JOURNAL OF VIROLOGY, Oct. 1987, p. 3235–3240
0022-538X/87/103235-06$02.00/0

Vol. 61, No. 10

Temporal Distribution of Transmissible Mink Encephalopathy Virus in Mink Inoculated Subcutaneously

WILLIAM J. HADLOW,* RICHARD E. RACE, AND RICHARD C. KENNEDY†

Laboratory of Pathobiology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840

Received 6 April 1987/Accepted 16 June 1987

Information was sought on the temporal distribution of transmissible mink encephalopathy virus in royal pastel mink inoculated subcutaneously with $10^{3.0}$ 50% intracerebral lethal doses of the Idaho strain. As determined by intracerebral assay in mink, extremely little replication of the virus occurred during the preclinical stage of infection. It seemed largely limited to lymph nodes draining the site of inoculation. Virus first appeared in the central nervous system (CNS) at 20 weeks, when all mink were still clinically normal. Early spongiform degeneration, limited to the posterior sigmoid gyrus of the frontal cortex, was first found at 28 weeks, or a few weeks before onset of clinical disease in most of the mink. Once virus reached the CNS, where greater concentrations occurred than elsewhere, it appeared in many extraneural sites (spleen, liver, kidney, intestine, mesenteric lymph node, and submandibular salivary gland). These seemingly anomalous findings, especially the limited extraneural replication of virus as a prelude to infection of the CNS, suggest that mink are not natural hosts of the virus. The results of this study support the generally held view that transmissible mink encephalopathy arises from chance or inadvertent infection of ranch mink with an exogenous virus, most likely feed-borne wild scrapie virus.

Transmissible mink encephalopathy (TME) is an invariably fatal neurodegenerative disease of adult ranch mink (*Mustela vison*) (21). A rare disease, it has occurred only in sporadic outbreaks in a few of the many countries where mink are raised since it first appeared 40 years ago in Wisconsin (5, 7, 13, 15, 21, 22). Despite its rarity, TME has attracted attention because of its striking similarities to scrapie, the prototype of transmissible subacute spongiform encephalopathies (11, 32). In both diseases, the neurohistologic changes and physicochemical and biological properties of the causal viruses are essentially the same (3, 8, 28, 33, 35, 37, 42). These similarities have given rise to much speculation about the origin of TME (36). Present evidence, both epidemiologic and experimental, strongly suggests that it represents chance or inadvertent infection of ranch mink with wild scrapie virus contaminating their feed, supposedly from organs of infected sheep used to prepare it.

Although the feed no doubt is the source of the causal virus, mink may not become infected simply by eating it. For example, under experimental conditions the incubation period in mink exposed orally to TME virus, even repeatedly, often is much longer than that presumed in most naturally occurring instances of the disease (3, 7, 22, 31, 34). Hence, mink probably become infected in other ways as well. Experimentally, they are readily infected by several parenteral routes, although the incubation period is still many months even by the intracerebral route (3, 7, 22, 31, 34; unpublished data). Of these ways, intradermal inoculation has been suggested as the most likely one under natural conditions (36). Such exposure is thought to come about during the fighting that commonly occurs among littermate kits, especially at feeding time. So, kits exposed by bites of needle-sharp teeth before they are caged individually, when about 3 months old, become affected with the disease as yearlings. Whatever the main route of natural exposure may be, it was highly effective in those herds in which 100% of adult mink succumbed during a single outbreak of TME (21).

Little is known about the pattern of the infection in mink—the sequence of virologic events that leads to fatal neurologic disease. Virus has been found in the brain, spleen, and mesenteric lymph node as early as 9 weeks after intracerebral inoculation (38). In mink severely affected with TME after parenteral inoculation, virus is widespread; it has been found not only in the brain, where the concentration is greatest, but also in the mesenteric lymph node, spleen, liver, kidney, lung, heart, intestine (and feces), submandibular salivary gland, skeletal muscle, and urinary bladder (34; unpublished data). Yet, except for these few preliminary findings, nothing has been reported about extraneural replication of the virus or about its temporal relation to the appearance of virus in the central nervous system (CNS) and the onset of clinical disease. We sought such information about the infectious process in a study of pastel mink inoculated subcutaneously with the Idaho strain of TME virus. Our findings are recounted here.

