Granular Ependymitis

Occurrence in Myxovirus Infected Rodents and Prevalence in Man

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Intracerebral inoculation of neuropathic and nonneuropathic strains of mumps virus into adult hamsters resulted in an acute focal infection of ependymal cells followed by focal ependymal denuding. Similar lesions followed defective infection with influenza A in both hamsters and mice; when a large amount of influenza A was inoculated, aqueductal stenosis and hydrocephalus developed in some adult animals as a sequela of widespread ependymal cell loss. A survey of 100 consecutive adult human brains showed that 65% had granular ependymitis which had many of the characteristics found in the focal ependymal lesions produced in rodents. These studies suggest that human CNS mumps infections may produce granular ependymitis (Am J Pathol 67:511–526, 1972).

THE EPENDYMA, which lines the cerebral ventricles and covers the choroid plexus, becomes recognizable early in fetal life and continues to be morphologically and functionally distinct from the underlying brain parenchyma.[1,2] Ependymal cells also appear to differ from other central nervous system (CNS) cells in their susceptibility to viral infection.

In previous studies, a neuropathic strain of mumps virus [3] specifically adapted to grow in the CNS of hamsters was shown to replicate widely in neurons as well as ependymal cells when inoculated intracerebrally into newborn animals.[4] This infection was fatal in 9 to 18 days. When a nonneuropathic strain of mumps virus was employed, the infection was limited to the ependyma and occasional neurons and was abolished with the appearance of neutralizing antibodies in serum. Although the ependyma was destroyed, few acute deaths occurred. Later, as a delayed sequela to the acute infection, stenosis of the aqueduct of

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Sylvius developed, leading to obstructive hydrocephalus in 100% of animals.[5,6] Similar studies, employing two other human myxoviruses, influenza A and parainfluenza II, showed that they also caused a limited but destructive infection of ependymal cells of newborn hamsters, leading to aqueductal stenosis and hydrocephalus in over 90% of exposed animals.[7]

In this study both neuropathic and nonneuropathic mumps virus and varying doses of influenza A virus were inoculated into adult hamsters and mice to investigate the effect of host age and viral dose on the cellular location of infection and the ultimate pathologic change. The findings are compared with the character and prevalence of ependymal lesions noted in humans to form a hypothesis for the mechanism responsible for common ependymal lesions in man.

Materials and Methods

Viruses

The viruses used and their sources are shown in Table 1. All were assayed in primary monkey kidney tissue culture (MKTC) using hemadsorption with washed guinea pig erythrocytes to detect virus after 5 days incubation. Infectivity is expressed as the reciprocal of the dilution causing infection in 50% of cultures ($TCID_{50}$).[8]

Animal Inoculation

In studies employing neuropathic mumps virus, 3-week-old outbred golden Syrian hamsters were used. All other studies employed hamsters or Harvard white mice of at least 8 weeks of age. All animals were anesthetized lightly with ether and inoculated intracerebrally. Hamsters infected with both strains of mumps virus received approximately 100 $TCID_{50}$ contained in 0.02 ml volumes. For influenza A virus studies, hamsters or mice were inoculated with tenfold dilutions of stock virus so that the final doses ranged from 10,000 to 10 $TCID_{50}$, also contained in 0.02 ml volumes. Virus dilutions were made in cold Hanks' solution. Control animals received plain Hanks' solution intracerebrally.

At varying intervals, random animals were anesthetized with ether and exsanguinated; brains were collected for virus assay, fluorescent antibody staining and conventional histologic study.

Table 1—Virus Strains Used

Virus	Strain (reference)	Cells for virus stock	Titer of stock virus in MCTC*
Mumps			
Neuropathic	Kilham[9]	MKTC	$10^{4.5}$
Nonneuropathic	Lepow[4]	MKTC	$10^{5.0}$
Influenza A	PR-8[10]	Adult mouse lungs	$10^{5.5}$

