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Pathogenesis of Herpes Simplex Virus Types 1 and 2 in Mice After Various Routes of Inoculation

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The pathogenesis of herpes simplex virus (HSV) types 1 and 2 was compared after inoculation of mice by different routes. Intravaginal inoculation of HSV-1 and HSV-2 produced a local infection, with virus recovery from the vagina through 5 days. Virus was recovered from the spinal cords 4 to 5 days after inoculation but not from liver, kidney, lung, spleen, or blood. Intravenous or intraperitoneal inoculation of HSV-2 produced a focal necrotic hepatitis similar to that described previously (S. C. Mogenson, B. Teisner, and H. K. Andersen, 1974). The viral etiology of the liver lesions was confirmed by virus isolation (through 4 days) and electron microscopy. No evidence of infection of the kidney, lung, blood, or spleen was observed, although virus was isolated from spinal cord homogenates 7 days after inoculation. HSV-1 inoculation by the intraperitoneal or intravenous route resulted in virus isolation from the kidney during the 7-day harvest period, without producing overt pathological changes. Virus was isolated from spinal cord homogenates 2 to 3 days after HSV-1 inoculation but not from homogenates prepared from spleen, lung, or blood. Increases in serum transaminase activity were observed after systemic (intravenous) inoculation of HSV-2 but not after HSV-1 inoculation.

Several biological differences exist between herpes simplex virus type 1 (HSV-1) and type (HSV-2). Included in these differences are serological properties (14), neurovirulence for animals (15), virus yields from rabbit kidney monolayers (6), and ability to plaque on chicken embryo cells (10). Recently it has been shown that intraperitoneal (i.p.) inoculation of HSV-2 produces a focal necrotic hepatitis in mice, whereas with HSV-1 infection involvement of the liver was a less frequent and less severe occurrence (11).

During our studies on the pathogenesis of HSV-1 and HSV-2 in mice using various routes of virus inoculation, we observed a similar tropism of HSV-2, but not HSV-1, for liver after systemic inoculation. Intravenous (i.v.) and i.p. inoculation of HSV-2 results in liver necrosis. After HSV-1 infection, virus can be recovered from the liver at 24 h but rapidly clears, and there is infrequent necrosis. Intravaginal inoculation of HSV-1 or HSV-2 produces a vaginitis that ultimately proceeds to a central nervous system disease, terminating in death. Only rarely is the liver involved, and virus is not usually recovered from kidney homogenates after intravaginal inoculation of either virus.

This paper summarizes our studies relating to the pathogenesis of HSV-1 and HSV-2 infection of mice using different routes of virus inoculation.

METHODS

Mice. Upj:TUC(ICR)spf mice of both sexes were obtained from the Upjohn colony. The animals were housed in stainless steel cages and given food and water ad libitum. At the time of inoculation they weighed 18 to 20 g.

Viruses. The herpesviruses used in these studies were propagated in and assayed by the plaque method on rabbit kidney monolayers. The virus titer of HSV-1 (MRS) was 1.7×10^7 plaque-forming units (PFU)/0.5 ml, and the mean lethal dose was $10^{2.9}$/0.05 ml after i.v. injection in mice. The HSV-2 (35D, obtained from L. Chien, University of Alabama, Birmingham) had a titer of 2×10^6 PFU/0.5 ml and $10^{2.4}$/0.05 ml after i.v. injection in mice.

Additional strains of HSV-2 (S-23, E-294, E-304, and E-685) were generously provided by Andre J. Nahmias, Emory University, Atlanta, Ga. Lawrence T. Chien, University of Alabama, provided additional HSV-1 strains (42D, 114A, and 113A) and HSV-2 strains (35C and 115A). Strains GM and NR were isolated in primary rabbit kidney monolayers in our laboratory from patients with genital lesions, whereas MRS was isolated from a lip lesion. Using plaque formation on chicken embryo monolayers as the primary criterion (10), strains GM and NR formed plaques and were classified as type 2, whereas MRS was classified as type 1.

Virus inoculation. For i.v. or intravaginal inoculation, 0.05 ml of the virus was used. Thirty minutes prior to intravaginal inoculation the vaginas were swabbed with a dry cotton swab. Virus (0.1 ml) was used to inoculate mice i.p.

Virus titrations. Mice were decapitated and whole blood or serum was collected. The kidney, brain, spinal cord, spleen, and lung were surgically removed and stored frozen. At the time of assay, 10% homogenates (wt/vol) of the organs were prepared in Eagle medium containing 3% fetal bovine serum and centrifuged prior to titration on rabbit kidney monolayers (18).

