Acta neuropath. (Berl.) 12, 103—115 (1969)

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Familial Spongy Degeneration of the Central Nervous System (Van Bogaert-Bertrand Disease)

An Ultrastructural Study*

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Received April 27, 1968

Summary. Light and electron microscopy study of skeletal muscle and cerebral biopsies from a case of spongy degeneration of central nervous system is reported. The multiple vacuoles present in cerebral gray and white matter correspond to (a) clefts within myelin sheaths resulting from splitting at the intraperiod line and (b) swollen astrocytic perikarya and processes. Unusual mitochondria containing crystalline-like material were observed only in astrocytes. The ultrastructural findings are consistent with cerebral edema. It is suggested that the astrocytes play a primary role in the fluid accumulation while the myelin swelling is a secondary lesion. The possible role of the abnormal astrocytic mitochondria is discussed.

Zusammenfassung. Es wird über licht- und elektronenoptische Untersuchungen an Muskel- und Hirnbiopsien eines Falles von spongiöser Degeneration des ZNS berichtet. Die in der grauen und weißen Hirnsubstanz enthaltenen Vacuolen entsprechen a) Spalten in den Markscheiden infolge Aufsplitterung an der intraperiodischen Linie und b) geschwollenen Astrocytenperikaryen und -fortsätzen. Ungewöhnliche Mitochondrien mit Gehalt an kristallinem Material fanden sich nur in Astrocyten. Die ultrastrukturellen Befunde entsprechen denen des Hirnödems. Es wird angenommen, daß die Astroglia eine primäre Rolle in der Flüssigkeitsansammlung spielt, während die Markscheidenschwellung als eine Sekundärläsion aufgefaßt wird. Die mögliche Bedeutung abnormer Astrocyten-Mitochondrien wird diskutiert.

Key-Words: Spongy Degeneration of the CNS — Van Bogaert-Bertrand-Disease — Electron Microscopy — Astrocytic Mitochondria — Abnormal Cerebral Edema.

The first ultrastructural study of the spongy degeneration of the central nervous system (Van Bogaert-Bertrand disease) was published by ADACHI *et al.* (1966). These authors reported that the vacuoles which characterize the light microscopic feature of this disease correspond to: a) large spaces within myelin sheaths resulting from "splits" of the sheath at the intraperiod line, and b), swollen astrocytic perikarya and processes. They also observed large numbers of unusual mitochondria in cortical astrocytes.

The present ultrastructural study of a cerebral biopsy from a 6 year old boy with spongy degeneration of the central nervous system confirms these findings. Details of the changes observed in astrocytes and the fine structure of unusual inclusions in skeletal muscle are also presented. The significance of the structural alterations in the pathogenesis of this disease is discussed.

* The present investigation was supported by Research Grants HD 00588, NB-05572-04 and NB-04613-05 and the Widener Fund.

** Post-Doctoral Fellow of the National Multiple Sclerosis Society.

Case Report

The patient is a 6 year old white male of Jewish ancestry. The parents are normal. A female sibling, now 13 months old, has failed to develop beyond a 2 month level; she has diffuse muscle flaccidity and now has poor responses to visual stimuli.

The patient was born after a full term pregnancy; forceps were used during delivery.

The first symptoms of the present illness were reported at the age of 1 month, when it was noted that the patient was inattentive to visual stimuli. At 3 months of age, the child was spastic with diffuse hyperreflexia and clonus. At the age of 3 years, he began having generalized motor seizures with both tonic and clonic components; these have recurred with intercurrent illnesses, but otherwise were controlled with medication.

Physical examination at 6 years of age revealed a dwarfed (92 cm; mean for age, 114 cm), apathetic, megaloencephalic (head circumference 57 cm; mean for age 50.8, δ 1.4 cm) child, unable to walk, stand, sit or talk. Poor head control, marked spastic quadriparesis with bilateral extensor plantar response and hyperflexia were noted. The patient was unresponsive to visual stimuli; opthalmoscopic examination revealed severe optic atrophy with medullated nerve fibers.

Numerous laboratory tests, including urine, serum and spinal fluid electrolytes, serum cholesterol, phospholipids and total fatty acids were unremarkable. X-ray of the skull was consistent with hydrocephalus. The electroencephalogram showed diffuse slowing of electrical activity. Cerebral biopsy was performed at the age of 6.

Materials and Methods

A brain biopsy was obtained from the right frontal lobe; three muscle biopsies held in a special clamp, were taken from the right calf. Immediately following surgical removal, one portion of the biopsied brain and one of the muscle biopsies were processed for ultrastructural study according to techniques previously described (GONATAS *et al.*, 1968). For light microscopy, a second portion of the brain tissue was fixed in formol calcium. Frozen sections were stained with PAS, oil red O and Peiffer's stain for metachromasia. Sections from the portion of tissue embedded in paraffin were stained with H. E., PAS and processed for Nissl's, Weil's and Bodian's stains. The second and third muscle biopsies were treated for histology and enzyme histochemistry according to previously described methods (SHY *et al.*, 1966).