MATERIALS AND METHODS

Animals. Female royal pastel mink, used throughout the study, were obtained from a local Aleutian disease-free herd, housed individually in an outdoor isolation compound, and given a standard wet ration and water. None was vaccinated for canine distemper, mink (parvovirus) enteritis, or botulism. Most were at least 2 years old when used.

Virus. The TME virus used was the second mink passage of the Idaho strain prepared as previously described (18). As determined by intracerebral inoculation of mink (four per 10-fold dilution), this stock suspension contained $10^{6.8}$ 50% lethal doses of virus per g of brain tissue.

* Corresponding author.
† Present address: 519 S. Fifth St., Hamilton, MT 59840.

TABLE 1. Temporal distribution of TME virus in tissues of pastel mink inoculated subcutaneously with 1,000 50% lethal doses

Time after inoculation (wk)	Presence of clinical disease	Virus titer (log_{10} 50% intracerebral lethal dose/100 mg of tissue) in[a]: Regional lymph nodes	Peripheral lymph nodes	Mesenteric lymph node	Spleen	Thymus	Salivary gland	Kidney	Liver	Intestine	Cerebral cortex	Diencephalon	Cerebellar cortex	Cervical spinal cord	Lumbar spinal cord	Sciatic nerve
1	−	1.0	NV	NV	NV	NV										
2	−	2.0	NV	NV	NV	NV										
4	−	2.5	NV	NV	NV	NV										
8	−		3.0	NV	NV	1.0										
12	−		4.0	NV	NV	NV	NV					NV		NV	NV	
16	−		NV	NV	NV	NV	NV	NV	NV	NV		NV		NV	NV	
20	−		NV	NV	NV	NV	NV	NV	NV	NV	NV	2.0	NV	2.0	2.0	NV
24	−		3.5	NV	NV	NV	NV	NV	NV	NV	3.0	4.0	2.5	4.0	4.5	≥1.5
28	−		3.5	≥1.5	3.5	≥1.5	2.5	1.5	1.5	NV	5.0	4.5	3.5	3.5	4.5	≥3.5
32	+		3.0	4.0	3.5	≥1.5	≥1.5		1.5		3.0	4.0	4.0	4.5	4.0	
33	+		3.5	2.0	2.5	1.0	NV	NV	NV	≥2.0	5.5	5.0	3.5	5.5	4.5	2.5
35	+		3.0	3.0	1.5	≥1.5	≥2.5	2.0	≥2.5	2.0	5.5	4.0	4.5	5.0	6.0	
36	−		3.5	≥1.5	≥1.5		NV		2.5	2.0	1.0	4.0	1.5	3.0	4.0	

[a] After 4 weeks, regional lymph nodes were included with peripheral lymph nodes. Virus was not detected in bone marrow when assayed at 8, 12, 16, 20, and 32 weeks. Undiluted serum was assayed for virus at 16, 20, 24, 28, and 32 weeks; a trace was detected at 28 weeks. Blank spaces indicate that the tissue was not assayed for virus. NV, Virus not detected.

Histologic methods. Frozen sections of brain and spinal cord fixed in Formalin-ammonium bromide were impregnated by the Cajal gold sublimate method and evaluated for astrocytosis and spongiform degeneration—the main neurohistologic changes of TME. In a few instances, these sections were supplemented with paraffin sections of Formalin-fixed brain and spinal cord stained with buffered azure-eosinate and with luxol fast blue–periodic acid-Schiff.

Viral assay. Suspensions (10%) of tissues collected at necropsy were prepared (with a mortar and pestle) in Eagle minimal essential medium containing 10% heat-inactivated fetal bovine serum and antibiotics. With a specially designed jig, mink anesthetized with ether were inoculated in the left parietal area of the cerebrum with 0.1 ml of serial 10-fold dilutions of the tissue suspensions. Two mink were used for each 10-fold dilution. The presence of TME virus in the inoculum was based on the occurrence of typical TME in these mink during a 24-month period. Endpoints in the titrations were estimated by the Spearman-Kärber method (6).