Serum enzymes. Serum glutamic oxalacetic acid transaminase (SGOT) and serum glutamic pyruvic acid transaminase (SGPT) activities were determined using a colorimetric assay (17). The sera from pools of blood (10 mice) were used for the assay.

Microscopy. For light microscopy, tissues were fixed using buffered 10% formalin and processed by standard procedures. The sections were stained with hematoxylin-eosin, von Kossa stain, hematoxylin-phloxine, or azure-eosinate. For electron microscopy, the tissues were collected and fixed in 2% glutaraldehyde, followed by a second fixation in 1% osmium tetroxide in phosphate (Millonig) buffer. Subsequent processing of the tissues was by standard procedures.

RESULTS

In the initial studies mice were inoculated i.v. or i.p. with HSV-1 or HSV-2. On days 3 or 4 and again on 6 or 7, three to four mice were autopsied and the livers were examined for evidence of abnormalities. In confirmation of Mogensen et al. (11), lesions were observed in those groups inoculated with HSV-2 but not HSV-1. Additional studies showed that liver lesions resulted in response to the i.v. inoculation of eight additional strains of HSV-2, but none was observed with three additional strains of HSV-1.

The data (Table 1) show that virus was isolated from liver homogenates of mice inoculated by the i.v. route with HSV-2. These data further show that the time of appearance and the quantity of virus isolated were dependent upon the size of the inoculum; with an inoculum of 1.3×10^4 PFU, virus was isolated from the liver beginning at 24 h, whereas with the smaller inoculum (1.3×10^3 PFU) virus was not isolated until 72 to 96 h. The titer of the virus in liver homogenates was increased with the higher inoculum (2.5 logs as opposed to 1.3 logs at 96 h with the lower inoculum). With neither inoculum was virus isolated after 96 h. Virus was first detectable in spinal cord homogenates at 96 h and persisted through 120 and 168 h. Virus was not isolated from blood or homogenates of lungs or spleens at any time. In this study 15 of 15 mice died within 10 days after inoculation with both dilutions of virus.

A comparison of the mortality of mice infected with HSV-1 or HSV-2, using the intravaginal or i.p. routes for inoculation, is shown in Fig. 1. With HSV-2, 75% of the mice inoculated vaginally and 100% of those inoculated i.p. died. With HSV-1 inoculation, 85% of the mice inoculated by the i.p. or vaginal routes died; however, those inoculated i.p. died earlier. The mean survival time was about 6.5 days and about 9 days after i.p. and intravaginal inoculation, respectively.

Table 2 shows the pathogenesis of HSV-1 and HSV-2 in mice after intravaginal inoculation of virus. The mice seemed to be especially sensitive to inoculation with HSV-1 in that the vaginal virus titers were about 1 log greater through the 120-h sampling period compared with HSV-2, and in both cases the titers were decreased at 168 h. At 168 h, virus was isolated

TABLE 1. *Virus isolation from liver and spinal cord homogenates of mice inoculated i.v. with HSV-2*

Time after inoculation (h)	Virus titers (mean log PFU/ml)							
	Liver				Spinal cord			
	N(V)/N(T)[a]	A[b]/mean[c]	N(V)/N(T)	B/mean	N(V)/N(T)	A/mean	N(V)/N(T)	B/mean
24	3/5	0.88 (0.95)[d]	0/5	<1.0	0/5	<1.0	0/5	<1.0
48	3/5	1.49 (1.37)	0/5	<1.0	0/5	<1.0	0/5	<1.0
72	5/5	2.23 (0.75)	1/5	0.21 (0.47)	0/5	<1.0	0/5	<1.0
96	5/5	2.55 (0.94)	4/5	1.34 (0.99)	3/5	1.40 (1.65)	4/5	2.45 (1.42)
120	0/5	<1.0	0/5	<1.0	3/5	1.24 (1.14)	4/5	1.18 (0.75)
168	0/2	<1.0	0/4	<1.0	2/2	4.19 (0.15)	4/4	4.38 (0.33)

[a] N(V) refers to the number of virus isolations, and N(T) refers to the total number of mice in the group.
[b] Mice were inoculated with 1.3×10^4 (A) or 1.3×10^3 (B) PFU in 0.05 ml into the tail vein. With both inocula, 100% of the mice died within 10 days.
[c] Means of the logs of the virus titers with standard deviations.
[d] Numbers in parentheses represent standard deviations.