Results

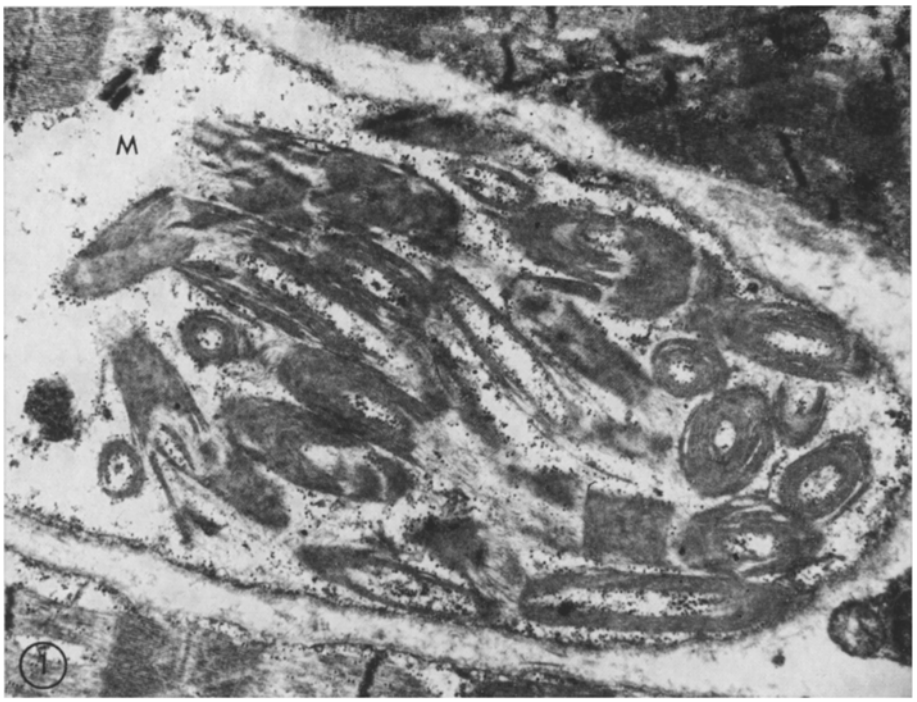
Muscle

Light Microscopy. Routine histology revealed no architectural abnormalities. The muscle cells of one fasciculus appeared mildly atrophied with an average size of 10 μ as compared to the general size of 20 μ. The sarcolemmal nuclei were normal. Central nuclei were not seen. Endomysial connective tissue and fat were not increased. Nerves and vessels were normal. The cytochemical study for succinic dehydrogenase, cytochrome A_3, reduced adenine nucleotide (NADH), myosin ATPase, and amylophosphorylase (SHY *et al.*, 1966), was unremarkable. The conclusion was: mild degree of neurogenic atrophy.

Electron Microscopy. The majority of the muscle cells were abnormal. The most common abnormality consisted of reduction in width of the fibrils, particularly at the level of the I band. The different components of the sarcomeres were, however, well recognized. Isolated triads composed of the T tubule and adjacent

Fig. 1. The peripheral portion of a muscle cell (*M*) contains numerous rounded or oval profiles. All specimens for electron microscopy were fixed in 2% osmium (DALTON, 1955). ×11,000

Fig. 2. Higher magnification of Fig. 1. The rounded or oval profiles appear to consist of sheets of parallel paired linear densities (double arrow: each arrow points to a single density) arranged concentrically. *g* glycogen. ×44,000



