Experimental Infection of Sheep and Goats with Transmissible Mink Encephalopathy Virus

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ABSTRACT

In a study to learn more about the pathogenicity of transmissible mink encephalopathy virus for the natural hosts of scrapie, 20 Cheviot sheep and 19 dairy goats were inoculated intracerebrally with the Idaho strain of the virus. Five sheep and nine goats became affected with a progressive neurological disease. The incubation period in the sheep varied from 45 to 80 months (mean, 65 months) and in the goats from 31 to 40 months (mean, 35 months). Except for degeneration of the cerebral cortex (neocortex), the disease was indistinguishable clinically and neurohistologically from scrapie. During two more passages of the virus in goats, the incubation period was shortened to 12 to 15 months, the morbidity rate rose to 100% (6/6 dairy goats and 3/3 African pygmy goats), and the cortical lesion became constant and more pronounced. By the intracerebral inoculation of pastel mink, transmissible mink encephalopathy virus was detected in the brains of several affected sheep and goats but not in extraneural sites (lymphoid tissues and intestine), except for a trace amount in the proximal colon of one goat. Even after two passages in goats, the virus remained nonpathogenic for the laboratory mouse. Despite the essential likeness of the experimental disease and scrapie, the common identity of their causal viruses remains to be determined. Even so, the results of this study are still compatible with the view that transmissible mink encephalopathy virus almost certainly is scrapie virus whose biological properties became altered by chance passage in mink, a carnivore and an aberrant host.

Key words: Central nervous system diseases, goats, mink, mink encephalopathy virus, scrapie virus, sheep.

RÉSUMÉ

Cette expérience portait sur l'inoculation intracérébrale de la souche du virus de l'encéphalopathie transmissible du vison de l'Idaho, à 20 moutons Cheviot et à 19 chèvres laitières, afin d'en connaître davantage sur la pathogénicité de ce virus, chez des hôtes naturels de la tremblante. Cinq moutons et neuf chèvres développèrent une maladie nerveuse progressive. La période d'incubation varia de 45 à 80 mois, chez les moutons, et de 31 à 40 mois, chez les chèvres; elle afficha donc une moyenne de 65 mois, chez les premiers, et de 35 mois, chez les deuxièmes. Les signes cliniques et les lésions microscopiques du cerveau s'avérèrent tout à fait semblables à ceux de la tremblante, à l'exception de la dégénérescence du néocortex de l'écorce cérébrale. À la suite de deux passages additionnels du virus chez des chèvres, la période d'incubation ne dura que 12 à 15 mois; le taux de morbidité atteignit 100%, à savoir: six chèvres laitières sur six et trois chèvres pygmées africaines sur trois; les lésions corticales devinrent par ailleurs constantes et plus marquées. L'inoculation intracérébrale de visons pastel permit de démontrer la présence du virus précité dans le cerveau de plusieurs moutons et chèvres affectés, mais non dans leur tissu lymphoïde ou intestinal, à l'exception du côlon proximal d'une chèvre qui en recelait une très faible quantité. Même après deux passages chez la chèvre, le virus demeura inoffensif pour la souris de laboratoire. En dépit d'une ressemblance essentielle entre cette maladie expérimentale et la tremblante, l'identité commune de leur agent causal respectif demeure toujours à déterminer. Les résultats de cette expérience sont tout de même compatibles avec l'hypothèse selon laquelle le virus de l'encéphalopathie transmissible du vison correspondrait à celui de la tremblante, mais dont les propriétés biologiques auraient subi une altération à la faveur d'un passage fortuit chez le vison, animal carnivore et hôte aberrant.

Mots clés: maladies du système nerveux central, chèvres, visons, virus de l'encéphalopathie du vison, virus de la tremblante, moutons.

INTRODUCTION

Transmissible mink encephalopathy (TME) has striking neuropathological similarities to scrapie of sheep and goats (1,2). Indeed, this resemblance, first recognized in 1963, prompted studies that demonstrated the transmissibility of this previously undefined rare neurological disease of ranch mink (*Mustela vison*) (3,4). This likeness to scrapie was strengthened when the physical and chemical properties of the mink virus were found identical with those of scrapie virus (5). (Although the exact nature of these infectious agents is still in question, we refer to them as viruses largely because we think of them in that way.) But the experimental host ranges of the two viruses indicated differences in their biological behavior. For instance, both cause encephalopathy in the Syrian hamster, Chinese hamster, squirrel monkey, goat, and mink (6,7,8,9,10, 11,12,13). Yet unlike scrapie virus, TME virus is not pathogenic for the

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laboratory mouse (1,9,14,15). Nevertheless, despite this and other differences, present evidence strongly suggests that TME virus almost certainly is scrapie virus whose biological properties became altered by chance passage in mink, a carnivore and an aberrant host (14).

To learn more about the pathogenicity of TME virus for the natural hosts of scrapie, we inoculated sheep and goats with the Idaho strain of the mink virus. Our findings are recounted here.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Female and castrated male sheep and goats, all from scrapie-free stock, were either obtained locally or raised at Rocky Mountain Laboratories. Kept in groups of three to eight in an indoor isolation unit, they were given alfalfa hay, mineralized salt, and water. When inoculated, they were 6 to 12 months old.

Yearling female royal pastel mink used to assay TME virus were obtained locally, kept individually in an outdoor isolation compound, and given a standard wet ration and water.

Female random-bred Swiss mice (Rocky Mountain Laboratories stock) 21 to 24 days old used in pathogenicity studies were kept in disposable plastic boxes in a maximum security unit not used for housing other animals. They had free access to pelleted mouse chow and water.

VIRUS AND INOCULATION

Transmissible mink encephalopathy virus came from a naturally infected pastel mink obtained in August 1963 from a commercial herd in southeastern Idaho (4). A 500 nm Millipore filtrate of a 5% suspension of its brain tissue, in phosphate-buffered saline containing 10% fetal bovine serum, was inoculated intraperitoneally into a pearl mink. When it became affected with encephalopathy, about ten months later, a 300 nm Millipore filtrate of a 10% suspension of its brain tissue was inoculated intraperitoneally into four pastel mink. Pieces of brain from each of these mink, collected after they became sick with the encephalopathy, were pooled and made into a 10% suspension in phosphate-buffered serum-saline. This second passage virus is our stock preparation of the Idaho strain. As determined by intracerebral inoculation of pastel mink (four per tenfold dilution), it contained $10^{6.8}$ LD_{50} of virus per g of brain tissue when used in this study.

Under local (procaine) anesthesia, the sheep and goats were inoculated into the left parietal area of the cerebrum. Through a short incision in the scalp and underlying periosteum, a 2.5 cm 20 gauge needle was inserted to its full length in a small hole bored in the calvaria with an electric drill.