from two of four kidney homogenates prepared from mice inoculated with HSV-2 and one of five mice inoculated with HSV-1. Both HSV-1 and HSV-2 inoculation resulted in infection of the spinal cord. HSV-2 appears to enter the central nervous system earlier than HSV-1 in that, at 96 h, HSV-2 was isolated from four of five spinal cord homogenates whereas with HSV-1 inoculation, virus was isolated from one of five at 48, 72, and 96 h and from three of five spinal cord homogenates at 120 h. Virus isolation from the liver was not a common occurrence after intravaginal inoculation with either virus, nor was there any evidence of liver lesions. The mortality data (Fig. 1) show that those mice inoculated by the vaginal route with HSV-2 died earlier than those inoculated with HSV-1.

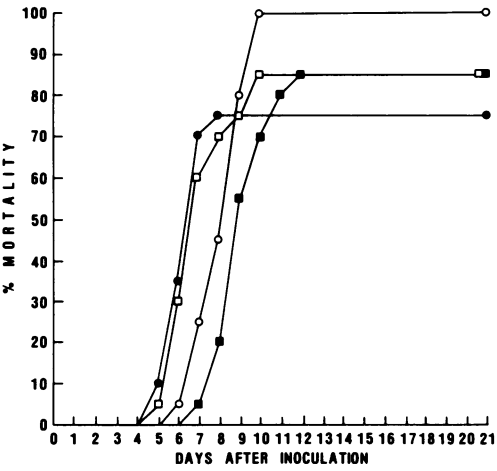

FIG. 1. *Mortality for mice of HSV-1 (squares) and HSV-2 (circles) inoculated intravaginally (solid symbols) or i.p. (open symbols).*

Table 3 compares the pathogenesis of HSV-1 and HSV-2 in mice after i.p. inoculation. As with i.v. inoculation of HSV-2 (Table 1), virus was isolated from liver homogenates during 96 h after i.p. inoculation of HSV-2 but not in samples taken thereafter. When mice were inoculated with HSV-1, high titers from all mice were found in the liver at 24 h, after which the titer and the number of mice yielding virus decrease so that after 72 h virus was no longer detected. Virus can be isolated from the kidneys of mice infected with HSV-2 only occasionally, i.e., virus was isolated from a total of two mice inoculated with HSV-2, whereas after i.p. inoculation with HSV-1 virus was isolated in high titer throughout the observation period. HSV-1 appeared to enter the spinal cord earlier than did HSV-2. An i.p. inoculation of mice with HSV-1 resulted in earlier deaths than that observed with HSV-2 inoculation (Fig. 1).

Microscopically, the lesions resulting after HSV-2 inoculation were scattered throughout

TABLE 2. *Pathogenesis of HSV-1 and HSV-2 in mice after intravaginal inoculation*

Time after inoculation (h)	Vaginal swabs		Liver		Kidney		Spinal cord	
	N(V)/N(T)[a]	Mean[b]	N(V)/N(T)	Mean	N(V)/N(T)	Mean	N(V)/N(T)	Mean
Vaginal inoculation of HSV-1								
24	5/5	4.47 (1.24)[c]	0/5	<1.0	0/5	<1.0	0/5	<1.0
48	5/5	4.97 (0.71)	0/5	<1.0	0/5	<1.0	1/5	0.34 (0.76)
72	5/5	4.41 (0.07)	0/5	<1.0	0/5	<1.0	1/5	0.36 (0.80)
96	5/5	4.09 (0.48)	0/5	<1.0	0/5	<1.0	1/5	0.47 (1.06)
120	5/5	3.69 (1.09)	0/5	<1.0	0/5	<1.0	3/5	1.74 (1.77)
168	2/5	0.59 (0.90)	0/5	<1.0	1/5	0.50 (1.13)	4/5	2.06 (1.85)
Vaginal inoculation of HSV-2								
24	3/5	1.81 (1.8)	1/5	0.66 (1.48)	0/5	<1.0	0/5	<1.0
48	5/5	3.56 (0.5)	0/5	<1.0	0/5	<1.0	0/5	<1.0
72	3/5	1.71 (1.63)	0/5	<1.0	0/5	<1.0	0/5	<1.0
96	5/5	3.72 (0.31)	0/5	<1.0	0/5	<1.0	4/5	1.73 (1.27)
120	5/5	3.65 (0.78)	0/5	<1.0	1/5	0.32 (0.72)	4/5	2.62 (1.60)
168	2/4	1.64 (1.64)	0/4	<1.0	2/4	0.94 (1.28)	4/4	2.93 (1.26)

[a] N(V) refers to the number of mice from which virus could be isolated, and N(T) refers to the total number of mice in the group.