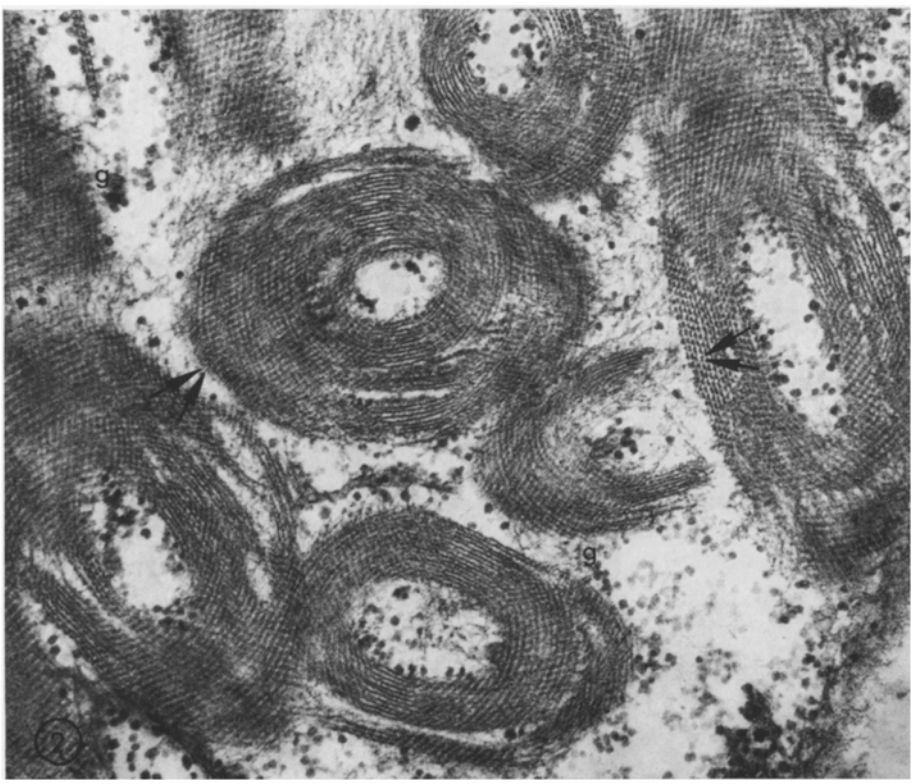


Fig. 1 and Fig. 2

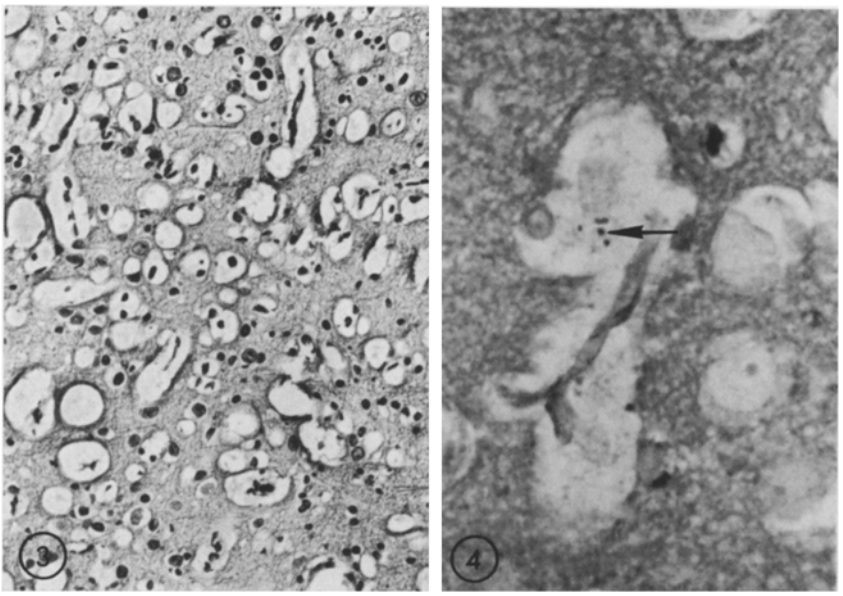


Fig. 3. Spongy degeneration of the cortex. Paraffin embedding; Bodian. ×350

Fig. 4. PAS-positive granules (arrow) in the cytoplasm of a perivascular swollen glial cell, probably astrocyte. Paraffin embedding. ×1,150

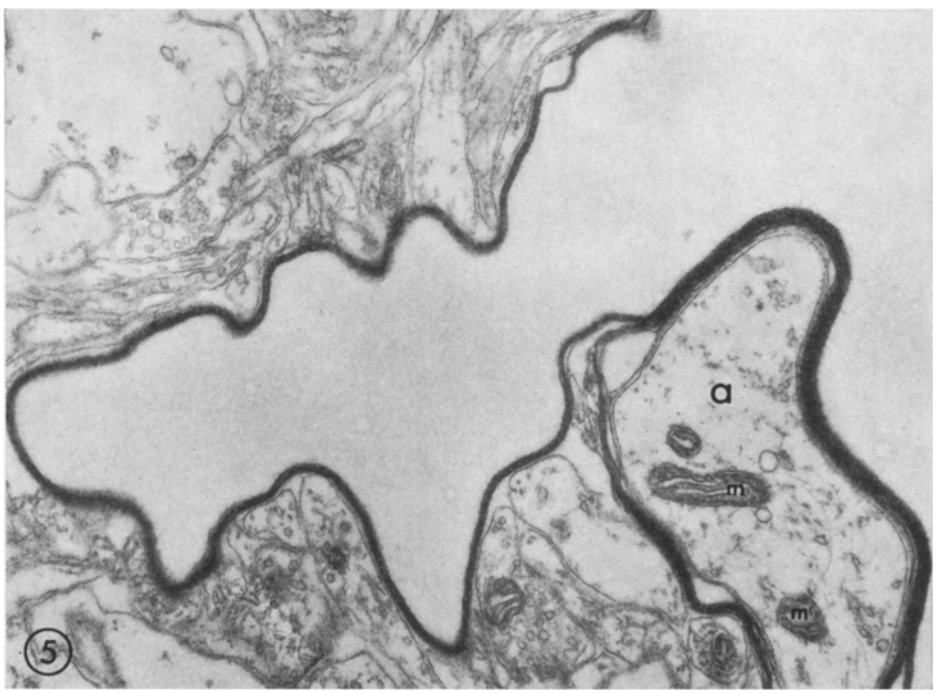


Fig. 5. Large electron-clear vacuole circumscribed by myelin lamellae. *a* axon; *m* mitochondria ×22,500

terminal cisterns were noted in the interfibrillary spaces. Also, abundant glycogen and mitochondria of normal appearance were present. Rarely, a complete disorganization of segments of the fibrillar component of the muscle cell was seen. Bundles of filaments crossed and intertwined with each other at random; fragments of Z-line, collapsed cisterns of sarcoplasmic reticulum and mitochondria in various stages of disintegration were also present.