Design of experiment. Twenty-six mink were inoculated subcutaneously behind the left elbow with $10^{3.0}$ 50% intracerebral lethal doses of TME virus. Beginning 1 week later, and at intervals of 1 to 4 weeks through 28 weeks, one mink chosen at random was killed for viral assay. At 32, 33, and 35 weeks, mink showing clinical signs of TME were selected. One mink killed at 36 weeks was selected as the most normal appearing of those remaining. So that the occurrence of TME in this group of mink could be studied further, the 13 not used for viral assay were allowed to become affected; they either died from the disease or were killed at an advanced stage of it.

At each interval, the mink was anesthetized with ether and exsanguinated by cardiac puncture. A complete necropsy was done, and serum and many tissue samples were collected for viral assay. Intestinal samples were pools of samples from three levels exclusive of the colon. Bone marrow was obtained from a whole femur. During the first 4 weeks after inoculation, lymph nodes draining the site of inoculation (left prescapular and axillary) were collected separately. After that, they were pooled with all other peripheral lymph nodes (submandibular, medial retropharyngeal, prefemoral, inguinal, and popliteal). Serum and tissue specimens were stored in sealed ampoules kept at −65°C until prepared for viral assay.

RESULTS

Clinical observations. During the first 20 weeks after inoculation, the seven mink killed for viral assay were clinically normal. TME first appeared at 24 weeks, when one of the 19 remaining was ataxic in the hindquarters and could not climb the sides of the cage without falling. It died 5 weeks later, after a typical clinical course during which the incoordination became worse and the mink spent much time biting the cage. A second mink became affected at 27 weeks and died about 6 weeks later, again after a typical clinical course manifested by long periods of somnolence, compulsive biting of the cage, and increasingly severe incoordination of gait. Between 31 and 34 weeks after inoculation, 11 more became affected. Three in early stages of the disease, at 32, 33, and 35 weeks, were killed for viral assay. In the others, the disease progressed to terminal prostration or death, usually in 2 to 3 weeks. A week after onset of rapidly progressive disease at 37 weeks, one mink chewed off its tail, a not uncommon event in TME, and was killed. Another became affected at 43 weeks and died 3 weeks later. The last mink in the group was clinically normal until 51 weeks after inoculation. It was killed 3 weeks later, when in an advanced stage of encephalopathy.

Thus, of the 19 mink remaining when TME first appeared, 16 became affected with the disease. The other three were still clinically normal when killed for viral assay at 24, 28, and 36 weeks. But in our experience with the inoculum used, they too would have become affected had they been allowed to live longer.

Temporal distribution of virus. One week after inoculation, only lymph nodes draining the site of inoculation had virus, a small amount (Table 1). At 2 and 4 weeks, they had slightly more, but again it was not detected in other lymphatic organs. After that, when all peripheral lymph nodes were pooled, moderate amounts ($10^{3.0}$ to $10^{4.0}$ 50% lethal doses) were found in them at 8 and 12 weeks. Because the thymus

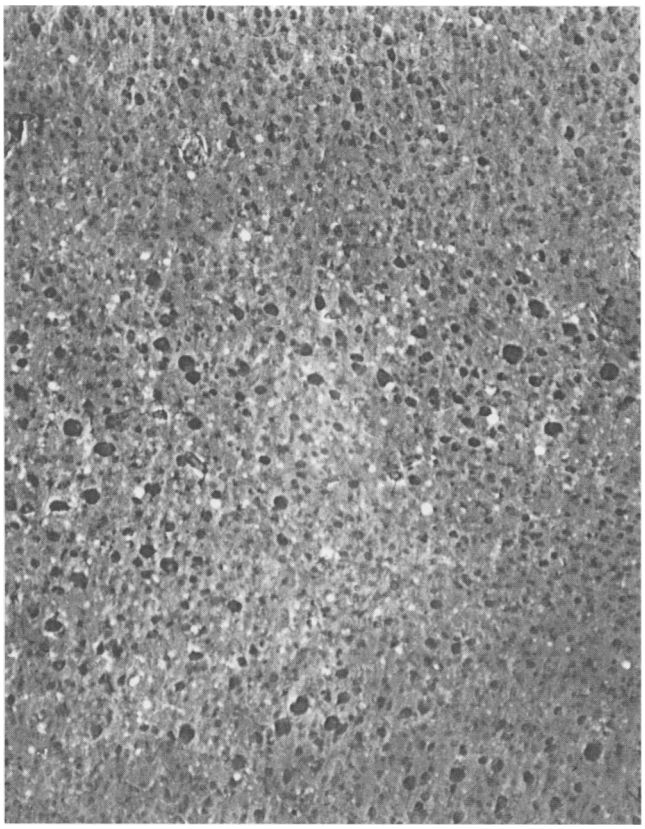

FIG. 1. Early spongiform degeneration in middle and deep layers of the posterior sigmoid gyrus at 28 weeks. The meningeal surface of the cruciate sulcus is beyond the top. Cajal gold sublimate; magnification, ×85.