[b] Virus titers are given as the means of the log of the virus titers (PFU per milliliter).

[c] Numbers in parentheses represent standard deviations.

TABLE 3. *Pathogenesis of HSV-1 and HSV-2 in mice after i.p. inoculation*

Time after inoculation (h)	Liver		Kidney		Spinal cord	
	N(V)/N(T)[a]	Mean[b]	N(V)/N(T)	Mean	N(V)/N(T)	Mean
i.p. inoculation of HSV-1						
24	5/5	3.01 (0.42)[c]	4/5	3.07 (2.00)	0/5	<1.0
48	2/5	1.09 (1.54)	3/5	2.56 (2.48)	2/5	0.51 (0.71)
72	0/5	<1.0	4/5	4.37 (2.45)	2/5	0.64 (0.96)
96	0/5	<1.0	4/5	4.13 (2.37)	3/5	1.48 (1.43)
120	0/5	<1.0	5/5	4.15 (0.49)	4/5	1.99 (1.31)
168	0/3	<1.0	3/3	1.97 (0.66)	1/3	0.50 (0.86)
i.p. inoculation of HSV-2						
24	4/5	2.00 (1.15)	0/5	<1.0	0/5	<1.0
48	2/5	1.25 (1.45)	0/5	<1.0	0/5	<1.0
72	3/5	1.76 (1.65)	1/5	0.58 (1.30)	0/5	<1.0
96	5/5	3.03 (0.54)	0/5	<1.0	0/5	<1.0
120	0/5	<1.0	1/5	0.76 (1.69)	0/5	<1.0
168	0/3	<1.0	0/3	<1.0	2/3	2.14 (1.90)

[a] N(V) refers to the number of mice from which virus could be isolated, and N(T) refers to the total number of mice in the group.

[b] Virus titers are given as the means of the log of the virus titers (PFU per milliliter).

[c] Numbers in parentheses represent standard deviation.