An isolated but striking finding was the presence within a muscle cell of round or oval profiles (Fig. 1 and 2). The exact structure of these profiles was difficult to establish. They consisted of sheets of parallel paired linear densities. Each dense

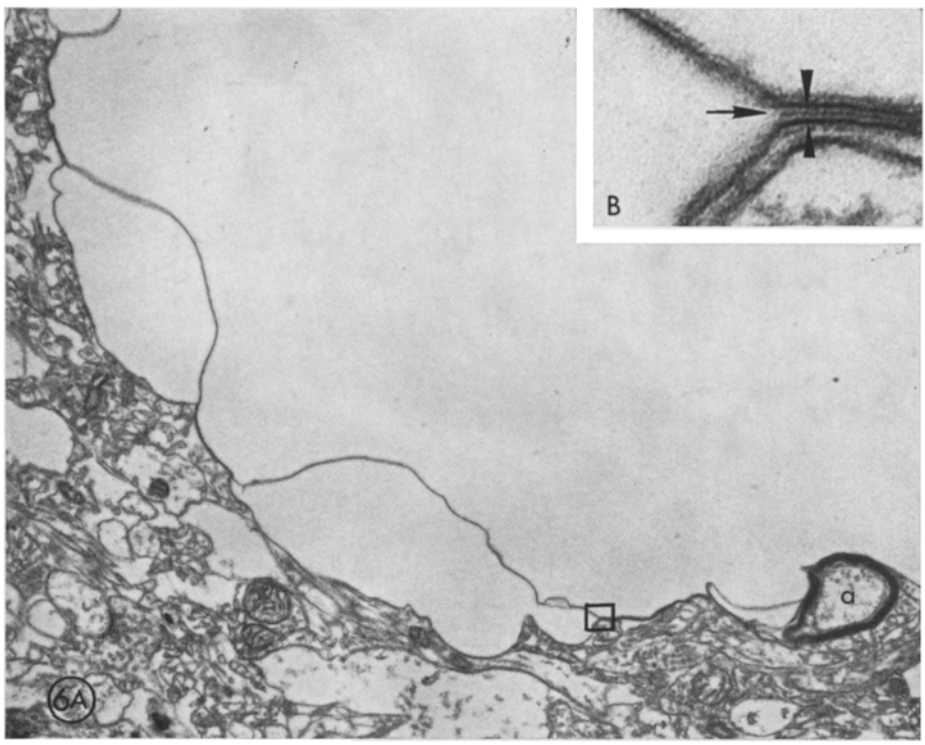


Fig. 6. A Large electron-clear vacuole circumscribed by membranes. *a* axon. ×14,000. B Higher magnification from the area of Fig. 6 A marked by square shows structure of myelin and split of the intraperiod line (arrow). Arrow head: major dense line. ×199,000

line, about 45 Å thick, was separated by an electron clear zone, 25 Å thick. Commonly, numerous sheets were arranged concentrically, forming round or oval profiles. The images of these profiles, however, varied considerably depending on the angle of the section.

Schutta has observed identical structures in a case of hyperkalemic periodic paralysis and in a case of myoglobinuria[1]. Structures similar to those observed in the muscle, have been reported in cerebellar hemangioblastoma by Cancilla and Zimmerman (1965), and in presynaptic endings from cerebral cortex near

[1] Probably the same structures have been observed in abnormal muscle cells also by Luft *et al.* [J. clin. Invest. **41**, 1776–1804 (1962)].

tumors by Ramsey (1967). Whether the observed structures represent products of altered cellular metabolism or a peculiar rearrangement of fragmented contractile material is not clear. The other constituents of the muscle cell, mainly the sarcolemma and basement membrane were well preserved. No abnormalities were observed in the endomysial collagen and fibrocytes. Short segments of nerve included in the muscle biopsy were also studied. No abnormalities were seen in the axons. Myelin sheaths were well preserved and had a periodicity of 130—140 Å. Schwann cells also appeared normal.