EXPERIMENTS

Three experiments were done: In the first, 20 Cheviot sheep and 19 goats, mainly of Saanen breeding, were each inoculated intracerebrally with 1 mL of a 10^{-2} dilution of the stock preparation of TME virus. In the second, six goats, mainly of French Alpine breeding, were each inoculated intracerebrally with 0.5 mL of a 10% suspension of brain tissue (pool of diencephalon, midbrain and medulla) from one affected goat of the first experiment. In the third, three African pygmy goats were each inoculated intracerebrally with 0.5 mL of a 10% suspension of a similar pool of brain tissue from one affected goat of the second experiment. When in an advanced stage of the neurological disease that ensued, the animals were anesthetized with sodium pentobarbital and exsanguinated.

At necropsy, tissues for passage and viral assay were sealed in ampoules and stored at -70°C until processed. Tissues for neurohistological examination were fixed in neutral-buffered 10% formalin and in Cajal's formalin-ammonium bromide. Large paraffin sections of brain stained with buffered azure-eosinate and sometimes also with luxol fast blue-PAS were evaluated by a scheme described previously (16).

DETECTION OF TME VIRUS

Transmissible mink encephalopathy virus was sought in neural and extraneural tissues of several affected sheep and goats. By means of a specially designed jig, 0.1 mL of 10% tissue suspension, prepared in phosphate-buffered serum-saline, was inoculated into the left parietal area of the cerebrum of mink anesthetized with ether. Each suspension of brain tissue was inoculated into four or six mink and each suspension of extraneural tissues into two mink. The occurence of typical TME in these mink during an 18 month period was taken as evidence of virus in the inoculum.

PATHOGENICITY OF TME VIRUS FOR THE MOUSE

Whether passage of TME virus in sheep or goats had made it pathogenic for the mouse was determined as follows: Groups of 24 mice each (lightly anesthetized with ether) were inoculated intracerebrally with 0.03 mL of 10% suspensions of diencephalon or cerebellar cortex from four affected sheep and four affected goats of the first experiment. Eighteen mice were similarly inoculated with a 10% suspension of brain tissue (pool of diencephalon, midbrain and medulla) from one affected goat of the second experiment. All mice were observed for 24 months for clinical signs of progressive neurological disease.

RESULTS

CLINICAL OBSERVATIONS

Five of the 20 sheep became affected with a progressive neurological disease, clinically indistinguishable from scrapie, 45 to 80 months (mean, 65 months) after inoculation. The common clinical feature was increasingly severe incoordination of gait. Stepping movements of the fore limbs became deliberate or stilted; those of the hind limbs usually resulted in a hopping gait. When made to move rapidly or turn sharply, severely affected sheep fell. Tremor, notably of the head, was common. One sheep became excitable and another, somnolent. Three circled or wandered about aimlessly. As the disease progressed, the sheep often preferred lying down to standing. Patchy loss of wool occurred in two, but the scratching and rubbing that led to it were seldom noticed. Impaired vision was presumed in three that regularly bumped into objects and sustained trauma about the head. Although they continued to eat, all sheep lost weight; four were extremely thin at necropsy. Two died. The others were

killed when near death. In all five sheep, the course of the disease was unremitting and short — lasting about six weeks.

Of the other 15 sheep, four succumbed to cerebrocortical necrosis (as did several of comparable age in the source flock) during the first 48 months after inoculation. Another four died from other intercurrent diseases. The remaining seven were clinically normal when killed and discarded 86 months after inoculation.

A similar progressive neurological disease also occurred in nine of the 19 goats in the first experiment 31 to 40 months (mean, 35 months) after inoculation. During that period, one of the other ten suffered from the disabling consequences of an abscess in the lumbar spinal cord and was killed. The remaining nine were clinically normal when killed and discarded 55 to 60 months after inoculation. All six goats in the second experiment became affected with the neurological disease. The incubation period was 12 to 15 months in five and 24 months in the other. In the third experiment, all three goats likewise became affected 12 to 15 months after inoculation.

The clinical pattern of the disease was the same in all three experiments. Behavioral changes, such as increased excitability or apprehension, characterized the insidious onset. With ears forward, tail up, and a dull facial expression, the goats often stood staring into space. Tremor of the head was common from the start and usually persisted. Scratching the neck and withers with hind feet and horns occurred in six goats, but it neither dominated the clinical picture nor resulted in much loss of hair. The outstanding feature was increasingly severe disturbances in gait. In some goats, it was wobbly, in others, deliberate or stilted. Most goats stumbled while walking. Seemingly unaware of their surroundings, the goats usually wandered about aimlessly in a swaying gait. At rest, they stood with their hind limbs well under the body.

As the disease progressed, the goats preferred lying down, and they had difficulty rising without help. A few became drowsy. Impaired vision, seemingly near blindness in some, commonly supervened. Toward the end, a few slobbered ruminal contents. Except for one in each experiment, all goats remained in a good to excellent state of nourishment. They were killed when they could no longer stand or had great difficulty doing so. Unlike that in the sheep, the tempo of clinical progression varied widely. In the first experiment, the course of the disease was 3 to 23 weeks (mean, 10 weeks), in the second it was 5 to 25 weeks (mean, 14 weeks), and in the third, 7 to 12 weeks (mean, 10 weeks).

NEUROHISTOLOGICAL CHANGES

In both sheep and goats, the neurohistological changes, too, were like those of scrapie, not only in kind but for the most part also in topographic distribution. Thus, the essential degenerative lesion comprised: shrinkage and increased basophilia of neurons; cytoplasmic vacuolation of neurons, mainly in the brain stem; astrocytosis; and spongiform alteration of the neuropil. With some variation in the relative prominence of each, the degenerative lesion occurred regularly in the corpus striatum, diencephalon, brain stem, and cerebellum. In two sheep and most goats, the cerebral cortex was affected as well. Because the extent and severity of the neurohistological changes were much the same in both hosts, a common description of the changes will suffice in most instances.

The cerebral cortex (neocortex) had undergone mild to moderate degeneration in two sheep and five goats of experiment 1 and in four goats of experiment 2. In one goat of experiment 2 and in all three goats of experiment 3, the degeneration was severe and widespread. All main lobes of the cerebrum were affected, and no particular gyri seemed favored. When mild, the degeneration often occurred irregularly in patches of varied intensity. When severe, it was usually uniformly so throughout the cortex.