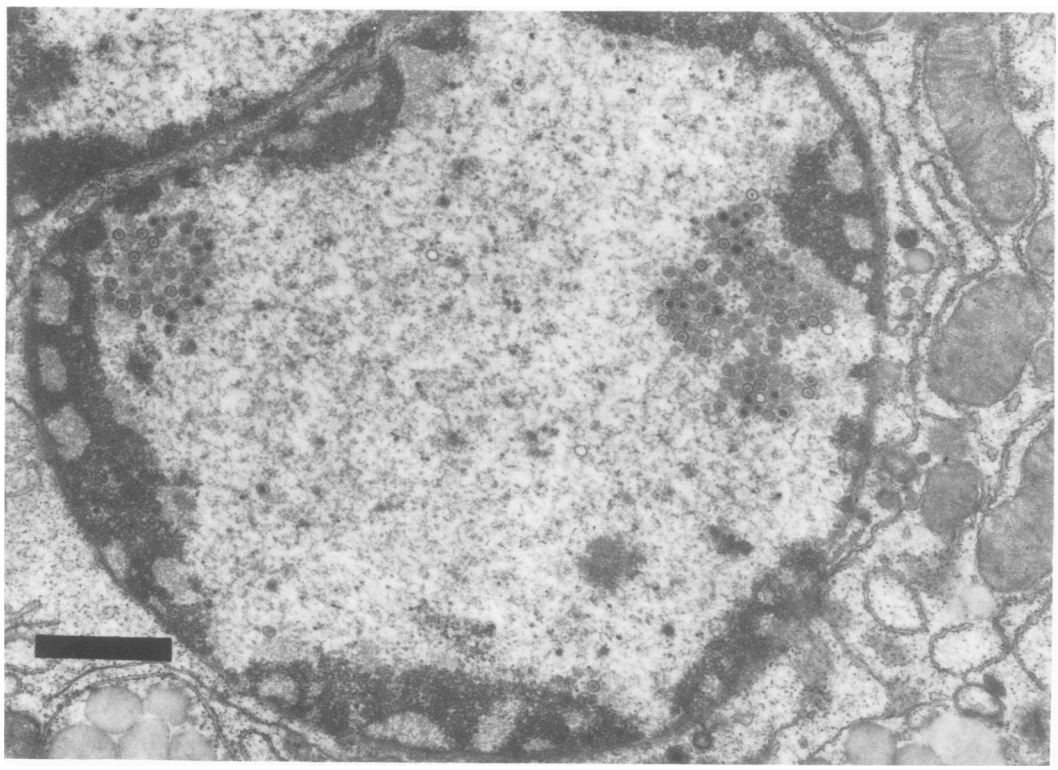

FIG. 2. *Two clusters of nonenveloped virions in the nucleus of a hepatocyte. Many free ribosomes are present in the cytoplasm, whereas only a few ribosomes remain attached to the rough endoplasmic reticulum. Chromatin is marginated. Bar = 1 μm.*

the parenchyma of the liver. Lesions were not present in liver or kidneys from HSV-1-infected mice or in kidneys from mice infected with HSV-2. Necrotic areas resulting from HSV-2 inoculation were similar to those described by Mogensen et al. (11) and consisted of a caseous center with dense accumulation of calcium salts and clusters of lipid droplets in hepatocytes peripheral to the lesion. The hepatocytes surrounding the lesion were more intensely stained than those in less involved areas. Intranuclear inclusions were also found occasionally in peripheral cells. Electron microscopy of liver cells revealed swollen mitochondria, glycogen depletion, and proliferation of the rough endoplasmic reticulum. Free ribosomes were more numerous than in normal hepatocytes. Nuclei contained marginated chromatin and nonenveloped virions (Fig. 2). Enveloped virions were found in the cytoplasm (Fig. 3) and in intranuclear pouches formed from the inner nuclear membrane (Fig. 4). Figure 5 shows that there was an infiltration of an electron-dense material (presumably calcium salts) into the degenerate mitochondria.

The pooled sera from groups of 10 mice infected i.v. with HSV-1 or HSV-2 were assayed for SGPT and SGOT (Fig. 6). Increased enzyme activities were detected 72 and 96 h after HSV-2 inoculation and decreased at 120 h. The changes in enzyme activity correlated well with the HSV-2 virus titers in the liver. After HSV-1 inoculation, there appeared to be a decrease in the SGOT activity through 72 h, followed by a gradual increase. The SGPT activity did not change in response to HSV-1 inoculation. The ranges of SGPT and SGOT levels in the pooled sera of noninfected mice from six separate experiments were 17 to 35 and 93 to 197 U/ml, respectively.

DISCUSSION

Except for the intravaginal route of virus inoculation, other routes of virus inoculation with HSV-2 produced a severe liver disease that was characterized by focal necrotic lesions. All other organs appeared normal. That the production of liver disease is specific for HSV-2 is shown by the fact that eight different strains of HSV-2 inoculated i.v. resulted in focally ne-

