The Effect of Mouse Hepatitis Virus Infection on the Microcirculation of the Liver

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Mouse hepatitis virus type 3 infection results in strain-dependent liver disease. The effects of mouse hepatitis virus type 3 on the microcirculation of the liver in both fully susceptible (Balb/cJ) and fully resistant (A/J) mice were studied. In Balb/cJ mice, 6 to 12 hr following infection, abnormalities in liver blood flow were observed which consisted of granular blood flow in both terminal hepatic and terminal portal venules. In addition, sinusoidal microthrombi were present predominantly in periportal areas. By 24 to 48 hr, liver cell edema and small focal lesions were prominent. At 48 hr, thrombi and hepatocellular necrosis were widespread, and blood was shunted from damaged areas into patent sinusoids. In sharp contrast to these abnormal findings, normal streamlined blood flow was present in the resistant A/J animals at all time points following infection. Since large amounts of virus were demonstrated by immunofluorescene in and by recovery and growth from livers of both resistant and susceptible strains, the presence of the virus *per se* cannot explain the abnormalities observed.

The liver serves many biological functions including the synthesis of a variety of essential compounds, detoxification of the blood, and the elimination from the body of potentially harmful substances. Although it is supplied by a double circulation, namely arterial and venous, the liver is extremely sensitive to changes in blood flow. The hepatic microcirculation regulates the nutrition and function of the parenchyma. The terminal portal venule (TPV) with its accompanying arteriole and bile ductule, the liver sinusoids, and the terminal hepatic venules into which these sinusoids drain comprise the microcirculatory unit (1, 2). The area of liver supplied by this unit is referred to as the simple liver acinus with the arterioles its functional center (3). Any agent or process that results in the disruption of the integrity of the microvascular unit will result in a disease state.

Coronaviruses, a group of pathogenic RNA viruses, cause a broad spectrum of disease in their natural hosts (4, 5). One of the coronaviruses, mouse hepatitis virus type 3 (MHV-3), produces three distinct patterns of disease in genetically dissimilar inbred strains of mice (6). Normal adult mice of the A strain are fully resistant to MHV-3 whereas Balb/cJ, NZB, C57, and DBA/2 mice are all fully susceptible and die of fulminant hepatic necrosis (7). C3H/eBFeJ mice develop acute hepatitis which progresses to chronic viral persistence with focal hepatic inflammation and granulomatous disease (7) Although the disease produced by this virus has been attributed to direct viral cytopathic effects, these cannot by themselves explain the disease observed (8). It has been suggested that cellular elements of the immune system are important in both resistance to and propagation of the disease (9–11). The spectrum and severity of these diseases vary with age, genetic background, route of infection, and virus titer (12). Resistance to acute infection depends at least in part on the potential for viral replication in cells of the reticuloendothelial system, a genetically restricted event (13, 14).

It has been recently recognized that cellular elements of the immune system are extremely important to the host's survival and the elimination of this virus. Specifically, T cells and macrophages play important roles in the defense of the host against MHV-3 infection (15).

A second host system activated in response to infection is the coagulation system. The deposition of fibrin in the vicinity of inflammatory lesions is evidence for this (16). The perivascular deposition of fibrin is one of the earliest observations in experimental encephalomyelitis (17), hyperacute renal allograft rejection (18, 19), and acute

proliferative glomerulonephritis (20). In addition, coagulation has been shown to be an important component of the delayed cutaneous hypersensitivity reaction (21, 22). Anticoagulation by warfarin abrogates the induration of the skin lesions and blocks the generation of procoagulent activity by mononuclear cells (23).

We have previously reported that MHV-3 induces the generation of procoagulant monokines (PCA) in a strain-dependent fashion which parallels disease activity (7, 24). Monocytes/macrophages are the cellular source of this PCA, but direct lymphocyte collaboration is required for the full expression of this activity. This present study was designed to determine whether MHV-3 induction of monocyte PCA and activation of the coagulation system are associated with abnormalities in the hepatic microcirculation.

MATERIALS AND METHODS

VIRUS

MHV-3, obtained from the American Tissue Type Culture Collection, Rockville Md. (ATTCC VR-262), was plaque-purified on monolayers of DBT cells. Stock virus was grown to a titer of 1.2×10^7 plaque forming units (PFU) per ml on 17 CL1 cells and was used for all subsequent experiments. Virus was assayed on monolayers of L2 cells in a standard plaque assay as previously described (24).

PURIFICATION OF VIRUS

MHV-3 was purified to homogeneity by a method described previously (25). Viruses were pelleted through polyethylene glycol and purified on sequential K-tartrate gradients. The purified virus at a titer of 1×10^9 PFU per ml was resuspended in normal saline. Viral protein was determined in a modified Lowry assay as described previously (26).

SOLID-PHASE RADIOIMMUNOASSAY

Animals were screened for antibody to MHV in a solid-phase radioimmunoassay as described previously (27). Flexible 96 well microtiter plates were coated with purified MHV-3 diluted in 100 μl of carbonate buffer (pH 9.6). The antigens were cross-linked with 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide HCl. Mouse sera were added, and then an aliquot of ^{125}I-labeled affinity purified goat antimouse immunoglobulin (specific activity of 4.3 to 7.2 μCi per μg) was added as a probe, and the individual wells were counted with appropriate positive and negative sera included in all assays as controls. All samples were assayed in triplicate.

MICE

Balb/cJ and A/J male mice, 4 to 6 weeks of age, were obtained from Jackson Laboratories, Bar Harbor, Maine. All mice were screened for the presence of antibody to
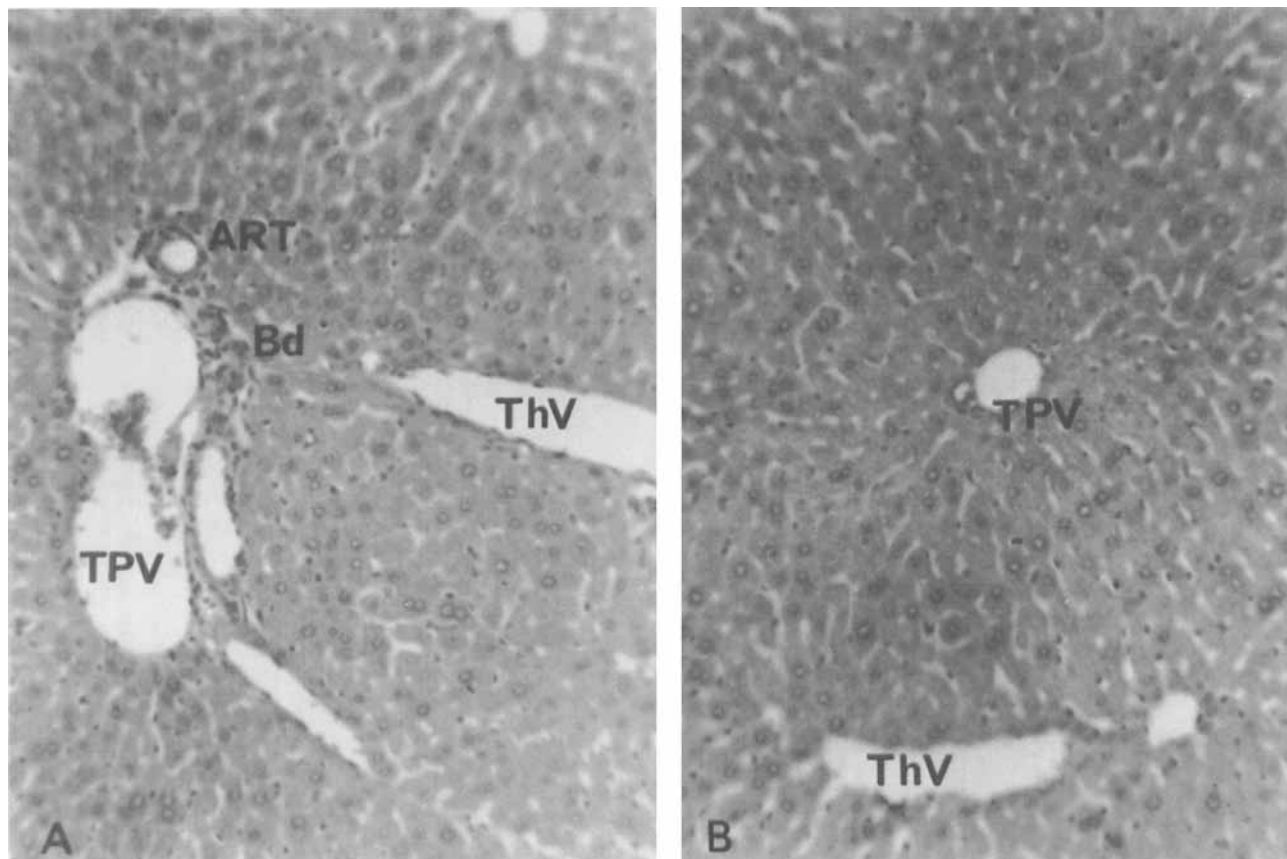

