Visna Virus Infection of American Lambs

Abstract. *Random-bred fetal and 4-week-old American lambs, inoculated intracerebrally with visna virus, developed a persistent infection in the brain and sometimes in the lung. The pathologic changes present in these lambs were similar to the early lesions of visna in Icelandic sheep, thus providing a possible model for the study of virus-induced demyelinating disease.*

Visna is a chronic persistent infection ("slow virus infection") of the brains of Icelandic sheep caused by an RNA virus which shares many biological properties with the RNA tumor viruses (*1*). The disease was first described by Sigurdsson *et al.* (*2*), and subsequent experimental studies in sheep have been reported by investigators in Iceland (*3*). Although sheep failed to develop clinical disease for periods up to 4 years after intracerebral inoculation of the virus, lymphocytic infiltration of the leptomeninges and choroid plexus was detected within 1 month of inoculation. Later in the infection, extensive pathologic changes were seen in the parenchyma of the brain. Widespread proliferation of glial and mononuclear cells was accompanied by demyelination in white matter. The neuropathological changes of visna in Icelandic sheep show some features in common with two human demyelinating diseases, postinfectious encephalomyelitis and multiple sclerosis (*4*). Although an immunopathologic mechanism for this virus-induced demyelination has been postulated (*5*), the pathogenesis of this slow demyelinating disease has remained unknown.

Studies of visna have been limited by (i) the inability to infect laboratory animals with visna virus (*6*), (ii) the generally held belief that breeds other than Icelandic sheep are not susceptible to the disease, and (iii) the stringent isolation conditions required to work with this agent in sheep in this country (*7*).

A strain of visna virus (*7*) which had undergone several passages in tissue culture was inoculated onto confluent monolayers of primary sheep choroid plexus cells, grown in Roswell Park Memorial Institute medium (RPMI 1640) plus lamb serum (10 percent) and maintained with medium plus 2 percent serum. Cytopathic effects (CPE) consisting of multiple syncytia were seen in 3 days. Supernatant fluids, removed at daily intervals for the next 4 days and stored at −70°C, were pooled, clarified at 5000*g* for 10 minutes, and concentrated 100-fold by centrifugation at 100,000*g* for 60 minutes. The infectivity of this concentrate measured 10^7 tissue culture infectious doses per milliliter in sheep choroid plexus cells. Degenerating infected cells from two 75-cm^2 flasks were scraped into a 2-ml portion of concentrate; this was used for the initial inoculation of fetal lambs.