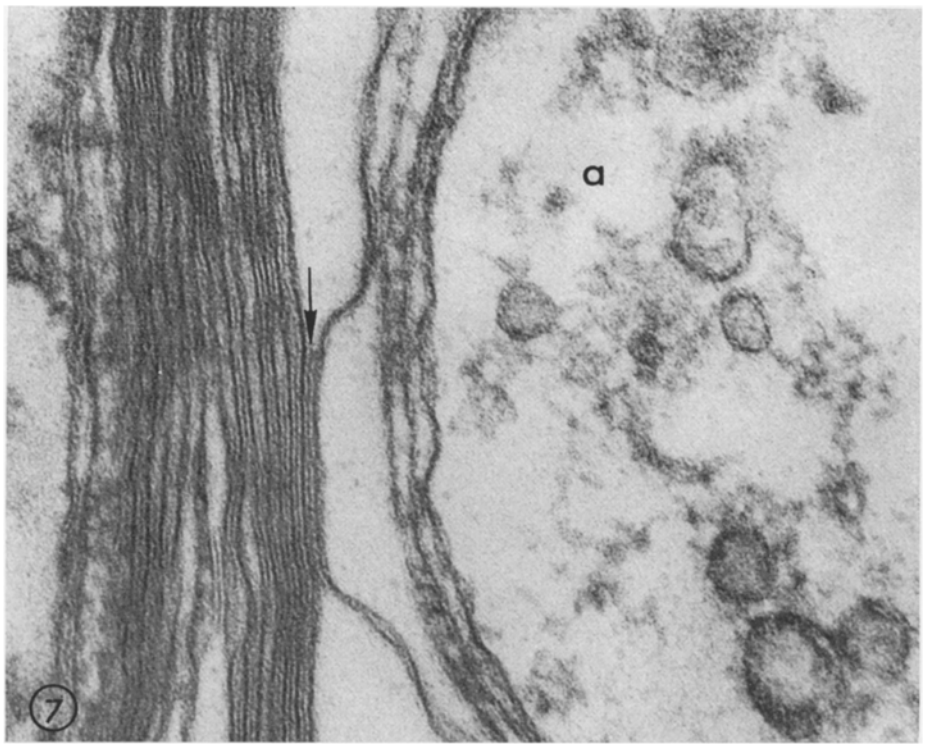


Fig. 7. Small vacuoles within a sheath of a myelinated axon, resulting from splitting at the intraperiod line (arrow). *a* axon. ×160,000

Brain

Light Microscopy. A segment of cortex with a portion of adjacent subcortical white matter was examined. The meninges were thin and no cellular infiltration was present in the subarachnoid and Virchow-Robin spaces. All cortical layers, except for the first layer, and the subcortical white matter showed numerous vacuoles (Fig. 3). In the cortex, the vacuoles were less numerous than in the subcortical white matter. Some of the vacuoles of the cortex were adjacent to the perikaryon of neurons, others surrounded glial nuclei or small vessels. Several of these vacuoles contained small PAS-positive granules (Fig. 4), which probably corresponded to the abnormal mitochondria observed in the electron microscope. The neurons appeared normal and were not diminished in number. The astroglial

cells were moderately increased in number; a few of them were binucleated. In the subcortical white matter, the vacuolization was marked. The oligodendroglial cells appeared to be increased in number. With the Weil stain only traces of myelin were seen. The Bodian stain revealed fragmentation of axons in the subcortical white matter. The absence of oil red O positive material indicated that there was no accumulation of triglycerides and cholesterol esters. In both white matter and gray matter, only a few scattered astrocytes of the type II of Alzheimer were noted.

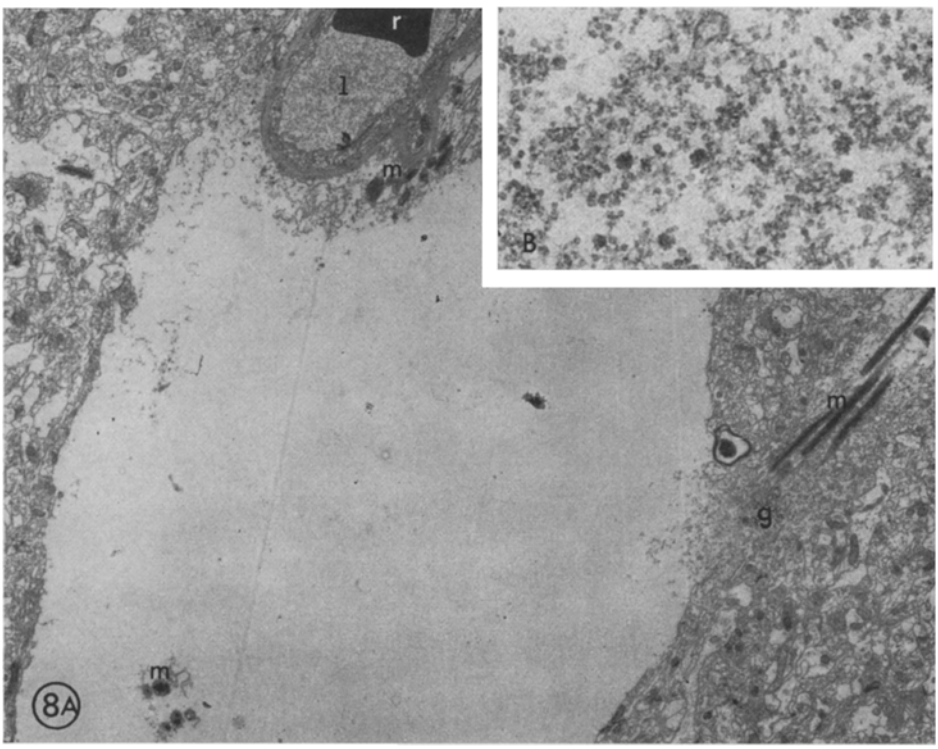


Fig. 8. A Swollen astrocytic perivascular foot surrounded by apparently normal, neuropil. The vacuole contains mitochondria (*m*) and membranous material. On the right of the figure a non-swollen process of the same astrocyte containing elongated abnormal mitochondria (*m*) and granular material (*g*). *l* lumen of vessel; *r* red blood cell. ×4,200. B High magnification of granular material. ×33,000

Electron Microscopy. The multiple vacuoles observed in the light microscope were of two types. The vacuoles of the first type were usually very large; they contained no cellular organelles or any electron dense material and were usually circumscribed by membranous lamellae, identifiable as myelin (Fig. 5, 6 A and B). Splitting of the lamellae at the intraperiod line were frequently observed (Fig. 6 B). Certain electron micrographs showed localized splits of myelin sheaths, resulting in small clear spaces; these images were suggestive of an early stage of the vacuole formation. Here again the splitting of the myelin involved the intraperiod line (Fig. 7). The myelin lamellae, circumscribing the vacuoles had a periodicity of

100—130 Å, identical to that found in the rarely observed normal myelin sheaths surrounding axons.