FIG. 1. MHV-3 infected A/J livers at 24 (A) and 72 hr (B) postinoculation showing normal histology. Portal spaces with TPVs, arterioles (ART), and bile ductules (Bd) are within normal limits as are ThVs (H & E, × 280).

MHV by a standard radioimunoassay (27) and plaque reduction assay (7).

STUDIES OF MICROCIRCULATION

Mice were injected intravenously with 1,000 PFU of purified MHV-3 in 100 μl of normal saline through the dorsal tail vein. Control mice were injected with 100 μl of normal saline. Each mouse was then anesthetized with nembutal sodium (6.4 mg per 100 gm body weight) by intraperitoneal injection. The animals were immobilized on a surgical table and their temperature controlled at 37°C. A tracheotomy was performed and each animal then was intubated with PE-10 intramedic tubing (Clay-Adams, NY) and ventilated with humidified 4.78% CO_2, balance O_2. The abdomen was then opened and the abdominal cavity irrigated with Ringer's lactate solution (0.42 gm per liter KCl, 0.5 gm per liter NaHCO$_3$, 9.0 gm per liter NaCl, and 0.24 gm per liter CaCl$_2$ in distilled water). A parafilm apron was inserted under the margin of the liver and over the viscera to maintain intraabdominal pressure and temperature. Tubocurarine (0.02 ml of 3 mg per ml solution) (Burroughs Welcome Ltd. Quebec, Canada) was injected intramuscularly into the right crus of the diaphragm. The liver was transilluminated with a quartz rod (Department of Physics, University of Toronto, Toronto, Canada) and connected to a fiberoptic

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A heterologous antibody to MHV-3 was produced in New Zealand white rabbits by repeated injections of 10^9 PFU of purified MHV-3 (50 μg) in complete Freund's adjuvant. The antibody was found to be suitable for use at a dilution of 1:40. Immunofluorescence was by an indirect method using a fluorescein-labeled goat anti-rabbit IgG as the second reagent.

light source (IntraLux 150H Volpi, Zurich Switzerland). The quartz rod was positioned under the liver margin and the microcirculation studied under a microscope (E. Leitz GmbH Wetzlar, Germany).

RESULTS

LIVER HISTOLOGY

Groups of Balb/cJ and A/J mice were injected intravenously with 1,000 PFU of MHV-3 or with saline and sacrificed at 6, 12, and 24 hr and then daily thereafter for 21 days. Liver tissue was obtained for immunofluorescence, viral titer, and histology. No lesions were noted in the livers of A/J mice up to 21 days following infection (Figure 1). This confirmed previous reports that this strain is entirely resistant to the pathogenic effects of the virus. In contrast, lesions were seen 24 hr after infection in the livers of Balb/cJ mice. These lesions
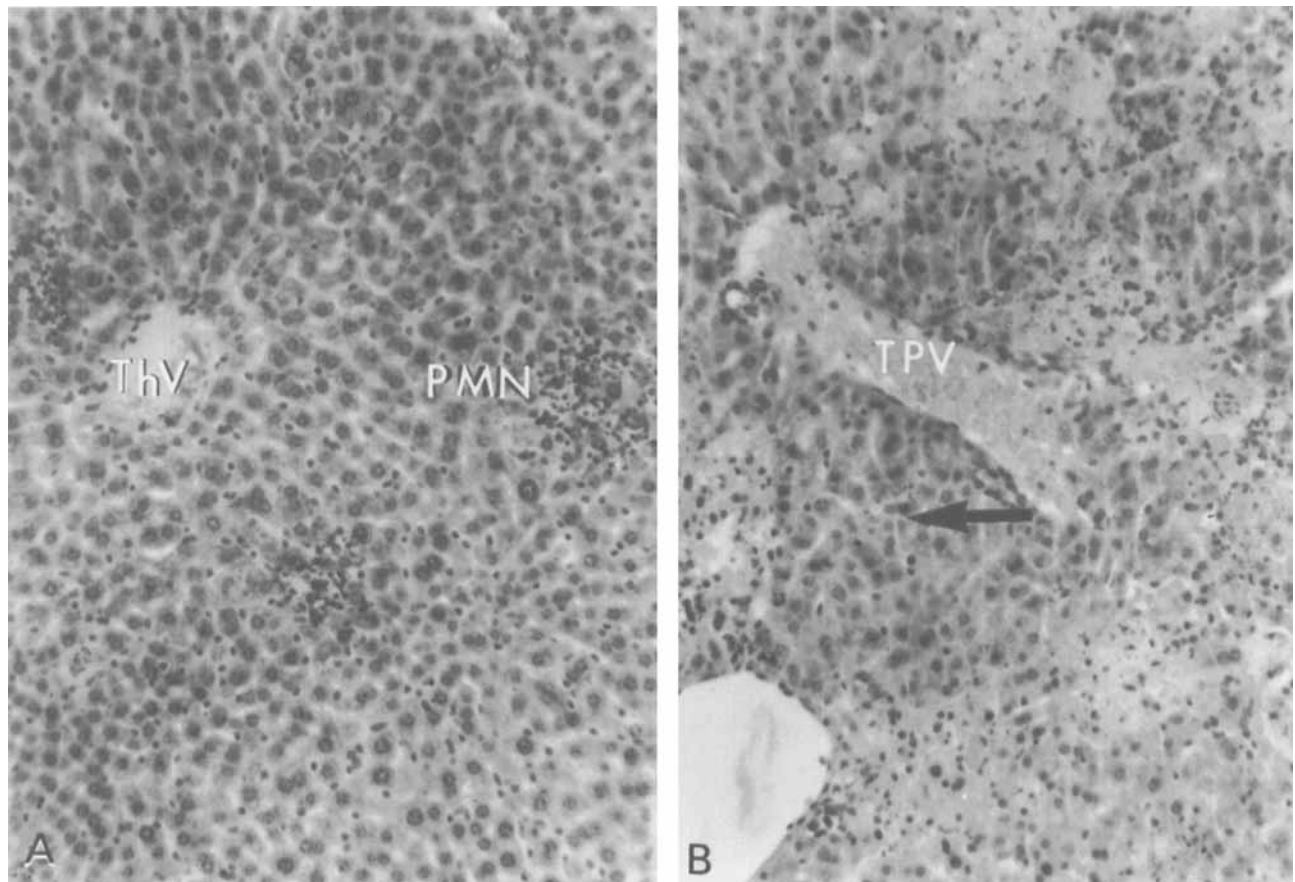