The spongiform change, mostly tiny holes, often extended throughout the thickness of the cortex, but it usually was most pronounced in the middle and deep layers (Fig. 1). Under low magnification, this distribution gave the cortical lesion a pseudolaminar pattern (Fig. 2). Except in the molecular layer underlying sulci, the sponginess tended to be faint in the more

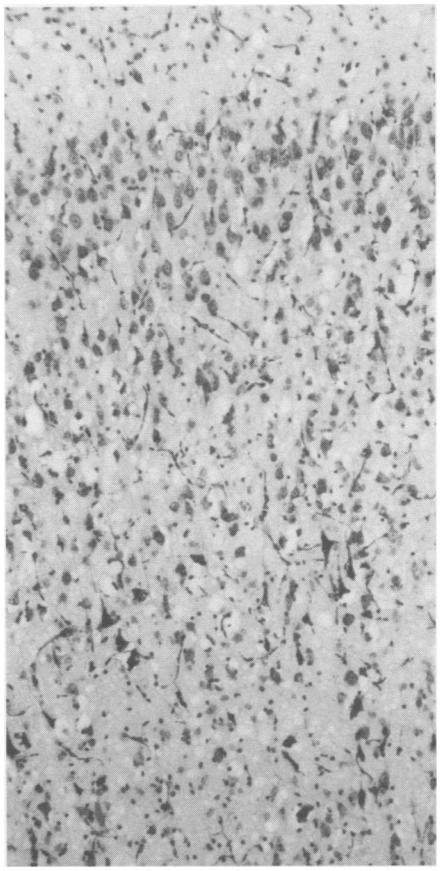

Fig. 1. Mild spongiform change, astrocytosis, and degeneration of giant pyramidal neurons in superior frontal gyrus of goat (Exp. 2). Azure-eosinate. X85.

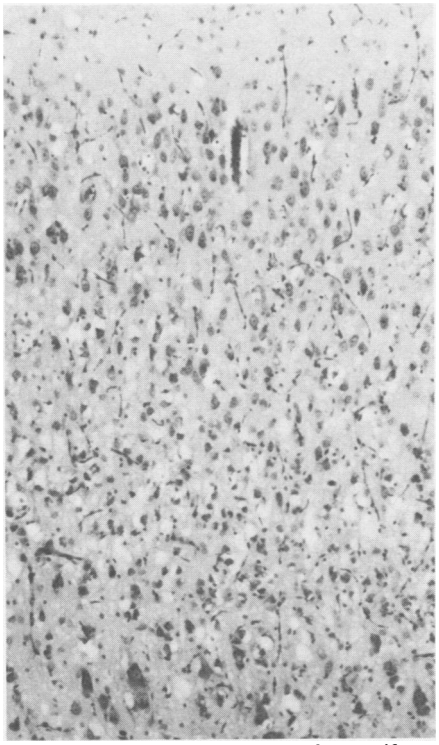

Fig. 2. Pseudolaminar pattern of spongiform change in frontal lobe gyrus of goat (Exp. 2). Azure-eosinate. X85.

superficial layers. Indeed, the spongiform change never caused the diffuse rarefaction commonly seen in subcortical gray masses. The sponginess was seldom accompanied by much neuronal shrinkage, which was mostly confined to the middle layers and included some giant pyramidal neurons in the superior frontal gyrus. As demonstrated by Cajal's gold sublimate impregnation, the astrocytosis was often more conspicuous than the spongiform change. When mild, it was most apparent in the middle and deep layers (Fig. 3). When more intense, it extended throughout the thickness of the cortex, not always in company with the spongiform change.

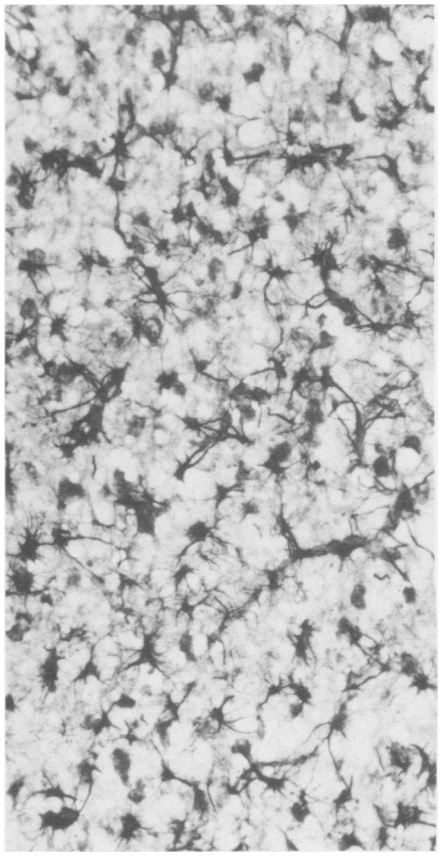

Fig. 3. Severe spongiform change and astrocytosis in middle layers of superior frontal gyrus of goat (Exp. 2). Cajal's gold sublimate. X175.

Even when absent or mild in the neocortex, the spongiform change and astrocytosis were often moderate to severe in phylogenetically older parts of the cerebral cortex, including the cingulate gyrus (juxtallocortex) (Fig. 4). Usually, the olfactory gyrus and stria were severely affected, especially by the spongiform change (Fig. 5). So

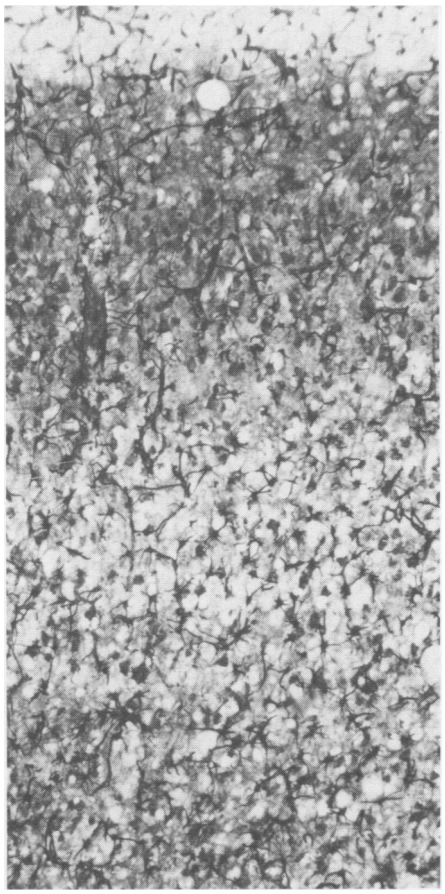

Fig. 4. Spongiform change and astrocytosis in cingulate gyrus of goat (Exp. 2). Cajal's gold sublimate. X85.

