Myelin Deficiency (md)

A Neurologic Mutant in the Wistar Rat

C. K. Csiza, DVM, PhD, and A. de Lahunta, DVM, PhD

Myelin deficiency (md), a newly discovered neurologic mutation in the Wistar rat, is transmitted by an X-linked, recessive lethal gene. Male rats are affected, and the first symptom is a head tremor recognizable at 12 to 15 days of age. The tremors become generalized within a few days and disappear when the animal is at rest. In the later stages, from 17 to 21 days of age, the slightest disturbance will precipitate a generalized seizure. Pups die within 30 days after birth. The only gross postmortem change is a gray color of the spinal cord instead of its normally white appearance. Microscopic findings reveal total lack of myelin formation at all levels of the central nervous system. (Am J Pathol 95:215–224, 1979)

IN 1957 the Wistar rat strain was received at our laboratory from Walter Reed Army Medical Center and has been random bred. Aside from sporadic cases of hydrocephalus in rat pups, no inherited abnormality was recognized previously. In March 1977, three young in a litter of seven exhibited whole body tremors. Two of the affected animals had an obvious hydrocephalus, but the brain of the third rat was grossly normal. Paraffin sections of brain were stained with hematoxylin and eosin and luxol fast blue and were examined for pathologic changes. Aside from very little stainable myelin, no abnormality was noted. At the same time, the mother rat with four of its clinically normal young (three females and one male) and three adult males (possible sires of the litter) were isolated from the main colony and set up as a nucleus of breeders for genetic study.

Materials and Methods

The mother rat was mated with three adult males (F_0) at 3-week intervals. The brother-sister system of breeding was used on the littermates of the affected young (F_1) when they reached breeding age. The same system of breeding was then employed in subsequent generations. The breeding pairs are housed in polycarbonate cages (19 × 10.5 × 8 inches). The bedding material is sterilized pine shavings. Cages are sanitized weekly. Rats have free access to drinking water (from bottles) and food (commercially available rodent feed containing 6% crude fat). All pups kept for breeding or research are ear-notched at 18 to 21 days of age. All cages are identified with a card which serves as a pedigree record. Records for all animals are kept in a breeder ledger according to cage number, dam and sire

From the Division of Laboratories and Research, New York State Department of Health, Albany, New York, and the New York State College of Veterinary Medicine, Cornell University, Ithaca, New York.

Accepted for publication December 5, 1978.

Address reprint requests to Dr. Charles K. Csiza, Division of Laboratories and Research, New York State Department of Health, Albany, NY 12201.

0002-9440/79/0411-0215$01.00

number, generation, number of young born, weaned, sex, ear notch, clinical signs (if any), weight, destiny, and the appropriate dates. Animals are examined daily. Tremors, convulsions, and other data are recorded on the date observed.

Histologic Studies

Tissues were fixed in 10% (v/v) buffered formalin by the perfusion procedure. Ten-micron sections were cut and stained separately with hematoxylin and eosin, luxol fast blue and cresyl echt violet, and a silver procedure (Holmes). Comparable sections of control and diseased nervous tissue were stained together in the same staining medium.

Results

Clinical Findings

Genetic studies are summarized in Table 1. The mother rat did not produce any additional young. All three of her daughters produced litters, but only one of them had pups with the neurologic abnormality. Six of ten females in the F_2 generation are carriers of the *md* gene. The data indicate an X-chromosome–linked recessive mutation. Clinical disease occurs in male pups hemizygous for the defective gene (md/Y).

The first symptom recognizable at 12 to 15 days of age is slight trembling of the head. In a day or so, tremors become generalized and more obvious. If pups are placed on a smooth surface, the hind limbs tend to slide sideways when they move. The tremors disappear when the animals are at rest. From 17 to 21 days of age, the slightest disturbance will precipitate a generalized seizure lasting up to a minute. The seizure is characterized by rigid extension of all four limbs. The hind limbs stretch backward and the front limbs forward as the animal falls on its side; the back becomes arched, respiration becomes rapid and abdominal and may be open-mouthed. In some animals the feet quiver and slowly move back and forth several times. Gradually the limbs are brought back to normal position, breathing returns, and the animal sits up. The symptoms start and end abruptly. Between attacks the animal experiences a mandatory

Table 1—Genetic Studies in the Wistar Rat Strain With Myelin Deficiency

Generation	md female carriers	Litters born to md female carriers	Total number of young (weaned)	Phenotypically diseased young*	
				Males	Females
F_0	1	1	7	3/4†	0/3
F_1	1/3	3	21	5/11	0/10
F_2	6/10	19	165	38/71	0/94

* Phenotypically diseased/total number of pups
† Two of three trembling males were also hydrocephalic.

rest period, which could be as short as 2 to 5 minutes toward the end of the animal's lifespan. Rats die within 30 days after birth. The mean age at mortality for 43 affected pups was 24.2 ± 2.7 days.