FIG. 2. (A) Livers from Balb/cJ mice at 24 hr after infection with focal areas of necrosis with a leukocytic infiltrate which is primarily of polymorphonuclear cells (PMN). (B) At 72 hr postinoculation, liver cell necrosis is confluent with few surviving hepatocytes (*arrow*) (H & E, × 280).

consisted of multiple well-defined necrotic foci with a polymorphonuclear cell infiltrate (Figure 2A). By 48 hr, these lesions had become more pronounced, and by 72 to 96 hr they were confluent and the liver almost entirely necrotic (Figure 2B).

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In order to detect viral antigens, a pooled heterologous antiserum to MHV-3 was produced by repeated injections of purified MHV-3 in New Zealand white rabbits. By radioimmunoprecipitation, the antibody was found to react primarily with the nucleocapsid protein, but in addition had weak E2 and E1 glycoprotein specificity. By indirect immunofluorescence, viral antigens could be detected at 24 hr in liver tissues from both A/J and Balb/cJ mice (Figures 3 and 4). At 24 hr, in the A/J mice, there were large amounts of viral antigen deposited in both the parenchyma and reticuloendothelial cells (Figure 3). By 48 to 72 hr postinfection, most of the antigen
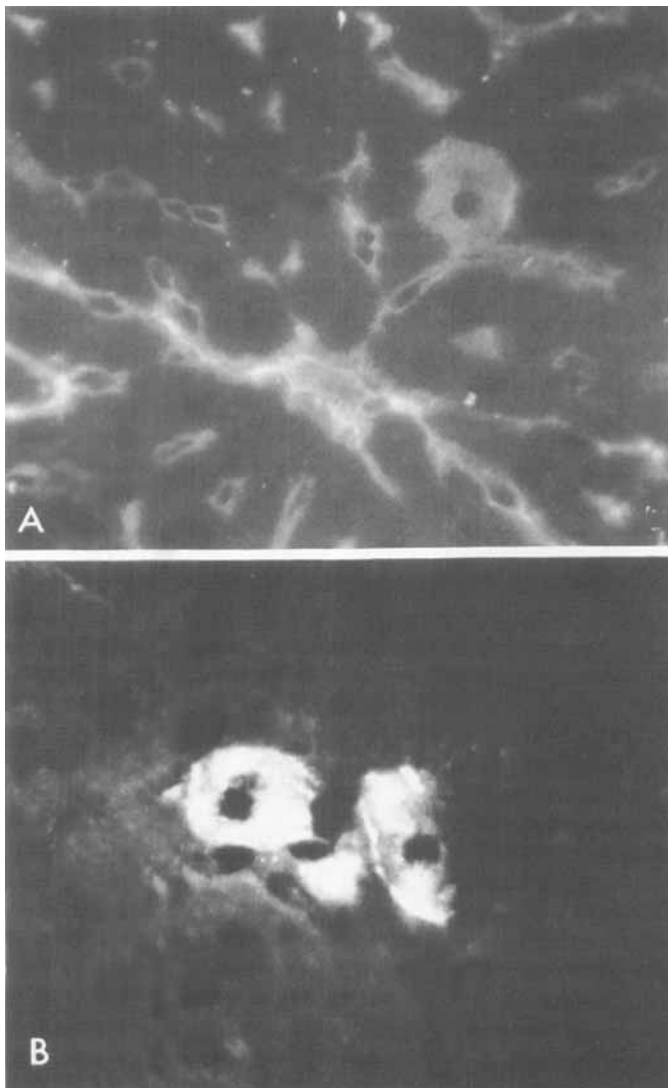

FIG. 3. Immunofluorescence of MHV-3 infected A/J livers demonstrating (A) viral antigens diffusely distributed throughout both the endothelial cells and the cells of the reticuloendothelial system. (B) At 48 hr, the viral antigens are localized to the hepatic parenchymal cells with both cytoplasmic and surface antigens demonstrable (× 500).

was found in the liver parenchymal cells (Figure 3). Although localized mainly to the cytoplasm of the hepatocytes, there were considerable deposits of antigen at the cell surface. In A/J mice, viral antigens were detected in large amounts for 4 days and then the titers decreased until they were no longer detected at 7 days. The antigens were diffusely deposited throughout the liver parenchyma, and large surface deposits were visualized. In Balb/cJ mice, viral antigen was detected as early as 18 hr following infection (Figure 4). In contrast to the A/J mice, the viral antigens were localized to the liver parenchymal cells with only minor amounts in the reticuloendothelial cells (Figure 4). By 24 to 48 hr, the viral antigens were found in cells surrounding the areas of necrosis but also in areas where no histologic abnormalities could be seen. Viral antigens were detected in the livers of Balb/cJ mice until the time of death, 96 to 120 hr following infection.

We were also able to show by immunofluorescence using a rabbit antifibrinogen/fibrin kindly provided by Dr. E. F. Plow, the deposition of fibrin in the sinusoids adjacent to areas of focal necrosis in the MHV-infected Balb/cJ mice at 12 to 18 hr postinfection (data not shown).

VIRAL TITERS

Livers were homogenized in a small volume of Dulbecco's modified essential medium, adjusted to a 10% homogenate and subjected to three bursts of sonication (Braun Sonik, Labline Instruments, Melrose Park, Ill.) at 4°C. Viral titers were determined on monolayers of L2 cells in a standard plaque assay as described previously (7). At 12 to 18 hr following infection, virus was not detected in the livers of either A/J or Balb/cJ mice. By 24 hr high viral titers could be measured in liver homogenates from both strains, however, the titer was one log greater in the Balb/cJ mice (Figure 5). By 48 hr, viral titers in the two strains differed significantly with a greater than 3-fold log increase in the Balb/cJ mice (Figure 5). By 72 hr, 80% of the Balb/cJ mice had expired and by 96 hr all were dead. High viral titers were detected in all of the animals studied (Figure 5). Virus could be recovered from the livers of the A/J mice for up to 6 days following infection. Maximal titers, at Day 5, reached 86% of those recorded in the Balb/cJ mice. The viral titers decreased rapidly and were undetectable by Day 7.

NORMAL MICROCIRCULATORY UNIT

In both strains studied, normal streamlined blood flow was observed in the in vivo transilluminated liver of the uninfected animals. The margin of the liver featured alternating terminal hepatic venules (ThVs) and TPVs (Figure 6). Arterioles were seen emptying into the TPVs. Sinusoidal flow could be easily followed from the TPVs to the ThVs. Hepatic plates were seen to be 1 to 2 cells thick and the hepatocytes had semitranslucent cytoplasm.

