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Leptomeningitis and Polycaryocyte Formation in the CNS of Rats Inoculated Subcutaneously with Herpes Simplex Virus

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Wistar rats were inoculated subcutaneously with either type 1 (HSV_1) or type 2 (HSV_2) Herpes simplex virus at 5 days of age. Animals were killed in extremis or at the end of the 14-day observation period postinoculation. Acute destructive meningoencephalitis with hemorrhage and leukocytic infiltration was observed in both groups. Polycaryocytes comprised of cells of the internal granular layer of the cerebellum were observed in some animals inoculated with HSV_1. These multinucleated cells appeared to be formed by fusion of virus-infected cells, and intranuclear inclusion bodies were observed. Lesions in the leptomeninges were particularly striking in animals inoculated with HSV_2. Viral replication in resident cells of the leptomeninges was demonstrated by electron microscopy.

INTRODUCTION

A variety of patterns of diseases including encephalitis are associated with herpes simplex virus infections. In developed countries of the world, herpes simplex virus is considered to be the single most important cause of fatal encephalitis (Longson, 1973). These cases are usually associated with type 1 herpes simplex (HSV_1) infection (Nahmias and Roizman, 1973), although type 2 herpes simplex virus (HSV_2) has been isolated from patients with herpetic leptomeningitis (Craig and Nahmias, 1973). In addition, about 50% of reported cases of neonatal herpes infection have had a concomitant encephalitis (Nahmias and Roizman, 1973). In one survey, approximately 70% of these clinical cases of neonatal herpes were attributed to HSV_2 and 30% were attributed to HSV_1 infection (Nahmias and Visintine, 1976). A variety of animal models have been used to study the pathogenesis of herpetic encephalitis (Nahmias and Roizman, 1973; Stevens, 1976). In this communication, we report on the characteristic lesions seen in suckling rats inoculated subcutaneously with HSV_1 or HSV_2.

MATERIALS AND METHODS

Animals and inoculation procedures. Wistar rats (Canadian Breeding Laboratories, Montreal) were housed in conventional shoebox-type cages at 72°F throughout the study. Rats were inoculated with one of the following strains of HSV: the Mayo strain (obtained from the Laboratory Center for Disease Control, Ottawa, Ontario) or the EX 1966 strain (isolated from a case of herpetic keratitis by L. A. Hatch) of HSV_1, and the MS or 075/72 (Percy and Hatch, 1975) strain of HSV_2. Animals were inoculated subcutaneously in the interscapular region at 5 days of age. Each animal received approximately 25–50 50% tissue culture infective doses ($TCID_{50}$) of virus, then were observed for a period of 2 weeks postinfection (pi). Clinically affected animals which died or were killed *in extremis*

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by chloroform inhalation were necropsied and tissues were routinely collected for light microscopy, fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin. In selected animals, brain tissue and eyes were collected, using aseptic technique, for viral isolation and titration studies. Tissues were also collected and fixed in 3% glutaraldehyde and processed for scanning (Cohen, 1974) or transmission (Percy *et al.*, 1980) electron microscopy. Only those litters with clinically affected animals, and with confirmed positive HSV isolations from one or more animals were included in the study. The animals reported in this communication were HSV-inoculated, nontreated control littermates of animals utilized in an antiviral treatment study. Viral culture, isolation, titration, and identification procedures have been previously described (Percy and Hatch, 1975; Nahmias *et al.*, 1971).

RESULTS

In general, clinically affected animals usually exhibited detectable nervous signs by 4 to 6 days pi and were killed *in extremis* at this time. In the HSV_1-inoculated rats, 8 of 23 animals survived; in the HSV_2-inoculated animals, 19 of 50 were clinically normal at 14 days pi and were killed for histological examination (Table I). At necropsy, there was no macroscopic evidence of cutaneous lesions at the site of virus inoculation, and there were no gross lesions evident in the visceral organs of animals studied in this series.