Weight gain in pups varies greatly among littermates in many, but not all, litters. Runtiness affects females as well as males and is found in large and small litters. Recognition of clinical signs and mortality occurs a few days earlier in rapidly growing affected pups compared with the runty littermates. In the *md* rat, growth rate decreases as tremors become worse and the seizures more frequent.

The only gross postmortem change in the *md* rat is a gray color of the spinal cord instead of its normal white appearance. This finding is present at the time the first tremors are observed.

Microscopic Findings

Microscopic study of the central nervous system of all the clinically affected rats revealed a significant lack of myelin staining at all levels (Figures 1 to 3). This was especially evident where neural processes were grouped together in the absence of cell bodies such as internal capsule, corpus callosum, central semiovale, corona radiata, rostral commissure, hippocampal fornix, and lateral olfactory stria of the cerebral hemispheres; the optic tract, stria habenularis thalamus, and internal capsule of the diencephalon, crus cerebri of the mesencephalon; pyramids, cerebellar peduncles, trigeminal spinal tract of the medulla; medulla and folial white matter of the cerebellum and all funiculi of the entire spinal cord. Spinal rootlets appeared to be normally myelinated. There was no evidence of any active degeneration of myelin in the central nervous system. In the control animals, the central portion of the hippocampal fornix was less myelinated than other adjacent white matter pathways.

Discussion

The myelin deficiency in the Wistar rat is the result of an X-linked recessive mutation. It is characterized clinically by whole body tremors, seizures, and death by 30 days of age. The pathologic process is limited to the myelinated fibers of the central nervous system. The defect appears to be due to lack of myelin formation and may be the result of a metabolic disturbance in the oligodendroglial cells. There is no evidence of inflammatory reaction or myelin destruction. Myelination of the peripheral nerves is normal. The condition is probably similar to the jimpy [1] and myelin synthesis deficiency [2] mutations of mice and type A III [3] congenital tremor syndrome of piglets. The inheritance of hypomyelination in all three species is similar, with the disease manifested only in males.

Growth and development of the central nervous system of rats proceeds in a sequential, caudocranial manner, reaching maturity at approximately 60 days of age.[4,5] The spinal cord is functionally mature at birth. The myelin of the ventral spinal roots is stainable at 2 days of age. Cranial nerves of the medulla and the medial longitudinal fasciculus are myelinated at 7 days. At 21 days, myelination in the medulla, pons, and midbrain is nearly complete, and it is well advanced in the mesencephalon.

Mortality due to *md* mutation occurs from 21 days. Deaths occur first in pups with the most rapid growth rate. Correlation of brain weight to total body weight in these animals ranges from 3.5 to 4.5% and is within normal limits for our rat colony. In pups with poor growth rates, this relationship is 5 to 6%.

It is interesting to note that in the original litter, two of three pups with tremors also had hydrocephalus. Since then, we have found eight young with hydrocephalus (six males and two females) but only two of the male pups had both abnormalities.

Electron microscopy and chemical analysis of lipid composition of the central nervous system of normal and *md* rats are under investigation.

There are several hereditary metabolic disorders in children, caused by hypomyelination or depletion of myelin.[6] One of these, ie, Pelizaeus-Merzbacher disease, is inherited as an X-linked recessive trait. In the congenital form there is no myelin; perivascular patches of myelin are exhibited in the infantile disease. Other disease conditions with faulty myelination have variable genetic expression and neuropathologic features. These have been termed "presumed Pelizaeus-Merzbacher disease,"[7] "sudanophil leukodystrophy,"[8] "leukodystrophy due to congenital deficiency of myelin,"[9] and "Schilder's disease (sudanophilic leukodystrophy)."[10] Many of the features of the above cases are similar to those in the *md* rat. We feel this model should be useful in comparative investigations of hereditary human disorders of defective myelination.