PATHOLOGIC MICROCIRCULATORY UNIT

Within 6 to 12 hr postinfection in the Balb/cJ mice, granular blood flow caused by the clumping of erythrocytes was observed in both the TPV and ThV. The edema
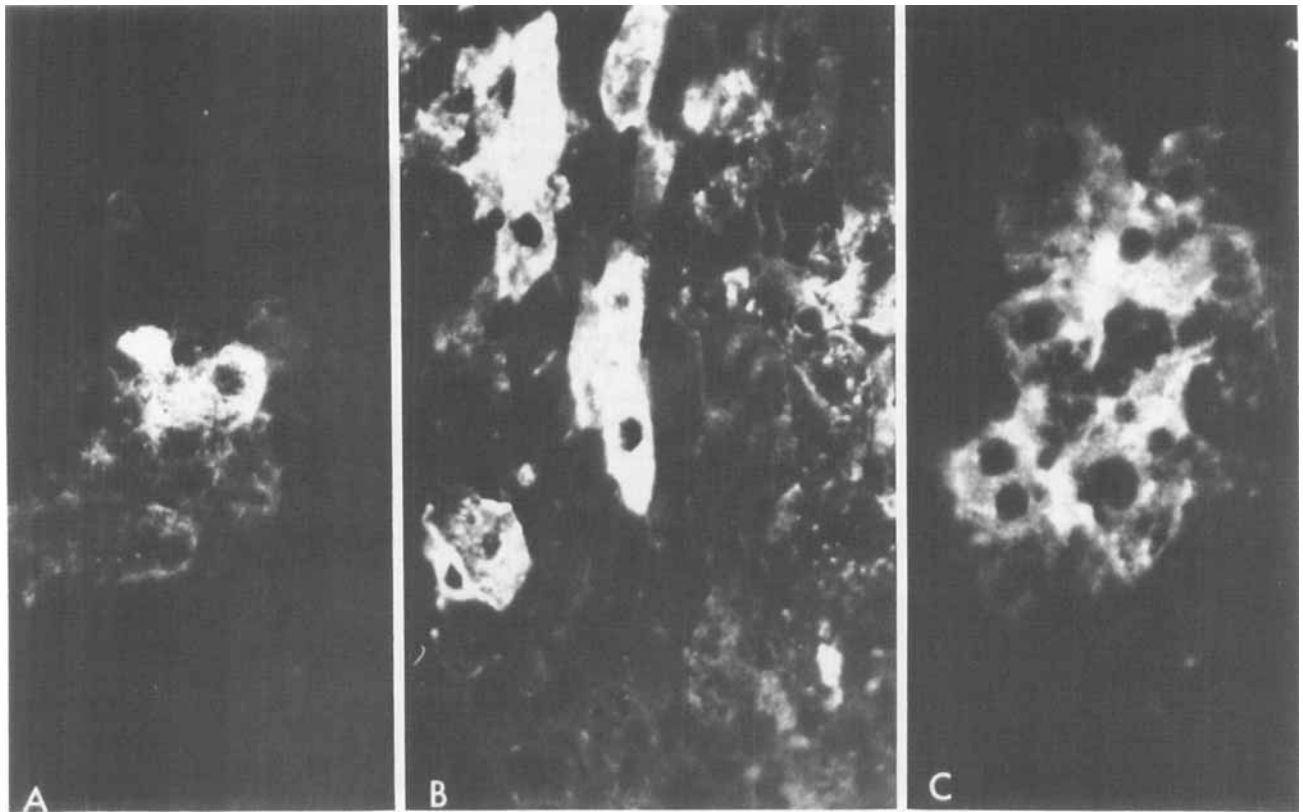
FIG. 4. Immunofluorescence of MHV-3 infected Balb/cJ livers at (A) 24 hr postinoculation showing deposits of antigen in areas of necrosis as well as in normal hepatic tissue. (B) By 48 to 72 hr, there are widespread deposits in all areas of the liver. (C) By 84 hr, there is diffuse hepatic necrosis, but in the surviving hepatocytes there are viral antigens at both the surface and within the cytoplasm ($\times$ 500).

that was observed was especially prominent in the cells abutting the vessels giving them a crenulated outline (Figure 7). In addition, the velocity of flow was obviously diminished. By 24 hr, granular flow was more prominent and was accompanied by liver cell edema (Figure 8A) and sinusoidal microthrombi (Figure 8B). The microthrombi were diffusely scattered, with some tendency to be localized periportally. By 24 hr and up to 36 hr, focal areas of pale swollen cells with little or no sinusoidal blood flow were scattered throughout the liver (Figure 8). As well, clearly circumscribed necrotic lesions appeared in areas (Figure 8B) where microthrombi had apparently obliterated a group of sinusoids. By 48 hr, more severe necrotic lesions were noted sometimes involving numerous acini (Figure 9A). Blood flow was diverted around necrotic tissue and was prominent by 60 hr postinfection (Figure 9B). By 72 hr, the liver was almost totally necrotic and blood flow was markedly reduced.

In contrast, no discernible abnormalities were noted in the livers of A/J mice. In spite of the presence of viral antigens in these livers as demonstrated both by the recovery and growth of virus from liver homogenates and by immunofluorescence, blood flow remained streamlined throughout the course of infection and no microthrombi or vascular abnormalities could be detected.

DISCUSSION

The liver is composed of polygonal cells arranged in plates of one or two cell thickness between the sinusoids,

the terminal branches of the microvascular lifeline (28). These cells can be considered to be in three zones dependent on their relationship to the afferent vessels: the TPV and arteriole (29). Cell sensitivity to injury induced by drugs, nutritional deficiency states, and toxins varies from zone to zone (30, 31). Damage is characterized in the early stages by clumping of erythrocytes (granular flow) and cellular edema followed by liver cell necrosis.

This study demonstrates that MHV-3 infection induces severe and progressive hepatic microcirculatory abnormalities in susceptible Balb/cJ mice which are not observed in the resistant A/J strain. Normal flow in small vessels is swift and streamlined with fastest flow at the parabolic front of the streaming erythrocytes. Red and white blood cells traverse the sinusoids as single cells so quickly that they cannot be seen clearly. Granular flow is defined as a deceleration in flow resulting from an aggregation of erythrocytes. The aggregation observed is a result of increased blood viscosity due to a loss of plasma fluid. Under electron microscopy, an electron dense material (fibrinogen) has been noted to coat the surface of the erythrocytes. This causes them to lose their negative surface charge and results in adherence of these cells to one another (32). Arteriolar flow can be seen as intermittent pulsatile ejections into the sinusoids.