was the only other lymphatic organ containing virus then, that in the peripheral lymph nodes probably came from the regional lymph nodes. At 16 weeks, virus was not detected in any organ, including the CNS. It first appeared in the CNS at 20 weeks, when all mink were still clinically normal. The diencephalon and cervical and lumbar spinal cord each had a small amount, $10^{2.0}$ 50% lethal doses. But virus was absent elsewhere.

In the clinically normal mink at 24 weeks, virus was present in all parts of the CNS, usually at titers of $10^{3.0}$ to $10^{4.5}$ 50% lethal doses, and for the first time in the sciatic nerve. The only extraneural virus was the moderate amount in the peripheral lymph nodes. In the clinically normal mink at 28 weeks, virus was again found in all parts of the CNS and in the sciatic nerve, mostly at moderate titer. It was also detected in the peripheral lymph nodes and in many extraneural organs (spleen, thymus, kidney, liver, mesenteric lymph node, and submandibular salivary gland), where it had not been earlier. Except in the peripheral lymph nodes and spleen, titers were low. At that time, undiluted serum had a trace of virus; TME occurred in one of the two assay mink after an exceptionally long incubation period of 82 weeks. This was the only time when virus was detected in serum.

The infection had a similar pattern in mink that were in early stages of encephalopathy at 32, 33, and 35 weeks. At 32 weeks, the titers and distribution of virus were much like those at 28 weeks. When the disease was somewhat more advanced clinically, at 33 and 35 weeks, titers in the CNS were slightly higher, reaching $10^{6.0}$ 50% lethal doses in the lumbar spinal cord. At that time, the intestine had a small amount of virus. So too did the intestine of the clinically normal mink at 36 weeks, when TME had already occurred in most mink. Otherwise, the titers and distribution of virus then were about like those at 28 weeks. Bone marrow was assayed for virus at 8, 12, 16, 20, and 32 weeks, but none was detected at any time.

Neurohistologic findings. Although the brain and spinal cord had small amounts of virus at 20 weeks, neurohistologic changes were not seen until 28 weeks, when all parts of the CNS had moderate amounts. The only lesion then was a linear patch of spongiform degeneration in the posterior sigmoid gyrus bordering the cruciate sulcus (Fig. 1). Affecting mainly the middle and deep layers of the neocortex in a pseudolaminar pattern, it was accompanied by a few shrunken neurons and a few enlarged astrocytes. Similar degenerative changes were found at the same site in the mink affected with early TME at 32 weeks, when the CNS had like amounts of virus. Astrocytes in the molecular layer underlying the adjacent cruciate sulcus were enlarged, but histologic changes were not seen elsewhere in the CNS.

At 33 and 35 weeks, when the disease was somewhat more advanced clinically and the CNS had more virus, neurohistologic changes were widespread. Frontal and parietal gyri were diffusely spongy. In the more severely affected frontal cortex, some larger neurons were shrunken. More caudally in the neocortex, the sponginess was less obvious. In the phylogenetically older cortex, including the hippocampus and the subcallosal, medial olfactory, and cingulate gyri, the degeneration varied from mild to moderate and was sometimes patchy. Except at the depths of sulci, little astrocytic response accompanied the cerebrocortical sponginess. The corpus striatum and septal area had undergone mild to moderate degeneration with mild astrocytosis. Sponginess was mostly mild in the diencephalon. In the midbrain tegmentum, it was mild but diffuse, especially in the periaqueductal gray matter. The cerebellar cortex was unchanged. So too were the more caudal parts of the CNS, except for mild astrocytosis in the gray matter of the spinal cord, mainly the cervical and thoracic segments. Neurohistologic changes of TME were not found in the clinically normal mink at 36 weeks, even though virus was then widespread in the CNS.