* Expressed as reciprocal of dilution containing 1 $TCID_{50}$

Virus Assay, Immunofluorescent Study, Histologic Evaluation and Serology

These methods have been described in detail previously.[4,5,7] Briefly, virus growth was assayed in MKTC cultures using tenfold dilutions of a 10% or 20% clarified brain suspension and three or more tubes dilution. For fluorescent antibody studies, sections from rapidly frozen brains were cut on a cryostat, dried and fixed in room temperature acetone. Using an indirect technic, sections were exposed first to guinea pig serum hyperimmune to mumps or influenza A virus, then to goat anti-guinea pig globulin fluorescent conjugate. Conventional histologic slides were prepared from formalin-fixed, paraffin-embedded brains and were stained with hematoxylin and eosin (H&E) or phosphotungstic acid hematoxylin (PTAH). Neutralizing antibody determinations were made by incubating serial twofold dilutions of inactivated serum with approximately 100 $TCID_{50}$ of the appropriate virus for 1 hour prior to assay in tube cultures of MKTC.

Human Neuropathic Studies

One hundred adult brains from consecutive autopsies performed at Cleveland Metropolitan General Hospital during 1969 were examined histologically for ependymal cell abnormalities. Sections of the lateral ventricles, temporal horn, third ventricle, upper aqueduct and fourth ventricle were examined in all cases. Hematoxylin and eosin, PTAH, myelin and Bodian stains were used. Only ependymal lesions consisting of focal ependymal cell loss with subependymal gliosis or loosening were included in the analysis to exclude postmortem artifacts. The age of each patient and the neuropathologic diagnosis were tabulated.

Results

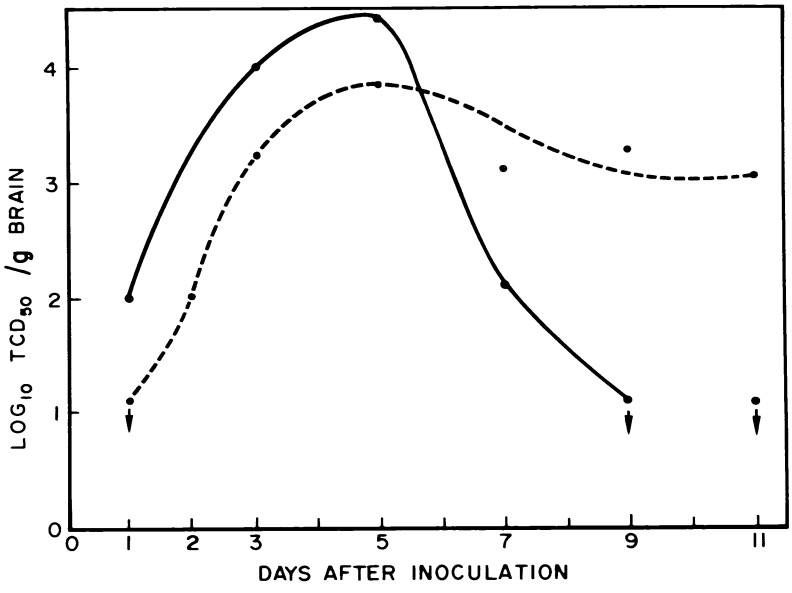
Studies of Adult Hamsters Infected with Neuropathic Mumps Virus

Three-week-old hamsters inoculated intracerebrally with neuropathic mumps virus showed no evidence of clinical disease during 14 or more days of observation.

Virus developed rapidly in the brains of 3-week-old hamsters, reached a peak titer on day 5 and soon disappeared. Text-figure 1 shows the growth of neuropathic mumps virus in 3-week-old hamster brain compared with the growth curve [4] of the same virus in newborn hamster brain inoculated with the same dose.

Viral antigen was detected in 3-week-old hamster brains by fluorescent antibody methods between days 3 and 9, with the most intense accumulation occurring on day 7. Antigen was present only in ependymal cells and involvement was always focal in small patches of contiguous cells (Figure 1A).