Despite the presence of viral antigens and infectious particles within the liver of the resistant A/J mice, there were no abnormalities of blood flow or parenchymal disease observed. Although the viral titers in the early phase of disease were substantially less than in the
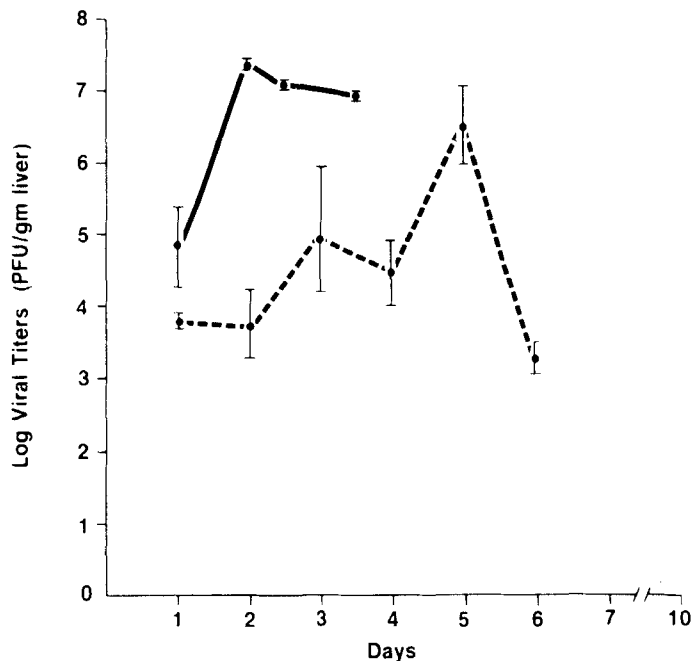
FIG. 5. Recovery and growth of viral antigens from infected A/J (---) and Balb/cJ (——) livers. By 48 hr, there is a 3-fold log increase in viral titers from the livers of Balb/cJ mice. At Day 5, maximal viral recovery was 86% of that recovered from the Balb/cJ mice.

susceptible Balb/cJ mice, 5 days following infection the titers of virus were the same in the two strains. Furthermore, by immunofluorescence, we were able to demonstrate that the virus was primarily localized to the hepatocytes in both strains. Thus, the mere presence of viral "infective" antigens does not in itself cause either microcirculatory or cellular abnormalities.

Previously, we reported that monocytes following MHV-3 infection *in vitro* express a PCA which can cleave prothrombin to thrombin with resultant fibrin formation (7). The induction of this activity correlates with disease susceptibility in that there is no monocyte PCA expressed in the resistant A/J mice while there is a large increment in the expression by monocytes from susceptible Balb/cJ mice. The induction of this PCA, which requires direct lymphocyte collaboration, is rapid and is seen as early as 4 to 6 hr following infection *in vitro* (7).

We have now extended these observations to show that monocytes assayed immediately out of the blood from MHV-3 infected Balb/cJ mice express a spontaneous total content PCA of 14,500 milliunits per 10^6 monocytes, a 140-fold increase over basal PCA from mock-infected Balb/cJ mice at 4 hr postinfection. The monocyte PCA rises to 32,750 milliunits per 10^6 monocytes at 24 hr and peaks at 78,000 milliunits per 10^6 monocytes at 48 hr. Approximately 30 to 45% of this
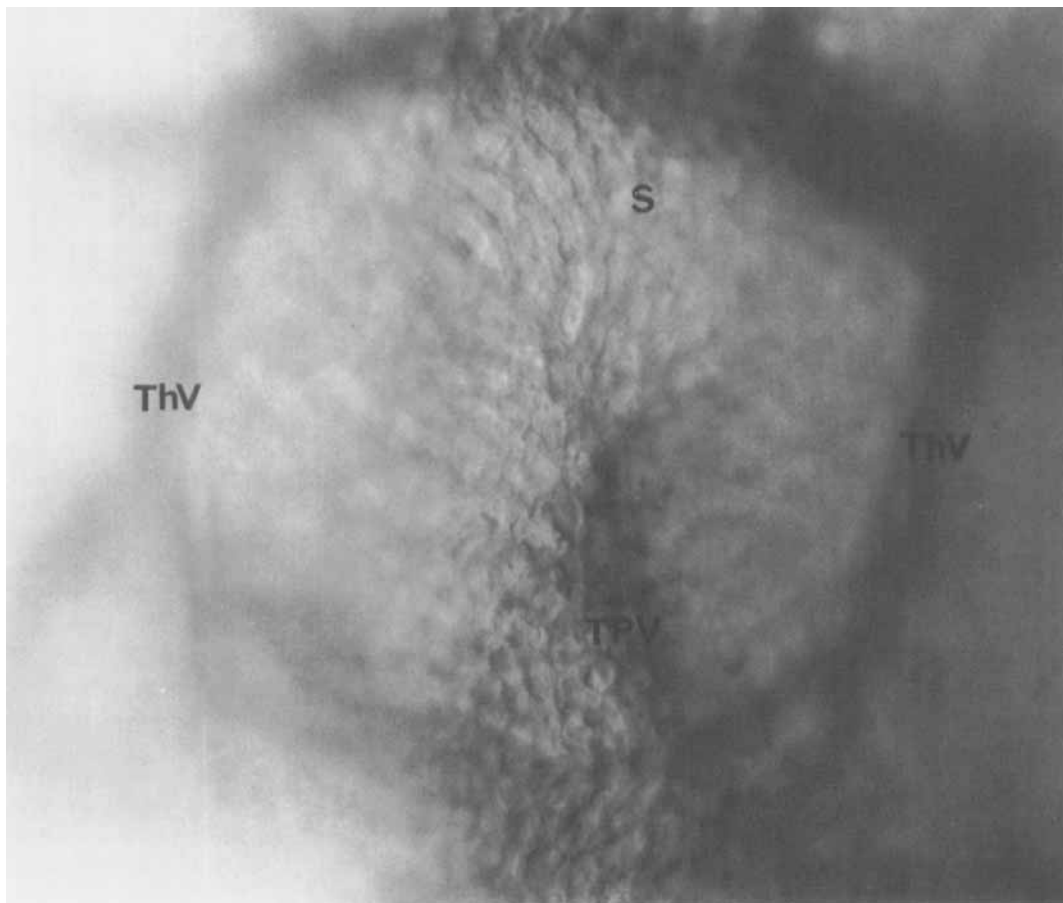

FIG. 6. Normal aspects of the microvascular hepatic circulation *in vivo* with alternating ThVs and TPVs at the liver margin. Sinusoids (S), the finest ramifications of the microvasculature are easily seen ($\times$ 1,000).
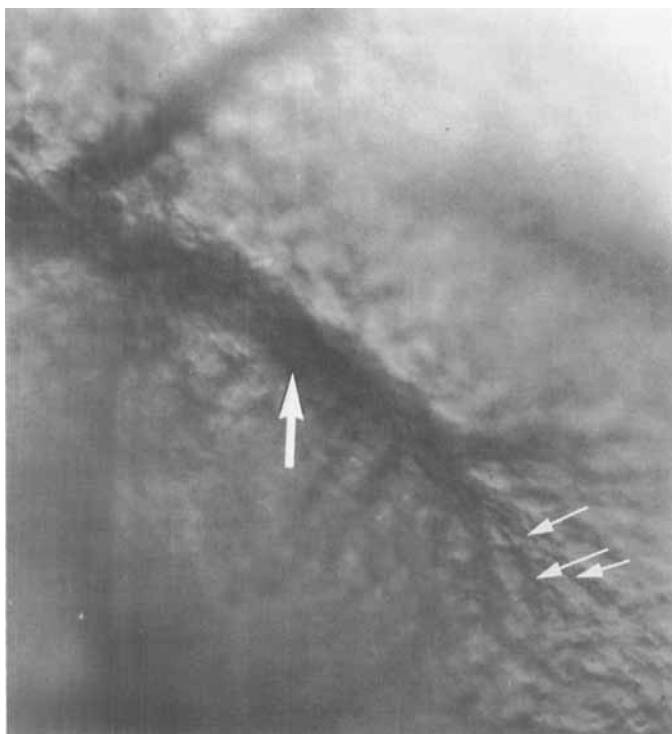
FIG. 7. At 8 hr postinoculation, blood flow in the sinusoids and the TPVs is granular (arrows). Liver cell architecture is normal, and viral antigens cannot be detected. The cells adjoining the vessel are edematous (pale), and their outline is jagged due to swollen hepatocytes (× 900).

