FINE STRUCTURE OF SPONGY DEGENERATION OF THE CENTRAL NERVOUS SYSTEM (VAN BOGAERT AND BERTRAND TYPE) *†

MASAZUMI ADACHI, M.D.,
BARBARA J. WALLACE, Ph.D.,
LARRY SCHNECK, M.D.,

AND

BRUNO W. VOLK, M.D.

(Brooklyn, N. Y.)

Spongy degeneration of the central nervous system of infants is a rare disorder. Since 1928, only 30 pathologically verified cases of this disease have been reported (1–17). During this period, the characteristic features of the disease have been gradually elucidated. The earliest cases reported were considered to be Schiller’s disease or Krabbe’s type of diffuse sclerosis (1–4, 7). The first reported instance of this disease was one described in 1928, by Globus and Strauss (1). The main symptoms displayed by their patient, a 6½ month old infant, were: apathy, seizures, and generalized rigidity. The major pathological change was diffuse destruction of convolutional myelinated fibers. The characteristic megalencephaly was stressed by Canavan in 1931 (2) although she considered the case to be one of Schiller’s disease. Eiselsberg (3) first described familial characteristics of spongy degeneration, but considered her cases to be instances of Krabbe’s disease. The clinical and pathological entity was established by van Bogaert and Bertrand (5) in 1949. More recently, genetic (5, 11, 13, 17), biochemical (8, 11, 13, 14, 17) and enzymatic (17) studies have been reported.

The disease, which is usually a familial, autosomal recessive disorder, involves both sexes and is observed predominantly in patients of Jewish origin. The initial symptoms which become clinically evident during the first few months of life, consist of progressive apathy, poor head control and generalized flaccidity. The later stages of the disease are characterized by progressive mental and motor deterioration, enlargement of the head, blindness, and spasticity terminating in pseudobulbar symptoms and decorticated or decerebrate rigidity. The pathologic features are characterized by widespread vacuolation of the subcortical white matter and the deep cortical lamina as well as demyelination involving the convolutional portions of the cerebrum. Another typical feature is the occurrence of Alzheimer type II astrocytosis in the cerebral cortex, subcortical white matter and deep gray matter.

Nothing is as yet known, however, of the fine structural aspects of this pathologic change. The present paper presents an electron microscopic study of

* From the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, New York.
† Supported by grants from the National Institutes of Health (B-2977) and the National Tay-Sachs Association.

508
a cerebral biopsy from a one year old patient with spongy degeneration in an attempt to aid in more fully understanding the nature of the pathologic changes in this disease.

CASE REPORT

History: The patient is a one-year-old white boy who was born of Jewish parents of Eastern European descent. The child had no siblings, and the family history was non-contributory. The infant was reported as having developed normally until the fourth month. At this age, he still had poor head control, was apathetic, and exhibited generalized flacid weakness. Two months later, his head was reported to be enlarged, and there was decreased response to visual stimuli. He reached for and held objects, and smiled on appropriate non-visual stimuli. By 10 months of age, the patient was spastic, and the head circumference had increased to 50 cm. (normal, 45 cm.).

Examination: Physical examination at one year of age revealed an apathetic megalencephalic patient (head circumference 55 cm., normal, 47 cm.) (fig. 1), with poor head control and internal strabismus. The child did not appear to see, although the pupils did respond to light. There were occasional episodes of rapid ocular oscillations. Ophthalmoscopic examination revealed pale discs, but no macular degeneration or abnormal retinal pigmentation. Auditory or tactile stimuli precipitated opisthotonic posturing. Tendon reflexes were 3+ and the Babinski response was positive.

Pneumoencephalograms performed at 10 months of age were negative. The electroencephalogram at 10 months was read as being within normal limits. The cerebrospinal fluid protein was within normal limits. A diagnostic cortical biopsy was performed when the patient was 1 year old.

Fig. 1. Photograph of patient with spongy degeneration showing enlarged head and decorticate posture.
Methods

A biopsy was obtained from the right frontal lobe. Immediately following surgical removal, one portion of the tissue was fixed in cold (0-4°C.) 2 per cent buffered osmium tetroxide for 90 minutes, rapidly dehydrated in ethanol, and embedded in epon for study with the RCA EMU-3G electron microscope. Ultrathin sections were obtained with a Porter-Blum microtome using glass or diamond knives. Sections were stained with lead according to the method of Reynolds (18) and with uranyl acetate. A second portion of the biopsy specimen was divided into two groups for enzyme histochemical studies. One group was immediately fresh frozen in isopentane. The other group was fixed for 3 hours in 4 per cent glutaraldehyde buffered to pH 7.2 with 0.2 M cacodylate buffer. Following fixation this tissue was rinsed in sucrose for 72 hours and frozen in isopentane cooled to −178°C, by liquid nitrogen. It was used to demonstrate activity of adenosine triphosphatase (ATPase) using a modification of the Wachstein and Meisel method (19).