The first pathologic change occurred 3 days after inoculation when a mild meningeal inflammatory reaction appeared. By day 5 there was a slight perivascular reaction, particularly around vessels in the subependymal area. At 7, 9 and 11 days an intense perivascular inflammatory response was evident, mainly in vessels near ependymal sur-



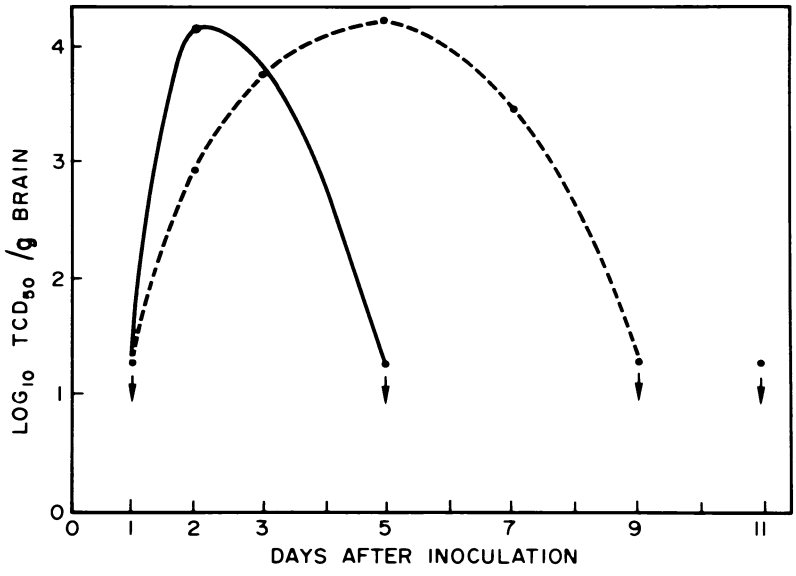
TEXT-FIG. 1—Growth curves of the neuropathic strain of mumps virus in 3-week-old (*solid line*) and newborn (*broken line*) hamster brain after inoculation intracerebrally.

faces. No inflammation was noted in the cerebral or cerebellar cortex. On day 7, focal areas of ependymal denuding appeared and some intact ependymal cells were seen floating free in the ventricular lumen. These denuded foci became more numerous on days 9 and 11. By day 14, the inflammatory response was markedly diminished and patchy ependymal loss was noted in the lateral, third and fourth ventricles and the aqueduct. No neutralizing antibodies to mumps virus were detected in pooled serum collected 3, 5 or 7 days after inoculation. Serum collected on days 9, 11 and 14 showed neutralization at dilutions of 1 : 10 or greater.

Infection of Adult Hamsters with Nonneuropathic Mumps Virus

Infection of adult hamsters with a nonneuropathic strain of mumps virus was similar to that with the neuropathic strain. No animals developed clinical disease. Virus developed even more rapidly in brains of adults and disappeared by 5 days. Text-figure 2 shows the growth rates of nonneuropathic mumps virus in adult and newborn hamsters [4] inoculated with an identical dose.

Viral antigen developed only in ependymal cells; it was first noted in minute focal cellular collections 2 days after inoculation. These increased moderately in size and intensity on day 5 (Figure 1B), even



TEXT-FIG 2—Growth curves of the nonneuropathic strain of mumps virus in adult (*solid line*) and newborn (*broken line*) hamster brain after intracerebral inoculation.

though virus was no longer recoverable. No viral antigen remained by day 7.

The histologic response was accelerated but otherwise similar to that noted with neuropathic mumps virus. By day 5, an intense perivascular response with predominantly mononuclear cells was present, and located almost exclusively in vessels adjacent to ependymal surfaces with no inflammation in cortical areas. Inflammatory cells invaded the parenchyma but were oriented between vessels and ependymal surfaces (Figure 2A), with little migration in other areas. By day 7, active ependymal and ventricular inflammation was present and focal areas of the ventricular surface were denuded of ependymal cells. The inflammatory response had almost disappeared by day 11 and distinct focal ependymal denuding was evident. Brains from animals sacrificed 21, 40 or 70 days after inoculation contained similar focal ependymal denuding (Figure 3A); however, there was no inflammation and histologic evidence of any regeneration of the ependymal lining. The brain underlying the denuded areas frequently had a loosened fibrous appearance. No ependymal lesions were observed in control brains. Serologic assays showed that no neutralizing antibody to mumps virus was present in pooled serum 2 or 5 days after inoculation. On day 7, a 1 : 4 dilution of serum neutralized virus; the necessary titer rose to 1 : 8 on day 11, and to 1 : 16 on day 21.