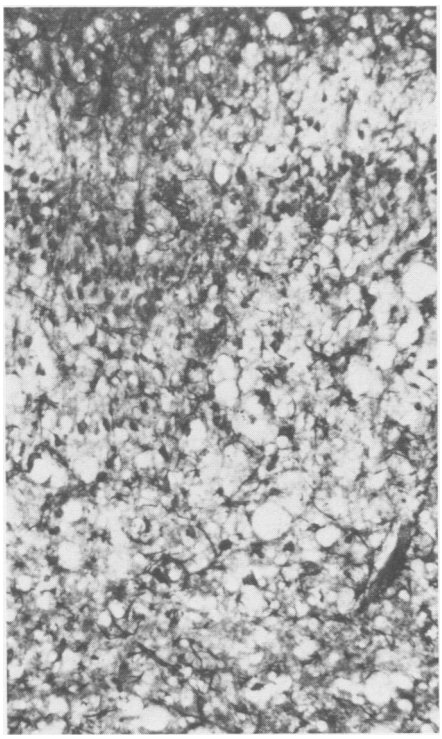

Fig. 5. Severe spongiform change with little astrocytosis in lateral olfactory gyrus of goat (Exp. 3). Cajal's gold sublimate. X85.

too in some sheep and goats were the subcallosal and parahippocampal gyri and the pyriform lobe generally (Fig. 6). Sponginess was common in the hippocampus, notably in the polymorphic cell layer (Fig. 7). Astrocytosis was usually intense, especially in the continuous molecular layers of the hippocampus and dentate gyrus (Fig. 8). In contrast to their naked appearance elsewhere in the brain, the enlarged nuclei of astrocytes found there and in the dorsal end of the parahippocampal gyrus were often surrounded by small amounts of faintly eosinophilic cytoplasm. Pyramidal neurons of the hippocampus were shrunken in some sheep and goats, but the granular layer of the dentate gyrus invariably was intact.

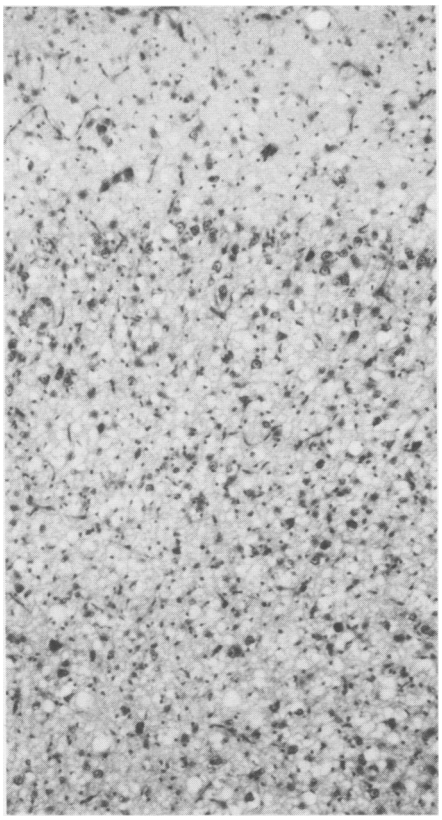

Fig. 6. Diffuse spongiform change in parahippocampal gyrus of goat (Exp. 3). Azure-eosinate. X85.

Elsewhere in the brain, the topographic distribution of the degenerative lesion was the same in all sheep and goats; it was no different from that characterizing scrapie as a neuropathological entity. Only the intensity of the degeneration in particular parts of the brain varied from one animal to another.

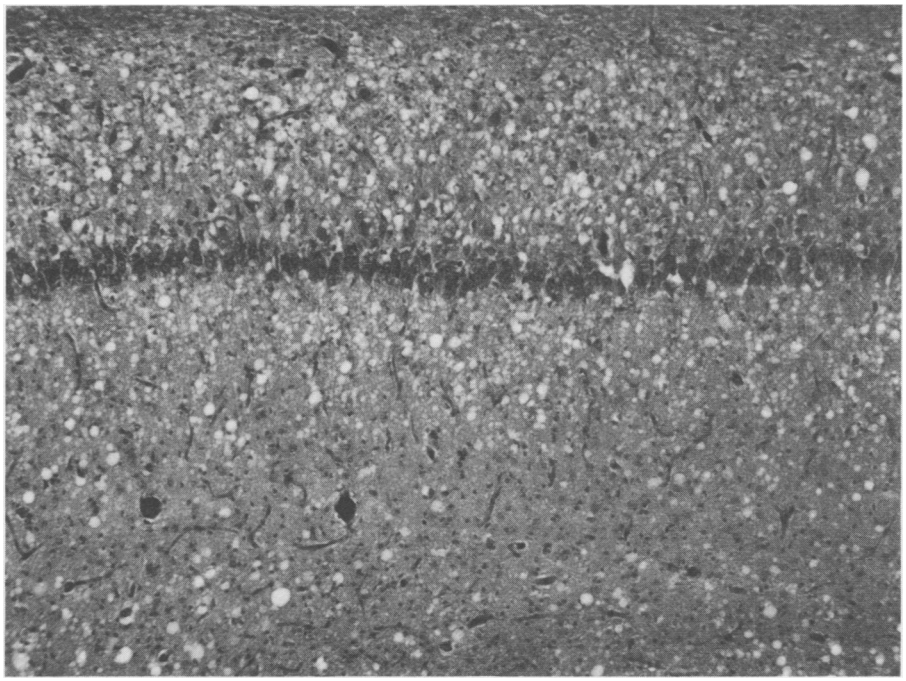

Fig. 7. Severe spongiform change in hippocampus of goat (Exp. 3). Alveus at top. Luxol fast blue-PAS. X85.

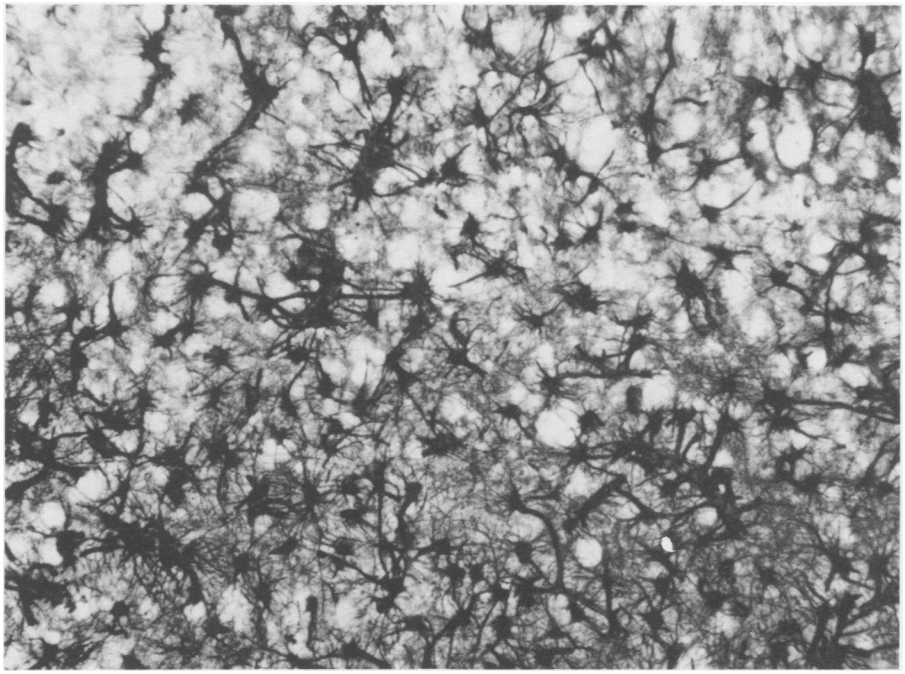

Fig. 8. Astrocytosis in molecular layer of hippocampus of goat (Exp. 3). Cajal's gold sublimate. X175.