Virology and histopathology. In this study, titers of virus tended to be higher in specimens of brain sampled than in eyes. The titers varied from approximately 60 $TCID_{50}$/g to 10^{-4} $TCID_{50}$/g (Table I). In general, lesions in the CNS seen on histological examination were confined to animals which succumbed during the 14-day observation period pi (Table I). Lesions were observed in various areas of the prosencephalon, mesencephalon, and rhombencephalon, but tended to be concentrated in the mesencephalon and rhombencephalon in both the HSV_1- and HSV_2-infected animals. The intensity and appearance of lesions varied from foci of microgliosis to areas of acute hemorrhagic encephalitis characterized by destruction of grey and white matter and polymorphonuclear and mononuclear cell infiltration. Hypertrophy of endothelial cells lining vessels in affected areas, and margination of leukocytes within vessels were frequently observed. Eosinophilic

TABLE I
Experimental Herpetic Encephalitis in the Rat
Incidence of Lesions and Positive Viral Isolations

Type and strains of HSV	Mortality rate	Tissue examined	No. with lesions	Virology					
					Titers of virus (infectious particles/g)				
				No. positive	60	10	10	10	10
HSV_1 (Mayo or Ex 1966)	15/23 (65%)	Brain	9/23	3/4			1	1	1
		Eye	0/4	3/4	1		1	1	
HSV_2 (MS or 075/72)	31/50 (62%)	Brain	32/50	36/50	1	6	10	15	4
		Eye	2/50	18/32	7	1	5	3	2

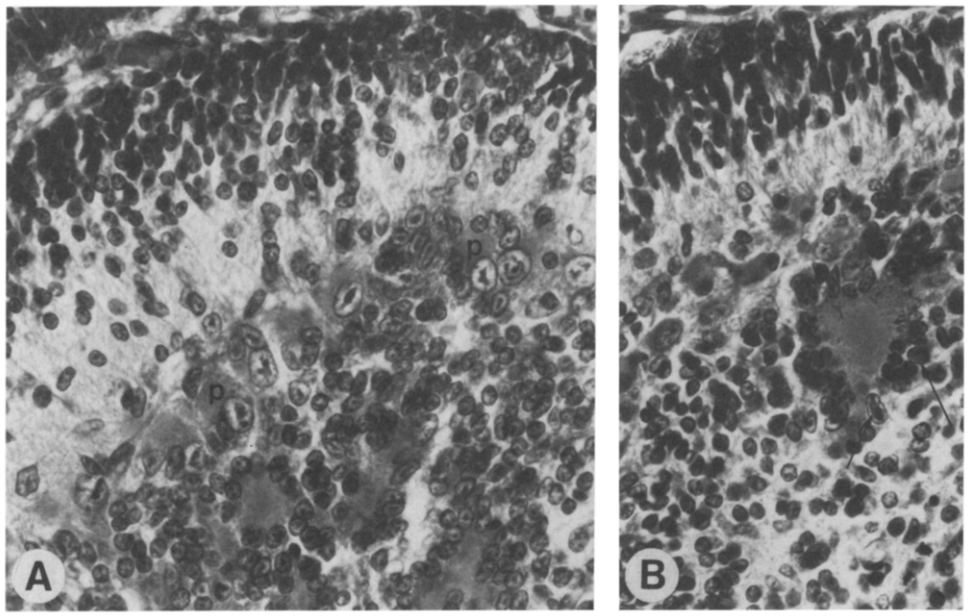

FIG. 1. Sections of cerebellum from two Wistar rats inoculated subcutaneously with the Mayo strain of type 1 Herpes simplex virus (HSV_1) at 5 days of age and examined at 4 days postinoculation (pi). Several polycaryocytes are present in the internal granular layer in 1A, (A), and one large polycaryocyte is illustrated in (B). Note the reactive Purkinje cells (P), and the intranuclear inclusion bodies (arrows) within polycaryocyte nuclei. Hematoxylin & Eosin stain, ×348.

to amphophilic, homogeneous to finely granular intranuclear inclusions were observed within astroglial cells and neurons in these areas. Lesions were frequently most extensive in the grey and white matter of the metencephalon. Segmental destructive encephalitis, with obliteration of the internal granular and molecular layers were particularly common in HSV_1-infected animals. Multinucleated giant cells were sometimes observed in HSV_1-inoculated rats. They were concentrated in the internal granule cell layer with extension into the underlying white matter, and were comprised of cell nuclei surrounding homogeneous to finely granular, eosinophilic cytoplasm. Intranuclear inclusions were present in some nuclei (Fig. 1). These syncytial giant cells were interpreted to have been derived from neurons of the internal granular layer.