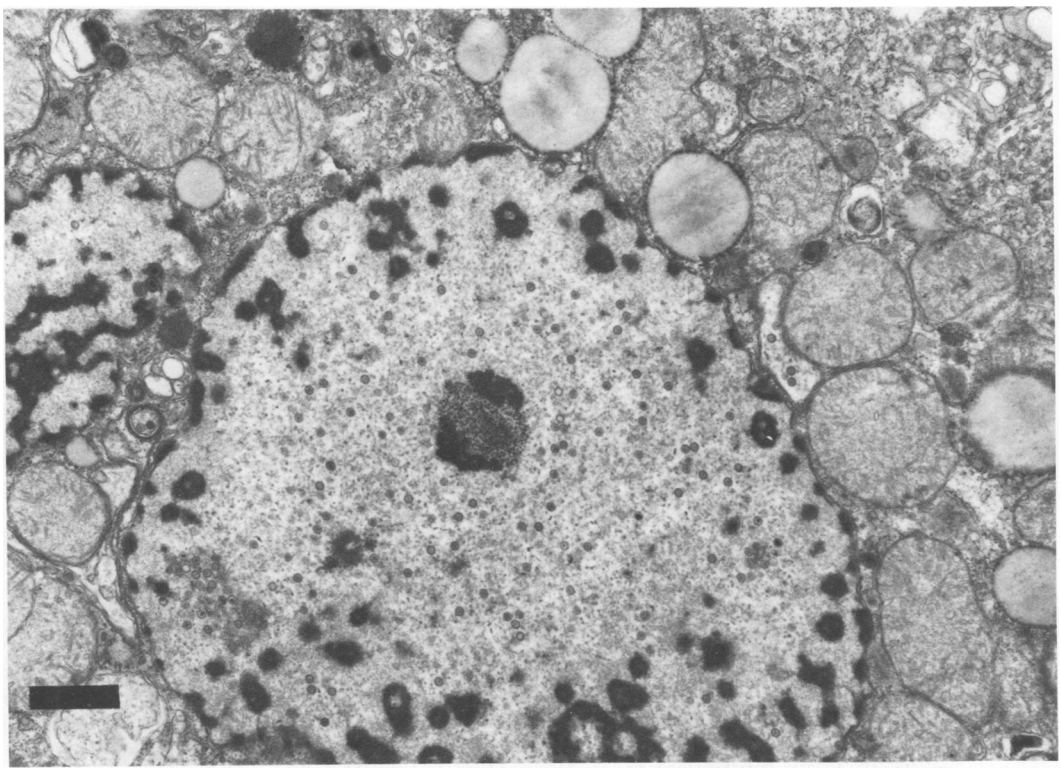

FIG. 3. *Early phase of infection of a hepatocyte. Features to be noted are chromatin clumping and margination, presence of nonenveloped virions in the nucleus, lack of glycogen deposits, mitochondrial swelling, loss of mitochondrial matrix, and presence of numerous perinuclear lipid droplets. Bar = 1 μm.*

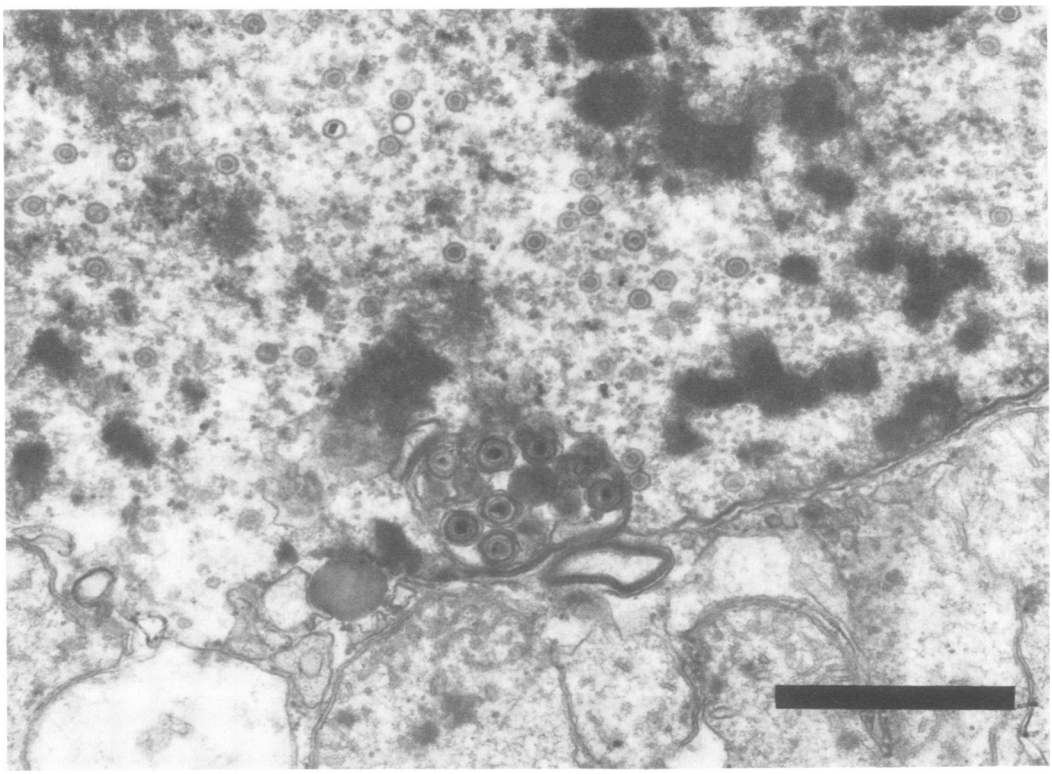

FIG. 4. *Portion of hepatocyte nucleus containing loose, nonenveloped virions. In the center is a sac or pouch formed from the inner nuclear membrane. This pouch contains enveloped virions, each of which consists of a central core plus three distinct membranes. Bar = 1 μm.*

crotic livers, whereas none of three HSV-1 strains produced any evidence of liver disease. Mogensen et al. (11) described a focal necrotic hepatitis in mice infected with HSV-2 that was characterized by a central amorphous eosinophilic mass surrounded by liver cells that contained typical Cowdry type A intranuclear inclusions typical of herpesvirus etiology and suggested this as a biological marker for differentiating HSV-1 from HSV-2 after i.p. inoculation. The replication of HSV-2 in hepatocytes has been confirmed by electron microscopy (Fig. 2 to 5).

