Virus-Induced Hydrocephalus: Development of Aqueductal Stenosis in Hamsters after Mumps Infection

Abstract. *Hydrocephalus developed as a sequela of mumps virus infections of suckling hamsters. The initial infection after intracerebral inoculation was limited largely to ependymal cells lining the ventricles. This infection was clinically inapparent but later resulted in a noninflammatory stenosis or occlusion of the aqueduct of Sylvius.*

Hydrocephalus has occasionally been observed in experimental animals inoculated intracerebrally with viruses that cause chronic infection or induce neoplasia (*1*). However, virus-induced hydrocephalus resulting from aqueductal stenosis has not been described heretofore. Furthermore, unlike previous observations, the hydrocephalus caused by mumps virus was consistently reproducible and occurred not because of continued viral replication but as a noninflammatory sequela of a preceding, clinically inapparent infection. This experimental model was found by chance during studies of a neuroadapted strain of mumps virus that produces acute encephalitis in suckling hamsters (*2*). A nonadapted strain of mumps virus was inoculated intracerebrally into hamsters for a control. With few exceptions, these animals failed to show clinical signs of disease during the initial 2- to 3-week observation period, but over subsequent months severe hydrocephalus developed.

The nonadapted strain of mumps virus was isolated, in primary cultures of rhesus monkey kidney cells, from cerebrospinal fluid of a patient with mild encephalitis. This strain was passed once in similar cultures to provide seed virus for all studies. Virus was assayed in monkey kidney cultures by use of hemadsorption with washed red blood cells from guinea pigs to detect virus after 5 days of incubation. Syrian hamsters, 1 to 4 days old, were inoculated intracerebrally with 0.01 ml of inoculum containing 100 tissue culture doses of virus, 50 percent effective (TCD_{50}). Animals were killed at intervals for titration of the virus content of the brain, conventional histology, fluorescent antibody staining, and assay of serum neutralizing antibodies. Tissues for titration were ground in cold mortars and prepared as 20-percent suspensions for virus assays. Fluorescent antibody staining was performed on frozen sections of tissue with the use of guinea pig antiserum prepared against the egg-adapted (Enders) strain of mumps virus and an indirect method. Direct staining with fluorescein-conjugated rabbit antiserum against hamster globulins was also performed to detect fixation of homologous antibody. Neutralizing antibody was determined with serum inactivated at 56°C for 20 minutes and mixed with 100 TCD_{50}'s of virus at 37°C for 1 hour prior to inoculation of monkey kidney cultures. Control animals were inoculated with virus pretreated with guinea pig antiserum against the Enders type strain of mumps virus, which had never been propagated in simian cells. Multiple uninoculated monkey kidney cultures were included in all assays of virus and antiserum to control for hemadsorption by indigenous simian agents.

Growth of virus and the histologic changes resulting from it were limited to the central nervous system. Virus was not recovered from brains 24 hours after inoculation, but growth of virus was demonstrable on the 2nd day, maximum titers being reached by the 5th day. A decline of virus content to undetectable levels by the 9th day was coincident with the development of neutralizing antibody (Table 1). The most prominent histologic feature of this infection was perivascular inflammatory reactions of mononuclear cells. This was first detectable on the 4th day, reached a maximum on the 9th to 11th days, and resolved by the 14th to 16th days. Small perivascular hemorrhages, a denuding of ependymal cells lining the ventricular surfaces, and an increase in perivascular and subependymal microglia cells were found during the height of the inflammatory reaction. Fluorescent antibody staining showed virus antigen limited almost entirely to ependymal cells of the ventricles and choroid plexus. This fluorescence, in the form of small cytoplasmic granules, was first seen on the 3rd day, with a marked increase until the 7th to 9th days, when almost all ependymal cells showed confluent cytoplasmic fluorescence (Fig. 1). Very few neurons showed evidence of virus antigen, and no antigen was detected in glial or vascular endothelial cells. No fixation of homologous antibody was detected with the decrease in viral fluorescence.