Studies of Adult Hamsters and Mice Infected with Influenza A Virus

Adult hamsters inoculated intracerebrally with doses of influenza A virus ranging from 10 to 10,000 $TCID_{50}$ developed no observable acute disease. Previous studies [7] had shown that influenza virus produces a "defective" infection in the hamster CNS, during which viral antigen develops in the ependyma but no complete infectious virus is formed. This was also true in adult hamsters, for virus could not be recovered from brain beyond 2 hours after inoculation.

Immunofluorescent studies showed that virus infected only ependymal cells, that viral antigen was maximal 24 hours after inoculation and had disappeared by 7 days. The number of ependymal cells which contained antigen at 24 hours was directly proportional to the dose of virus given and approximately the same proportion of cells contained antigen when observed on days 3 and 5 as on day 1, indicating that there was no secondary spread of virus among ependymal cells. Viral antigen developed in a diffuse cytoplasmic pattern in focal areas of the ependyma or in isolated cells when lesser quantities of virus were given.

An intense meningeal and subependymal perivascular inflammatory response was evident 48 hours after inoculation. By 5 days, an active ventriculitis also occurred and focal areas of ependyma had disappeared. By day 9, the inflammatory response had faded.

To evaluate possible delayed effects of influenza A infections of adult hamsters, 28 animals given 10,000 $TCID_{50}$ were observed for up to 90 days. Groups of 8 to 10 hamsters given 1000, 100 and 10 $TCID_{50}$ of virus were also observed for 60 days. No deaths occurred during prolonged observation; however, massive hydrocephalus was evident on sacrifice in 11 of 28 hamsters, or 39.2%, given a maximal dose of virus (Table 2). Hydrocephalus was not evident in any animals given lower doses.

Table 2—Gross Hydrocephalus After Intracerebral Inoculation of Adult Hamsters with 10,000 $TCID_{50}$ of Influenza A Virus

Time after inoculation (Days)	Hydrocephalus (lesion present no. sacrificed)	Percent hydrocephalic
7	0 2	0
14	1 2	50
30	1 2	50
60	0 2	0
90	9 22	40.9
Total	11 28	39.2

Chronic histologic changes could be related to the dose of influenza A virus given. In all animals which received 10,000 $TCID_{50}$ ependymal denuding was almost complete. In hydrocephalic animals, the aqueduct of Sylvius was occluded or severely stenotic in the region of the posterior commissure; ependymal cells when identified were found in the form of tiny rosettes or aqueductals. Proximal and distal to the site of occlusion, the aqueduct was mildly dilated and usually free of ependymal cells. Histologically, the brain surrounding the aqueduct had a loose fibrous appearance in H&E sections. Myelin was decreased in this area and a moderate increase in glial nuclei and fibers was observed in PTAH stained sections (Figure 2B). Brains of animals receiving 1000 $TCID_{50}$ of virus contained focal areas of ependymal denuding similar to the areas noted in animals infected with mumps virus. Pathologic changes in animals who had received 100 or 10 $TCID_{50}$ were slight, equivocal or absent. No evidence of any chronic inflammatory response was noted, regardless of the dose of virus given.

Adult mice given similar doses of influenza A virus developed an infection almost identical to that in hamsters. Figure 1C shows the area of the fourth ventricle of a mouse given 1000 $TCID_{50}$ of virus; numerous ependymal and choroid plexus cells contain antigen. Figure 1D shows a similar area from a mouse inoculated with approximately 10 $TCID_{50}$; only 3 infected cells are noted. Foci of ependymal cell loss were noted in areas similar to those which contained virus during the active stage of infection (Figure 3B) in mice sacrificed 30 days after inoculation. Adult mice given maximal doses of influenza A virus occasionally developed aqueductal stenosis and hydrocephalus; however, no systematic studies were made of the phenomena.

Neuropathologic Changes in Human Autopsy Brains

Brains from 100 consecutive autopsies contained a large variety of neuropathological lesions, including vascular, degenerative, neoplastic and infectious lesions. There seemed to be no direct correlation between ependymal lesions and other disease processes. When lesions of the ependyma were analyzed independently, 65 brains were abnormal and showed a stereotyped lesion commonly called granular ependymitis. The lesion was always multiple and consisted of a focal loss of ependymal cells together with an abnormality in the subependymal area; either an increased accumulation of glial fibers or loosening of glial fibers, or both, indicated that it was a premortem lesion and not a postmortem artifact. Granular ependymitis was probably even more

frequent since such a small percentage of the total ependymal surface was examined.