In the present studies, virus was isolated from liver homogenates of mice inoculated i.v. or i.p. with HSV-2 and preceded the appearance of virus in spinal cord homogenates. Increases in serum transaminases, indicative of liver damage, were observed after HSV-2 but not HSV-1 inoculation. It has been shown (9) that intranasal inoculation of newborn mice with HSV-2 results in virus multiplication in the lung, which is then disseminated through the blood to the liver and spleen and finally to the brain by both viremia and neural transmission. Although no mention was made of visual changes occurring in the liver, virus has been recovered from the livers, adrenals, and brains of newborn mice from mothers who were infected intravaginally with HSV-2 after becoming pregnant (1).

The pathogenesis of HSV-1 and HSV-2 after intravaginal inoculation of female mice whose vaginas were preswabbed was quite similar with both types of virus and very much like that after the intravaginal inoculation of pregnant mice (13) or female hamsters with HSV-2 (18). Virus was isolated from the vaginas of all mice during the initial 5 days and from the central nervous system, with virus recovery from the spinal cord of four of five mice on day 4 after HSV-2 inoculation and three of five mice on day 5 after inoculation with HSV-1. Virus isolations from liver or kidney after intravaginal inoculation were infrequent, and evidence of viremia was not detectable with either HSV-1 or HSV-2. These data are in agreement with those previously presented on the intravaginal inoculation of pregnant mice with HSV-2 (13).

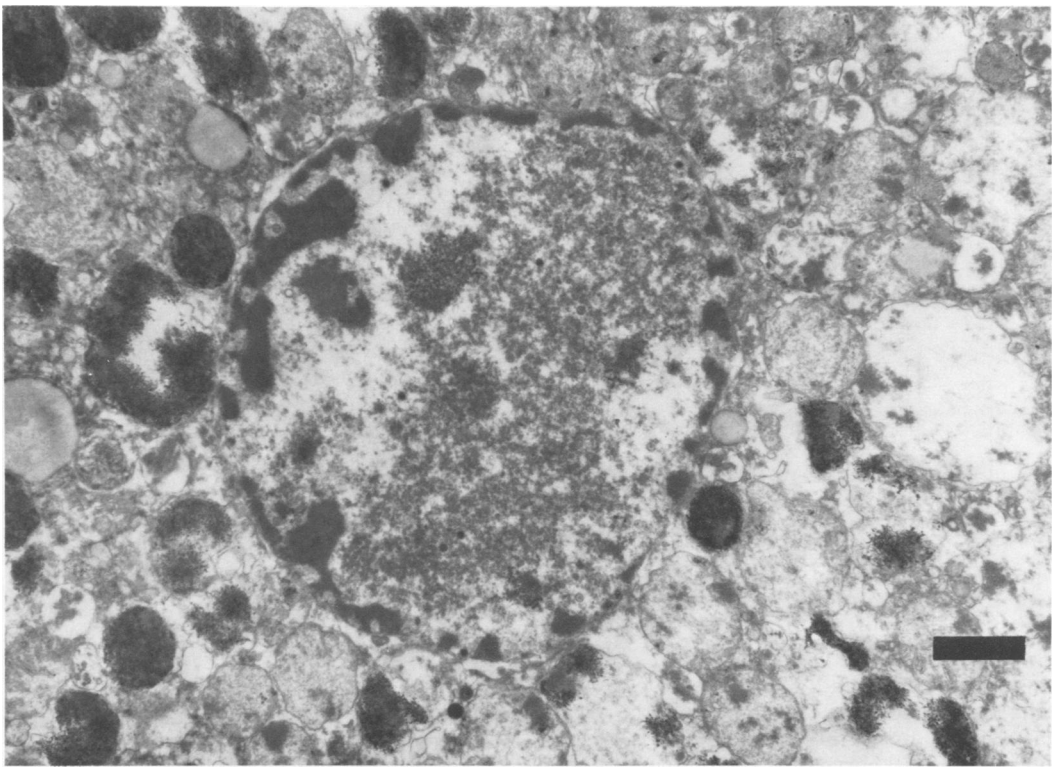

FIG. 5. *Early phase of infection of a hepatocyte. Note chromatin margination, perinuclear lipid droplets, mineralization of mitochondria, and almost complete loss of cytoplasmic organization. Bar = 1 μm.*