TABLE 1. MICROCIRCULATORY ABNORMALITIES FOLLOWING MHV-3 INFECTION

Abnormalities	Stages[a]			
	I	II	III	IV
Time (postinoculation) (hr)	6	12	36	60
Virus	−	−	+	+
Granular flow	+	+	+	+
Edema	−	+	+	+
Microthrombi	−	+	+	+
Focal necrosis	−	−	+	+
Confluent necrosis	−	−	−	+

[a] +, present; −, absent.

total content PCA was expressed at the surface of the cell (viable PCA). The expression of this monocyte PCA preceded viral replication and histologic abnormalities. Monocytes from MHV-3 infected A/J animals expressed no increases in PCA above normal background levels (in preparation).

In the susceptible Balb/cJ mice, the earliest morphological abnormalities noted were in the microcirculation. Prior to recovery and growth of virus from infected livers marked granular, flow was observed.

Therefore, monocytes expressing surface PCA could initiate microcirculatory flow abnormalities (granular flow) with resultant microthrombi, endothelial cell injury, and hepatocellular necrosis (Table 1). In addition, monocytes expressing PCA could attract cells of the lymphoid series into areas of virus-infected tissues with resultant cell-mediated liver injury. Finally, monocyte PCA, being a serine protease, could in itself alter the hepatocellular membrane with resultant hepatocellular necrosis. Activation of the coagulation system is important both in inflammation and expression of the immune response. The importance of fibrin deposition to tissue injury has been described in a number of disease processes (16–20). Furthermore, fibrin deposition is prominent in delayed cutaneous hypersensitivity reactions, and a direct correlation exists between the expression of monocyte PCA and delayed cutaneous hypersensitivity (33). Holdsworth has shown that in experimental glomerulonephritis, macrophages are prominent during the

development of injury and that depletion with a specific antimacrophage serum was able to prevent fibrin deposition and glomerular injury (34). Our studies support the concept that alterations in the coagulation and vascular systems precede tissue damage in MHV-3 infection and suggests that intravascular blood coagulation may be the primary event in the pathogenesis of parenchymal damage.