The corpus striatum and associated structures were commonly affected. In all animals except two goats of experiment 1, mild to moderate spongiform change and astrocytosis were diffuse in the caudate nucleus and putamen (Fig. 9). Nearly always, the astrocytosis was more intense in the putamen. Neither severe nor widespread, neuronal degeneration was confined to the larger cells in both structures. Shrunken neurons, mild to moderate sponginess, and astrocytosis were common in the septal area. Rarely, a vacuolated neuron appeared among the shrunken ones of the septal nuclei. In about half the animals, most neurons of the globus pallidus were shrunken amid enlarged astrocytes. The amygdaloid body was often the site of intense astrocytosis and mild spongiform change without appreciable neuronal degeneration.

Consistently more severely affected than the corpus striatum was the thalamus. Indeed, neuronal degeneration and astrocytosis were generally more severe and widespread in the thalamus than elsewhere in the brain. The intensity of the astrocytosis often seemed disproportionately greater than that of neuronal shrinkage (Fig. 10). For the most part, the spongiform change was mild to moderate; in some nuclei, however, it occasionally was focally severe. Although seemingly diffuse at first glance, the degenerative changes in the thalamus did not affect all nuclei, at least equally. Those that regularly had the most severe changes included the lateral dorsal, medial dorsal, posterior ventral, lateral and medial geniculate (Fig. 11), pretectal, and pulvinar (Fig. 12).

The mammillary body and portions of the hypothalamus abutting on the third ventricle usually shared the astrocytosis and neuronal shrinkage occurring in the thalamus. Other than scattered holes, spongiform change was minimal. In one sheep and four goats, neurons of the supraoptic nucleus were pale-staining and frayed, but otherwise the main hypothalamic nuclei were largely intact in all animals. The subthalamic nucleus in two sheep and four goats had undergone severe neuronal degeneration with little associated spongiform change or astrocytosis.

Midbrain structures were regularly affected. Moderate to severe astrocytosis accompanying generally mild sponginess invariably occurred in the rostral colliculus (Fig. 13). Astrocytosis was always intense in the caudal colliculus, where vacuolated neurons were common in the goats. Also especially in the goats, a few large neurons of the red nucleus were chromatolytic — a neuronal change rarely found elsewhere. In two sheep and all goats, the compact part of the substantia nigra was spongy, and its neurons were shrunken. Occasionally, astrocytosis was diffuse in the tegmentum.

In the pons and medulla, the spongiform change and astrocytosis were much less conspicuous than in more

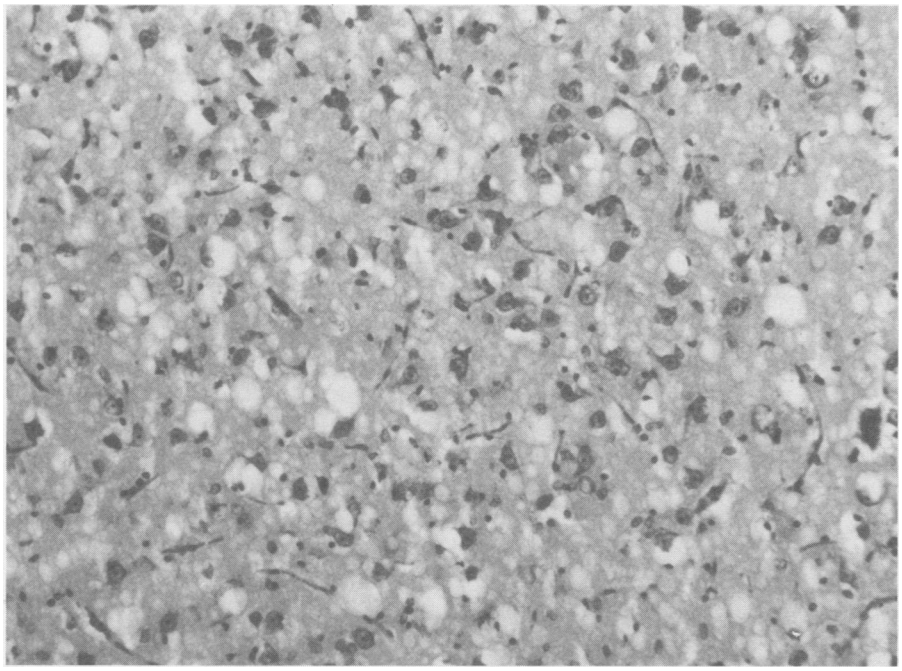

Fig. 9. Moderate spongiform change in caudate nucleus of goat (Exp. 2). Azure-eosinate. X85.

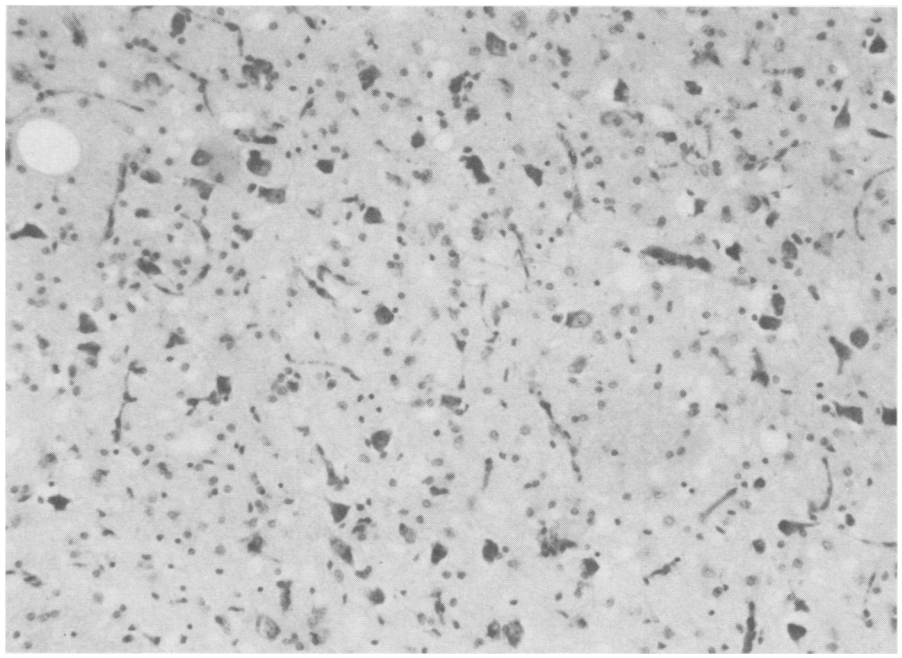

Fig. 10. Severe neuronal degeneration and astrocytosis with mild spongiform change in lateral dorsal nucleus of thalamus of goat (Exp. 1). Azure-eosinate. X175.

