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Replication of the scrapie agent in ocular neural tissues

(hamster/slow virus infection/retinopathy)

NED BUYUKMIHCI*†, MARIE RORVIK*, AND RICHARD F. MARSH‡

*Department of Urban Practice, University of Tennessee, Knoxville, Tennessee 37901; and ‡Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT **Optic nerves and retinas removed from hamsters experimentally inoculated with the scrapie agent contain a high titer of infectivity. Ophthalmoscopic examination of these animals revealed gross lesions of retinopathy as early as 3 weeks before the onset of clinical signs of brain degeneration. These results suggest that the scrapie agent may spread centrifugally in nerve fibers after intracerebral inoculation and that the scrapie-associated retinopathy seen in hamsters is directly induced by the agent rather than the result of retrograde degeneration from central neural damage.**

Scrapie is a natural slow virus infection of sheep and goats and is classified with Creutzfeldt–Jakob disease, kuru, and transmissible mink encephalopathy as a subacute spongiform encephalopathy. Pathologic evidence of disease is limited to the brain and spinal cord in all species susceptible to spongiform encephalopathies with the exception of the Syrian hamster in which degeneration of optic nerve and retina has been detected by light microscopy in animals killed during the terminal stages of scrapie disease (1).

This study reports the increasing titer of scrapie infectivity in hamster optic nerve and retina during the preclinical stages of infection, the appearance of gross lesions of retinopathy 3 weeks before the onset of clinical signs of disease, and the description of ultrastructural changes in affected retinas.

MATERIALS AND METHODS

Animals. Syrian hamsters used in these experiments were outbred (LVG:LAK), weanling males purchased from Lakeview Hamster Colony (Newfield, NJ).

Inoculation. To inoculate animals with scrapie, 0.05 ml of a 5% saline suspension of hamster brain containing an infectivity titer of $10^{7.5}$ times the 50% lethal dose ($10^{7.5}$ LD_{50}) was injected into the right cerebral hemisphere. Control animals were similarly injected with a 5% saline suspension of normal brain.

Tissue Collection. At weekly intervals after inoculation, hamsters were anesthetized with pentobarbital and exsanguinated by opening the right auricle. Sensory (neural) retinas to be tested for infectivity were dissected from enucleated eyeballs, weighed, and placed on dry ice. The brain was then removed and sections of optic nerve (from the chiasm 1.5 cm rostrad) and left cerebrum were aseptically collected (by using separate instruments and containers), weighed, and placed on dry ice. These tissues were then triturated in saline and bioassayed by intracerebral inoculation of weanling hamsters (2). End-point titers were calculated 16 weeks after inoculation by the method of Spearman and Kärber (3). Hamsters used for pathology studies were infused, simultaneously with exsanguination, with 7 ml of 2.5% glutaraldehyde in phosphate or cacodylate buffer (pH 7.4). The eyes were enucleated, the corneas were slit, and the globes were further fixed in glutaraldehyde. After washing in buffer, the anterior segments of the eyes were removed and the remaining posterior eyecups were postfixed in osmium tetroxide, dehydrated in increasing concentrations of ethanol, and embedded in an epoxy resin (Epon 812).

RESULTS

Ophthalmoscopic Examination. The appearance of the normal hamster ocular fundus consists of a homogeneous brown-grey background with four to six retinal arterioles and venules converging on an ill-defined pink optic disk. The vessels appear to be floating in space and cast shadows on the fundus background. A dark brown-black conus papillaris emanates from the center of the optic disk. As early as 3 weeks before the onset of clinical signs in scrapie-inoculated hamsters (5 weeks after infection), ophthalmoscopic examination revealed subtle changes consisting of mottling of the fundus and slight attenuation of the vasculature. These changes progressed with time. When the animals were severely affected clinically, the fundus background was extremely mottled and most of the vessels appeared as thin grey-white lines with only a few having a blood column. These vessels lacked the normal aerial appearance and did not cast shadows. The optic disk at this time was pale grey although the conus papillaris appeared to be unaffected.