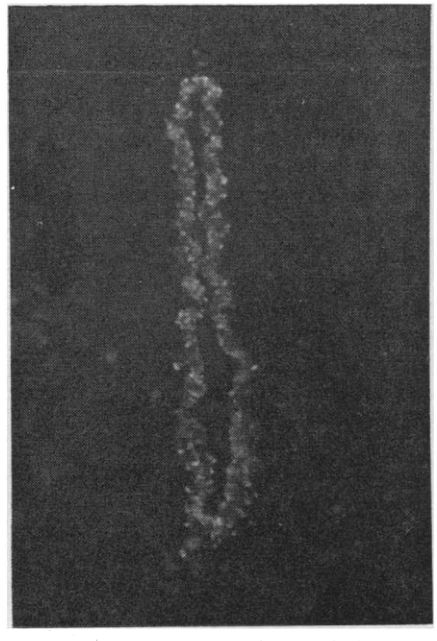

Fig. 1. Horizontal section of hamster brainstem, 7 days after intracerebral inoculation with mumps virus, stained with fluorescent antibodies. Virus antigen is limited to the ependymal cells lining the aqueduct. (× 100)

Signs of clinical disease only rarely accompanied this infection. Animals developed normally until the 3rd to the 6th weeks after inoculation, at which time the occipital curvature of the skull became prominent. Failure to gain weight and unsteadiness of movement usually accompanied the progressive bulging of the skull. Seizures were not observed. Gross dilatation of the lateral and third ventricles was first apparent after the 2nd week, and by the 3rd week after inoculation severe hydrocephalus was consistently found (Fig. 2). Dilatation of the fourth ventricle did not occur.

In order to study the course of disease, 14 litters of hamsters (86 animals) were inoculated and observed daily over a 3-month period. Four died during the period of acute infection. Of the remaining 82 hamsters, 78 (95 percent) developed clinical signs of hydrocephalus (between the 14th and 62nd days after inoculation), and 61 (74 percent) died during the observation interval (between the 2nd and 8th weeks after inoculation). All of the 21 animals that survived the 3-month observation period, including three that showed no clinical signs, had a marked degree of hydrocephalus when they were killed. Histologic studies, including serial sections of brains of 10 animals killed 21 to 90 days after inoculation, showed a marked loss of ependymal cells with an increase of astrocytic nuclei adjacent to the denuded ventricular and aqueductal surfaces.

Table 1. Course of the clinically inapparent mumps virus infection of the nervous system of suckling hamsters that had been inoculated intracerebrally with 100 TCD_{50}'s. +, Definite but mild reaction; ++, moderate; and +++, severe reaction.

Assay method	Days after inoculation							
	1	2	3	5	7	9	11	14
Virus content in $\log_{10}$ TCD_{50} per gram of brain	< 1.5	3.0	3.7	4.2	3.5	< 1.5	< 1.5	< 1.5
Intensity of perivascular inflammatory reaction	0	0	0	+	++	+++	++	+
Intensity and extent of virus antigen in ependymal cells	0	0	+	++	+++	+++	++	0
Neutralizing antibody (titer)				< 1 : 4	< 1 : 4	1 : 4	1 : 20	