Table 3 shows the distribution of granular ependymitis in adults by age at death. Approximately the same number of persons aged 20 to 40 had the lesion as persons 60 or over, suggesting that the lesion dated from preadult life and was not a pathologic process associated with old age.

Discussion

Variation in viral susceptibility of specific cell types within a tissue or organ often determines the form and extent of pathologic change which will occur during or after infection. This study demonstrates that the ependyma of rodents remains susceptible to infection with influenza A, neuropathic and nonneuropathic strains of mumps virus, while neurons susceptible to neurotropic mumps virus in the newborn period lose this susceptibility with age. This localized viral susceptibility accounts for the morphologic changes noted as delayed sequelae of an acute infection.

In a previous study,[4] neuropathic and nonneuropathic strains of mumps virus were shown to produce markedly different infection and pathologic change in newborn hamsters. The neuropathic strain caused a fatal subacute infection involving neurons in addition to ependyma. In the present study, the same agent produced only a localized infection of ependyma in 3-week-old hamsters, indicating that the neuronal population had developed resistance to infection, while the ependyma remained susceptible. The focal cell loss which resulted from the localized infection of ependyma appeared to be of no clinical significance but did suggest a mechanism for the production of a common human neuropathologic lesion, granular ependymitis.

When nonneuropathic mumps virus was inoculated intracerebrally into newborn hamsters, a nonfatal progressive infection of ependyma occurred which finally involved almost every cell. While the animals

Table 3—Survey of 100 Consecutive Adult Autopsy Brains for Granular Ependymitis

Age (yrs)	Female	Male	Total	Normal	Abnormal	Percent abnormal
20–40	5	4	9	3	6	66
40–60	12	16	28	11	17	61
60 & over	25	38	63	21	42	66
Total	42	58	100	35	65	65

tolerated the active infection, the destruction of the ependyma led to aqueductal stenosis and hydrocephalus in 100% of animals as a delayed sequela.[5] In the adult hamster, the infection with nonneuropathic mumps virus was accelerated, yet for unknown reasons it remained focal, producing only a patchy loss of ependyma rather than complete denuding of the ventricular spaces and no hydrocephalus occurred.

The effect of viral dose as well as age could be studied in the influenza A virus infected animals. Because of the defective nonpropagating nature of the infection, only the ependymal cells initially exposed to virus developed viral antigen and were destroyed. Thus the extent of ependymal denuding could be controlled. When large doses of virus were inoculated ependymal cell loss was complete, and approximately 40% of hamsters developed aqueductal stenosis and hydrocephalus. Ninety-three percent of newborn hamsters given approximately the same dose became hydrocephalic.[7] The reason for this discrepancy may simply be the difference in surface area of ependyma available for virus adsorption. The influenza A infections in mice were essentially the same as in hamsters, indicating that the process was not a unique and isolated phenomena of one species.

When hydrocephalus did occur in the adult animals, it was accompanied by periventricular and periaqueductal gliosis, a finding also noted in many cases of human hydrocephalus secondary to aqueductal lesions.[11] Similar gliosis was not found in the newborn animals, indicating that reaction to infection changes with age as well as the selective vulnerability of cell populations.

These studies illustrate how a variety of clinical and pathologic states may result when specific factors such as age and virus dose are altered. The influenza A studies may have no counterpart in man, for there is little evidence that influenza A virus can directly invade the human nervous system. In contrast, mumps is the virus most commonly associated with viral meningitis and encephalitis,[10,12] and even in patients with parotitis and no clinical signs of CNS involvement as many as 35% have a pleocytosis.[13] Thus, mumps virus invades the human CNS with great frequency and might well result in analogous pathologic sequelae.

The analysis of human brains collected randomly from autopsies demonstrated the common occurrence of granular ependymitis. If more sections had been observed, probably even more of the "normal" brains in the series would have contained ependymal lesions. The pathologic change was as prevalent in young as in old adults suggesting that whatever the cause, it usually occurred prior to adult life.

