Transmissible Mink Encephalopathy: Experimental Transmission to the Squirrel Monkey

Abstract. *A progressive, fatal spongiform encephalopathy developed in three squirrel monkeys 11 months after inoculation with primate-passaged transmissible mink encephalopathy agent. The clinical symptoms and histopathologic and electron microscopic findings suggest that this naturally occurring disease of mink has been transmitted experimentally to squirrel monkeys.*

The etiologic agents of scrapie disease of sheep and transmissible encephalopathy of mink (TME) have been shown to be filterable and to have unusual physical and chemical stabilities (*1*, *2*). Hadlow (*3*) in 1959 pointed out the remarkable similarities in the epidemiology, clinical symptoms, and neuropathology between scrapie and a disease of man called kuru. Similarities of kuru and another chronic degenerative disease of man, Creutzfeldt-Jakob disease, had also been suggested (*4*). Subsequently, kuru (*5*) and Creutzfeldt-Jakob disease (*6*) were transmitted to lower primates. These four diseases are similar in that they can all be transmitted experimentally to one or more hosts in which the incubation period is many months long, the neuropathology is similar, differing primarily in the distribution of the lesions, and the clinical findings in both natural and experimental infections are those of a progressive, fatal neurologic disease. These similarities led Gibbs (*6*) to propose for them the descriptive term "subacute spongiform viral encephalopathies."

In the study of experimental host range of TME by Marsh *et al.* (*7*), a 1-year-old rhesus monkey, inoculated (subcutaneously, intramuscularly, intravenously, and orally) with a 10 percent suspension of TME-infected mink brain, remained asymptomatic for 33 months. The animal was killed, and light microscopic examination of the brain revealed a polioencephalopathy with vacuolization of the gray matter similar to that seen in the brains of mink with TME. Suspensions of the rhesus monkey brain inoculated intracerebrally back into mink produced a disease indistinguishable from TME. Visceral tissues were also infective for mink. A stumptail macaque inoculated intracerebrally with homogenate of rhesus monkey brain developed a severe encephalopathy 16 months postinoculation (*8*). Second passage in rhesus monkeys has been initiated; one of these rhesus monkeys has developed a severe encephalopathy 19 months postinoculation.

Three female squirrel monkeys between 3 and 4 years of age were inoculated with the rhesus monkey brain homogenized in TenBroeck grinders and suspended in medium 199 containing 10 percent calf serum, and antibiotics (250 international units of penicillin and 250 μg of streptomycin per milliliter). Squirrel monkey No. 68 was inoculated intracerebrally in the right cortex with 0.2 ml and intravenously with 0.3 ml of the brain homogenate. Squirrel monkeys No. 23 and No. 69 were inoculated in the right cerebral cortex with 0.2 ml and intraperitoneally with 0.5 ml of the brain homogenate. These three animals were housed together in a single cage in an isolation room with other species of primates inoculated with similar materials.

Eleven months postinoculation, all three TME-inoculated squirrel monkeys developed a clinically similar, rapidly progressive, neurologic disease. Squirrel monkey No. 69 died 3 weeks later. Monkeys No. 23 and No. 68 were electively killed in terminal stages of the disease 4 weeks after the onset of clinical symptoms. Retrospectively, the earliest sign of clinical illness was an alteration of normal behavior which occurred at 10 months postinoculation. The normal and predictable reaction of these squirrel monkeys to the approach of a man was a rapid retreat to the upper rear part of the cage followed by frenzied escape attempts if the cage was touched or its door opened. During the 10th month postinoculation, a closer approach to the cage was required to elicit this response. This tolerance eventually reached the point that the animals would remain on the floor of the cage, or, rarely, climb the front screen to meet the approaching man. This progressively raised threshold for the perception of environmental threat preceded the acute phase of the illness by several weeks.

To facilitate a description of the clinical observations in squirrel monkeys, the neurological disease has been divided into three major categories: (i) impaired consciousness and cognition; (ii) motor dysfunction; and (iii) tremor. Many of these signs of dysfunction developed simultaneously and must be presumed to be interdependent expressions of a diffuse process.

Apathy, drowsiness, diminished attention span, and decreased activity were noted at 11 months postinoculation. The animals became somnolent, but were intermittently alert and attempted to eat and drink. These signs were progressively more severe until the animals became unresponsive in the terminal stages of the disease. In addition, squirrel monkey No. 69 experienced two episodes of unconsciousness, one at 11 months postinoculation and again 1 week later. The first episode was followed by apparent recovery. The second episode occurred during early signs of apathy and decreased activity.

There appeared to be impairment of body-spatial orientation and perception, as evidenced by abnormal body positions and limb postures which were maintained for extended periods, and searching motions of the feet and hands. All the monkeys had difficulty balancing when attempting to drink from a bowl on the bottom of the cage and would rock gently back and forth. In order to eat, the elbows and forearms were placed together on the floor, the food was held with both hands and the head was lowered to reach the food. The process of eating was inefficient, and most of the chewed food was left on the floor.