The freshly frozen tissue was sectioned in the cryostat (−14°C.) at 20 microns and fixed one half hour in 3 per cent neutral formalin (0-4°C.) with 1 per cent calcium chloride. Sections were rinsed rapidly in ice cold distilled water and incubated (37°C.) for 15 minutes in the substrate solution. Control sections were incubated in the solution without substrate. The ATPase solution consisted of 20 ml. of DH2O with 3.75 gm. sucrose, 20 ml. of 0.2 M Tris-maleate buffer, 5 ml. of 0.1 M MgSO4, 25 mg. ATP, 2 ml. of 2,4-dinitrophenol (5 mM), and 3 ml. of 2 per cent PbNO3 adjusted to pH 7.2. After incubation the sections were washed in 0.25 M sucrose (0-4°C.) and were fixed in buffered 2 per cent osmium for 1½ hours. After fixation the sections were rinsed in 0.25 M sucrose (0-4°C.), dehydrated in ethanol, and embedded in epon. A third portion of the biopsy was fixed in 10 per cent neutral formalin and in absolute ethanol for paraffin embedding or frozen sections. The histologic sections were stained with hematoxylin and eosin, Luxol fast blue-periodic acid-Schiff (PAS), Best's carmine, Alcian blue, Sudan black B, toluidine blue, cresyl violet, Bodian, Romanes, Holzer, phosphotungstic acid hematoxylin, trichrome and Naumenko-Feigin stains (20). The remaining fragments of tissue were fresh frozen in isopentane for biochemical studies.

RESULTS

Gross Findings: The surgically removed specimen included the cerebral cortex and white matter and measured 3 cm. x 2.8 cm. x 2.3 cm. in its greatest dimensions. The leptomeninges were not remarkable. On section, the demarcation between the cortex and white matter was indistinct. The deeper cortical laminae and subcortical white matter were extremely edematous, soft, gray and somewhat glassy in appearance. The remainder of the white matter was a pale white.

Light Microscopy Findings: The leptomeninges were thin and delicate. No inflammatory cells were present. The most striking features were multiple vacuoles in the subcortical white matter which varied considerably in size (fig. 2). In the fusiform layer of the cortex, similar vacuoles were present, although less in number than in the subcortical white matter. In the ganglionic layer of the cortex the vacuoles were rather small and were present mainly adjacent to the astrocytic nuclei, neuronal cytoplasm, and perivascular spaces (fig. 3). The remainder of the cortical layers showed similar but less pronounced changes. In the subcortical white matter, the vacuoles were bordered by the axonal fibers as seen in Romanes and Bodian stains with a tendency for their long axes to lie parallel to the nerve fibers (fig. 4). The vacuoles appeared
Fig. 2. Characteristic multiple vacuoles in deep cortical laminae and in subcortical white matter. Hematoxylin and eosin stain; × 40.

Fig. 3. Cerebral cortex (ganglionic layer) showing vacuoles, adjacent neurons (N) as well as capillary blood vessels (C); Alzheimer type II astrocytes are present (arrows). Hematoxylin and eosin stain; × 375.

Fig. 4. Vacuoles in subcortical white matter bordered by axonal fibers. Bodian stain; × 95.
empty in PAS, Best's carmine, Alcian blue, and Sudan black B stains. In Luxol fast blue-PAS preparations remarkably few myelinated fibers were observed. The axonal fibers were relatively well preserved as contrasted to the myelin loss. Alzheimer type II astrocytes were abundant in the subcortical white matter and deep cortical lamina. These astrocytes were characterized by enlarged pale nuclei with no visible cytoplasm as seen in most of the stains used (fig. 3). The glial fibers were not increased in Holzer, phosphotungstic acid hematoxylin, or trichrome stains. The astrocytic cytoplasm was markedly pale and swollen in the Naoumenko-Feigin stain. A few macrophages containing PAS-positive material were noted near the perivascular spaces. No metachromasia was demonstrated in toluidine blue and cresyl violet stains.

**Electron Microscopic Findings:** In the subcortical white matter the multiple vacuoles, observed by light microscopy, were present within the myelin sheaths lying between split lamellae of the myelin spirals (figs. 5, 6). The split formation was noted between the major dense lines, and the intraperiod lines were obscured (figs. 6A, 6B). The major dense lines were focally disintegrated. Occasionally, numerous vacuoles were noted within the same myelin lamellae with adhesions between the loops (fig. 7). A majority of the myelin membranes were attenuated by these large spaces and some of these membranes were often focally ruptured. The vacuoles communicated with widened extracellular spaces through the ruptured membranes (figs. 8, 9). Some of the old sites of rupture exhibited curling of the myelin membranes and formation of nodules (fig. 9). The axis cylinders were compressed by the myelin vacuoles, and their fibrillar and mitochondrial contents were moderately decreased in amount (fig. 5). The cytoplasm and processes of the astrocytes in the white matter were "watery" and almost all of the cell membranes were ruptured (fig. 10). The mitochondria were markedly decreased in number and many vesicles were present (fig. 10). There were occasional macrophages in the perivascular areas containing myeloid figures. The oligodendroglial cells were not remarkable. The blood vessels were normal. There was no fenestration between capillary endothelial cells, nor were there alterations of the basement membranes.