Timed-gestation, random-bred sheep

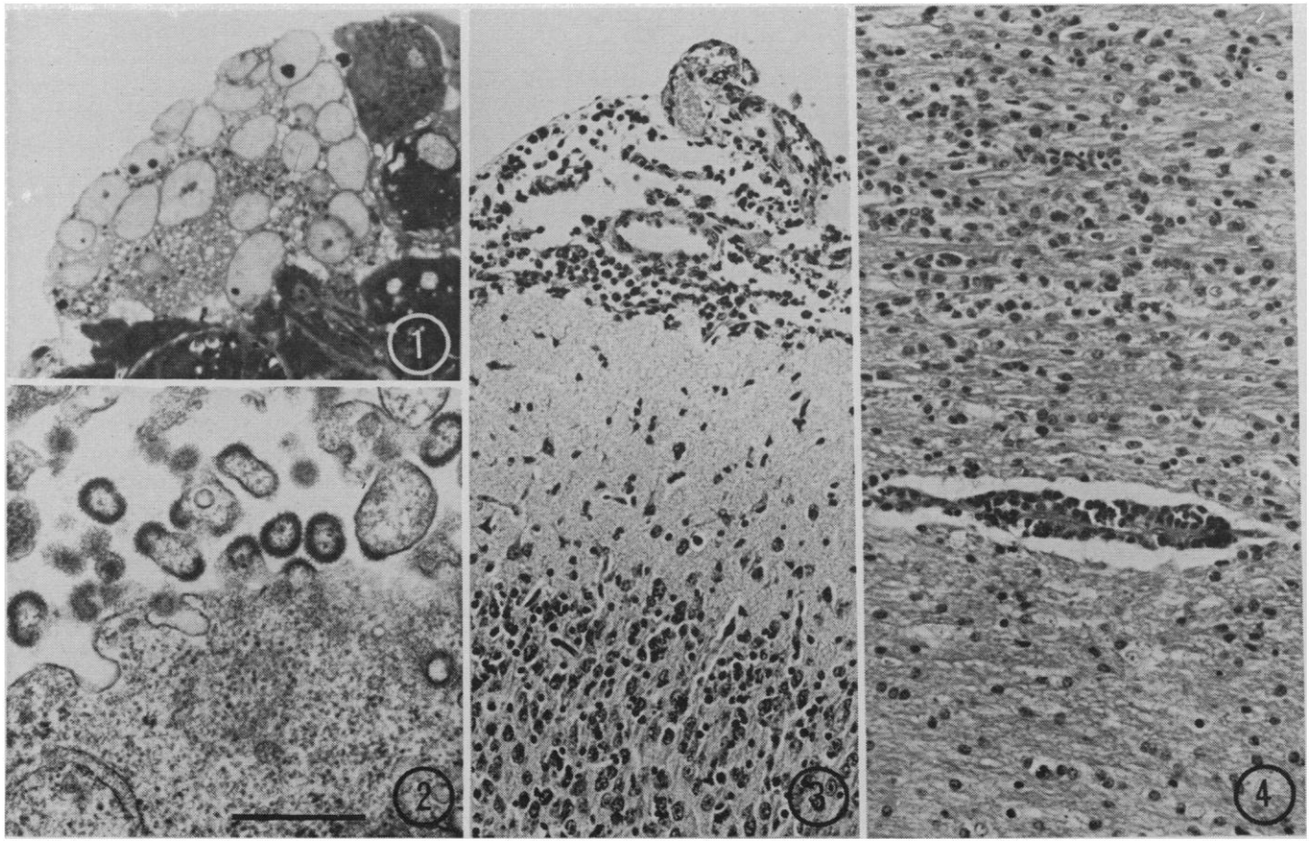

Fig. 1. Light micrograph of brain tissue culture made from lambs infected with visna virus. Multiple nuclei are present in the giant cell occupying center of field. Epon-embedded section stained with toluidine blue (× 800). Fig. 2. Electron micrograph of tissue culture of brain cells derived from lamb infected with visna virus. Note particles budding from plasma membrane of cell (scale, 0.5 μm). Fig. 3. Cerebral cortex, fetal animal, inoculated on day 65 of gestation and killed 45 days later. An infiltrate of lymphocytes and mononuclear cells is present in the subarachnoid space. Hematoxylin and eosin (× 185). Fig. 4. Cerebral white matter, newborn lamb, inoculated at 4 weeks of age and killed 9 days later. Lymphocytes and mononuclear cells surround the blood vessel. Note lesion in white matter associated with increase in cellularity. Some of these cells are blood elements, others may represent reactive glia. Stained with hematoxylin and eosin (× 240).

were used in these studies. After laparotomy of the mothers (*8*), two sets of twin fetal lambs were inoculated intracerebrally through the uterine wall on the 65th day of the 150-day gestational period. Twins of one set received 0.1 ml of cell-free virus inoculum; the others received 0.1 ml of virus and cells. One fetus was surgically removed from each ewe 7 days after inoculation and the remaining fetuses were examined 5 weeks later. The first two fetuses removed had mild focal collections of lymphocytes in the leptomeninges; the other two showed meningitis and accumulations of inflammatory cells around blood vessels. Virus was not recovered when brain homogenates of the four lambs were inoculated onto sheep choroid plexus cells. However, tissue fragments or trypsinized cells from all four brains, grown in vitro in RPMI medium and 10 percent lamb serum, developed characteristic CPE within 2 weeks. The CPE consisted of formation of syncytia (Fig. 1). Electron microscopic studies of these syncytia disclosed particles indistinguishable from those described in other cell cultures inoculated with visna virus (*9*) (Fig. 2).

The virus isolated from the first infected fetal lamb brains was grown in sheep choroid plexus cells, and a pool of concentrated virus and cells was prepared for all subsequent inoculations. Two further mid-gestational fetuses were inoculated and killed 8 and 36 days later; both brains showed inflammatory cells in the meninges and perivascular spaces, and virus was recovered from both brains, but only by explant methods. Three fetal sheep were inoculated at 115 days gestation; one was killed 2 weeks later and the remaining two were killed 2 weeks after birth (7 weeks after inoculation). The brain of the fetus taken at 2 weeks, and that of its twin, excised 2 weeks after birth, had no histological lesions, and both failed to yield virus. The other lamb infected at 115 days gestation and killed 2 weeks after birth showed histopathological lesions in brain, and virus was recovered from explants. Subsequently, four 5-week-old lambs were inoculated intracerebrally with 0.2 ml of virus and were killed at 1, 2, 4, and 10 weeks after inoculation. Lambs killed at 1 and 10 weeks had multiple focal lesions in the central nervous system and virus was recovered from brain explant cultures. The lamb killed 4 weeks after inoculation had occasional lesions in the brain, but no virus was recovered from cultures of brain cells. However, visna virus was isolated from cultures prepared from the choroid plexus of this lamb. The lamb killed 2 weeks after inoculation had no histopathological abnormalities, and no virus was recovered.

