Spontaneous Spongy Degeneration of the Mouse Brain

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Abstract. A spontaneously-occurring spongy disorder of the white matter of the central nervous system was discovered in the Charles River strain of Swiss-Webster mice and is described in this report. The disorder was transmitted with an autosomal recessive pattern of inheritance. Clinical characteristics of the affected animals included enlargement of the cranium, failure to thrive and tremor of the hind limbs when held by the tail in a suspended position. Maintenance of the colony with propagation of the disease was achieved by selective in-breeding of litter mates. Light microscopic examination of the central nervous system revealed a spongy degeneration of the white matter of the entire neuraxis. Ultrastructural studies localized the abnormality to the cell body and processes of the astrocyte which appeared distended and enlarged with dispersion of cytoplasmic organelles. Hemidesmosomes were prominent in the foot processes of astrocytes. This animal model bears a similar morphology and pattern of inheritance to Canavan's spongy degeneration of the white matter in humans and should provide a base for future investigations aimed at gaining insight into the pathogenesis of the human and this animal neurological disorder.

Key Words: Animal model; Astrocytes, swelling; Autosomal recessive inheritance; Canavan's disease; Spongy encephalopathy; Status spongiosus.

INTRODUCTION

Swiss-Webster mice obtained from either Bio-Labs or Charles River colonies were being utilized in our laboratories in a series of experimental studies when it was discovered that some animals from the Charles River colony did not behave normally. Examination of these animals revealed cranial enlargement, failure to thrive and tremor of the hind limbs that became more pronounced the longer the mouse was held by the tail in a suspended position. Histological examination of the central nervous system revealed a spongy degeneration of the white matter that resembled Canavan's disease in humans. This report describes studies of the clinical, genetic and morphological aspects of the disease and the establishment of a colony of these animals to serve as a reservoir for future studies of this disorder.

MATERIALS AND METHODS
Breeding and Morphological Studies

The Swiss-Webster mice utilized in this study were obtained from either Charles River or Bio-Lab stock. The National Research Council and the Institutions' guides for care and use of laboratory animals were followed. The experimental breeding group from Charles River Laboratory consisted of 40 females over 100 days old and 12 females over 50 days old that were bred to 12 males over 50 days old. Six males and six females from the Bio-Lab colony

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were bred as controls. Other matings included Charles River females and Bio-Lab males and Bio-Lab females with Charles River males. The mice were numbered and were mated in a defined sequence. Because of limited numbers, males were utilized to impregnate several females. Twenty females and males from the first generation Charles River colony were selected for future breeding experiments.

Animals were killed according to one of the following procedures. Offspring 10, 20, 30 and 40 days old, respectively, were anesthetized with intraperitoneal chloral hydrate, exsanguinated and the brains were fixed by immersion in 10% neutral buffered formalin. A coronal section of the right hemisphere was processed for paraffin embedding and hematoxylin and eosin (H&E) staining. All older mice were killed by cardiac perfusion of 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. The descending aorta was clamped to ensure optimal perfusion of the brain although some mice were fixed by whole body perfusion in order to preserve other organs for study. The brains were removed and immersed in fixative for two to three hours after which sections of the right hemisphere, cerebellum, pons, medulla and spinal cord were placed in 10% formalin for histology. Coronal sections of the left hemisphere or sections from other regions of the neocortex were cut with a Sorvall Tissue Chopper at 200 micrometers and processed for electron microscopy with flat embedding in Epon.

Some offspring of the second generation were kept for additional breeding. All others were killed as adults following an observation period. Several matings were identified that produced diseased offspring. These animals or their unaffected offspring were kept as breeding stock. Those animals that did not produce affected offspring were deleted from the breeding program.

Each animal carried a number that identified its lineage from the original stock. Accurate records of each mating, duration and result were kept. The number of offspring born and the number weaned were also recorded. All the offspring that died postnatally were fixed in 10% formalin for further histological study and statistical analysis.