There was generalized weakness and marked slowing of all motor behavior such as eye blink, gait, and turning of the head. Movements of the extremities were at times groping and awkward and were suggestive of apraxic deficits. Spontaneous movements were infrequent as the disease progressed, and stimulation by prodding resulted in broad-based ataxic movement of a short distance followed by a return to the sitting position with the head bent forward almost touching the floor. The tail was carried in horizontal position close to the floor.

Two types of tremors were observed. Intermittent, brief (1 to 2 seconds in duration) episodes of very rapid tremors, involving one or both feet, were observed throughout the clinical period.

There was no associated alteration in consciousness or interruption of other concurrent motor function. The second type of tremor was occasional episodes of slow, rhythmic, side-to-side rocking movements.

Brain, cord, and visceral organs of the squirrel monkeys were collected for viral assay in mink and for light microscopy. In addition, brain tissues from squirrel monkeys Nos. 23 and 68 were fixed for electron microscopy. Suspensions of brain and spleen from these clinically affected squirrel monkeys inoculated intracerebrally into mink produced a disease that was clinically and pathologically indistinguishable from TME.

There were no gross pathological alterations of the central nervous system or of the visceral organs of the three TME-inoculated squirrel monkeys or of an uninoculated control squirrel monkey. In preliminary light microscopic studies of the central nervous system, the most striking finding was a severe spongiform polioencephalopathy (Fig. 1) involving most of the gray matter of the brain. In the spinal cord, a spongiform poliomyelopathy was observed; however, it was intense only in the posterior horns. These changes were consistent in all three TME-inoculated squirrel monkeys but were not seen in the control animal. Vacuoles varying in size chiefly from 10 to 20 μm, with occasional sizes up to 45 μm, were located in the neuropil. Rare vacuoles of a smaller diameter than the cell nucleus were located within the cytoplasm of nerve cell bodies. Loss of nerve cells was not conspicuous. In the cerebellum, vacuolization was limited to the granular layer of the cortex, with focal involvement of the subjacent white matter of the folia. Extensive proliferation and hypertrophy of astrocytes was observed in the areas of most intense vacuolization. Demyelination, microglial proliferation, and inflammatory infiltrates were not observed.

The lesions in the central nervous system of TME-inoculated squirrel monkeys, as seen by light microscopy, were similar to those that have been seen in mink with TME, except for an involvement of cerebellum and spinal cord which has so far not been encountered in mink.

Preliminary electron microscopic studies of the cerebral cortex of two TME-inoculated squirrel monkeys revealed a focal and presumably hydropic swelling of dendrites and axons. They also confirmed the presence of vacuoles within neuronal perikarya and nerve cell processes as seen by light microscopy. The large vacuoles in the neuropil (Fig. 2A) were found to be derived from several neighboring disrupted cell processes and were filled with variously shaped membranes (Fig. 2B). Similar findings have been obtained in cortical tissues of TME-infected mink (*9*) and also in the cortex of monkeys inoculated with brain suspensions of patients suffering from kuru (*10*) and from Creutzfeldt-Jakob disease (*10*).

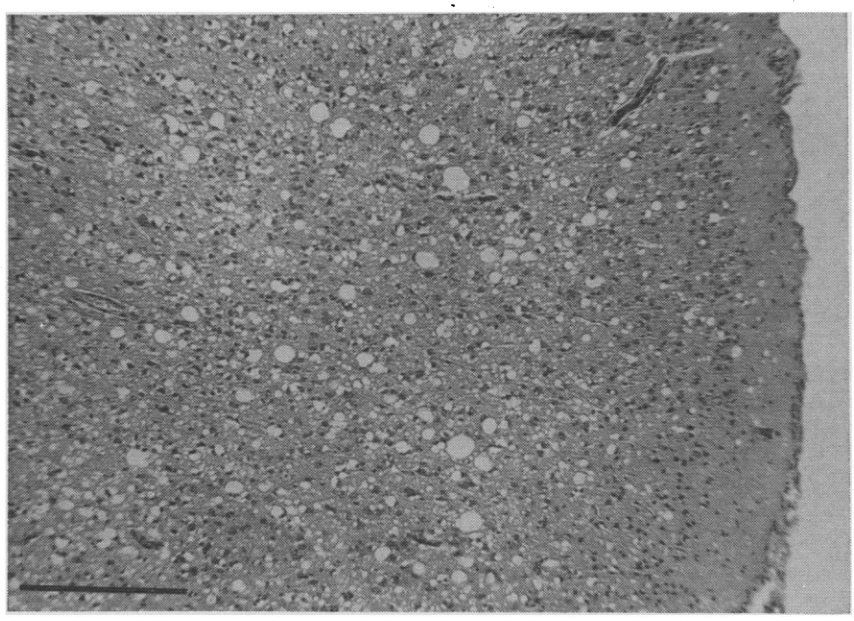

Fig. 1. Spongiform polioencephalopathy of cerebral cortex of squirrel monkey inoculated with primate-passaged TME agent. Hematoxylin and eosin stain; bar equals ¼ mm.