Ultrastructure of Retinal Lesions. Fig. 1 illustrates the typical ultrastructural appearance of the outer portion of sensory retina in clinically affected hamsters as compared with normal. The number and length of the photoreceptor inner and outer segments were decreased. Most of the outer segment material was absent; that remaining showed nonspecific vesiculation and disorganization of the lamellar membranes. The inner segments were more broad than normal and were undergoing degeneration. Although not shown in the illustration, the outer nuclear layer (photoreceptor nuclei) was decreased to just a few nuclei, many of which were pyknotic.

Infectivity Titration. Hamsters inoculated with scrapie had an incubation period of 8 weeks before the onset of clinical signs of disease. The results of end-point titrations for infectivity in brain, optic nerve, and retina at 3, 6, and 10 weeks after infection are shown in Table 1.

DISCUSSION

Gross retinal degeneration was found, by ophthalmoscopic examination, as a scrapie-induced diagnostic change in a living animal showing no incoordination, ataxia, or other sign of encephalopathy. No attempt was made to evaluate impairment of visual acuity, but the severity of the retinal lesions would

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† Present address and address for reprint requests: University of California, School of Veterinary Medicine, Department of Surgery, Davis, CA 95616.

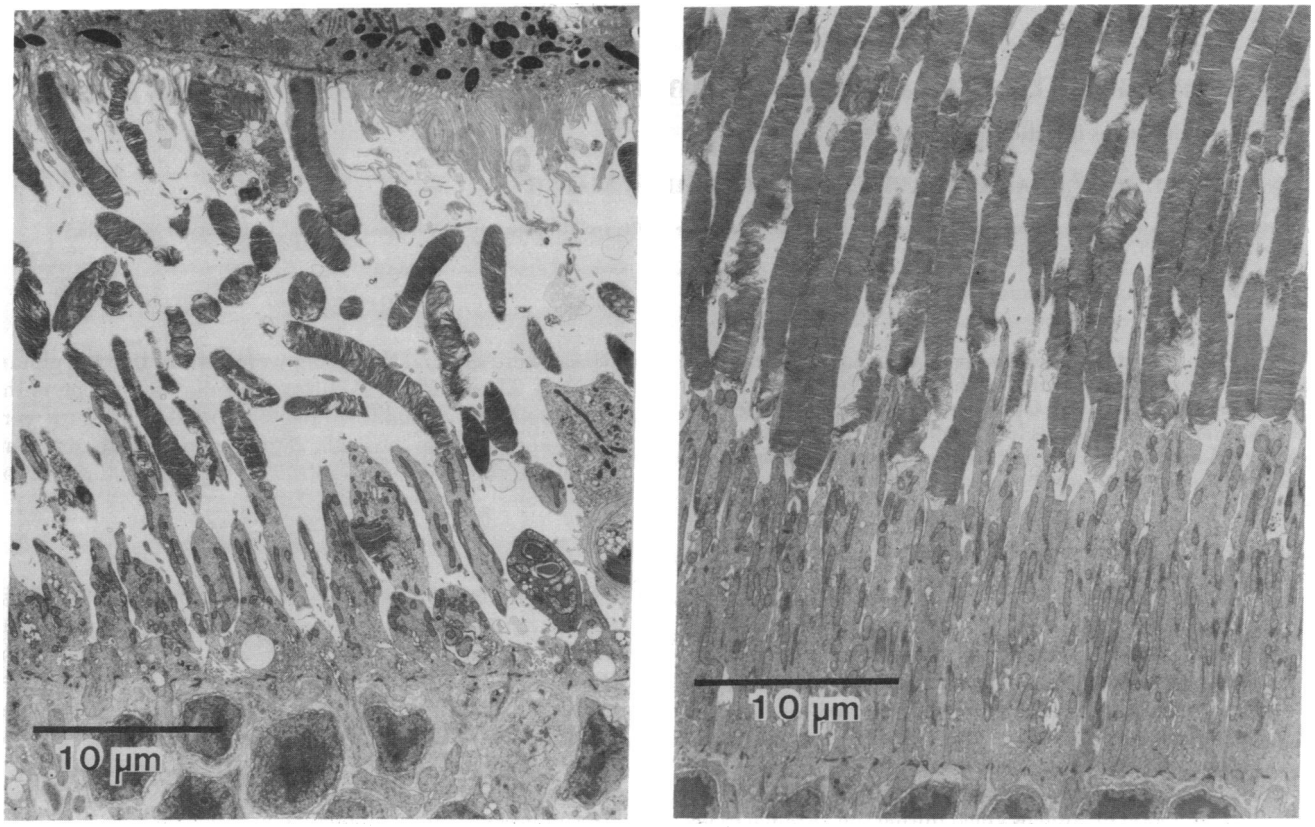

FIG. 1. Outer sensory retina. (Uranyl acetate and lead citrate stain.) (*Left*) From a clinically affected hamster 10 weeks after scrapie inoculation. Note the disorganization and degeneration of the photoreceptor inner and outer segments. (*Right*) From a control animal.

indicate that these animals did not have normal eyesight. This can perhaps be better studied in humans with Creutzfeldt–Jakob disease in which, at least in the early stages, more careful clinical examination may be possible.

The high titers of infectivity in the optic nerve and retina suggest active replication of the scrapie agent in these tissues, but two alternative explanations must be considered. The first is contamination from infectivity in brain tissue or from residual inoculum remaining after the intracranial injection. The close apposition of the optic nerve to the brain could allow for surface contamination of this tissue. However, because the scrapie agent is highly cell-associated with little or no infectivity in body fluids (including the cerebral spinal fluid), the contamination would be expected to be of relatively low titer rather than of the high titers observed. Also, the high titer in the retina, a tissue with no direct physical contact with the brain, suggests that this is an unlikely explanation for infectivity in the optic nerve. The possibility that infectivity comes from contamination by residual inoculum also seems unlikely because the optic tissues showed an increasing titer of infectivity during the incubation period.