A number of investigators have described the effects of viral infection on endothelial cell function and integrity. These include the findings of viral particles within endothelial cells (35), as well as morphologic changes within these cells (36–38). By the fourth day following parvovirus infection, cushion-like swellings of the endothelial cells were observed which produced focal narrowing of the vascular lumen. Following this stage, the vessels were lined by pyknotic desquamating endothelial cells or denuded endothelium. During the early stages of vascular obstruction, leukocyte aggregates were seen in capillaries and venules with plugs adherent to the vessel walls. Subsequently, aggregated erythrocytes and fibrin thrombi occluded the parenchymal arterioles (39). These findings were noted in the vessels of the neural tissues but also in the liver and other gastrointestinal organs including the spleen and pancreas. Other investigators have demonstrated by immunofluorescence, viral particles within the endothelial cells with resultant vasculitis, microthrombi, and circulatory abnormalities (40). In a study in patients with infectious hepatitis, deposits of hepatitis B surface antigen in endothelial cells and flow abnormalities were observed during the early stages of infection (41, 42). These studies are extremely important in that endothelial cells are known to secrete a number of mediators which regulate the integrity of the vascular compartment including factor VIII, plasminogen activator, and activators of the contact system (43, 44). Thus, injury of these cells could result in the activation of the coagulation system, microvascular abnormalities, and tissue injury. Reports have suggested that the secretion of mediators by endothelial cells is regulated in part by the cells of the immune system (45, 46). Monocytes expressing PCA could induce endothelial cell injury directly by activation of the coagulation system or induce the secretion of other procoagulants which could result in further compromise of the integrity of the vascular tree.
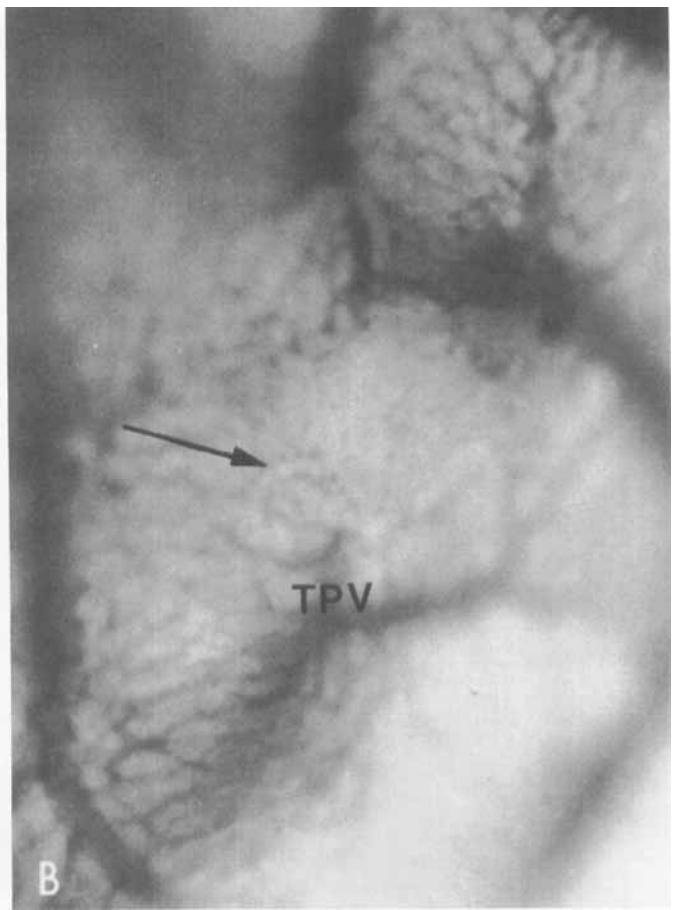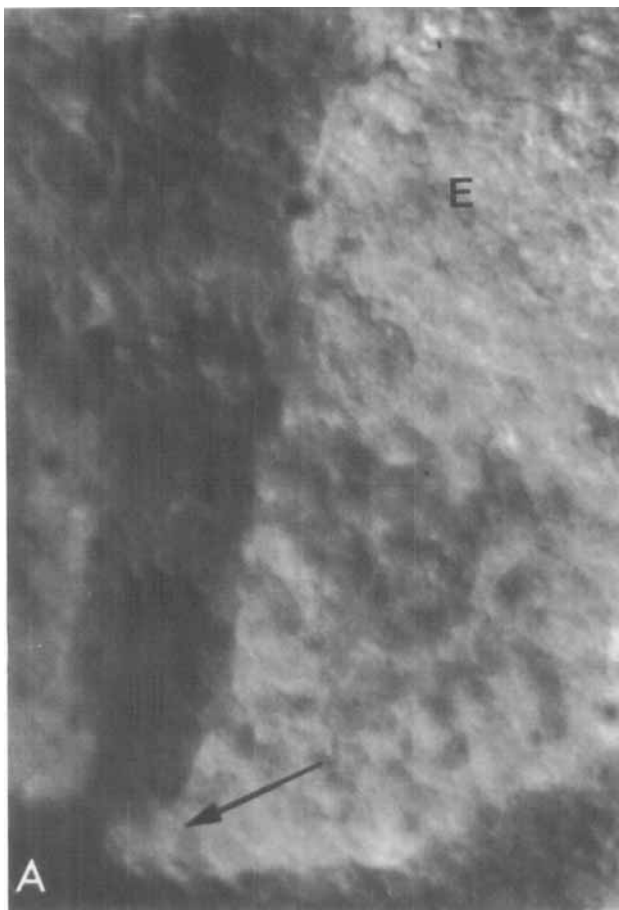
FIG. 8. (A) By 24 hr postinoculation, there is generalized granular flow with no sinusoidal flow through areas of edematous hepatocytes (E); there is narrowing of the vascular lumen (*arrow*) (× 900). (B) Numerous microthrombi (*arrow*) are visible particularly at the outflow of the TPVs (× 450).
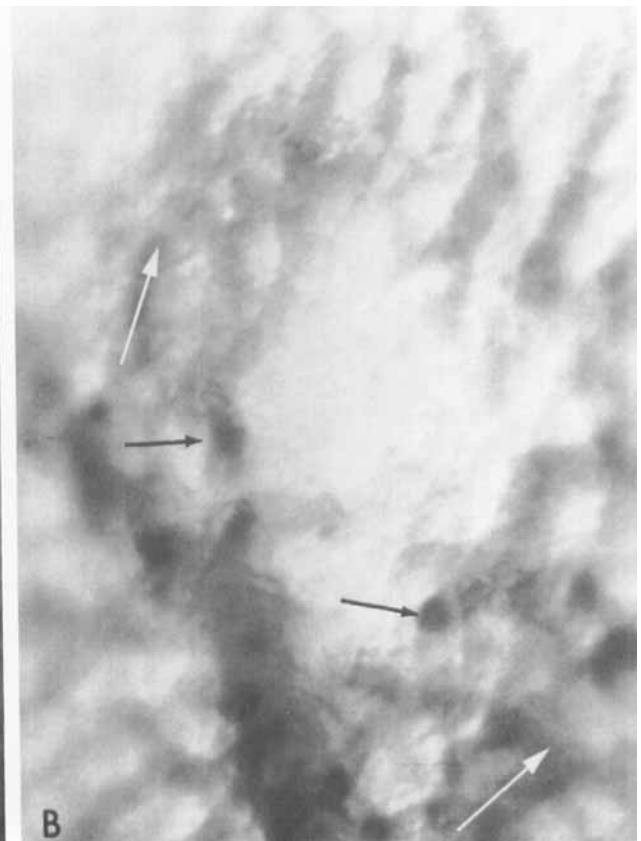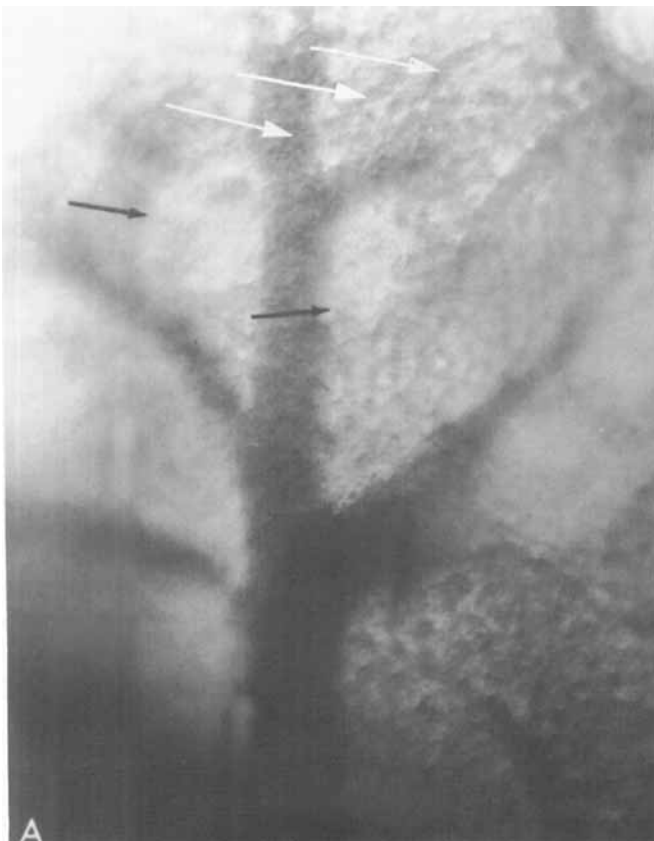

FIG. 9. (A) By 48 hr, widespread necrotic foci (*black arrow*) are prominent as well as marked granular flow (*white arrows*) and poor sinusoidal perfusion (× 225). (B) At 72 to 96 hr, necrotic lesions involving numerous acini are evident with blood flow in the sinusoids bypassing (*white arrows*) the areas of necrosis. The microthrombi (*black arrows*) blocking flow are visible at the base of the obstructed vessels (× 900).

In summary, we report that, following MHV-3 infection, striking abnormalities are seen in the *in vivo* hepatic microcirculation of susceptible Balb/cJ mice, while no abnormalities are seen in the microcirculation of resistant A/J mice. We postulate that these microcirculatory changes, which are preceded by virally induced synthesis of monocyte PCA, are a potential mechanism for the hepatocellular injury and necrosis seen in the susceptible Balb/cJ mice. Further investigations are now underway to determine the cellular and molecular mechanisms responsible for these abnormalities and to evaluate the effects on the hepatocellular injury of specific agents capable of reversing or blocking changes in the microvasculature.

