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Early adrenal infection by herpes simplex virus type-1 (Miyama + GC strain): special reference to inoculation dose and spread from the adrenal to the central nervous system

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Summary. Male C3H/HeN mice, aged 5 weeks, were inoculated intraperitoneally (i.p.) with different doses (1×10^3, 1×10^5, 5×10^5, 1×10^6 pfu) of the herpes simplex virus type-1 (HSV-1) (Miyama + GC strain). The LD_{50} of this virus was 10^2 pfu (i.p.) per mouse. All the mice in each group died 12 days after inoculation. Adrenal necrosis was found to be dose-dependent, the threshold dose being 5×10^5 pfu. In addition, encephalitis and inflammatory cell infiltration in abdominal ganglia appeared in 3–4 days after inoculation. By the plaque method, HSV-1 was detected first in the adrenal glands, then in neurons in the spinal cord and the brain. These findings suggest that in mice inoculated with doses of virus sufficient to infect the adrenal gland, HSV-1 spreads to the central nervous system through peripheral nerves after replication in the adrenal.

Key words: HSV-1 – Adrenal gland – Adrenal infection – Central nervous system

Introduction

It is believed that primary infection with herpes simplex virus (herpes virus hominis type-1; HSV-1) is usually followed by latent infection of various ganglia and that subsequent episodes of herpetic disease are due to reactivation of this latent infection (Burnet and Williams 1939; Scott 1954; Ellison et al. 1959; Baringer and Swoveland 1973; Stevens 1977; Corey and Spear 1986).

The strong affinity of herpes virus for tissues of ectodermal origin is reflected in the involvement of the central nervous system, peripheral ganglia, cornea and skin in herpetic infections (Frenkel 1960; Goodman et al. 1986). HSV-1 infection of the adrenal gland would be anticipated since the adrenal medulla is of the same embryological derivation as nervous tissue, and there are, in fact, many case reports of adrenal involvement in neonates, cancer patients and immunosuppressant adults with fulminant HSV-1 infection (Hass 1935; Bird and Garden 1959; Baharani et al. 1966; Haynes and Azimi 1968; Major and Foley 1970; Rosen and Hajdu 1971; Buss and Scharyj 1979; Goodman et al. 1986).

However, few experimental studies of extraneural herpetic infection with adrenal involvement have been undertaken (Goodpasture and Teague 1923; Smith 1931), compared with those concentrating on infection of the nervous system (Johnson 1964; Rabin et al. 1968; Baringer and Criffith 1970; Lascano and Berria 1980). Recently, some detailed studies of herpetic infection of the mouse adrenal gland have been published (Hill et al. 1975; Nachtigal and Caulfield 1984; Hill et al. 1986; Potratz et al. 1986), but questions such as the necessary inoculation dose of virus have remained unanswered.

In the present study, we have examined the pathological changes in the adrenal glands following intraperitoneal (i.p.) inoculation of HSV-1 in

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mice. We have obtained reliable new information on the specific relation between the amount of HSV-1 injected and pathological findings in the adrenal gland, as well as establishing a possible pathway for the spread of HSV-1 after replication in the adrenal.

Materials and methods

Animals. Five-week-old male C3H/HeN mice were used.

Virus. HSV-1, Miyama +GC strain (Nii and Kumahora 1961), was supplied by Professor Kumagai, Tohoku University, School of Dentistry, in Sendai. In our laboratory, the virus was passaged several times in green monkey kidney (GMK) cells and stored at −80° C.

Virus inoculation. The mice were inoculated i.p. with 0.2 ml medium containing 1×10^3, 1×10^5, 5×10^5 and 1×10^6 plague forming units (pfu). The control mice were inoculated i.p. with 0.2 ml saline.