DISCUSSION

The temporal distribution of virus in the mink differed greatly from that of scrapie virus in naturally infected Suffolk sheep (16) and in mice inoculated subcutaneously with the Chandler strain of the virus (9). The striking difference was the extremely limited replication of virus in lymphatic organs as a prelude to infection of the CNS after peripheral inoculation. This pattern of extraneural replication resembled that in goats inoculated subcutaneously with the Chandler strain (14). During the first 12 weeks, TME virus probably replicated to some extent in lymph nodes draining the site of inoculation but not in other lymphatic organs, except the thymus at 8 weeks. Even more impressive and unexpected was the complete absence of virus from all extraneural sites at 16 and 20 weeks. Then, 4 weeks after its seeming disappearance from the peripheral lymph nodes, virus appeared in the CNS, where its concentration increased thereafter as clinical disease evolved. Because the onset of

clinical disease occurred in most mink within about a 3-week period, these erratic virologic findings most likely cannot be explained as chance variations in the dynamics of extraneural replication when only one mink was examined at each interval.

The pathway by which virus reached the CNS is uncertain. Most likely it was blood borne from the regional lymph nodes, as its detection in the thymus at 8 weeks and in serum at 28 weeks suggests. But during the long period when virus persisted in the regional lymph nodes, it may have infected peripheral nerves and passed along them to the CNS—a possibility alluded to by others (38). This mode of entry has also been suggested in some models of scrapie virus infection in mice (30). After peripheral inoculation of mice, virus appears in the spinal cord before it does in the brain, as do the pathologic changes (4, 9). Whether this sequence supervened in the mink is not clear; virus was in both levels of spinal cord but in only one of three levels of brain when it was first found in the CNS at 20 weeks. Early lesions, however, did not occur in the spinal cord.

Nevertheless, because virus was apparently limited to the regional lymph nodes, it may have replicated at the inoculation site rather than in lymph nodes draining the site. Therefore, skin cannot be ruled out as the source of virus infecting the CNS. Possible replication of scrapie virus in skin has been mentioned (27). But the only reported study we found that examined this organ for scrapie virus failed to demonstrate it in a suspension of skin (face and forequarters) from a Cheviot sheep affected with the disease after intracerebral inoculation (41).

Virus appeared in the CNS at 20 weeks, or 4 weeks before clinical disease first appeared and about 13 weeks before it occurred in most mink. Others reported similar findings in mink inoculated intraperitoneally with the Wisconsin (Hayward) strain of TME virus (8). They first found virus in the brain at 16 weeks and then regularly after that. Clinical disease began appearing about 14 weeks later. Neurohistologic changes were first found at 24 weeks and from then on. And as in our study, early spongiform degeneration was limited to the posterior sigmoid gyrus bordering the cruciate sulcus in the frontal cortex—an exquisite example of selective vulnerability of nervous tissue to a viral infection (24).

The concentration of virus in the brain when lesions first occur and clinical disease supervenes is not known. Also unknown is the concentration when electroencephalographic changes appear that indicate the presence of encephalopathy several weeks before the onset of clinical disease (12). In our study, the concentrations were fairly high in most parts of the CNS at 28 weeks, when only minimal spongiform degeneration was present in the frontal cortex and the mink was still clinically normal. Similar concentrations of virus and minimal cortical changes were also found at 32 weeks, when the mink was in the early clinical stage of TME. Undoubtedly, a threshold concentration, which probably varies from one part of the CNS to another, is necessary for neurohistologic changes to occur (14, 29). But once that threshold is reached, higher concentrations may not necessarily increase the severity of the lesion; they probably indicate only how readily the virus replicates in a particular part of the CNS (4, 14).

The topographic distribution of the higher concentrations of virus did not parallel that of the neurohistologic changes. For example, the cerebellar cortex had moderate amounts of virus but was free of histologic changes, as is typical of TME (8, 23; unpublished data). Also, the spinal cord with similar amounts of virus had only minimal changes. Such discordance sometimes occurs also in sheep and goats affected with natural scrapie, in which the correlation otherwise is generally good (16, 17). Thus, even though high concentrations of virus occurred throughout the CNS, regional variations in its vulnerability to injury by the virus were still reflected in the topographic distribution of the neurodegeneration that distinguishes TME (8).