The vacuoles of the second type were generally smaller than the intramyelinic ones. They were mostly electron clear, but contained recognizable cellular organelles or granular or filamentous material and were bounded by a double membrane. These vacuoles were identified as swollen astrocytic perikarya or processes because of their preferential location around vessels (Fig. 8A), or because of the characteristics of their nucleus (Fig. 9). The non-swollen areas of these abnormal astro-

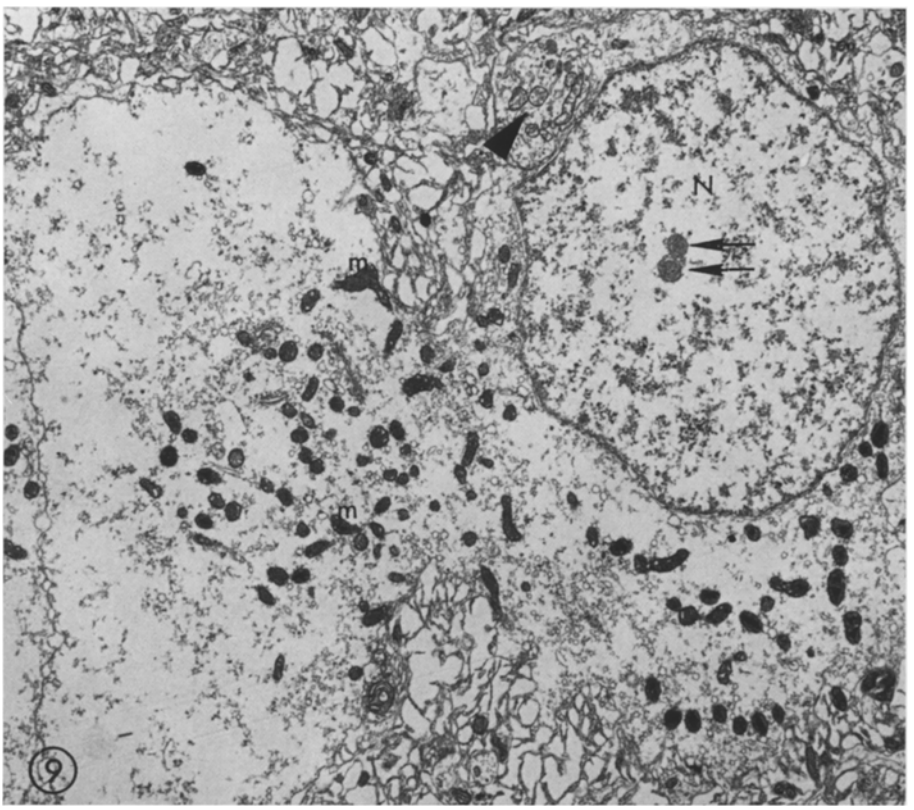


Fig. 9. Swollen astrocytic perikaryon containing abnormal mitochondria (*m*). Compare the abnormal mitochondria (*m*) with normal ones (arrowhead). Note two nuclear bodies (arrows) (BOUTEILLE *et al.*, 1967). ×7,000

cytes contained granular material (Fig. 8B) of various sizes, tentatively identified as glycogen. Most of the mitochondria of the swollen and non-swollen astrocytic perikarya and processes were abnormal (Fig. 9, 10). They were characterized by a very dense and packed matrix, which in its central part displayed a paracrystalline arrangement (Fig. 11A—C). The central material consisted of a dense core 0.10—0.12 μ wide, with a characteristic fine structure which appeared to be modified by the plane of sectioning. In certain sections, distinct parallel dense bands 60—70 or 180 Å wide (the 180 Å bands being probably the image of two of the smaller bands cut tangentially) were separated by lucent zones about 40 Å

wide; in other sections the layered appearance was indistinct and instead, closely packed longitudinal lines made the core of this unusual structure (Fig. 11 C). The thickness of the apparent unit was about 40 Å. The cristae, formed by peripheral infoldings of the inner mitochondrial membrane, were in regular alignment and perpendicular to the central core material; the core was never transected by cristae. The size of the abnormal mitochondria varied and some measuring up to 10 μ in length were seen (Fig. 11 A).

The nuclei of the astrocytes were normal and none of the astrocytes showed a pale nucleus suggestive of a type II cell of ALZHEIMER.