Table 1. Titer of scrapie agent in brain, optic nerve, and retina at intervals during the incubation period and at end stage

Tissue*	Titer,† $\log_{10}$ LD_{50}/g		
	At 3 weeks	At 6 weeks	At 10 weeks
Brain	7.3	8.6	9.6
Optic nerve	6.0	7.6	8.3
Retina	4.3	6.0	7.6

* Composite samples from three animals.
† Shown as $\log_{10}$ of 50% lethal dose per g.

The second explanation for infectivity in optic tissues is that it represents accumulation rather than active replication of the agent. This possibility cannot be resolved at this time. But whether infectivity in optic nerve and retina is due to accumulation or due to active replication, these findings encourage the interpretation that the optic lesions observed are caused by a direct effect of the agent on the retina rather than being the result of retrograde degeneration from injury to cells in the brain.

One of the major unanswered questions in unconventional slow virus infections is "How do these agents gain access to the central nervous system?" Lymphoreticular tissues appear to be the primary site of uptake of infectivity after natural exposure or peripheral inoculation, with the central nervous system becoming infected much later (4). Because of the inability to detect infectivity in serum or blood cell fractions at any stage of transmissable mink encephalopathy infection in minks, it has been speculated that these neuropathic agents migrate into the central nervous system via nerve fibers (5). This possibility is supported by the results of these experiments. The higher titer of infectivity in the optic nerve at each interval tested suggests that the retina is infected via nerve fibers from the brain (centrifugally). This possible migration of infectivity in nerve fibers is also a reasonable explanation for infection of corneal epithelium in hamsters infected with transmissable mink encephalopathy (6). Other neuropathic viruses such as rabies and herpes simplex are capable of infecting both centrifugally and centripetally. Therefore, the possibility of these unconventional agents infecting the central nervous system via nerve fibers becomes more likely.

The animals in this study are part of a larger group that is being used for study of degenerative changes in the retina and optic nerve at weekly intervals during the incubation period.

This report describes the ultrastructural lesions in sensory retinas of end-stage hamsters. Because of the severe and widespread degeneration in clinically affected hamsters, it is not possible to distinguish primary from secondary effects. Examination of tissues collected earlier in the disease process should provide more information on the sequential development of retinopathy. All previous studies on the neuropathology of the spongiform encephalopathies have been on brain and spinal cord where the intercellular intrarelationships and organization are more complex than in the retina.

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