Granular ependymitis is known to occur following tuberculosis and syphilis of the central nervous system[14,15] and probably also can follow other more acute CNS infections and subarachnoid hemorrhage. The prevalence of granular ependymitis in man and the similarity to the experimental lesions suggest that they may be the sequelae of a banal viral infection, localized to ependymal cells. Furthermore, if the infection progressed to involve and destroy most of the ependymal lining, more severe pathology may result, namely, aqueductal stricture and obstructive hydrocephalus.

No evidence exists that mumps replicates primarily in the ependyma of man, but the mild transient nature of most cases of CNS mumps[12] would be compatible with this localization. There is no definitive evidence that mumps infection in man can lead to aqueductal stenosis as a delayed sequela; however, 2 cases have been reported in which hydrocephalus did follow severe mumps meningoencephalitis,[16,17] suggesting that such a mechanism may be responsible for some cases of human hydrocephalus.

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[*Illustrations follow*]

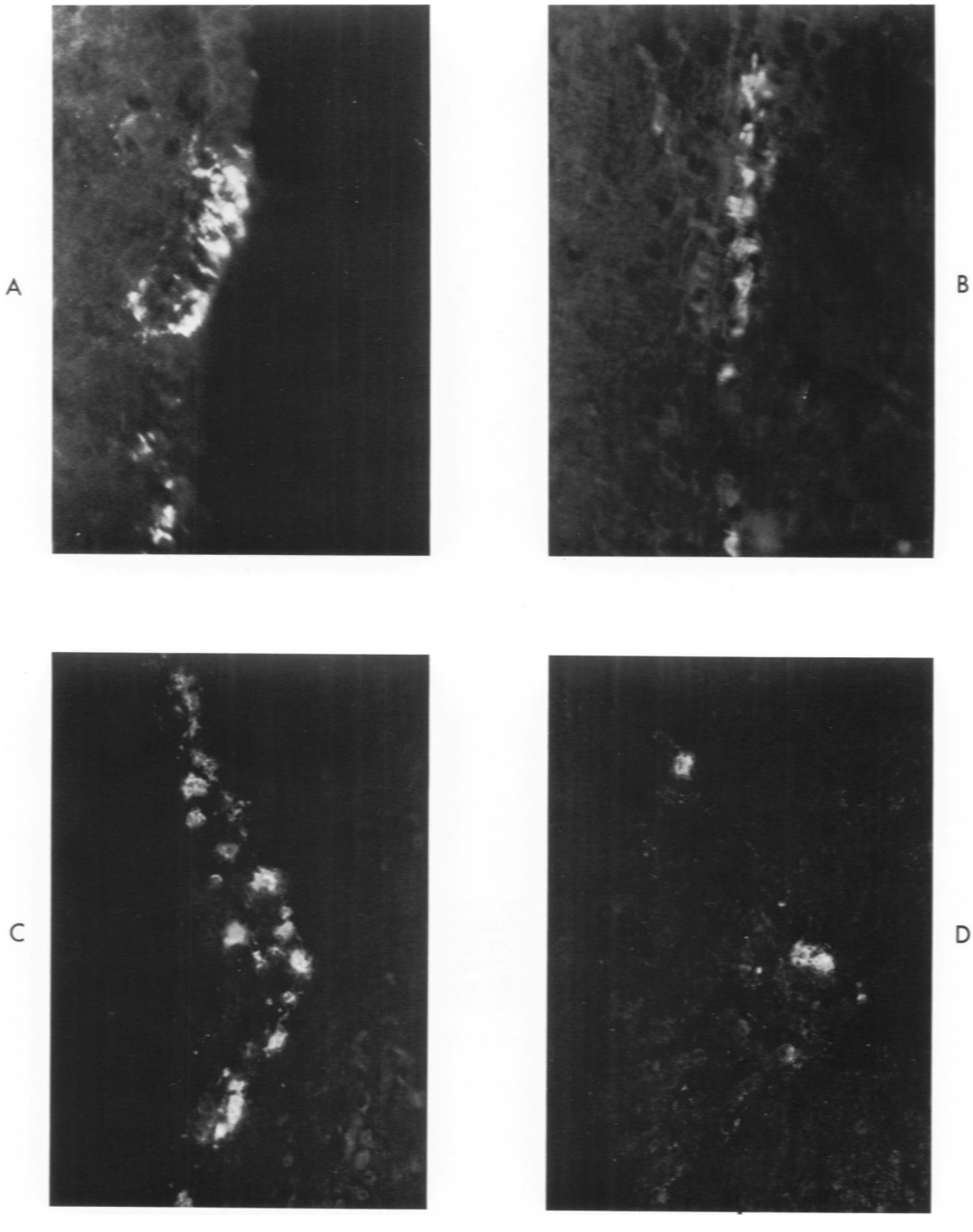


Fig 1—Fluorescent antibody stained sections of rodent brains after intracerebral inoculation of viruses. Virus antigen is limited to ependymal cells of the lateral ventricle in **A** and to ependymal cells of the fourth ventricle in **B, C** and **D.** Interval after inoculation represents time of maximal fluorescence. **A**—Neuropathic mumps virus, 100 $TCID_{50}$, 7 days (× 260). **B**—Nonneuropathic mumps virus, 100 $TCID_{50}$, 5 days (× 260). **C**—Influenza A virus, 1000 $TCID_{50}$, 1 day (× 100). **D**—Influenza A virus, 10 $TCID_{50}$, 1 day (× 100).

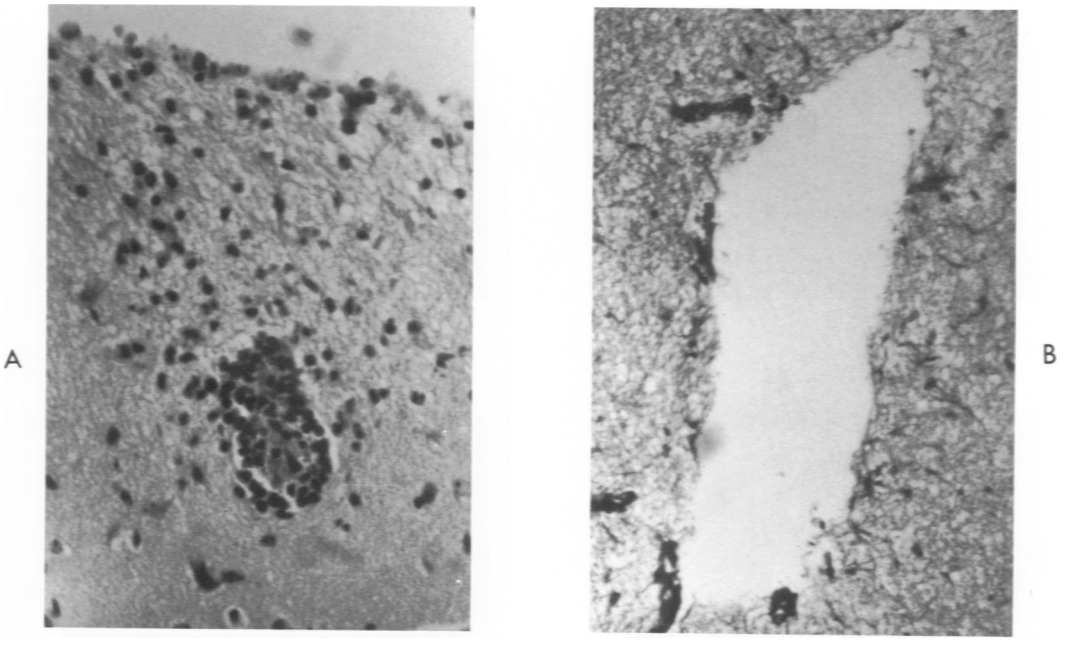
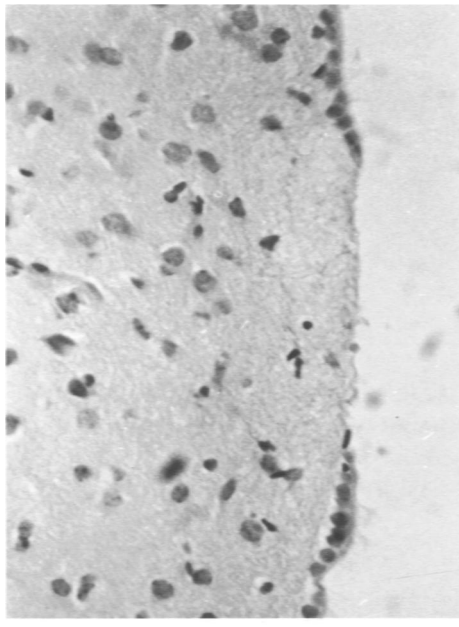
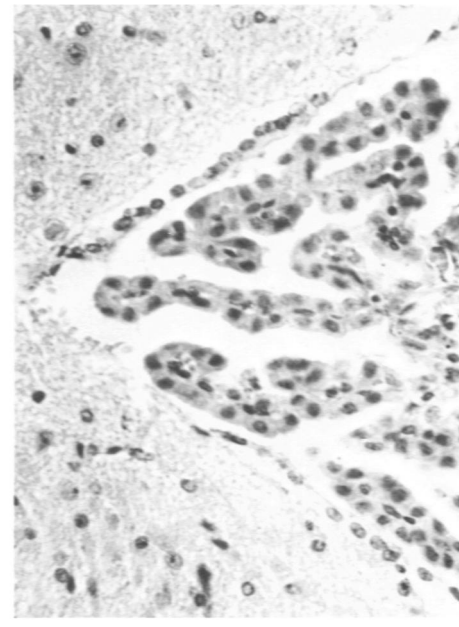


