus. Note the resemblance of these to the hyalinized Kupffer cell in Figure 2. Same animal shown in Figure 1. × 1,300.

FIG. 4. An acidophilic body contains irregular coarse carbon particles; similar material is present in Kupffer cells. Mouse liver, 48 hours after intraperitoneal inoculation with MHV3 virus. Undiluted India ink had been injected intravenously 5 days earlier. Section cut at 5 μ. × 1,500.



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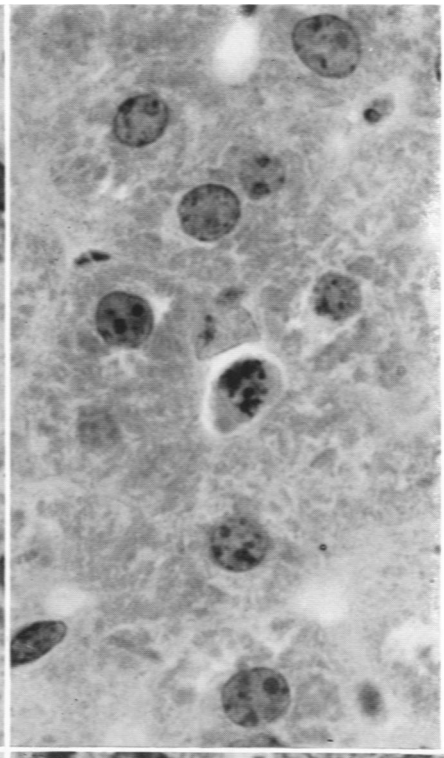

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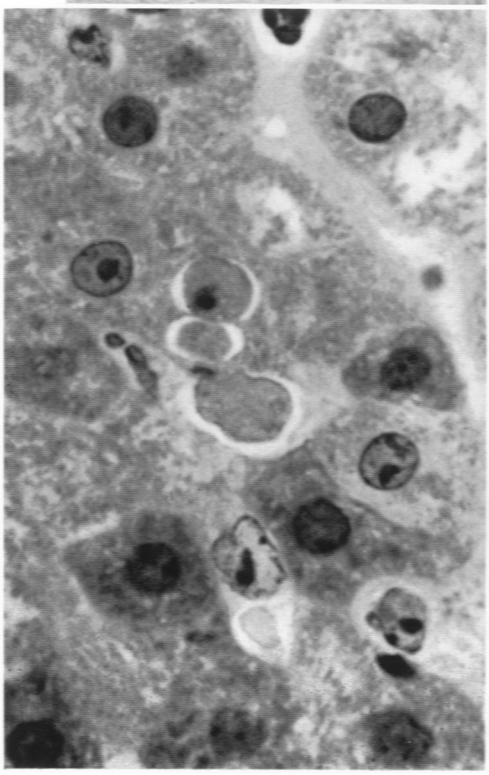

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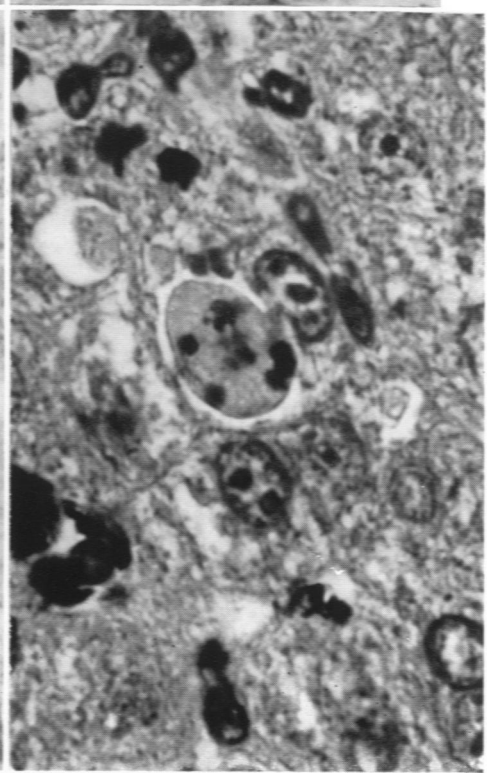