Only after secondary replication occurred in the CNS did virus appear in several extraneural organs (spleen, liver, kidney, intestine, salivary gland, and mesenteric lymph node), where we had expected to find it earlier but did not. Why virus became so widely disseminated late in the infection is puzzling. At first glance, it suggests that secondary viremia (assuming that the CNS became infected from a primary one) supervened by virus reentering the bloodstream from its site of replication in the CNS. The greater concentration of virus there than in the peripheral lymph nodes earlier might have had a bearing on this event. But the spread of virus from the CNS in this way seems unlikely, for ordinarily, virus replicating there does not reenter the blood circulation (10).

Perhaps more likely was the movement of virus from the brain stem and spinal cord down peripheral nerves to the salivary gland and abdominal viscera, much as in rabies (40). The occurrence of virus in the sciatic nerve, a common finding in scrapie-affected sheep and goats (14, 16, 17), no doubt resulted from extension of the infection in the spinal cord. Such centrifugal spread of virus along peripheral nerves has been found also in mice infected with scrapie virus (27) and in other viral infections of the CNS (25).

Still, the late dissemination of virus could have been simply delayed spread from the regional lymph nodes or even from where it might have been sequestered at the inoculation site. Then again, maybe the late dissemination was more apparent than real; it could have been a consequence of delayed replication in the many extraneural sites that virus had reached earlier during primary viremia, however feeble and fleeting it might have been.

Whatever the explanation, titers of virus in extraneural organs remained low—probably too low for them to be important sources of virus that might be shed to the exterior. The wide dissemination of virus outside the CNS once the mink became sick was like that found in mink severely affected with TME after intramuscular (34) or intraperitoneal (unpublished data) inoculation. After intracerebral inoculation, virus was less widely disseminated (34), as we found also during terminal disease in sheep and goats similarly inoculated with the Idaho strain of TME virus (18).

When compared with the virologic events in scrapie (16), the limited extraneural replication of TME virus that preceded infection of the CNS suggests that mink are not natural hosts of the virus. Ostensibly, the infection in mink is a dead-end one (39)—a conclusion consistent with the notion that TME virus is not indigenous to this carnivore. Moreover, most evidence indicates that it is not naturally transmissible, either horizontally or vertically (3, 7, 36). This is so despite the late appearance of virus in organs (intestine, kidney, and salivary gland) from which it could be shed to the exterior.

Supposedly, then, each occurrence of TME is a new infection with exogenous virus, putatively wild scrapie virus. Because of the biologic variability of scrapie virus (2), origin of TME in this way would explain slight variations in its expression in different outbreaks (36) and in the results of experiments with virus isolated from them (1, 18, 26, 28, 37). Moreover, such variability also explains why only some wild

strains of scrapie virus are pathogenic for mink, causing a neurologic disease indistinguishable from TME (19, 20, 36; unpublished data).

Thus, although our findings do not resolve the uncertainty about the origin and identity of TME virus, we think that they support the notion that it is not native to mink. This conclusion is consistent with the generally held view that it most likely is feed-borne wild scrapie virus to which mink, as aberrant hosts, are accidently or inadvertently exposed now and again.

ACKNOWLEDGMENTS

We thank Clarence Robinson for preparing the histologic sections, Lola Grenfell for assembling the data, Liza Hamby for translating from the Russian, and William Anderson for taking care of the mink. The late Carl M. Eklund contributed much to the design and early guidance of the study.