Transmission Study

A symptomatic mouse was killed by an intraperitoneal injection of 3.5% aqueous chloral hydrate and the brain was immediately removed. The right cerebral hemisphere was separated and processed for light microscopic examination, after immersion-fixation in 2.5% glutaraldehyde, to confirm the diagnosis of spongiform degeneration. The left hemisphere was minced, placed into 5 ml of sterile phosphate-buffered saline in a Dounce homogenizer, and homogenized using ten up and down strokes of the pestle. The homogenate was filtered once through a 210 μm nylon mesh screen supported in a Millipore filter holder and the residual material further diluted 1:1 with sterile phosphate-buffered saline. Thirty to 50 μl aliquots of this material were used for intracerebral test injections. The brain from a healthy adult Charles River female mouse was removed and processed in an identical fashion for control injections.

Mouse pups from three separate litters (two Bio-Lab and one Charles River), of one- and two-week-old mice were used for experimental and control inoculations. Using a 30 gauge needle, a total of 15 animals (approximately half of each litter) were given intracerebral injections of homogenate prepared from the diseased brain, while 13 (the other half of each litter) were inoculated with homogenate from normal mouse brain. The pups were then marked according to the inoculation administered, and replaced in cages with their mothers.

RESULTS

Consultation with the Charles River Laboratory staff following the discovery of the spongiform degeneration in the brain of their Swiss-Webster mice revealed that an outbred colony, which had been maintained by Charles River Laboratory for the National Institutes of Health for over 30 years, was the source of our animal stock.

Breeding Studies

Bio-Labs/Bio-Labs. Breeding of the Bio-Lab pairs was normal. The litter size ranged from 14–16 pups and all survived and were healthy. This established the
suitability of our animal room for breeding and the nutritional quality of the standard laboratory diet.

**Bio-Labs/Charles River.** The first generation of this breeding proved to be normal. However, litter mate breedings of the first or second offspring produced clinically affected animals that remained alive (Fig. 1A). The litter size was generally less than ten, especially if the female was from the Charles River stock. The number of affected offspring was never more than two per litter. This group of mice was the best suited for the production of normal, carrier and affected offspring. Litter mate breeding provided an ample supply of affected mice.

**Charles River/Charles River.** Some breeding pairs from these initial matings produced affected offspring in the first generation. The litter size was never larger than ten and no more than two affected animals were demonstrated in a litter. Litter mate matings from the first through third pregnancies of initial pairs produced affected offspring (Fig. 1B).

A clinical test for the disease was developed in order to identify diseased mice before they were killed. This consisted of holding a mouse by the tail in a suspended and unsupported position. Affected mice exhibited a gentle tremor of the hind limbs which became more pronounced the longer the animal was held in that position. The animals were rated on a scale of 0 to 4+, based on the onset and severity of the tremor.

Some of the diseased females that were under 90 days of age failed to conceive in spite of prolonged confinement with, or a subsequent change of, a mate. Other affected females produced only one to five pups. The pups would not be tended, were spread about the cage and died within a few days. This situation was repeated after a second or third pregnancy when the litter size might be reduced to a single pup. Some of the affected males failed to impregnate any female even after several weeks of confinement together. Thus, male and female mice that were rated 4+ on
the scale were no longer used for breeding. Several were allowed to survive and were followed for longer periods in order to observe the natural progression of the disease.

The offspring of the initial breeding pairs that did not exhibit tremor were utilized for brother-sister matings. Offspring of these matings were normal, carriers of the disorder or affected. The litter size of this group was usually eight or less and no more than two affected animals were identified in any litter.

Clinical Characteristics of Affected Animals

Some pairs of animals were identified that would produce affected offspring in every gestation. Sometimes, affected pups could be identified during the first two weeks postnatally. The affected animals were characterized by an enlargement and rounding of the cranium, failure to thrive even when nursed by normal females, sparseness of hair and a shorter than normal tail. Their gait was unsteady and they moved about the cage less than their litter mates. When startled from sleep, they would exhibit a stretched posture with complete rigidity that lasted for several seconds and continued after removal from the cage. These animals failed to conceive or to impregnate other animals. Most animals were killed before 150 days. They all lost weight, exhibited a paucity of spontaneous movement and often displayed spontaneous tremor that increased in severity when the animal was suspended or picked up by the tail.

Gross Features of the Brain

The perfused brains from affected animals were generally softer than those from normal animals. They initially floated rather than sank in fixatives and were difficult to section with the Sorvall Tissue Chopper due to their sponginess. The sectioned surface of the affected brains appeared granular and irregular in contrast to normal brains which were smooth and regular.