REFERENCES

1. Rappaport AM. In: Javitt NB, ed. Hepatic blood flow: morphologic aspects and physiologic regulation. Liver and biliary tract physiology. International Review of Physiology, Vol. 21. Baltimore: University Park Press, 1980: 1-63.
2. Rappaport AM. The microcirculatory acinar concept of normal and pathological hepatic structure. Beitr Pathol Bd 1976; 157:215-243.
3. Rappaport AM, Schneiderman JH. The function of the hepatic artery. Rev Physiol Biochem Pharmacol 1976; 76:130-159.
4. Robb JS, Bond CW. Coronaviridae. In: Fraenkel-Conrat H, Wagner RR, eds. Comprehensive virology, Vol 14. New York: Plenum Press, 1979: 193-247.
5. Tyrell DAJ. Coronaviridae. Intervirology 1978; 10:321-336.
6. Virelizier JL, Allison AC. Correlation of persistent mouse hepatitis virus (MHV-3) infection with its effect on mouse macrophage cultures. Arch Virol 1976; 50:279-285.
7. Levy GA, Leibowitz JL, Edgington TS. Lymphocyte-instructed monocyte induction of the coagulation pathways parallels the induction of hepatitis by the murine hepatitis virus. In: Popper H, Schaffner F, eds. Progress in liver disease, Vol 7. New York: Grune and Stratton, 1982: 393-409.
8. Le Prevost C, Levy-Leblond E, Virelizier JL, et al. Immunopathology of mouse hepatitis virus type 3 infection. I. Role of humoral and cell-mediated immunity in resistance mechanisms. J Immunol 1975; 114:221-225.
9. Le Prevost C, Virelizier JL, Dupuy JM. Immunopathology of mouse hepatitis virus type 3 infection. III. Clinical and virological observations of a persistent viral infection. J Immunol 1975; 115:640-643.
10. Virelizier JL. Pathogenicity and persistence of mouse hepatitis virus in inbred strains of mice. In: Ter Meulen V, Sidell S, Wege H, eds. Advances in experimental medicine and biology, Vol 142. Biochemistry and biology of coronaviruses. New York: Plenum Press, 1981: 349-358.
11. Stohlman SA, Frelinger JA. Macrophages and resistance to JHM virus CNS infection. In: Ter Meulen V, Siddell S, Wege H, eds. Advances in experimental medicine and biology, Vol 142. Biochemistry and biology of coronaviruses. New York: Plenum Press, 1981: 387-398.
12. Wege H, Sidell S, Ter Meulen V. The biology and pathogenesis of coronaviruses. In: Cooper M, ed. Current topics in microbiology and immunology, Vol 99. New York: Academic Press, 1982: 165-189.
13. Bang FB, Warwick A. Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility. Proc Natl Acad Sci USA 1960; 46:1065-1075.
14. Weiser WY, Bang FB. Blocking of *in vitro* and *in vivo* susceptibility to mouse hepatitis virus. Exp Med 1977; 146:1467-1472.
15. Stohlman SA, Frelinger JA, Weiner LP. Reistance to fatal central nervous system disease by mouse hepatitis virus, strain JHM. II.

16. Dvorak HF, Mihm MC, Dvorak AM, et al. Morphology of delayed type hypersensitivity in man. I. Quantitative description of the inflammatory response. Lab Invest 1974; 31:11-116.
17. Oldstone MBS, Dixon FJ. Immunohistochemical study of allergic encephalomyelitis. Am J Pathol 1968; 52:251-261.
18. Dempster WJ. The migrant cells in allotransplants of heart, kidney and skin. II. Monocyte-macrophage activity. Br J Exp Pathol 1977; 58:504-520.
19. Lindquist RR, Guttman RD Merrill JP. Renal transplant in the inbred rat. VI. Electron microscopic study of the mononuclear cells accumulating in rejecting renal allografts. Transplantation 1971; 12:1-14.
20. Vassalli P, McCluskey R. The pathogenetic role of the coagulation process in glomerular diseases of immunologic origin. Adv Nephrol 1971; 1:47-63.
21. Edwards RL, Rickles FR. Delayed Hypersensitivity in man: effects of systemic anticoagulation. Science 1978; 200:541-544.
22. Colvin RB, Johnson RA, Mihm ML, et al. Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. J Exp Med 1973; 138:686-677.
23. Colvin RB, Mossesson MW, Dvorak HF. Delayed type hypersensitivity skin reactions in congenital afibrinogenemia lack deposition and induration. J Clin Invest 1979; 63:1302-1309.
24. Levy GA, Leibowitz JL, Edgington TS. Induction of monocyte procoagulent activity by murine hepatitis virus type 3 parallels disease susceptibility in mice. J Exp Med 1981; 154:1150-1163.
25. Wege H, Wege H, Nagashima K, et al. Structural polypeptides of the murine coronavirus JHM. J Gen Virol 1979; 42:37-47.
26. Levy GA, Schwartz BS, Curtiss LK, et al. Plasma lipoprotein induction and suppression of the generation of cellular procoagulent activity *in vitro*. Requirement for cellular collaboration. J Clin Invest 1981; 67:1614-1624.
27. Leibowitz JL, Fung LS, Levy GA. A sensitive radioimmunoassay for the detection of antibody to mouse hepatitis virus. J Virol Meth; 3:255-265.
28. Rappaport AM. The microcirculatory hepatic unit. Microvasc Res 1973; 6:212-228.
29. Quistoff B, Chance B, Takeda H. Two and three dimensional vascular heterogeneity of rat liver. Effects of anoxia and alcohol on the lobular vascular patterns. In: Dutton PL, Leigh LS, Scarpa A, eds. Frontiers of biological energetics: from electrons to tissues, Vol. II. New York: Academic Press Inc., 1978: 1487-1524.
30. Greenway CV, Stark RD. Hepatic vascular bed. Physiol Rev 1971; 51:23-65.
31. Rappaport AM. Physio-anatomical basis of toxic liver injury. In: Farber E, Fisher MM, eds. Toxic injury to the liver, Chap 1. New York: Decker Publishers, 1979: 1-46.
32. Bloch EH. Microscopic observations of the circulating blood in the bulbar conjunctiva in man in health and disease. Ergebenisse der Anatomie und Entwicklungs Geschichte 1956; 35:1-98.
33. Hopper KE, Geczy CL, Davies WA. A mechanism of migration inhibition in delayed type hypersensitivity reactions. I. Fibrin deposition on the surface of ellicited peritoneal macrophages *in vivo*. J Immunol 1981; 126:1052-1059.
34. Holdsworth SW, Neale TJ, Wilson CB. Abrogation of macrophage-dependent injury in experimental glomerulonephritis in the rabbit: use of a specific anti-macrophage serum. J Clin Invest 1981; 68:686-698.
35. Friedman HM, Macarak EJ, MacGregor RR, et al. Virus infection of endothelial cells. J Infect Dis 1981; 143:266-273.
36. Vorbrodt AW, Lossinsky AS, Wisniewski M, et al. Ultrastructural cytochemical studies of cerebral microvasculature in scrapie infected mice. Acta Neuropathol 1981; 53:203-211.
37. Phinney PR, Fligiel S, Bryson Y, et al. Necrotizing vasculitis in a case of disseminated neonatal herpes simplex infection. Arch Pathol Lab Med 1982; 106:64-67.
38. Burch GE, Tsui CY, Harb JM. Pathologic changes of aorta and coronary arteries of mice infected with Coxsackie B4 virus. Proc Soc Exp Biol Med 1971; 137:657-661.
39. Margolis G, Kilham L. Parvovirus infections, vascular endothelium and hemorrhagic encephalopathy. Lab Invest 1970; 22:478-488.

40. Burch GE, Rayburn P. EMC viral infection of the coronary blood vessels in newborn mice: viral vasculitis. Br J Exp Pathol 1977; 58:565-571.

41. Jelev A. Microcirculation changes in virus hepatitis patients. Folia Medica 1980; 22:20-23.

42. Neumann HAM, Berretty PJM, Reinders Folmer SCC, et al. Hepatitis B surface antigen deposition in the blood vessel walls of urticarial lesions in acute hepatitis B. Br J. Dermatol 1981; 104:383-388.

43. Levin EG Loskutoff DJ. Serum mediated suppression of cell asso-

ciated plasminogen activator activity in cultured endothelial cells. Cell 1980; 22:701-707.

44. Collen D. On the regulation and control of fibrinolysis. Thromb Haemost 1980; 43:77-89.

45. Vassalli JD, Hamilton J, Reich E. Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors and cyclic nucleotides. Cell 1976; 8:271-281.

46. Hirschberg H, Bergh OJ, Thorsby E. Antigen presenting properties of human vascular endothelial cells. J Exp Med 1980; 152:249-261.