LITERATURE CITED

1. **Barlow, R. M., and J. C. Rennie.** 1970. Transmission experiments with a scrapie-like encephalopathy of mink. J. Comp. Pathol. **80**:75–79.
2. **Bruce, M. E., and A. G. Dickinson.** 1987. Biological evidence that scrapie agent has an independent genome. J. Gen. Virol. **68**: 79–89.
3. **Burger, D., and G. R. Hartsough.** 1965. Encephalopathy of mink. II. Experimental and natural transmission. J. Infect. Dis. **115**:393–399.
4. **Cole, S., and R. H. Kimberlin.** 1985. Pathogenesis of mouse scrapie: dynamics of vacuolation in brain and spinal cord after intraperitoneal inoculation. Neuropathol. Appl. Neurobiol. **11**: 213–227.
5. **Danilov, E. P., N. S. Bukina, and B. P. Akulova.** 1974. Encephalopathy in mink. Krolikovod. Zverovod. **17**:34. (In Russian.)
6. **Dougherty, R. M.** 1964. Animal virus titration techniques, p. 169–223. *In* R. J. C. Harris (ed.), Techniques in experimental virology. Academic Press, Inc., New York.
7. **Duker, I. I., V. I. Geller, V. A. Chizhov, V. M. Roikhel, V. V. Pogodina, G. I. Fokina, S. G. Sobolev, and M. B. Korolev.** 1986. Clinical and morphological investigation of transmissible mink encephalopathy. Vopr. Virusol. **31**:220–225. (In Russian.)
8. **Eckroade, R. J., G. M. Zu Rhein, and R. P. Hanson.** 1979. Experimental transmissible mink encephalopathy: brain lesions and their sequential development, p. 409–449. *In* S. B. Prusiner and W. J. Hadlow (ed.), Slow transmissible diseases of the nervous system, vol. 2. Academic Press, Inc., New York.
9. **Eklund, C. M., R. C. Kennedy, and W. J. Hadlow.** 1967. Pathogenesis of scrapie virus infection in the mouse. J. Infect. Dis. **117**:15–22.
10. **Fenner, F., P. A. Brachmann, E. P. J. Gibbs, F. A. Murphy, M. J. Studdert, and D. O. White.** 1987. Veterinary virology, p. 457–458. Academic Press, Inc., New York.
11. **Gajdusek, D. C.** 1985. Unconventional viruses causing subacute spongiform encephalopathies, p. 1519–1557. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman, and R. E. Shope (ed.), Virology. Raven Press, New York.
12. **Grabow, J. O., R. J. Eckroade, and R. P. Hanson.** 1971. Serial electroencephalographic studies of experimentally induced transmissible mink encephalopathy. Am. J. Vet. Res. **32**:457–464.
13. **Hadlow, W. J.** 1965. Discussion of paper by D. Burger and G. R. Hartsough, p. 303–305. *In* D. C. Gajdusek, C. J. Gibbs, Jr., and M. Alpers (ed.), Slow, latent, and temperate virus infections. NINDB monograph 2. U.S. Government Printing Office, Washington, D.C.
14. **Hadlow, W. J., C. M. Eklund, R. C. Kennedy, T. A. Jackson, H. W. Whitford, and C. C. Boyle.** 1974. Course of experimental scrapie virus infection in the goat. J. Infect. Dis. **129**:559–567.
15. **Hadlow, W. J., and L. Karstad.** 1968. Transmissible encephalopathy of mink in Ontario. Can. Vet. J. **9**:193–196.
16. **Hadlow, W. J., R. C. Kennedy, and R. E. Race.** 1982. Natural infection of Suffolk sheep with scrapie virus. J. Infect. Dis. **146**: 657–664.
17. **Hadlow, W. J., R. C. Kennedy, R. E. Race, and C. M. Eklund.** 1980. Virologic and neurohistologic findings in dairy goats affected with natural scrapie. Vet. Pathol. **17**:187–199.
18. **Hadlow, W. J., R. E. Race, and R. C. Kennedy.** 1987. Experimental infection of sheep and goats with transmissible mink encephalopathy virus. Can. J. Vet. Res. **51**:135–144.
19. **Hanson, R. P., R. J. Eckroade, R. F. Marsh, G. M. Zu Rhein, C. L. Kanitz, and D. P. Gustafson.** 1971. Susceptibility of mink to sheep scrapie. Science **172**:859–861.
20. **Hanson, R. P., and R. F. Marsh.** 1974. Biology of transmissible mink encephalopathy and scrapie, p. 10–15. *In* W. Zeman and E. H. Lennette (ed.), Slow virus diseases. The Williams & Wilkins Co., Baltimore.
21. **Hartsough, G. R., and D. Burger.** 1965. Encephalopathy of mink. I. Epizootiologic and clinical observations. J. Infect. Dis. **115**:387–392.