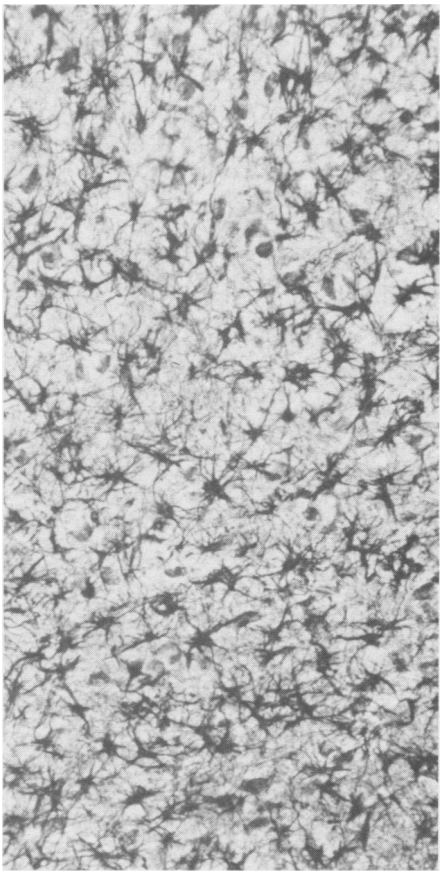

Fig. 11. Dense astrocytosis without spongiform change in medial geniculate nucleus of thalamus of sheep. Cajal's gold sublimate. X175.

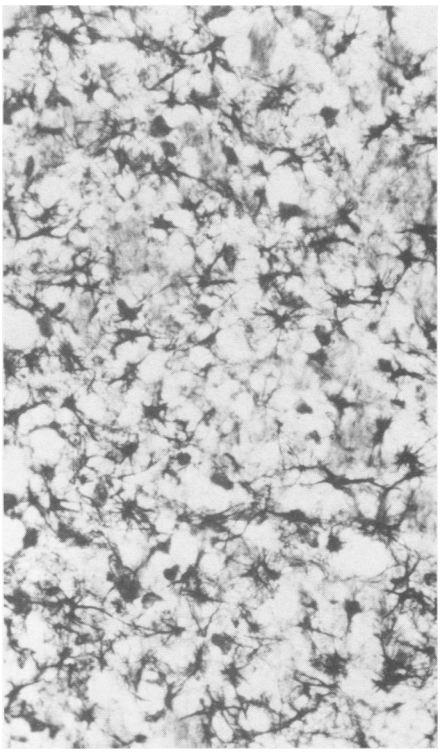

Fig. 12. Severe spongiform change and astrocytosis in pulvinar of goat (Exp. 2). Cajal's gold sublimate. X175.

rostral levels of the brain; neuronal degeneration was foremost. In the ventral pontine nucleus of every animal, neurons were shrunken or vacuolated (Fig. 14). The papillioform nucleus of the pons was similarly affected in most animals. Many nuclei of the medulla had undergone some degree of neuronal degeneration. Those most regularly and often most severely affected included the trapezoid, lateral and medial vestibular, lateral cuneate (Fig. 15), and lateral caudal. Vacuolated neurons were most numerous in the lateral

cuneate nucleus, lateral caudal nucleus, and reticular formation, especially in the goats. Neurons of the inferior olive were often shrunken, but their separation from fixation artifact was never certain. In one sheep and ten goats the caudal end of the spinal trigeminal nucleus, near its blending into the substantia gelatinosa of the spinal cord, was extremely spongy.

The cerebellum was affected in all animals. Purkinje cells were mostly unchanged, but in a few sheep and goats they had multiple small, vacuoles peripherally — never a single large vacuole typically found in neurons of pontobulbar nuclei. In about half the animals, proliferated nuclei of Bergmann glial cells formed focal aggregates or short bands that extended into the molecular layer of the cortex. Scattered holes were common in the granular layer, which otherwise looked normal in sections stained with azure-eosinate. But as demonstrated by Cajal's gold sublimate impregnation, astrocytosis was the distinctive change in the cortex (Fig. 16). Enlarged astrocytes formed a meshwork of variable density in the granular layer. Straight and crimped glial fibers radiated in the molecular layer. The intensity of such cortical astrocytosis was generally moderate to severe, but it varied among the folia; no particular lobe seemed favored. In most animals, all neurons of the fastigial and interposed nuclei were shrunken, whereas those of the dentate nucleus were seldom so.

Other than mild astrocytosis in the gray matter and an occasional vacuolated neuron in the intermediate cell columns in a few sheep and goats, the spinal cord was not affected.

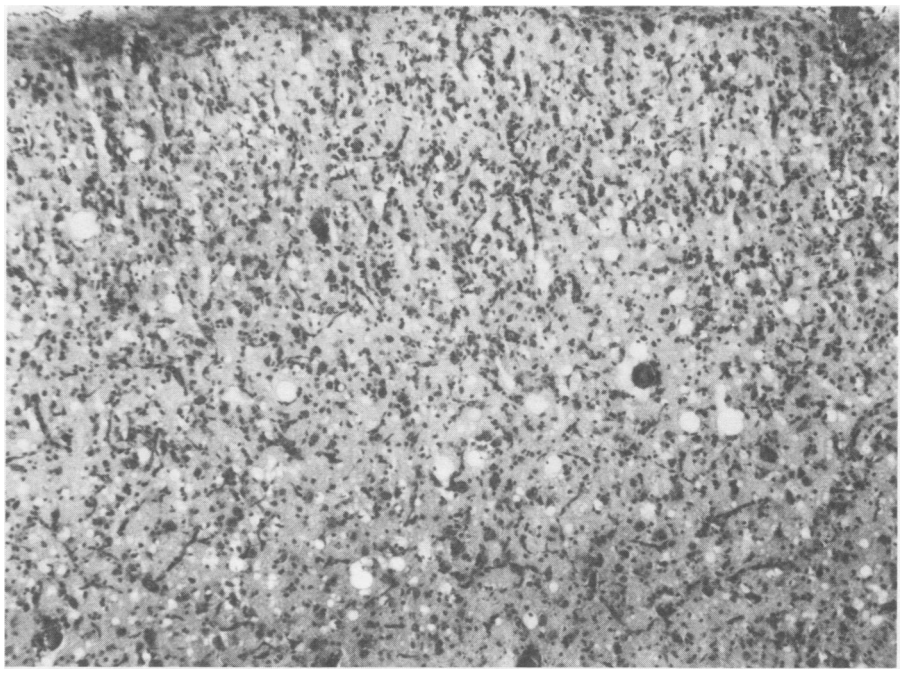

Fig. 13. Moderate spongiform change and dense astrocytosis in rostral colliculus of goat (Exp. 2). Meningeal surface at top. Azure-eosinate. X85.