Changes in the leptomeninges and associated vessels were most frequently observed in HSV_2-inoculated animals. These were most evident in the leptomeninges overlying the cerebellar folia and interpeduncular fossa. Lesions in these areas were characterized by marked hyperplasia and hypertrophy of fibroblasts and macrophages, polymorphonuclear and mononuclear cell infiltration, and the presence of erythrocytes and strands of densely eosinophilic, proteinaceous material (Fig. 2). Similar components were evident in selected specimens examined by scanning electron microscopy (Fig. 3). In tissues examined by transmission electron microscopy, polymorphonuclear cells, lymphocytes, and erythrocytes were interspersed within a network of reactive macrophages and fibroblasts (Fig. 4). In addition to identifiable strands of collagenous tissue, electron-dense fibrillar structures with the characteristic banding associated with fibrin deposition were observed (Fig. 4). Typical herpesvirus nucleocapsids were observed within the

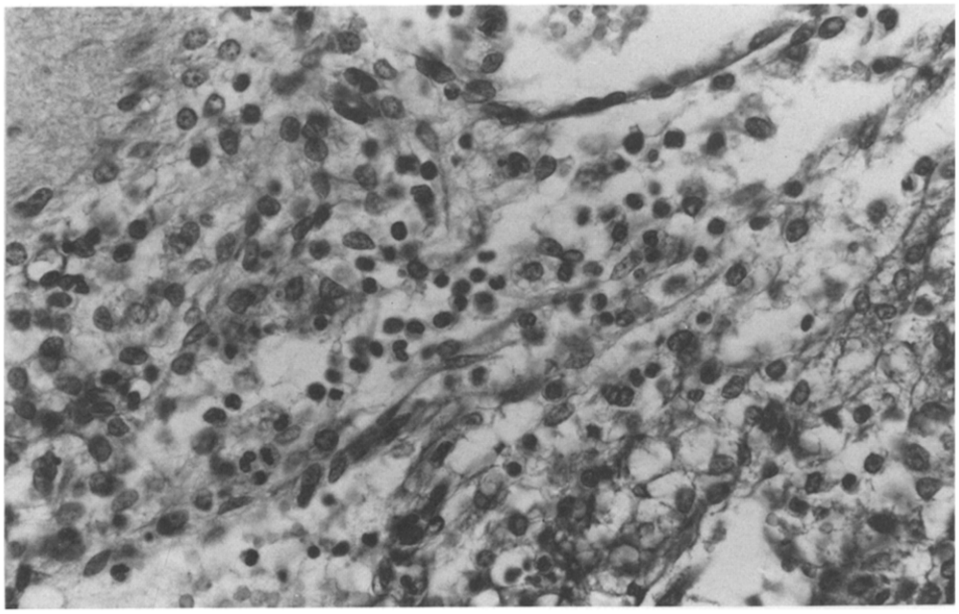

FIG. 2. Leptomeninges (interpeduncular fossa region of the cerebellum) in animal No. 204–8, inoculated with HSV_1 (Mayo strain) and killed at 4 days pi. There is hypercellularity, with concomitant mononuclear and polymorphonuclear cell infiltration. Hematoxylin & Eosin, ×352.