References

1. Sidman RL, Dickie MM, Appel SH: Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. Science 144:309–311, 1964.
2. Meier H, MacPike AD: A neurological mutation (msd) of the mouse causing a deficiency of myelin synthesis. Exp Brain Res 10:512–525, 1970
3. Patterson DSP, Done JT: Neurochemistry as a diagnostic aid in the congenital tremor syndrome of piglets. Br Vet J 133:111–119, 1977
4. Jacobson S: Sequence of myelinization in the brain of the albino rat: A. Cerebral cortex, thalamus and related structures. J Comp Neurol 121:5–29, 1963
5. Bass NH, Netsky MG, Young E: Microchemical studies of postnatal development in rat cerebrum. 2. Formation of myelin. Neurology 19:405–414, 1969

6. Altman PL, Katz DD (editors): Human Health and Disease. Bethesda, Md, Federation of American Society for Experimental Biology, 1977
7. Watanabe I, McCaman R, Dyken P, Zeman W: Absence of cerebral myelin sheaths in a case of presumed Pelizaeus-Merzbacher disease: Electron microscopic and biochemical studies. J Neuropathol Exp Neurol 28:243–256, 1969
8. Norman RM, Tingey AH, Harvey PW, Gregory AM: Pelizaeus-Merzbacher disease: A form of sudanophil leukodystrophy. J Neurol Neurosurg Psychiatry 29:521–529, 1966
9. Adachi M, Schneck L, Torri J, Volk BW: Histochemical, ultrastructural and biochemical studies of a case with leukodystrophy due to congenital deficiency of myelin. J Neuropathol Exp Neurol 29:601–614, 1970
10. Crome L, Zapella M: Schilder's disease (sudanophilic leukodystrophy) in five male members of one family. J Neurol Neurosurg Psychiatry 26:431–438, 1963

Acknowledgments

We are indebted to Dr. M. K. Abelseth, Director, Laboratories for Veterinary Science, for his interest in the development of the mutant rat colony; to Dr. D. McMartin, pathologist, Division of Laboratories and Research, for microscopic examination of the original animals; and to Drs. M. P. Dentinger and K. D. Barron, Veterans Administration Hospital, Albany, for their collaboration in the electron microscopic study of the myelination disorder in the *md* mutant rat.

[*Illustrations follow*]