Survival time. The mice were divided into four groups. Groups A and B consisted of 12 mice each, Group C of 16 mice and Group D of 15 mice. Each group received a different concentration of HSV-1; 1×10^3 pfu per mouse for group A, 1×10^5 pfu per mouse for Group B, 5×10^5 pfu per mouse for Group C and 1×10^6 pfu per mouse for Group D. The survival time was examined in each group.

Histological examination. The progression of the disease was observed daily in all four groups of animals. In Groups A and B, three mice were sacrificed at 1, 3, 5 and 7 days after inoculation. In Groups C and D, three or four mice were sacrificed at 1, 2, 3, 4 and 5 days after inoculation. The brain, spinal cord, ganglia, eyeball, liver, lungs, alimentary tract, heart, kidney, adrenal gland and testis were removed, fixed in 10% buffered formalin, embedded in paraffin and sections cut at 2 μm thickness. After haematoxylin-eosin (H&E) staining, the sections were observed by light microscopy.

The chi-square test was used for examining the relation between the frequency of pathological changes and inoculation concentration of HSV-1.

Immunohistological examination. Samples of the same tissues used in the histological study were sectioned and processed for immunohistological examination (peroxidase-antiperoxidase method). The specimens were digested with 0.25% trypsin in phosphate-buffered solution (PBS) for 30 min at 37° C. This was followed by the application of rabbit anti-HSV-1 serum, swine antiserum to rabbit immunoglobulin and PAP complex (horseradish peroxidase-rabbit horseradish peroxidase; Dakopatts, Accurate Chemical and Scientific Corp., USA). The control preparations consisted of HSV-1 infected and non-infected GMK cells, and infected and non-infected human lung tissue.

Assay of virus titre in organs. Mice were inoculated with 5×10^5 pfu per animal as described above. At 1, 4, 8, 12, 24, 48, 96 and 120 h after inoculation, three mice for each time point were killed. The brain, spinal cord and adrenal glands were

Table 1. Mean survival days of C3H/HeN mice in each group after intraperitoneal (i.p.) inoculation of HSV-1

Groups (pfu[a] per mouse)	Mean survival (days)
Group A (1×10^3)	6.4
Group B (1×10^5)	5.8
Group C (5×10^5)	4.7
Group D (1×10^6)	4.0

[a] Plaque forming units

Table 3. Progression of pathological changes in each group of mice after i.p. inoculation of HSV-1

		Days after i.p. inoculation						
		1	2	3	4	5	6	7
Adrenal necrosis	Group A	0/3[a]		0/3		0/3		1/3
	Group B	0/3		1/3		1/3		0/3
	Group C	0/3	3/3	2/3	3/3	3/3		
	Group D	0/3	2/3	3/3	3/3	3/3		
Inflammatory cell infiltration in abdominal ganglia	Group A	0/3		0/3		0/3		2/3
	Group B	0/3		1/3		2/3		2/3
	Group C	0/3	0/3	2/3	2/3	3/4		
	Group D	0/3	0/3	1/3	2/3	3/3		
Meningo-encephalitis	Group A	1/3		0/3		1/3		0/3
	Group B	0/3		0/3		2/3		2/3
	Group C	1/3	0/3	0/3	1/3	3/4		
	Group D	0/3	0/3	0/3	1/3	2/3		

[a] Values are expressed as the ratio of the number of mice showing pathological changes to the total number examined

Table 2. The frequency of pathological changes in the four groups of mice after i.p. inoculation of HSV-1

	Group A	Group B	Group C	Group D
Meningoencephalitis	2/12 (16.7)	4/12 (33.3)	5/16 (31.3)	3/15 (20.0)
Pneumonitis	9/12 (75.0)	4/12 (41.6)	5/15 (33.3)	3/15 (20.0)
Adrenal necrosis	1/12 (8.3)	2/12 (16.6)	11/15 (73.3)*	11/15 (73.3)*
Peritonitis	2/12 (16.7)	5/12 (41.7)	7/15 (43.7)	6/15 (40.0)
Lymphocyte infiltration in abdominal ganglia	2/12 (16.7)	5/12 (41.7)	7/15 (43.7)	6/15 (40.0)
Hepatitis	0/12 (0.0)	2/12 (16.7)	3/16 (18.7)	1/15 (6.7)