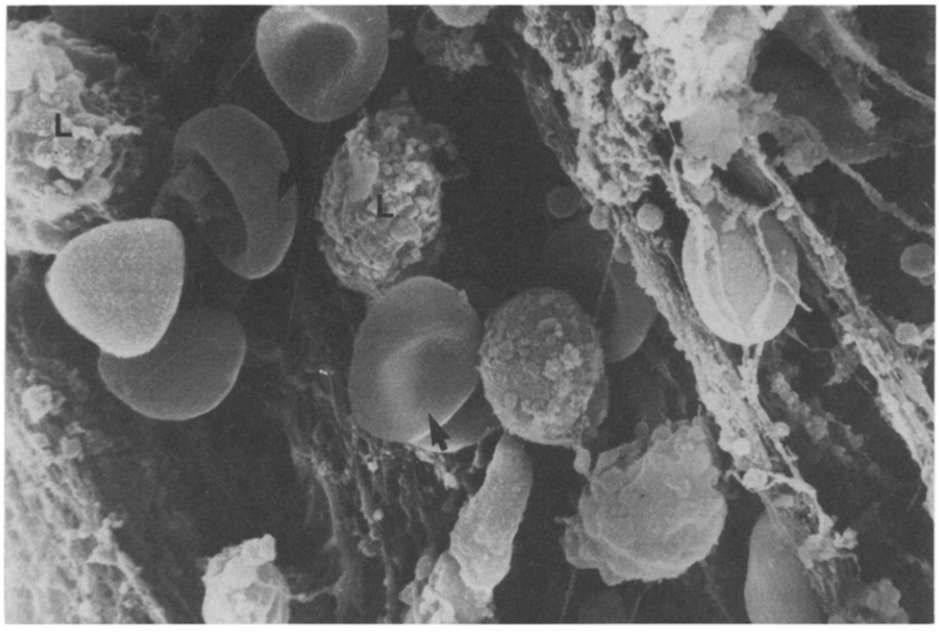

FIG. 3. Scanning electron photomicrograph of the leptomeninges (interpeduncular fossa region) from rat (No. 136–8) inoculated subcutaneously with the 075/72 strain of HSV_2, and killed in extremis at 5 days pi. Note the leukocytes (L), and the biconcave erythrocytes (arrows) enmeshed in strands of fibrinous exudate. ×14,100.