22. **Hartung, J., H. Zimmermann, and U. Johannsen.** 1970. Infektiöse Enzephalopathie beim Nerz. 1. Mitteilung: klinisch-epizootiologische und experimentelle Untersuchungen. Monatsh. Veterinaermed. **25**:385–388.
23. **Johannsen, H., and J. Hartung.** 1970. Infektiöse Enzephalopathie beim Nerz. 2. Mitteilung: pathologisch-morphologische Untersuchungen. Monatsh. Veterinaermed. **25**:389–395.
24. **Johnson, R. T.** 1980. Selective vulnerability of neural cells to viral infections. Brain **103**:447–472.
25. **Johnson, R. T.** 1983. Viral infections of the nervous system, p. 45. Raven Press, New York.
26. **Kimberlin, R. H., S. Cole, and C. A. Walker.** 1986. Transmissible mink encephalopathy (TME) in Chinese hamsters: identification of two strains of TME and comparisons with scrapie. Neuropathol. Appl. Neurobiol. **12**:197–206.
27. **Kimberlin, R. H., H. J. Field, and C. A. Walker.** 1983. Pathogenesis of mouse scrapie: evidence for spread of infection from central to peripheral nervous system. J. Gen. Virol. **64**:713–716.
28. **Kimberlin, R. H., and R. F. Marsh.** 1975. Comparison of scrapie and transmissible mink encephalopathy in hamsters. I. Biochemical studies of brain during development of disease. J. Infect. Dis. **131**:97–103.
29. **Kimberlin, R. H., and C. A. Walker.** 1978. Pathogenesis of mouse scrapie: effect of route of inoculation on infectivity titres and dose-response curves. J. Comp. Pathol. **88**:39–47.
30. **Kimberlin, R. H., and C. A. Walker.** 1980. Pathogenesis of mouse scrapie: evidence for neural spread of infection to the CNS. J. Gen. Virol. **51**:183–187.
31. **Marsh, R. F.** 1974. Slow virus diseases of the central nervous system. Adv. Vet. Sci. Comp. Med. **18**:155–178.
32. **Marsh, R. F.** 1976. The subacute spongiform encephalopathies, p. 359–380. *In* R. H. Kimberlin (ed.), Slow virus diseases of animals and man. Elsevier/North-Holland Publishing Co., Amsterdam.
33. **Marsh, R. F., D. Burger, R. Eckroade, G. M. Zu Rhein, and R. P. Hanson.** 1969. A preliminary report on the experimental host range of the transmissible mink encephalopathy agent. J. Infect. Dis. **120**:713–719.
34. **Marsh, R. F., D. Burger, and R. P. Hanson.** 1969. Transmissible mink encephalopathy: behavior of the disease agent in mink. Am. J. Vet. Res. **30**:1637–1642.
35. **Marsh, R. F., and R. P. Hanson.** 1969. Physical and chemical properties of the transmissible mink encephalopathy agent. J. Virol. **3**:176–180.
36. **Marsh, R. F., and R. P. Hanson.** 1979. On the origin of transmissible mink encephalopathy, p. 451–460. *In* S. B. Prusiner and W. J. Hadlow (ed.), Slow transmissible diseases of the nervous system, vol. 1. Academic Press, Inc., New York.
37. **Marsh, R. F., and R. H. Kimberlin.** 1975. Comparison of scrapie and transmissible mink encephalopathy in hamsters. II. Clinical signs, pathology, and pathogenesis. J. Infect. Dis. **131**:104–110.
38. **Marsh, R. F., J. M. Miller, and R. P. Hanson.** 1973. Transmissible mink encephalopathy: studies on the peripheral lymphocyte. Infect. Immun. **7**:352–355.

39. **Mims, C. A., and D. O. White.** 1984. Viral pathogenesis and immunology, p. 74. Blackwell Scientific Publications, Ltd., Oxford.
40. **Murphy, F. A., A. K. Harrison, W. C. Winn, and S. P. Bauer.** 1973. Comparative pathogenesis of rabies and rabies-like viruses. Infection of the central nervous system and centrifugal spread of virus to peripheral tissues. Lab. Invest. **29**:1–16.
41. **Stamp, J. T., J. G. Brotherston, I. Zlotnik, J. M. K. Mackay, and W. Smith.** 1959. Further studies on scrapie. J. Comp. Pathol. **69**:268–280.
42. **Zu Rhein, G. M., R. J. Eckroade, and J. D. Grabow.** 1973. Pathobiology of transmissible mink encephalopathy, p. 16–38. *In* W. Zeman and E. H. Lennette (ed.), Slow virus diseases. The Williams & Wilkins Co., Baltimore.