Numbers in parentheses indicate percentage of pathological changes

* Statistically significant ($\chi^2 = 20.06$, $P < 0.01$)

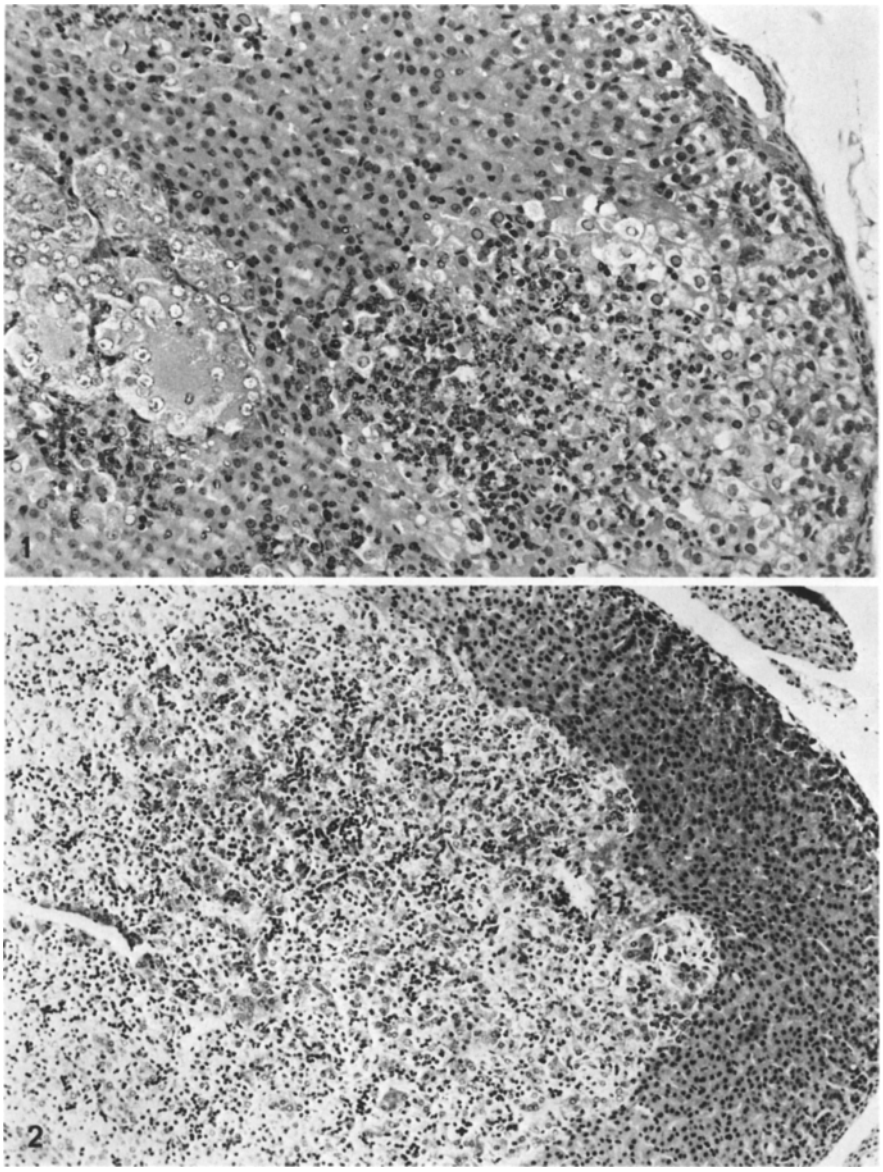


Fig. 1. Focal necrosis of the adrenal cortex extending the medulla 3 days after intraperitoneal (i.p.) inoculation of 5×10^5 pfu of HSV-1 (Group C). H&E, ×220