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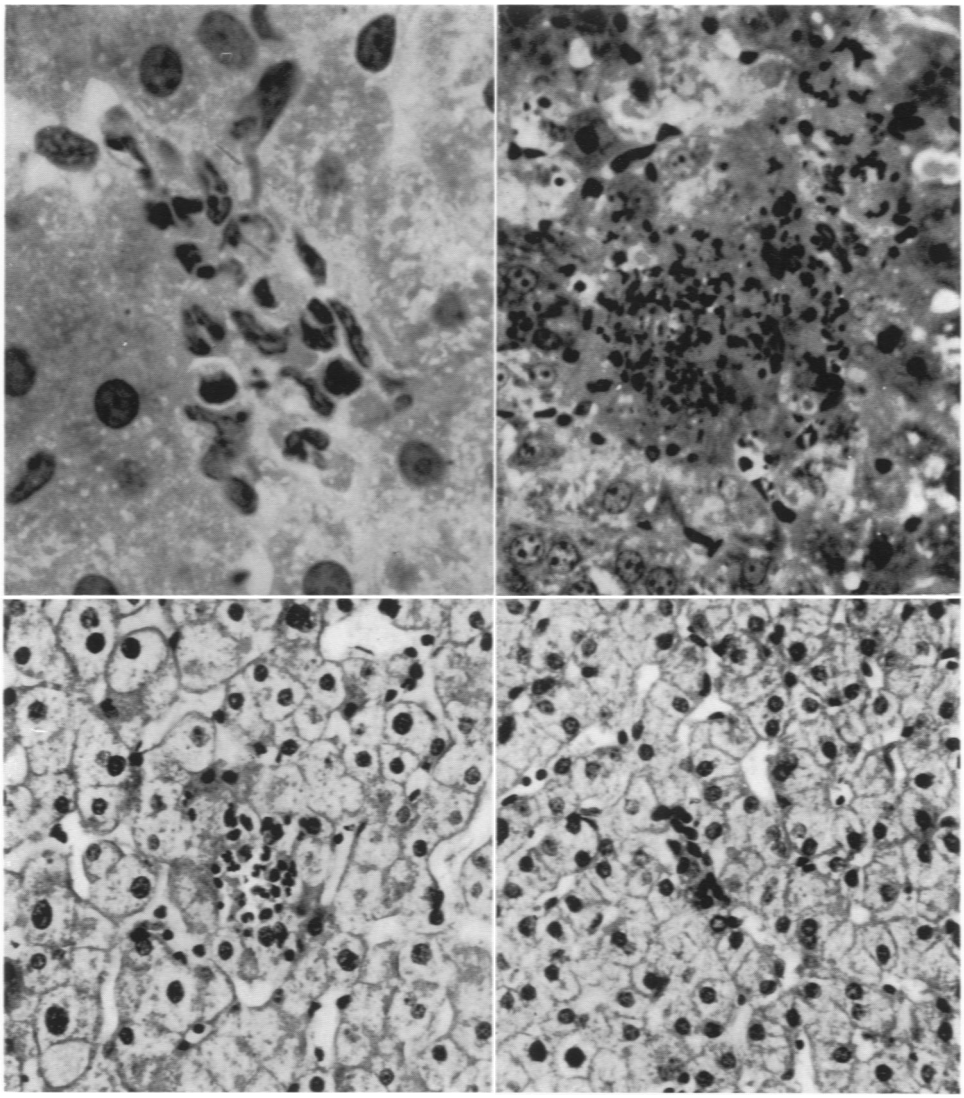
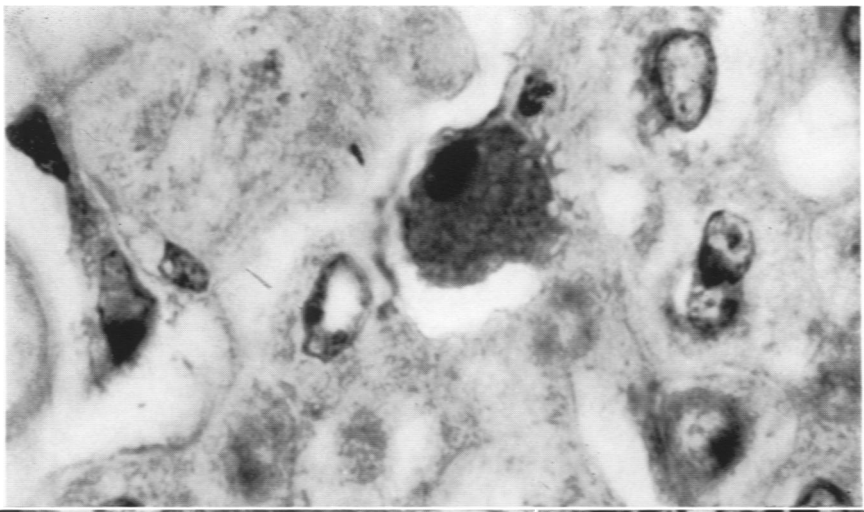