Light Microscopy

Conventional histological sections stained with H&E revealed a very extensive and pronounced degeneration of the white matter that resulted in a vacuolated appearance consistent with a spongy state (status spongiosus) (Fig. 2). This appeared to be less in younger animals and progressed with age (Fig. 3). The most conspicuous involvement was in the cerebral white matter (Fig. 2), although the white matter of the brain stem, cerebellum and spinal cord were also involved (Fig. 4). The grey matter of the cortex was initially spared, however the astrocytic process within the deep cortex was sometimes swollen (Fig. 5). The status spongiosus affected the white matter tracts within the basal ganglia (Figs. 2, 6). In some animals a mild but definite dilatation of the lateral ventricles was evident.

Examination of affected animals at all ages revealed intact neurons, even when vacuoles were demonstrated adjacent to the soma (Fig. 7). The vacuoles could be traced to a dilatation of the cell bodies and processes of cells with large, circular to ovoid nuclei that were situated close to blood vessels or within the neuropil. From their location and appearance, the affected cells were most likely astrocytes. Myelinated fibers were normal and dilatation of the extracellular space of the brain was not demonstrable.

Electron Microscopy

Sections from different regions of the central nervous system were examined in order to identify the cell or cells directly involved in the formation of vacuoles. In

Fig. 2. Coronal section of a mouse brain, 120 day old, showing an extensive status spongiosus. H&E. ×20.

all regions examined, the affected cell proved to be the astrocyte or its foot processes. The cytoplasm of these cells was electron-lucent with widely dispersed organelles (Fig. 8), which was prominent, especially when compared to a normal astrocyte (Fig. 9). Mitochondria were round or elongated with well-defined cristae. Cisternae of

Fig. 4. A mid-sagittal section of pons medulla and cerebellum in a 120-day-old animal with widespread involvement of white matter and minimal involvement of the cerebellar cortex. H&E. ×16.

Fig. 3. Lateral cerebral cortex and white matter in a 30-day-old mouse; early onset of status spongiosus affecting white matter, deep cortex and basal ganglia. The lateral ventricle and choroid plexus are in the upper right corner. H&E. ×50.
both rough and smooth endoplasmic reticulum were also present and loosely dispersed. A well-defined Golgi complex was noted. Bundles of astrocytic fibrils were identified within the cell cytoplasm and processes, however, the amount appeared to be less in the most dilated cells or processes. The nucleus of the astrocyte was round with finely dispersed chromatin that tended to condense near the nuclear membrane. Its appearance did not vary from that of the normal except for the loss of definition around the periphery. Nucleoli were often present. Large numbers of typical hemidesmosomes were observed in some of the astrocytes adjacent to blood vessels. These were more prominent during the earlier stages of swelling and tended to lose their detail and disperse with the advance in swelling (Fig. 10). The basement membrane shared by the astrocyte and the endothelium was homogenous and the endothelium and its junctions appeared normal. Other components of the neuropil including axons, myelin sheaths and synapses had no discernible abnormalities. In many of the vacuoles, there were membranous arrays and myelin figures. Some of the membranous arrays were derived from invaginations of adjacent cell processes or myelin (Fig. 11). Most of the neurons were intact and seemed to be unaffected by the presence of swollen astrocytic processes against their membranes.

Transmission Study

All pups were observed for signs of failure to thrive and neurologic dysfunction. Neither developed. A total of nine pups were cannibalized by the mothers. The
Fig. 6. Lateral cerebral cortex and white matter in a 150-day-old mouse. There is relative sparing of the superficial cerebral grey matter and involvement of the white matter and tracts of the basal ganglia. The lateral ventricle and choroid plexus are seen in the upper right corner. H&E. × 40.

Fig. 7. Spongy changes involving neuropil and white matter with preservation of neurons of pontine base. H&E. × 230.
Fig. 8. A swollen astrocyte from mid-brain with dispersed fibrils and organelles and a close association with a blood vessel. $\times 5,000$. Inset reveals numerous hemidesmosomes present along the astrocytic cell membrane adjacent to the vessel. $\times 8,500$.

Fig. 9. An astrocyte from the midbrain of a normal mouse with typical organelles and astrocytic fibrils. $\times 10,000$.
Fig. 10. This affected astrocytic process is characterized by dispersion of organelles and the appearance of membranous arrays and vacuoles. ×10,000.