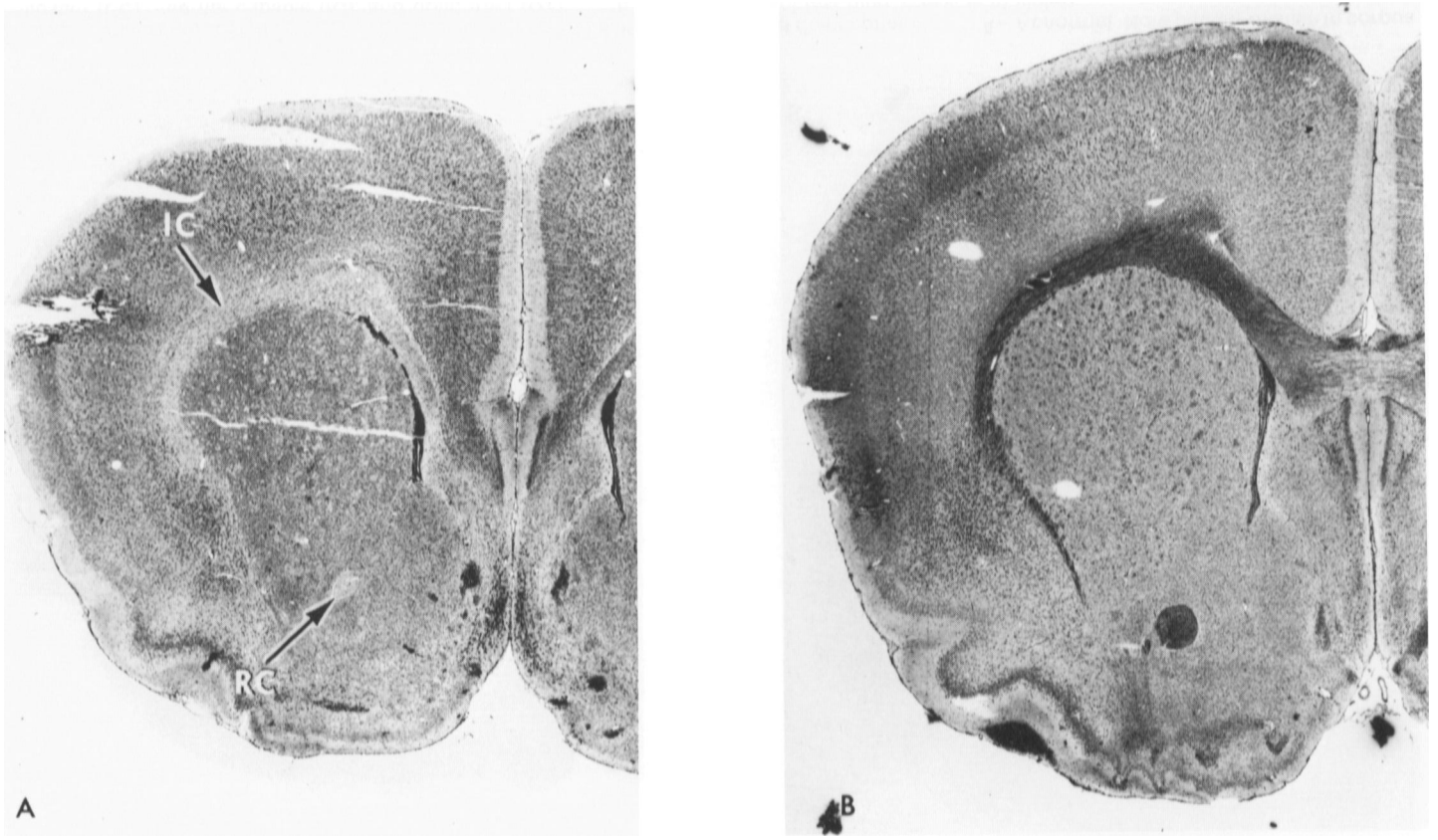


Figure 1—Transverse sections through cerebrum, frontal lobe. **A**—Abnormal. Note paucity of stain in internal capsule (*IC*) and rostral commissure (*RC*). **B**—Normal. (Luxol fast blue, cresyl echt violet)

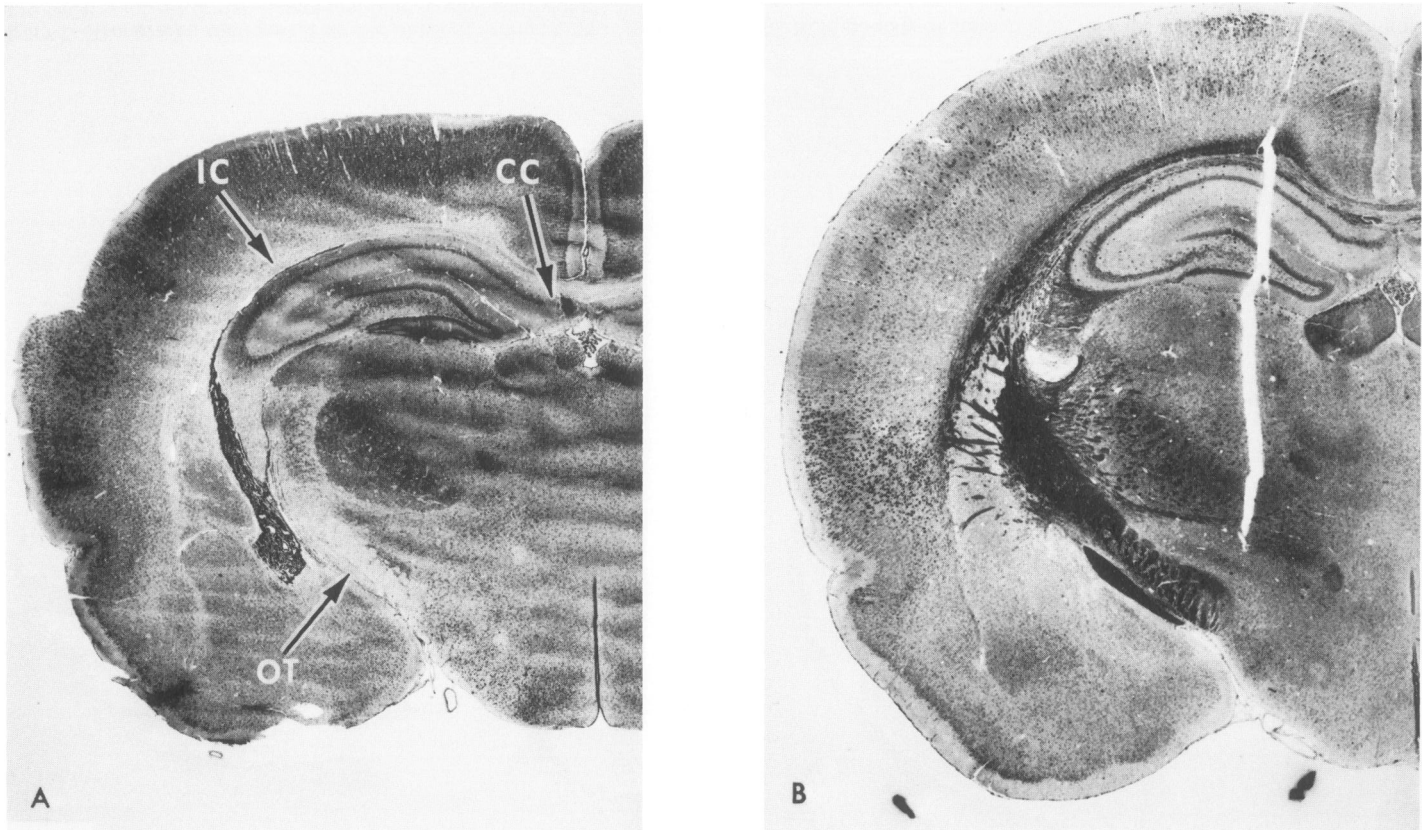
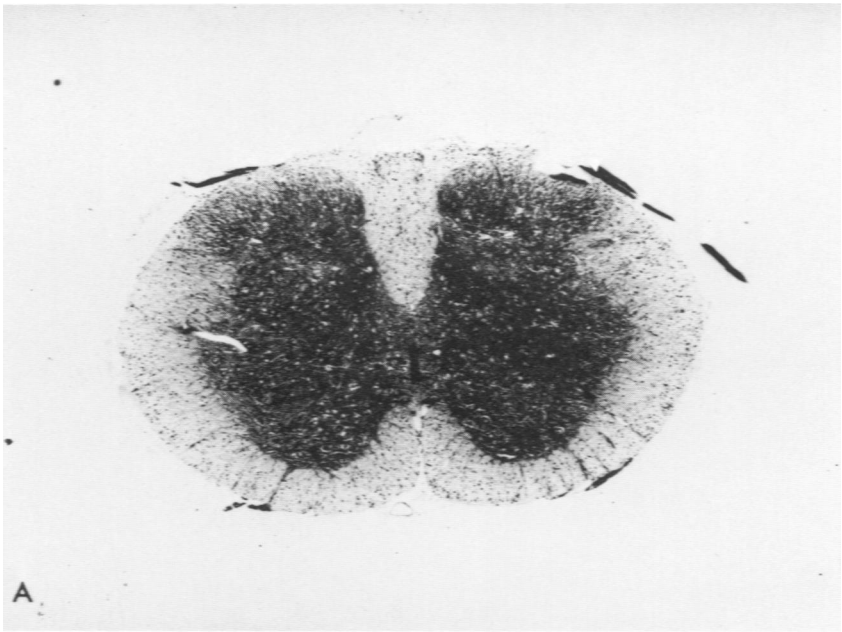