The experimental diseases in mink and squirrel monkeys were similar in their long incubation period, histo-

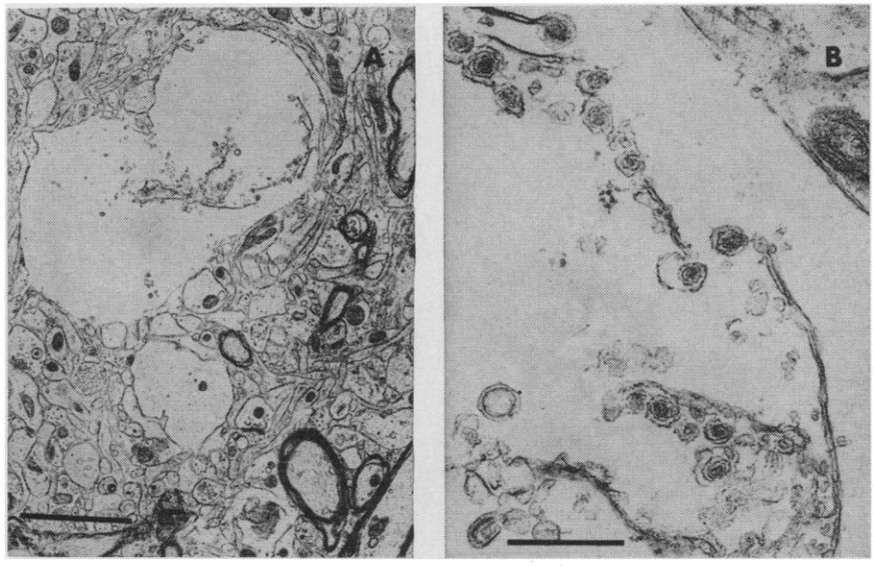

Fig. 2. Electron micrographs of cerebral cortex after immersion fixation of tissue in 1 percent osmium tetroxide, embedding in Epon/Araldite, and contrasting of sections with uranyl acetate and lead citrate. (A) Oblong vacuole with membranous contents surrounded by cell processes including myelinated axons. Bar equals 2 μm. (B) Detail of membranes revealing spiral, circular, and linear profiles. Bar equals ½ μm.

pathologic criteria, and in some of the clinical signs. Differences existed in the distribution of lesions in the gray matter. All the squirrel monkeys developed a similar disease at the same time after being held in isolation for a period of 11 months. Similar spontaneous neurologic disease in subhuman primates is not known to occur (*5, 6*). Specific efforts were not made to identify simian passenger viruses (*11*). The possibility that such agents would have contributed to these findings seems remote.

Transmissible encephalopathy of mink in squirrel monkeys provides a readily available inexpensive primate model for the study of "subacute spongiform viral encephalopathies." This investigation lends support to the suggested relationship between animal and human transmissible viral encephalopathies.

ROBERT J. ECKROADE
GABRIELE M. ZU RHEIN
Department of Pathology, University of Wisconsin Medical School, Madison 53706
RICHARD F. MARSH
ROBERT P. HANSON
Department of Veterinary Science, University of Wisconsin, Madison

References and Notes

1. D. C. Gajdusek and C. J. Gibbs, Jr., in *Infections of the Nervous System*, H. M. Zimmerman, Ed. (Williams & Wilkins, Baltimore, 1968), p. 260.
2. R. F. Marsh and R. P. Hanson, *J. Virol.* **3**, 176 (1969).
3. W. J. Hadlow, *Lancet* **1959-II**, 289 (1959).
4. I. Klatzo, D. C. Gajdusek, V. Zigas, *Lab. Invest.* **8**, 799 (1959).
5. D. C. Gajdusek, C. J. Gibbs, Jr., M. P. Alpers, *Nature* **209**, 794 (1966); *Science* **155**, 212 (1967).
6. C. J. Gibbs, Jr., D. C. Gajdusek, D. M. Asher, M. P. Alpers, E. Beck, P. M. Daniel, W. B. Matthews, *Science* **161**, 388 (1968); C. J. Gibbs, Jr., and D. C. Gajdusek, *ibid.* **165**, 1023 (1969).
7. R. F. Marsh, D. Burger, R. J. Eckroade, G. M. Zu Rhein, R. P. Hanson, *J. Infect. Dis.* **120**, 713 (1969).
8. Details are in preparation for publication elsewhere.
9. G. M. Zu Rhein and R. J. Eckroade, *Proc. 6th Int. Congr. Neuropathol.*, Paris, 31 August to 4 September 1970 (in press).
10. P. W. Lampert, K. M. Earle, C. J. Gibbs, Jr., D. C. Gajdusek, *J. Neuropathol. Exp. Neurol.* **28**, 353 (1969); P. W. Lampert, C. J. Gibbs, Jr., D. C. Gajdusek, Program and Abstracts, Joint Meeting, American Association of Pathologists and Bacteriologists and American Association of Neuropathologists, St. Louis, Mo., 7–10 March 1970.
11. N. G. Rogers, M. Basnight, C. J. Gibbs, Jr., D. C. Gajdusek, *Nature* **216**, 446 (1967).
12. This work was supported by grants (AI-NB 09023, and 5-FO2-HE-35, 844-04) from NIH and by a cooperative agreement with the USDA. We thank the Wisconsin Regional Primate Research Center for providing the squirrel monkeys and Dr. C. Hopper, Department of Neurology, for consultation on the clinical observations. The technical assistance of Mrs. Martha Blakis and Mrs. Kris Muetzel is gratefully acknowledged.