Fig. 2. Marked lymphocyte infiltration of the adrenal medulla without cortical necrosis, so-called adrenalitis, 4 days after i.p. inoculation of 5×10^5 pfu of HSV-1 (Group B). H&E, ×110

removed aseptically. After homogenization using a glass homogenizer, the tissues were sonicated. The supernatant obtained by centrifugation at 2500 rpm for 5 min was used as a primary solution. GMK cells were cultured on a 24-well plate to form a monolayer. The GMK monolayer was inoculated with serial dilutions of the viral solutions for 1.5 h. They were then maintained in minimum essential medium (MEM) containing 0.4% methylcellulose and cultured for 48 h. After staining with crystal violet, the number of pfu was counted.

Results

All the mice in all four groups became ill and died 3–12 days after i.p. inoculation of HSV-1. The mean survival time shortened from 6.4 to 4.0 day with the increase in dose from 1×10^3 to 1×10^6 pfu (Table 1).

Histologically, relatively high frequencies of peritonitis and pneumonitis were noted in Groups A and B. The frequency of adrenal necrosis was significantly higher ($P < 0.01$; Table 2) in Groups C and D than in Groups A and B. As shown in Table 3, adrenal necrosis occurred in almost all the mice in Groups C and D from 2 days after i.p. inoculation of HSV-1. The necrosis mainly involved the cortex, but occasionally also involved the medulla; the density of the associated inflammatory cell infiltration increased with time. Up to day 3 after inoculation, necrosis ranged from the zona fasciculata to the zona reticularis and extended to the medulla. Numerous intranuclear inclusion bodies were observed at the periphery of the necrotic zone. In the centre of the necrotic ar-