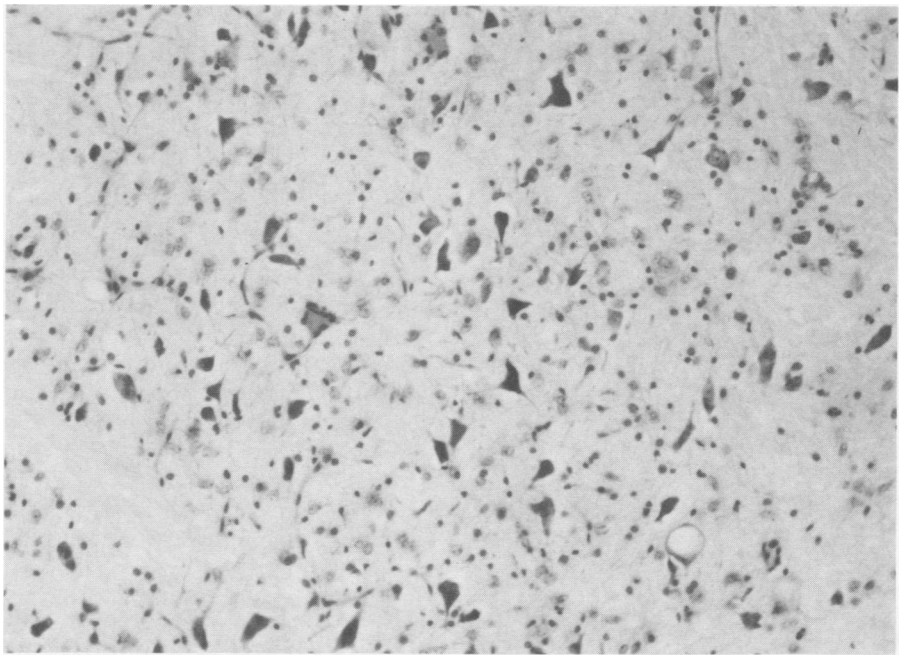

Fig. 14. Neuronal degeneration and vacuolation and astrocytosis in ventral pontine nucleus of goat (Exp. 1). Azure-eosinate. X175.

DETECTION OF TME VIRUS

Transmissible mink encephalopathy virus was detected in the diencephalon of two sheep and two goats in the first experiment. In each instance, all assay mink succumbed to TME after an incubation period that varied from 26 to 35 weeks (mean, 32 weeks). A pool of brain tissue (diencephalon, midbrain and medulla) from one goat had a virus titer of $10^{5.5}$ LD_{50} per g of tissue. Virus was sought also in the prescapular and mesenteric lymph nodes, spleen, terminal ileum, and proximal colon of the two goats. Only the proximal colon of one had virus — a trace amount as indicated by the occurrence of TME in one of the two assay mink after an incubation period of 52 weeks.

PATHOGENICITY OF PASSAGED TME VIRUS FOR MICE

As judged clinically, none of the mice inoculated with brain tissue from

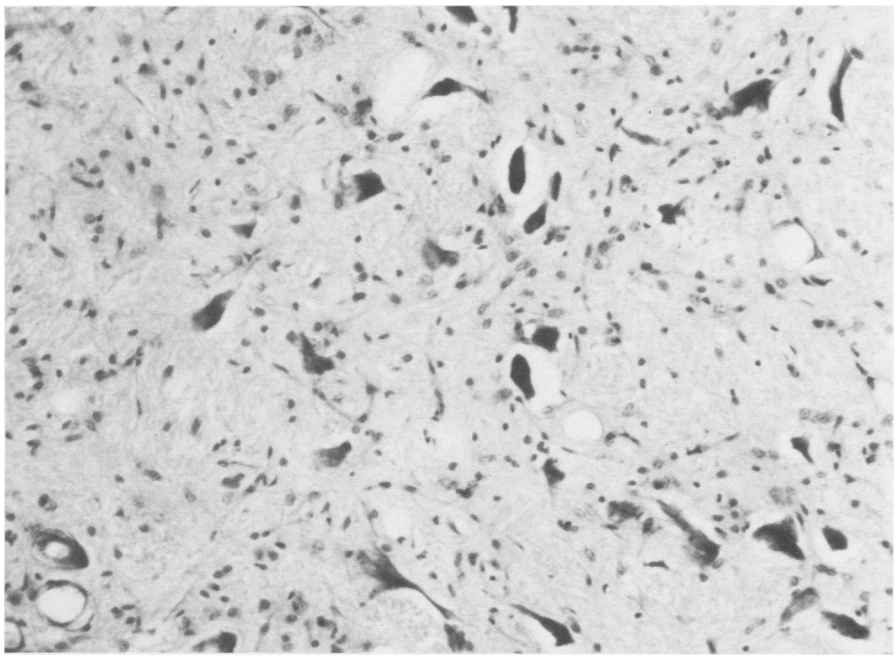

Fig. 15. Shrunken and vacuolated neurons in lateral cuneate nucleus of goat (Exp. 3). Azure-eosinate. X175.

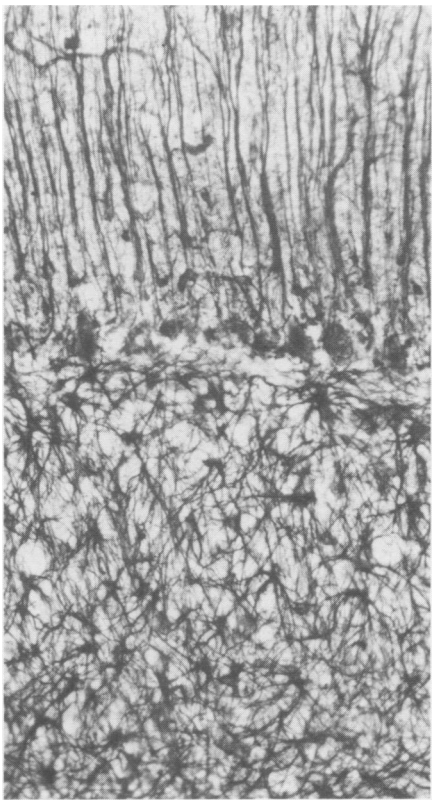

Fig. 16. Dense astrocytosis in cerebellar cortex of goat (Exp. 3). Cajal's gold sublimate. X175.

either sheep or goats succumbed to a neurological disease resembling murine scrapie (17).