The nuclei and perikarya of neurons were normal. Only a few neurons showed an irregular shape and dense perinuclear cytoplasm because of compression by

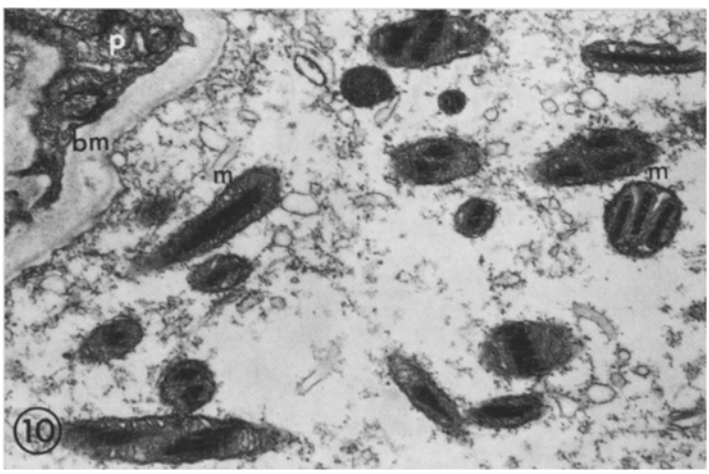


Fig. 10. Several abnormal mitochondria (*m*) in an astrocytic perivascular foot. *bm* basal membrane. *p* perithelial cell. ×34,000

adjacent vacuoles. Mild focal swelling of dendrites involving frequently the postsynaptic component of axo-dendritic synapses were present. Compressed axons, inside collapsed myelin sheaths, and empty myelin sheaths suggesting advanced axonal degeneration, were occasionally seen. The presynaptic endings were normal. Oligodendrocytes and blood vessels had normal appearance.

Discussion

VAN BOGAERT and BERTRAND (1949) originally suggested that the basic morphologic picture of familial spongy degeneration of the central nervous system is that of a chronic edema, involving gray and white matter. The present ultrastructural findings are consistent with this interpretation; they indicate that the edema fluid accumulates in the cytoplasm of astrocytes and in intrameylinic vacuoles resulting from the splitting of the myelin sheaths at the level of the intraperiod line, while the intercellular clefts are not enlarged.

Two hypotheses have been suggested to explain the pathogenesis of edema in the spongy degeneration. The first theory, proposed by WOLMAN (1958) suggests that the rapid catabolism of abnormal myelin might be the primary event. This