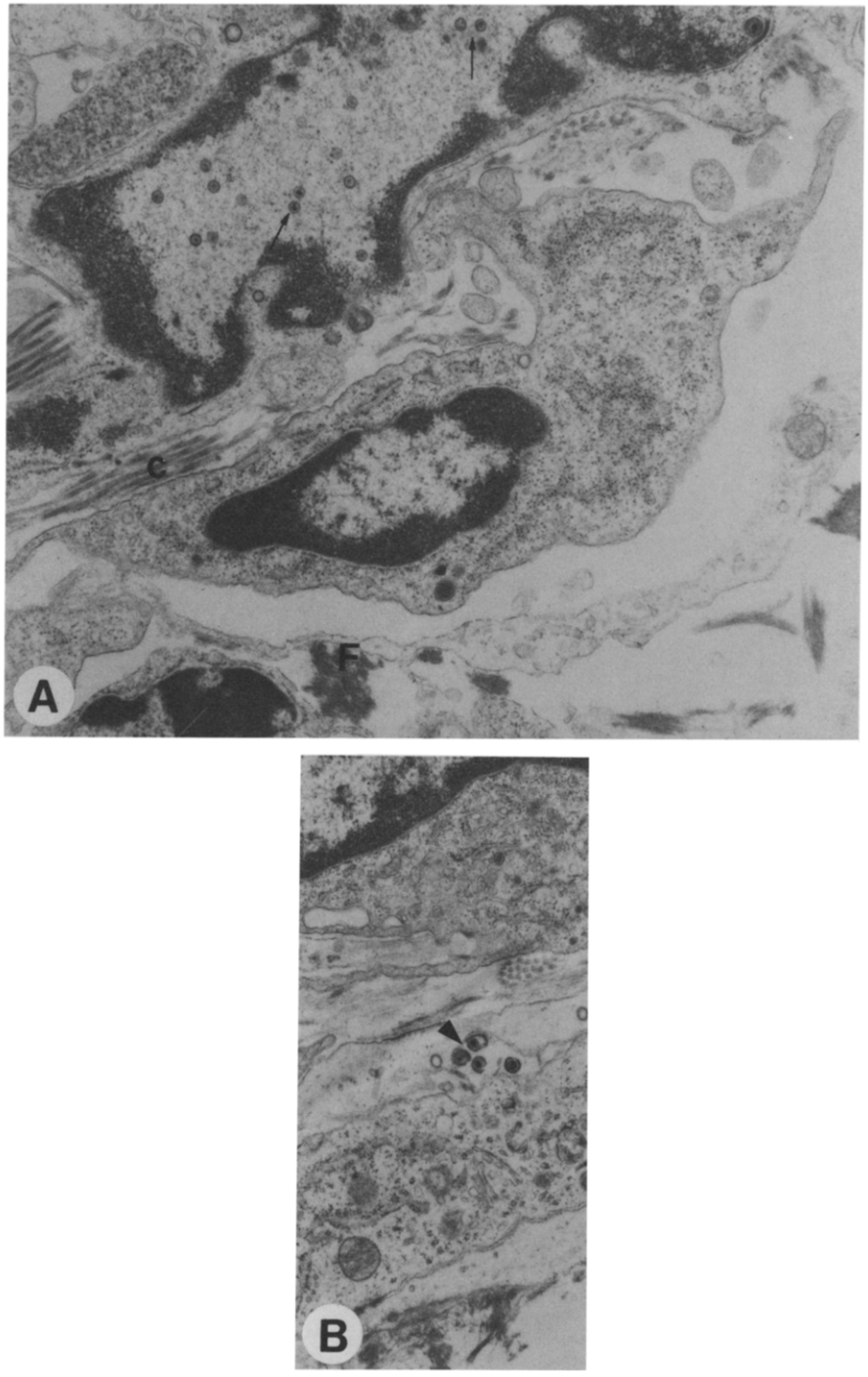

FIG. 4. Transmission electron photomicrographs of leptomeninges of animal No. 135–5, inoculated subcutaneously with the MS strain of HSV_2, and examined at 5 days pi. Typical herpes viral particles (arrows) are present within the reactive fibrocyte in (A). Note the variation in staining properties of collagen fibers (c), and the aggregations of electron-dense and banded fibrin strands (F). Several enveloped herpesvirus particles (arrowheads) are present in an intercellular space in (B). ×15,800.

nuclei of reactive fibroblasts, and enveloped viral particles were observed within intercellular spaces (Fig. 4). Viral particles were not demonstrated within infiltrating leukocytes. In other tissues examined by light microscopy, lesions were minimal to absent in eyes, lung, liver, kidney, and adrenal glands. Although the virus was isolated from the eyes of a number of animals in this study, there were relatively few animals with demonstrable ocular lesions (Table I). Focal iritis was most commonly observed, although focal retinitis and/or keratitis were also demonstrated on rare occasions.

DISCUSSION

The distribution of lesions in the CNS and the relative absence of ocular lesions in this study differed from a previous report, in which lesions were more extensive in the cerebrum and eye of rats of the same age inoculated intracerebrally with HSV_2 (Crawford *et al.*, 1979; Percy *et al.*, 1980). The failure to produce obvious herpetic vesicles following the subcutaneous inoculation of HSV_1 or HSV_2 was an unexpected finding. Cutaneous lesions were frequently observed in suckling rats inoculated intraperitoneally with HSV_2 (Percy and Hatch, 1975). Oh and Minasi inoculated newborn rabbits subcutaneously with either HSV_1 or HSV_2 and reported that severe skin lesions occurred only in the HSV_2-inoculated animals. Similarly, visceral, CNS, and ocular lesions were more common with HSV_2 than with HSV_1-infected animals (Oh and Minasi, 1980). On the other hand, in our study, extensive lesions were observed in the CNS in both HSV_1- and HSV_2-infected suckling rats, and lesions in the eye and viscera were sparse to absent. These variations may be a reflection of the strains of HSV utilized in the study, although the age at inoculation, and species variations between the rat and rabbit are among the other factors to be considered.

The presence of multinucleated giant cells seen in the cerebellar cortex of some animals inoculated subcutaneously with HSV_1 was of particular interest. These polycaryocytes were localized in the internal granular layer, and appeared to be comprised of neurons from this region. In the case illustrated in Fig. 1, the presence of virus was confirmed by the isolation of HSV_1 from the contralateral half of the sagitally sectioned brain. Multinucleated cells have been observed in other acute viral encephalitides. In hamsters inoculated intracerebrally with measles virus, polycaryocytes were observed in the hippocampus and cerebral cortex and were interpreted to be due to fusion of adjacent neurons (Baringer and Griffith, 1970). In another study, syncytial cells were observed in the CNS of mice inoculated intracerebrally with mouse hepatitis virus, a coronavirus (Lucas *et al.*, 1977). Depending on their location and appearance, multinucleated cells were interpreted to consist of astroglial cells, ependymal cells or inflammatory cells (Lucas *et al.*, 1977). On the other hand, the polycaryocytes seen in our study appeared to be confined to the cerebellar cortex, and were induced by a route other than intracerebral inoculation. It is known that a variety of enveloped viruses may induce syncytial giant cell formation by the alteration of cytoplasmic membranes and the fusion of virus-infected cells (Roizman, 1962). It has been suggested that the induction of polycaryocyte formation by measles virus may enhance viral spread within the CNS (Baringer and Griffith, 1970).

The presence of an acute destructive meningitis in numerous animals inoculated parenterally with HSV_2 is consistent with the clinical findings in human cases of

meningitis due to HSV. The virus isolated from these patients is normally HSV_2 and lesions have been attributed to blood-borne dissemination of the virus to the meninges (Craig and Nahmias, 1973; Nahmias and Roizman, 1973). In this study, virus was readily demonstrated within fibroblasts in the leptomeninges by electron microscopy. Thus, in this model, the virus appears to actively replicate *in situ* within resident cells of the leptomeninges.

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