The pathological lesions induced by visna virus differed, depending on the age of the animal. Fetal animals showed infiltration of the meninges with lymphocytes, monocytes, and plasma cells (Fig. 3). Perivascular infiltrates of inflammatory cells were present in the majority of these animals. Although the 4-week-old lambs showed mild meningitis, the most significant abnormality was in the cerebral white matter (Fig. 4). In patchy foci of parenchymal damage there were excessive numbers of cells, including lymphocytes, monocytes, and plasma cells. Other cells in these lesions may represent reactive glia. Inflammatory cells were present around blood vessels in proximity to these lesions. Frozen sections of brains were stained with fluorescein-labeled antibodies from sheep having high serum neutralizing antibody titers to visna virus (*10*), but no viral antigen was seen.

In addition to brain lesions, many fetal and postnatal lambs developed an interstitial round cell pneumonia of varying severity. In these animals there were lymphocytes and plasma cells in the peribronchial regions, and, in some instances, germinal centers were present in the lung. Virus was isolated by explant techniques from the lungs of five of these animals. Lymphoid hyperplasia with germinal center formation and plasmacytosis were present in spleen and lymph nodes. These findings suggest that visna virus infection produces a persistent infection in the brain and lung as well as a lymphoproliferative disease, possibly associated with the oncogenic potential of the virus. Whether activated lymphocytes play an immunopathologic role in the late demyelinating disease observed in Icelandic animals is unknown.

Although the visna-related virus of progressive pneumonia, a natural disease of American sheep, has been recovered from brain on one occasion (*11*), it has not been associated with disease of the central nervous system. Our findings establish that visna virus can infect American lambs and produce early pathological changes resembling those described in Icelandic sheep (*12*). The study was initiated in fetal lambs in hopes of adapting visna virus to North American sheep. However, the lesions produced in fetuses inoculated with "unadapted" virus were as frequent and severe as those induced by the virus after passage in fetal lamb brain. The demonstration of lesion in 10 of 13 inoculated lambs parallels the experience in Iceland where lesions and disease were not seen in all infected animals (*2*). The infection in American lambs is persistent, yet infectious virus was only recovered by cultivation of cells from brains. This finding, combined with preliminary failures to demonstrate viral antigen in brains of inoculated sheep, suggests that the virus in the sheep's brain was defective or was being replicated in very small quantities in vivo. The lesions were confined to the white matter in postnatal lambs, and, if the infection follows the course of natural visna, they may represent the prelude to demyelinating disease.

OPENDRA NARAYAN
Departments of Animal Medicine and *Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*
ARTHUR M. SILVERSTEIN
Department of Ophthalmology, Johns Hopkins University School of Medicine
DONALD PRICE
Departments of Pathology and *Neurology, Johns Hopkins University School of Medicine*
RICHARD T. JOHNSON
Departments of Neurology and *Microbiology, Johns Hopkins University School of Medicine*

References and Notes

1. F. H. Lin and H. Thormar, *J. Virol.* **6**, 702 (1970).
2. B. Sigurdsson, P. Palsson, H. Grimsson, *J. Neuropathol. Exp. Neurol.* **16**, 389 (1957).
3. M. Gudnadottir and P. Palsson, *J. Immunol.* **95**, 1116 (1965); M. Gudnadottir and K. Kristindottir, *ibid.* **98**, 663 (1967).
4. B. Sigurdsson, P. Palsson, L. Van Bogaert, *Acta Neuropathol.* **1**, 343 (1962).
5. P. W. Choppin, in *Textbook of Immunopathology*, P. A. Miescher and H. J. Muller-Eberhard, Eds. (Grune, New York, 1968), p. 337.
6. H. Thormar, *Z. Neurol.* **199**, 1, 151 (1971).
7. Virus was obtained from Dr. Kenneth Takemoto under U.S. Department of Agriculture Veterinary Permit 4983.
8. K. L. Kraner and C. J. Parshall, Jr., *Methods Anim. Res.* **3**, 211 (1968).
9. J. E. Coward, D. H. Harter, C. Mayarr, *Virology* **40**, 1030 (1970).
10. Serum, from a sheep with visna, having a neutralizing titer greater than 2000 to visna virus, was provided by Dr. Neal Nathanson and Dr. G. Petersson, Institute of Experimental Pathology, Reykjavik, Iceland.
11. N. G. Rogers, C. J. Gibbs, Jr., D. C. Gajdusek, S. W. Anderson, M. Basnight, *Bacteriol. Proc.* (1971), p. V72.
12. B. Sigurdsson and P. Palsson, *Br. J. Exp. Pathol.* **39**, 519 (1958).
13. We thank B. Schumann, J. Chase, S. Johnson, E. King, A. Stocks, and D. Walcott for technical assistance. Supported in part by research contract DN-49-193-MD-2460 from the U.S. Army Research and Development Command; program project grant NS 10920-01 to the Department of Neurology; NIH general research grant RR 5378; and by an Independent Order of Odd Fellows research professorship to A.M.S.

30 August 1973; revised 21 December 1973