Fig. 11. Enlarged astrocytic process in the white matter contain membranous profiles and myelin figures. Some of the membranous arrays seem to be derived from herniations of adjacent cell processes and myelin (arrows). ×6,000.
remaining pups (nine injected with diseased brain, ten with control) were killed by perfusion fixation with 2.5% glutaraldehyde, at three, four, and five weeks after intracerebral inoculation and tissue was processed for light microscopy as described above. All brains appeared normal histologically.

DISCUSSION

The first case of spongy degeneration of the white matter of the central nervous system in a human infant was described in 1928 by Globus and Strauss as a case of Schilder’s disease (1, 2). Other reports included pronounced megalencephaly and a familial tendency as conditions of the disease (3, 4). The disorder was established as a clinical and pathological entity by van Bogaert and Bertrand in 1949 (5). Although this form of spongy degeneration is not a common disorder, there has been a succession of studies reported in the literature including a review article citing 94 cases of the disease (6–18). The majority of the patients were juveniles; most were of Jewish extraction; many resided in Europe and all died within the first decade of life. Parental consanguinity was reported in seven instances (about 14%). The frequency of occurrence in non-Jewish families is increasing and some reports indicate that several nationalities have been affected by the disease (18).

When the genetic aspects of all the reported cases were analyzed, there appeared to be a higher frequency than expected for a purely autosomal recessive pattern of inheritance (18). This may have been due to sample size or a statistical bias. Males and females were equally affected. Recent biochemical and ultrastructural studies have demonstrated an accumulation of fluid in the cytoplasm of astrocytes and between myelin lamellae (18). Although the primary basis for the fluid accumulation is still unknown, histochemical and ultrastructural studies have suggested that there is a metabolic abnormality of the astrocytic mitochondria including a decrease in their ATPase activity (18, 19). Additionally, a number of toxins have been administered experimentally and have induced a similar type of spongy degeneration of the white matter. These toxins include cuprizone (20), isonicotinic acid hydrazide (21), hexachlorophene (22) and triethyl tin (23).

A number of genetically transmitted disorders affecting the central nervous system of mice have been described the latest of which include: stumbler, a mutant with cerebellar disease (24); cribriform degeneration, a recessive disorder with gray and white matter degeneration (25); and twitcher, a mutant with hereditary leukodystrophy (26). Thus, the discovery of a mutation in our stock from an outbred colony of Swiss-Webster mice was unexpected, but not an unusual occurrence. Inherited neurological disorders in laboratory animals provide convenient models for the study of disease processes and interest in such models is increased if the disorder resembles that of a human disease with unknown etiology and pathogenesis.

The morphological resemblance of our mouse brain disorder to human spongy encephalopathy of the white matter and in particular to Canavan’s disease was striking. The breeding experiments were essential for determination of the autosomal recessive nature of the disorder and the establishment of a colony of animals for further characterization of the disease. The success at maintaining the colony has been gratifying. More than 25 animals have been found to have severe spongy degeneration by histological examination.

Our initial attempts at demonstrating an infective agent, possibly a slow virus, have not been successful, further supporting the genetic basis of the disease. There are animal models of slow virus disease that bear a remarkable resemblance to the disorder described here (27–29).

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The astrocyte is a cell that responds to a variety of injuries with swelling of the cytoplasm and cellular processes. Thus, it was not surprising to find that the astrocyte in these mice was the first cell to be affected. This was evident with both light and electron microscopic evaluations. The progressive nature of the disease was suggested by different stages of involvement of the astrocyte and the range in size of the cell swelling. Large vacuoles may have formed from the fusion and coalescence of several smaller, swollen processes. The cause of the astrocytic swelling was not determined by our initial studies. Although the source of the fluid was likely from microvessels, there were no morphological changes in the endothelium. The increased prominence of the astrocytic hemidesmosomes may have served as attachments for support of the swollen astrocytes rather than as a response to alterations of the blood-brain barrier. It is tempting to suggest that an absent or abnormal membrane or mitochondrial ATPase is responsible for the disorder, however the exact cause of the disease must await definitive histochemical and biochemical evaluation.

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REFERENCES


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