FIG. 5. Early parenchymal cell necrosis is manifest in the vicinity of extensive sinusoidal cell involvement. Mouse liver. 24 hours after intravenous inoculation with MHV3 virus. × 1,300.

FIG. 6. A focus of necrosis consists largely of hyalinized parenchymal cells. Mouse liver, 48 hours after intravenous inoculation with MHV3 virus. × 460.

FIG. 7. This is an example of early parenchymal cell necrosis in human viral hepatitis. Needle biopsy specimen; section cut at 5 μ. × 320.

FIG. 8. Focal Kupffer cell proliferation; biopsy specimen in human viral hepatitis. Section cut at 5 μ. × 320.



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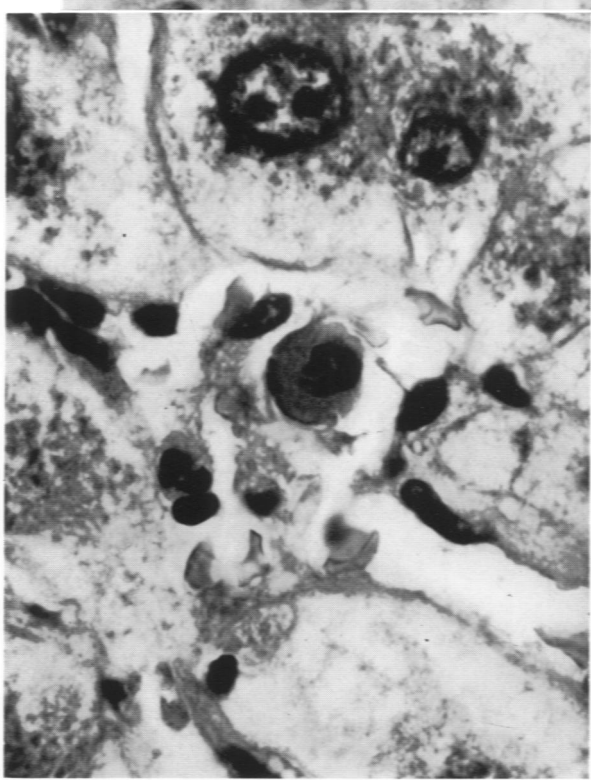

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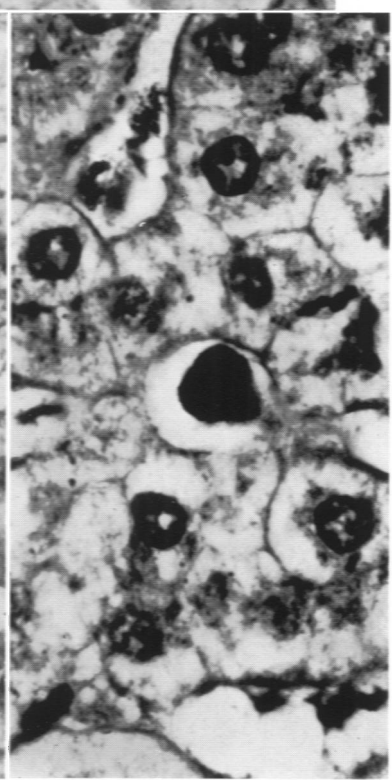

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FIG. 9. A swollen Kupffer cell appears in a sinusoid. Biopsy specimen in human viral hepatitis. Section cut at 5 μ; PAS stain after diastase digestion. × 1,500.

FIG. 10. A hyalinized sinusoidal cell exhibits a pyknotic nucleus; this is probably a Kupffer cell. Biopsy specimen in human hepatitis. Section cut at 5 μ. × 1,150.

FIG. 11. An acidophilic body is evident in a sinusoid. Biopsy specimen in human hepatitis. Section cut at 5 μ; PAS stain after diastase digestion. × 1,150.