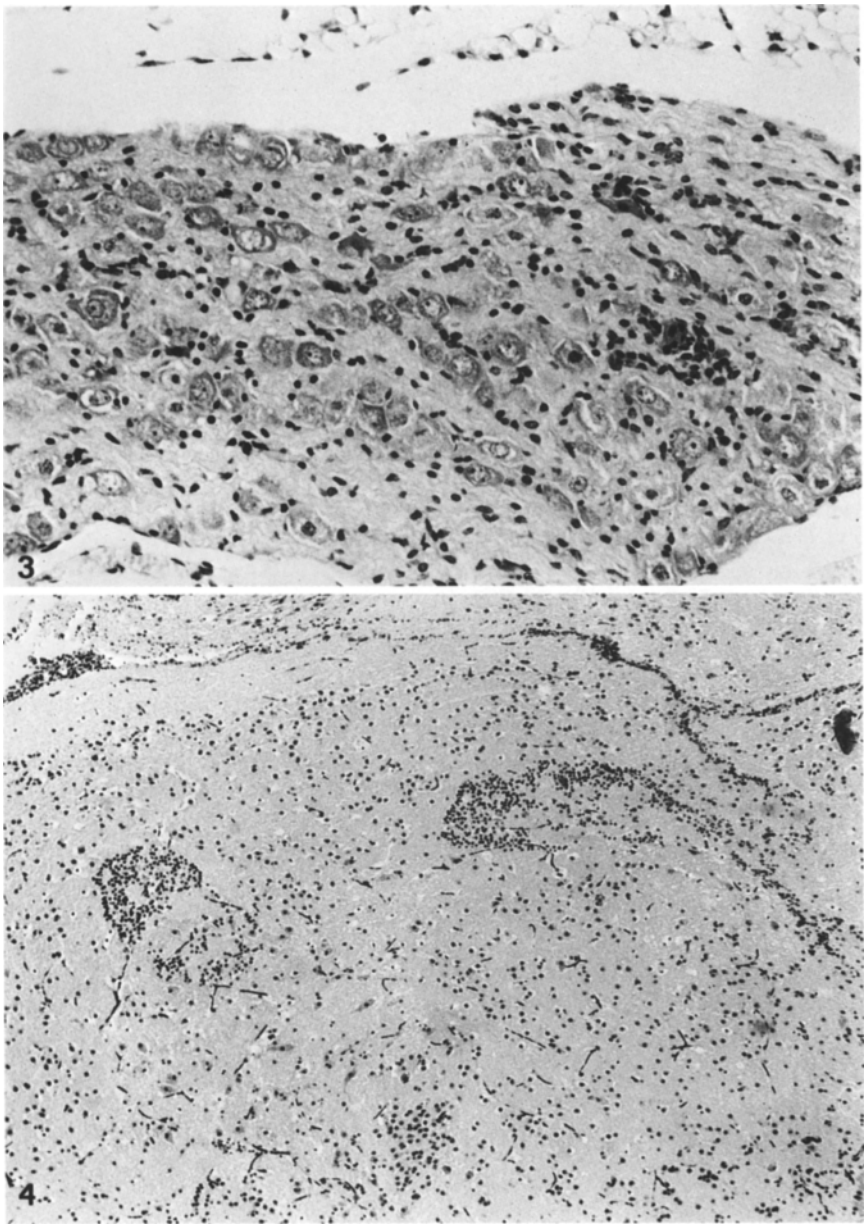


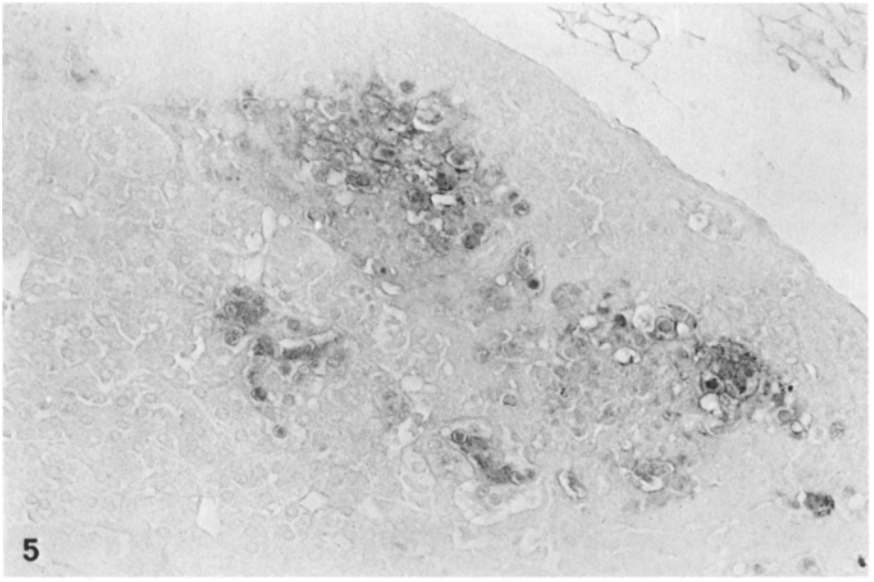
Fig. 3. Lymphocyte infiltration of abdominal ganglion. Third day after i.p. inoculation of 5×10^5 pfu of HSV-1 (Group C). H&E, ×220

Fig. 4. Herpes simplex encephalitis in a mouse 4 days after inoculation of 5×10^5 pfu of HSV-1 (Group C). H&E, ×110

eas, karyolysis and pyknosis were prominent (Fig. 1). The appearance of adrenal necrosis in Groups A and B occurred a day or two after that in Groups C and D. Furthermore, in two animals from Group B, a marked inflammatory cell infiltrate in the medulla without cortical necrosis, so-called adrenalitis, was observed (Fig. 2). Lymphocytic infiltration of abdominal ganglia (Fig. 3) appeared from 3 days after inoculation in Groups C and D (Table 3). Meningoencephalitis characterized by parenchymal and meningeal mononuclear cell infiltration, perivascular lymphocyte cuffing and petechial haemorrhage was also noted (Fig. 4).