DISCUSSION

In both sheep and goats, the experimental disease was indistinguishable clinically from either natural or experimental scrapie in these hosts (16,18,19,20). Functional disturbances that might have been attributable to the extraordinary cortical lesion were not recognized. Most likely this was largely so because such disturbances were overshadowed by those arising from degenerative changes in the corpus striatum, diencephalon, brain stem, and cerebellum — structures that have a greater bearing on gait and posture than the cerebral cortex in these ungulates (21). And besides, the correlation between clinical signs and neuropathological changes in sheep and goats affected with scrapie is only a broad, general one at best (16,18,22). [This may be partly so because such sheep and goats are seldom afforded more than a cursory neurological examination, inherent limitations in doing it notwithstanding (23).]

Except for occurring in the cerebral cortex (neocortex) in two sheep and most goats, the neurohistological changes also were indistinguishable from those of scrapie (16,20,22,24,25, 26). Although typically the site of spongiform degeneration and astrocytosis in TME (27), the neocortex is only mildly affected, if at all, in either natural or experimental scrapie in sheep and goats. Yet, as in the animals of this study, phylogenetically older parts of the cerebral cortex, such as the hippocampal formation and subcallosal and olfactory gyri, are often affected by degenerative changes. Apart from such degeneration in the allocortex, the essential neurohistological changes of scrapie characteristically occur in the corpus striatum, diencephalon, brain stem, and cerebellum — a topographic pattern that also distinguished the disease in the sheep and goats infected with TME virus.

Although the topographic pattern was the same in the sheep and the goats, its general extent was more regularly manifest in the goats, as is largely so in scrapie too (16,22). Nevertheless, in both species the degenerative changes in the brain were as obvious and as distinctive as those in Suffolk sheep and dairy goats affected with natural scrapie (20,26). The overtness of the changes in the sheep contrasts with their common obscureness in scrapie-affected Cheviot sheep, especially when the disease is rapidly progressive (24), as was the ovine disease reported here. Except for suggesting that the readily demonstrable neurohistological changes in our Cheviot sheep resulted from peculiarities of TME virus, we have no explanation for this striking difference.

The presence of TME virus in the brain and its near absence from extraneural tissues of the two goats suggests that the goat is not a natural host of the virus. Ostensibly, the infection was a dead end one (28), as TME virus infection in mink seems to be as well (unpublished observation). Yet, such limited replication of virus outside the central nervous system was found also in goats inoculated intracerebrally with mouse-passaged scrapie virus (25). Both these findings contrast sharply with the widespread replication of scrapie virus in extraneural sites (mainly lymphatic tissues) of naturally infected sheep and goats (20,26). Although possibly a reflection of the origin and passage history of the virus, these diverse patterns of replication most likely were a consequence of the route of exposure to it.

Thus, presumably in the sheep and goats inoculated intracerebrally with

TME virus, as in mice similarly inoculated with scrapie virus (29), extraneural replication was not essential to the evolution of florid neurological disease. Still, the virological findings in the two goats provided no basis for drawing conclusions about any pathogenetic differences between scrapie and the disease induced by TME virus.

Somewhat different results were reported by others who inoculated goats with the Wisconsin (Hayward) strain of TME virus (9,10,14). The clinical findings were less clear-cut, but diffuse spongiform degeneration of the cerebral cortex occurred in some goats, even in the absence of clinical signs (9,10). As in our study, the cortical lesion became more pronounced during successive intracerebral passage of the virus in goats (10). In one group of goats inoculated subcutaneously, small amounts of virus were detected in the regional lymph node and spleen in each of two, 7 and 27 months later (6). But neither goat had virus in the brain or histological evidence of neurological disease.

These apparent differences in pathogenicity of the Wisconsin and Idaho strains of TME virus for goats probably reflect variations in the biological behavior of the putative scrapie virus infecting the mink in the two widely separated geographic areas. Such variations in wild scrapie virus are known to exist, as exemplified by its diverse behavior in the laboratory mouse (30). This diversity, which may reflect biochemical differences (31), probably accounts also for slight variations in the expression of TME reported from one outbreak to another (14).

The continued lack of pathogenicity of TME virus for the mouse even after two passages in goats, offered no clues about its true identity. Nevertheless, such failure to cause progressive neurological disease in the mouse was found also with wild scrapie virus (Suffolk sheep and dairy goat origin) after one or more intracerebral passages in mink (14 and unpublished observations). Then too, scrapie virus of sheep or goat origin that had been passed in the mouse was not pathogenic for mink (1,14).

The essential likeness of the experimental disease and scrapie in sheep and goats has a bearing on the relationship of their causal viruses. They may be the same, or nearly so as other observations suggest (5,6). But given the technical limitations in distinguishing one from the other (12), the extent of their similarity, or indeed their common identity, will not be known until each is purified and its chemical structure determined. The same is true for the relation of TME virus and scrapie virus to other unconventional viruses causing spongiform encephalopathies (32). At present, these viruses are distinguished from one another mainly in the way they behave in a wide range of natural and experimental hosts. But such behavior can be changed simply by varying the passage history of the virus.

On the other hand, if TME virus and scrapie virus are not the same, then the nosological status of scrapie as a natural disease of sheep and goats might be questioned. Thus, it may be less a specific etiological entity than it is a clinical, or even a clinicopathological, one. This possibility, referred to before (33,34), is supported by the observation that brain tissue from persons dying of Creutzfeldt-Jacob disease caused an encephalopathy in goats that was indistinguishable clinically and neurohistologically from caprine scrapie (35). If this view of scrapie has credence, then virus from such diverse sources as mink brain and human brain can be said to have caused scrapie, at least clinically in the goat. Such a view would have epidemiological implications for both the human disease and the animal diseases.

Despite these considerations and the pitfalls in drawing conclusions about the cause of a neurological disease solely from clinicopathological observations (36), we think our findings are still compatible with the view that TME virus almost certainly is scrapie virus whose biological properties became altered by chance passage in an aberrant host. Indeed, an identical disease with widespread spongiform degeneration of the cerebral cortex also occurred in goats inoculated intracerebrally with mink-passaged scrapie virus of Suffolk sheep brain origin (unpublished observation).

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