Figure 2—Transverse sections through cerebrum, parietal and temporal lobes, and diencephalon. **A**—Abnormal. Note paucity of stain in corpus callosum (*CC*), internal capsule (*IC*), and optic tract (*OT*). **B**—Normal. (Luxol fast blue, cresyl echt violet)



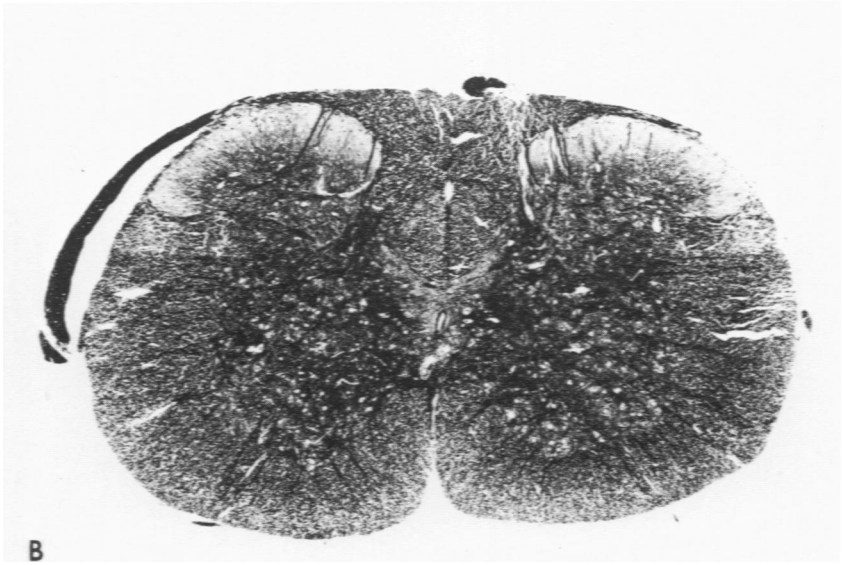


Figure 3—Transverse sections of cervical spinal cord. **A**—Abnormal. Note paucity of stain in funiculi of the spinal cord and the preservation in the spinal rootlets. **B**—Normal. (Luxol fast blue, cresyl echt violet)

[*End of Article*]