Fig 2A—Section of hamster brain 5 days after inoculation of nonneuropathic mumps virus showing perivascular inflammation and parenchymal spread of mononuclear cells toward the ependymal surface. Note disruption of ependyma and ependymal cell free in lumen of ventricle (H&E, × 260). **B**—Section of hamster brain 90 days after inoculation with 10,000 $TCID_{50}$ of influenza A virus showing the aqueduct of Sylvius just distal to area of occlusion. There is total loss of ependymal cells and increased periaqueductal gliosis (PTAH, × 130).





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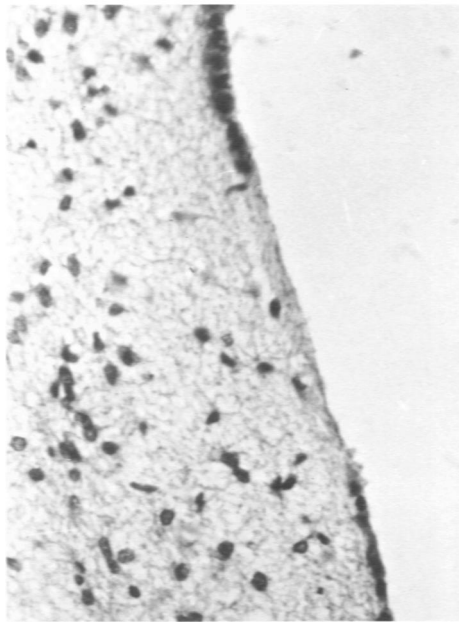

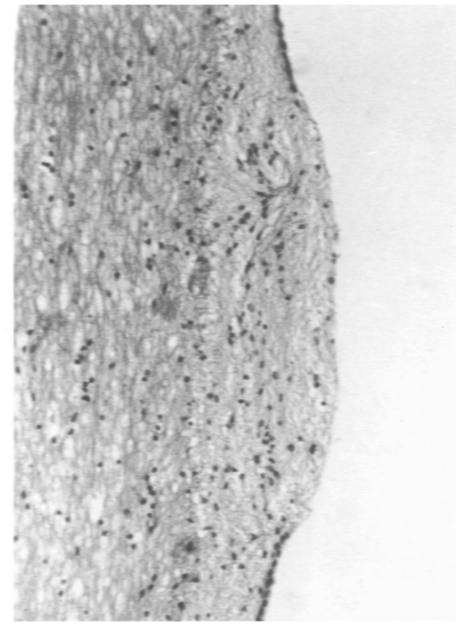

D

Fig 3—Sections of **A** hamster and **B** mouse brain after intracerebral inoculation of viruses and **C, D** human brains from random autopsies. **A**—Surface of ventricle 21 days after inoculation of nonneuroadapted mumps virus (× 260). **B**—Fourth ventricle 30 days after inoculation of 1000 $TCID_{50}$ of influenza A virus (× 100). **C**—(H&E, × 260). **D**—Granular ependymitis in man (H&E, × 100).

[*End of Article*]