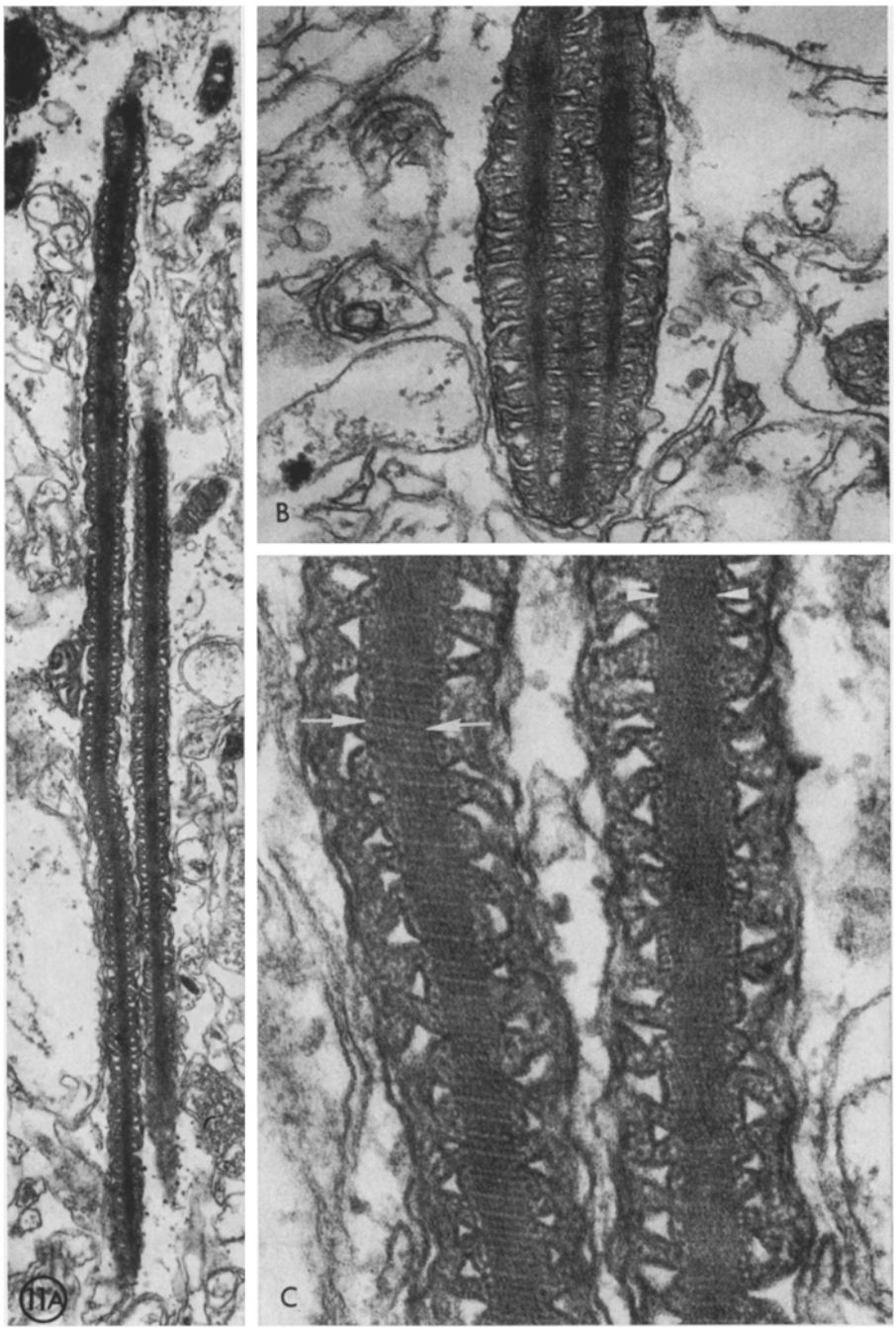


Fig. 11. A—C Abnormal mitochondria. A Two extremely elongated abnormal mitochondria with a dense core. ×16,500. B The abnormal mitochondria may contain two or more dense cores. ×50,000. C The material of the dense area varies according to the plane of sectioning. Parallel dense bands (arrows) are separated by lucent zones. In other areas (arrowheads) the core is made of closely packed lines in longitudinal direction. ×99,000

could lead to the production of increased amounts of myelin catabolites of low molecular weight, which in turn, could cause accumulation of water.

According to the second hypothesis, proposed by MEYER (1950) and ZU RHEIN *et al.* (1960), the morphological abnormalities of the myelin sheath, are secondary to fluid accumulation.

This second hypothesis has recently received strong support from a biochemical study by KAMOSHITA *et al.* (1968). These authors investigated the chemical composition of the white matter and of isolated central myelin from two cases of spongy degeneration; no accumulation of small molecular weight products of myelin breakdown was found. These results do not substantiate WOLMAN's theory. Furthermore, the chemical abnormalities of the isolated myelin were non-specific.

The ultrastructural findings seem to be in agreement with the hypothesis that myelin is secondarily involved. Splitting of myelin sheaths at the intraperiod line has been observed in several unrelated conditions associated with fluid accumulation in the central nervous system, where there is no evidence of primary involvement of myelin (GONATAS *et al.*, 1964; HIRANO *et al.*, 1965; ALEU *et al.*, 1966; VAN HARREVELD and KHATTAB, 1967; GAMBETTI and GONATAS). Therefore, vacuolization of myelin might be interpreted, at least in some instances, as secondary to certain types of edema. Furthermore, ROBERTSON (1958) has demonstrated in the peripheral nerve, that the material which constitutes the intraperiod line is strongly hydrophilic and when the myelin sheath is immersed in hypotonic medium, it undergoes passive swelling with splits at the intraperiod line. Beside the splitting of the myelin sheath, severe and widespread swelling of the astrocytes and peculiar changes of astrocytic mitochondria characterize the ultrastructural picture of the spongy degeneration. The astrocytes are considered to play a key role in the fluid balance of the central nervous system (FARQUHAR and HARTMANN, 1957; SCHULTZ *et al.*, 1957; DE ROBERTIS, 1965; FRIEDE, 1965). For these reasons, it seems likely that the astrocytes may be primarily responsibe for the fluid accumulation in the spongy degeneration, while the myelin swelling is a secondary lesion. If this is the case, the changes of the astrocytic mitochondria become of interest. Mitochondrial changes similar to those observed in the present disease have been occasionally reported in different cells and unrelated conditions (SUZUKI and MOSTOFI, 1967; NORRIS and PANNER, 1966; KLINKERFUSS, 1967), but, to the best of our knowledge, these mitochondrial changes have never been seen in normal or pathologic conditions of the central nervous system, other than the spongy degeneration.

The role and the significance of these abnormal mitochondria remain unclear. In cerebral cortex it has been shown that 30 to 40% of the energy produced by mitochondrial respiration is coupled to the ionic pump (Na-K dependent ATPase) (WHITTAM and BLOND, 1964), which regulates the permeability of the plasma membrane to ions and water (SKOU, 1965; VAN HARREVELD, 1966). It can be speculated that if the astrocytic mitochondria fail in their role of energy su pply a disturbance in the ionic pump mechanism with resulting accumulation of fluid may be expected. Conversely, a long-standing impairment of the ionic pump may produce changes in mitochondria. Isolation of the abnormal mitochondria, study of their biochemical properties and investigations on the Na-K dependent

ATPase activity in cerebral cortex from cases of spongy degeneration, may provide a clue to this unusual disorder of the central nervous system.

Acknowledgements. The authors wish to thank Dr. R. GROFF and the staff of Neurosurgery for performing the brain biopsy. The technical assistance of Mr. JOSEPH MARTIN, Miss IRENE EVANGELISTA, Mrs. MARY O'CONNOR and Miss DOROTHEA BETTINI is appreciated.

The light microscopic and cytochemical study of the muscle biopsy was performed in Dr. G. M. SHY's laboratory.

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