Though encephalitis appeared 1 day after i.p. inoculation in one mouse each in Groups A and C, in general, it appeared later than 4 days after inoculation (Table 3).

Immunohistochemically, a positive staining reaction for anti-HSV-1 was detected in areas of adrenal necrosis (Fig. 5). It was positive in both the nucleus and the cytoplasm of cortical cells. Positive HSV-1 staining was also present in the renal tubular epithelium in three mice from Group C and four mice from Group D. HSV-1 positivity in the nucleus and cytoplasm of nerve cells in the spinal cord and spinal ganglia was present in one mouse each from Groups C and D, 5 days after inoculation (Fig. 6).



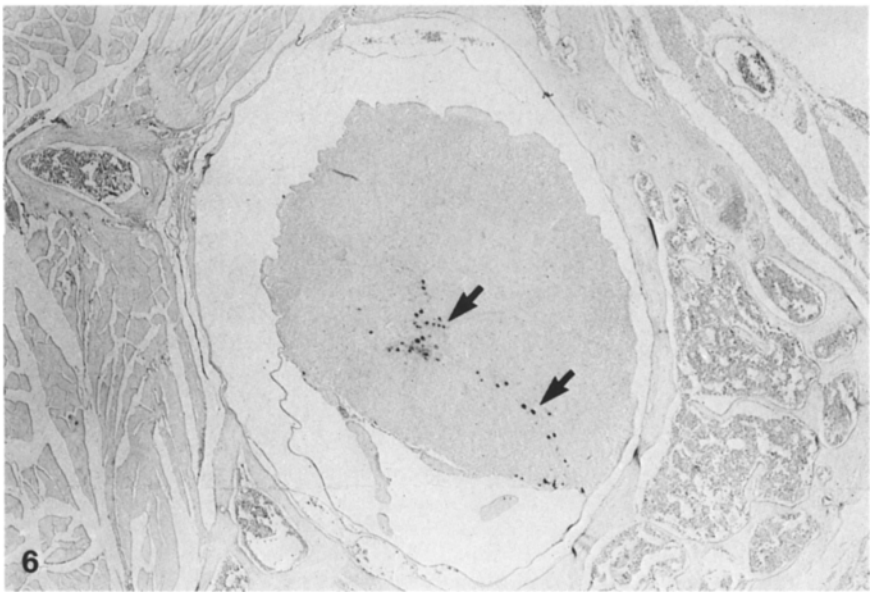


Fig. 5. Positive reaction of HSV-1 antigen in the adrenal necrosis in a mouse on 3rd day after i.p. inoculation of 5×10^5 pfu (Group C) of HSV-1. PAP stain, ×220

Fig. 6. Numerous nerve cells showing strong positive reaction for HSV-1 antigen (*arrows*) in the section of the spinal cord in a mouse on 5th day after i.p. inoculation of 5×10^5 pfu (Group C) of HSV-1. PAP stain, ×50

Pathological changes, such as necrosis, giant cell formation, lymphocyte infiltration etc., were not observed in the spinal cord and the renal tubuli. By the plaque method, the HSV-1 titre peaked after 3 days and then declined in the adrenal, whereas in the spinal cord and brain the HSV-1 titre began to increase after 3 and 4 days respectively.

In the control group, there were no appreciable changes in the various organs examined.