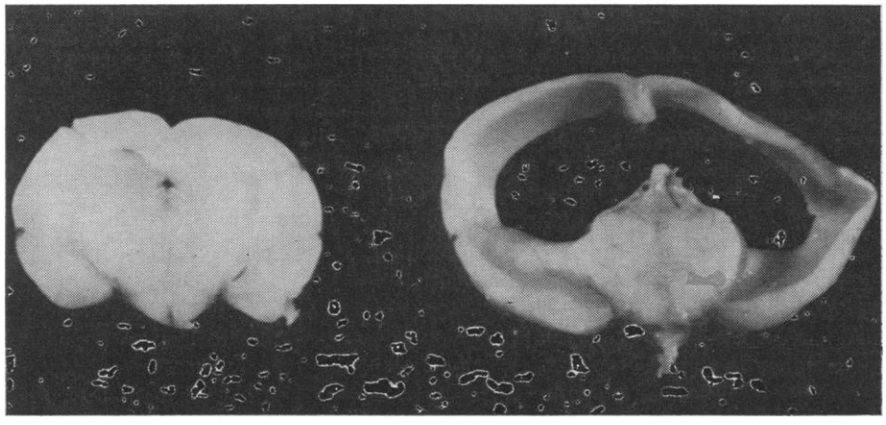

Fig. 2. Coronal sections of hamster brains 4 weeks after inoculation of mumps virus. Brain on left, inoculated with virus plus antiserum, appears normal; brain on right, inoculated with virus alone, shows typical hydrocephalus. (× 3)

Severe stenosis or total occlusion of the aqueduct of Sylvius was found in all cases; occlusions consistently occurred in the rostral portion of the aqueduct just caudal to the posterior commissure at the level of the superior colliculi.

Control animals, inoculated with virus mixed with antiserum against the Enders strain of mumps virus, developed neither perivascular inflammatory response nor antigen detectable by fluorescent antibody staining, and subsequently they developed neither clinical nor pathological evidence of hydrocephalus. These observations indicate that the infection and the sequela of hydrocephalus were not due to nonspecific effects of inoculation or to the presence of a simian agent in the inoculum. Two other strains of mumps virus, recently isolated from human cerebrospinal fluid, also induced hydrocephalus after intracerebral inoculation of 100 TCD_{50}'s, which indicates that this phenomenon was not peculiar to one strain of mumps virus.

The similarity of this disease to the aqueductal stenosis observed as a common cause of hydrocephalus in children and adults (*3*) suggests that the clinically inapparent infection of ependymal cells with a common respiratory virus might result in subsequent noninflammatory occlusion or stenosis of the aqueduct of Sylvius in man.

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Enclosed Bark as a Pollen Trap

Abstract. *Counts were made of pollen in traps formed by enclosed bark in two remnants of bristlecone pine,* Pinus aristata *Engelm., from the White Mountains of east-central California. The traps, dated by tree-rings at A.D. 350 and 1300 B.C., contained a major complex of pine-sagebrush pollen and traces of other species, representing the equivalent of the present vegetation.*

Sediments have been the primary source of pollen for palynological studies. Dating of sediments has been by radiocarbon determinations, extrapolation of sedimentation rates, or other methods. Another source of pollen of recent millenia is the enclosed bark or healed scars disclosed when bulk remnants of ancient bristlecone pine, *Pinus aristata* Engelm., are sectioned for tree-ring studies. The initiation and closure of such pollen traps can be dated by the tree-ring method. An analysis has been made of pollen from two such traps found in bristlecone pine trees from the White Mountains of east-central California.

Sample 1 was found in a section from a dead and downed snag in Methuselah Walk, a small valley containing 9 of the 17 trees over 4000 years old reported by Schulman (*1*). This remnant (TRL 63-43) grew at an elevation of about 2900 m (37°23′N, 118°10′W), in an area nearly devoid of understory vegetation. On adjacent slopes, however, grow relatively dense stands of curl-leaf mountain mahogany (*Cercocarpus ledifolius* Nutt.), big sage brush (*Artemisia tridentata* Nutt.), and Mormon tea (*Ephedra viridis* Coville).

The specimen contains tree rings dated from 3080 B.C. to A.D. 850. This high-quality ring record has been incorporated into the master chronology for the area, and units of dated wood have been distributed to three radiocarbon laboratories.

Bark formed over a scar area on the tree was subsequently trapped by the thumb-like lobe of wood which grew over it. The scar was formed about A.D. 311, and the bark grew and was trapped during the following century. Hence, any pollen included in the bark sample must date from A.D. 300 to A.D. 400.

Sample 2 was exposed in the cross section of a snag (VL 100) cut in 1963. The dead tree was located 150 m from the western margin of the bristlecone pine stand near the head of the North