28 April 1970; revised 26 June 1970

Mutation in Internode Length Affects Wheat Plant-Type

Abstract. *A mutant form was found in an M_2 population of wheat* Triticum aestivum *L. em. Thell. (aestivum group) 'Seneca'. The population was derived from soaked grains treated with 3.2 kilorads of gamma rays. The first and second internodes below the spike were reduced in length 33 and 15 percent, respectively, and the total height was 18 percent shorter than the prototype. The flag leaf sheath was normal in length resulting in spike placement below the flag leaf lamina. Segregation data suggest that one dominant gene controls this character. The canopy structure of a population of mutant plants is different from that of the normal type; therefore, this mutant can be used to evaluate light interception and physiological aspects of crop productivity.*

The study of canopy structure as it is related to productivity has led to a concept of plant-type by which production efficiency of crop plants is to be maximized (*1*). Such attributes as leaf angle and size, tillering ability, and plant height influence the amount and distribution of light intercepted in the canopy of cereal crops at a given plant population density and, therefore, are expected to affect photosynthetic efficiency. Photosynthesis in the culm, flag leaf, spike, and awn contribute variously to carbohydrate accumulation in the grain (*2*). Genetic modification of plant form through the use of isogenic lines provides information on the relative contribution of plant parts to grain yield (*3*). Thus the concept of plant-type has provided some previously unrecognized guidelines for the improvement of cereal crops. The major breakthroughs in increasing the yield of wheat (*4*) and rice (*5*) have been accompanied by major changes in phenotype, notably the development of short-statured wheat varieties and short-statured, erect-leaved varieties of rice. Full analysis of the concept of plant-type and whether it provides meaningful breeding objectives requires genetic variants with a common background genotype. We report here the finding of one such mutant of wheat. The mutant has reduced length of upper culm internodes with normal flag leaf lamina and sheath. This results in spike placement below the flag leaf lamina which in turn alters the canopy by placing the flag leaf in a more prominent position for light interception.

Four lots of grains of 'Seneca' wheat [*Triticum aestivum* L. em. Thell. (aestivum group)] were soaked in water for 24 hours, and each was given one of the following mutagenic treatments: 1.0 or 3.2 krad of gamma rays (^{137}Cs), or 100 or 320 rad of unmoderated fission neutrons. Similar lots were soaked for 24 hours in 0.01*M* or 0.100*M* ethyl methanesulfonate. The M_1 plants from all treated seeds and a control population were grown in reproductive isolation near Oak Ridge, Tennessee, in 1966. Approximately 850 M_2 plants, spaced 45 cm apart, of each of the six treated and the control populations were grown near Knoxville and Springfield, Tennessee, in 1967. Three plants (S636-1, S636-4, and S785-5) with reduced length of the upper internode were found in the population irradiated with 3.2 krad of gamma rays; no plants of this type were found in the other populations. Plants from the M_3 and M_4 generations derived by self-pollination from these putative mutant plants were observed when grown at

Table 1. Culm length of M_4 mutant and normal plants derived from S636-1. Internode 1 is the peduncle directly below the spike. Means of measurements of five culms each on nine homozygous mutant and 19 homozygous normal plants are given.

Internode	Culm				Percentage of normal
	Mutant		Normal		
	Length (cm)	Percentage of total length	Length (cm)	Percentage of total length	
1	19.9*	32	29.7	39	67.0
2	15.7*	25	18.5	24	84.9
3	10.9	18	11.9	16	91.6
4	8.8	14	9.4	12	93.6
5	5.8	9	5.8	8	100.0
6	1.0	2	0.7	1	
Total	62.1*	100	76.0	100	81.7

* Significant difference from normal ($P < .05$).