Discussion

Nachtigal and Caulfield (1984) observed adrenal necrosis after intranasal inoculation of HSV-1 and Kapoor et al. (1982) demonstrated replication of HSV-1 in the adrenal of nu/nu mice. Hill et al. (1986) detected HSV-1 of the relatively low virulence strain P_2C_6 in the adrenal 15 min after intravenous inoculation. Potratz et al. (1986) also identified HSV-1 in the adrenal gland of mice 1 h after i.p. inoculation and found histological evidence of adrenal necrosis 2 days after inoculation. However, the relation between the inoculation dose of HSV-1 and adrenal necrosis remains unclear. We have shown the adrenal gland to be a primary site of acute HSV-1 infection (Irie et al. 1986) and clarified the relationship between the appearance of specific adrenal necrosis and viral dose, 5×10^5 pfu being considered as the threshold dose for infec-

tion. In the present study, adrenal necrosis in groups A and B was less frequent and occurred a day or two later than in Groups C and D. Furthermore, HSV-1 was not identified in the adrenal gland of most mice in Groups A and B, by the plaque method (data not shown).

The relation between the number of cells sensitive to HSV-1 in the adrenal and the amount of virus may be an important factor. However, if HSV-1 infection of the adrenal gland is related solely to its ectodermal origin, similar necrosis indicating viral replication should occur in abdominal ganglia and other neural tissues. In fact, Slavin and Berry (1943) found inclusion bodies and necrosis in a few neurons and glial cells in mice infected with herpes virus. We also demonstrated HSV-1 antigen in the neural cells of the spinal cord and ganglia and observed inflammatory cell infiltration in abdominal ganglia. However, these changes were far milder than those seen in adrenal gland. This suggests that the structural peculiarity of the adrenal, which consists of cortex of mesodermal origin and medulla of ectodermal origin, may have a bearing on the changes observed. It is possible that the lining cells of the adrenocortical sinus may have a phagocytic function or that the high concentration of glucocorticoid hormones in the cortex may be well suited for viral replication (Goodpasture and Teague 1923; Frenkel 1960; Hill et al. 1986).

We isolated HSV-1 from the adrenal gland, spinal cord and brain during the progression of the disease. As shown in Fig. 7, peak levels of HSV-1 in the adrenal gland, spinal cord and brain occurred after different lengths of time. With regard to the histological findings and viral titres, our results give support to the theory of Hill et al. (1986) that axonal transport from the adrenal to the central nervous system, probably by the sympathetic nerves, in animals inoculated with a massive dose of virus. On the other hand, the occurrence of encephalitis 1 day after inoculation suggests the possibility of haematogenous spread of HSV-1 to the brain.

It is also possible that the adrenal gland is a site of latent HSV-1 infection, as reported by Cook and Stevens (1976). These authors isolated HSV-1 from the adrenal medulla of immunocompetent mice after intravenous infection by co-cultivation. As shown in Fig. 2, the marked lymphocytic infiltration observed in the adrenal medulla resembles the changes in trigeminal ganglion reported by Ishizaki (1972) and Warren et al. (1978a, b) in latent HSV-1 infection. Similar inflammatory cell infiltration of the adrenal medulla is occasionally seen in human cases at autopsy. In consideration

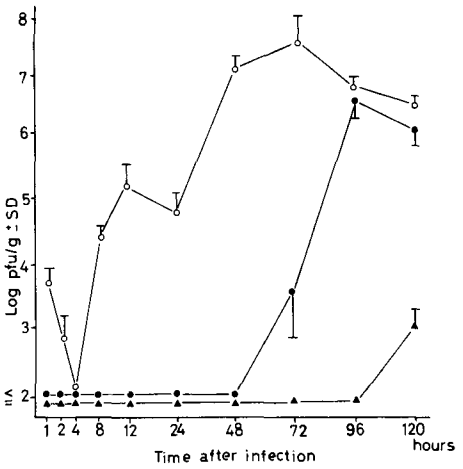


Fig. 7. Replication of HSV-1 in the adrenal (o), spinal cord (•) and brain (▲) after i.p. inoculation of 5×10^5 pfu of HSV-1

of these findings, the human adrenal gland requires detailed investigation as a site of latent infection of HSV-1 as well as HSV-2.

Adrenal necrosis was considered to be the cause of death in the mice studied. However, healing may be possible in mice with less severe necrosis, as observed by Nachtigal and Caulfield (1984).

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