When HSV-1 was inoculated i.p., virus was isolated from the kidney during the 7-day observation period, but histological changes were not apparent. Virus was isolated from the liver on day 1 and thereafter it cleared. Virus invasion of the central nervous system (spinal cord) began on day 2. In this regard, the pathogenesis of HSV-1 after i.p. inoculation was similar to that described previously (16). HSV-2 inoculated by the same route produced hepatitis, as demonstrated by virus isolations from the livers of all mice on day 4 and most mice at earlier times. HSV-2 isolations from the kidney were only infrequent, but by day 7 virus could be detected in the spinal cords.

Johnson (8) showed that virus antigen was largely limited to peritoneal macrophages after i.p. inoculation of herpesvirus. This "primary" barrier due to peritoneal macrophage could be overcome with high virus inocula, leading to subsequent infection of liver, spleen, and adrenal cortex, with subsequent invasion of the central nervous system. Antithymocyte serum enhanced HSV-1 infection after i.p. or intravaginal inoculation of mice (12), resulting in enhanced brain virus titers and encephalitis (23). If the mice were pretreated with silica or antimacrophage serum prior to i.p. inoculation, high and persistent virus titers were present in the livers together with severe, necrotizing hepatitis (23). These data, together with those showing that the transfer of adult macrophages protected young mice from i.p. inoculation with herpes simplex virus (7), suggest that the macrophage plays an important role in determining the outcome of herpesvirus infection. Destruction of macrophages by silica has been shown to increase the susceptibility of mice to herpesvirus (5) and cytomegalovirus (20) infection. It has been shown (21) that virus replicates in macrophages from young mice but not in those from older animals. This restriction of virus replication is presumably due to an error in deoxyribonucleic acid metabolism (21).

Treatment of mice with other immunosuppressive agents, such as cortisone (4) or cyclophosphamide (16), alters the pathogenesis of herpesvirus infections. Virus could be isolated from the livers of cortisone-treated animals (4), and inclusion bodies could be detected in the livers and adrenals of animals pretreated with cyclophosphamide (16). Increased virus titers

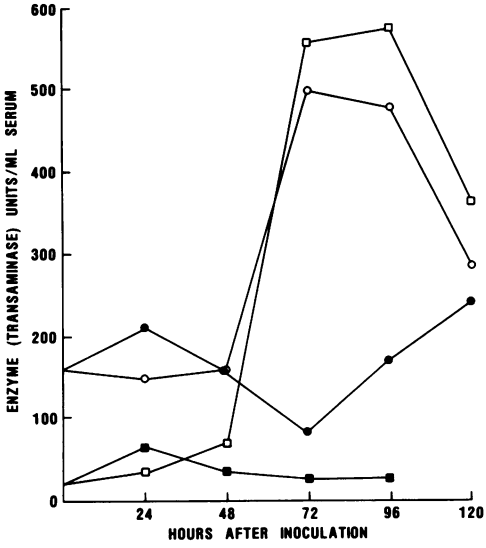

FIG. 6. *SGOT (circles) and SGPT (squares) activity in pooled sera from mice inoculated i.v. with HSV-1 (solid symbols) and HSV-2 (open symbols).*

were observed in the kidneys of cyclophosphamide-pretreated mice (16); however, the virus was confined to the interlobular connective tissue and to small nerve fibers accompanying the arcuate arteries. Cyclophosphamide treatment reduced the number of peritoneal macrophages as well as peritoneal lymphocytes (16).

From these studies, it is tempting to speculate that the invasion of the liver by HSV-2 but not HSV-1 may be due to a defect in the macrophage processing of HSV-2, allowing for the virus to replicate initially in the macrophage and establish foci of infection, leading to necrosis due to virus replication in hepatocytes and subsequently producing elevated serum transaminase levels.

It is of some interest to note that severe, disseminated herpesvirus infections in humans can involve the liver. For example, virus has been isolated from the liver of fatal HSV-2 infections of infants (6 and 8 days old) (2, 22). Two recent reports have described liver involvement of fatal varicella in an immunosuppressed 7 year old (19) and a fatal case of infectious mononucleosis in a 20 year old (3).

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