In the deep cortical layer, the fusiform layer adjacent to the subcortical white matter showed similar vacuoles within the myelin sheaths. Ruptured myelin lamellae and astrocytic cell membranes as well as distended extracellular spaces occurred here also. In the ganglionic layer, the major changes were seen in the astrocytes. What appeared to be vacuoles in the light microscope, was revealed by the electron microscope to be the markedly swollen and "watery" cytoplasm of astrocytes (fig. 11). Some of the "watery" astrocytic processes were seen adjacent to the neurons (fig. 12). The nuclei of the Alzheimer type II astrocytes appeared large and pale (fig. 13A) when visualized in the electron microscope under low power. Under higher magnification they were also enlarged with sparse nucleoplasmic granules and showed loss of chromatin. Their nucleoli were distinct and the nucleolomema was well preserved (fig. 13B). The mitochondria within the astrocytic processes showed enormous elongation (fig. 14A), and distention and distortion of the ribosomes, and
Fig. 5. Electron micrograph of subcortical white matter showing large vacuoles within the myelin lamellae (arrows) and dilatation of extracellular spaces (ES); × 8,800.

Figs. 6A and B. High power of the myelin lamellae showing separation between the major dense lines and the obscured intraperiod lines (arrows); × 36,100 and × 100,500.
Fig. 7. There are multiple vacuoles within the myelin sheaths with adhesions (arrows) between the loops; × 28,500.
Fig. 8. Montage of a vacuole (IV) within a myelin sheath (MS) showing communication with distended extracellular spaces (ES) through ruptured myelin lamella (arrows). AX = axon; × 2,010.
Fig. 9. Some of the ruptured myelin lamellae which form a nodulé (framed area shown in inset). Intracellular vacuoles (IV); Site of rupture (arrows); Extracellular spaces = ES; × 8,250. Inset: High power view of the nodular edge exhibiting altered myelin period lines (MP) with curling (CR); × 36,000.

Fig. 10. Astrocytes in subcortical white matter showing ruptured cell membranes (arrows) and many cytoplasmic vesicles (V). Astrocytic cytoplasm communicates with increased extracellular spaces (ES); × 6,600.
Fig. 11. Ganglionic layer of cortex showing marked distention of astrocytic cytoplasm (AC) with "watery" appearance; × 3,200.

Fig. 12. "Watery" astrocytic process (AP) adjacent to neuron; × 3,477.
Fig. 13A. Alzheimer type II astrocyte showing enlarged and pale nucleus; $\times$ 3,745.
Fig. 13B. High power view of another Alzheimer type II astrocyte showing pale and sparse nucleoplasmic granules (NG) with loose chromatin pattern. The nucleolus shows distinct nucleolonema (NL) without pores amorphous; $\times$ 7,700.
Fig. 14A. Mitochondria within astrocytic process showing marked elongation; × 4,280.
Fig. 14B. High power view of mitochondria showing distention (D) and attenuation (A) of cristae. Peculiar longitudinal striation within the matrix was present in large mitochondria (M1) as well as normal-sized mitochondria (M2); × 21,600.
Fig. 15. Astrocytic end feet in perivascular area showing marked dilatation; × 5,760.

striation in the matrix (fig. 14B). Similar alterations in the matrix were also observed in normal-sized mitochondria (fig. 14B). Astrocytic end feet at the perivascular areas were also markedly swollen (fig. 15). The remainder of the cortical layers revealed occasional enlarged astrocytic cytoplasm and proc-
Fig. 16A. Mitochondrion of normal brain showing intense ATPase activity (arrows) in the intercrystal matrix; × 62,400.
Fig. 16B. Mitochondrion of present case showing decreased ATPase reaction product (arrows) in the intercrystal matrix; × 64,800.

These, otherwise the neuronal and vascular structures were not remarkable. No increased extracellular spaces or ruptured cell membranes were present in the cortex except in the cells of the fusiform layer.

**Histochemical Findings:** Studies of mitochondrial ATPase showed decreased enzyme activity localized as occasional somewhat ill-defined dark granules in the intercrystal matrix (fig. 16B), while mitochondria of normal brain showed diffuse strong reaction in the same location (fig. 16A). No reaction was noted in control tissue which was incubated in the solution without substrate.

**Discussion**

The present study demonstrates that the formation of vacuoles in the white matter of spongy degeneration is the result of a change within the myelin sheath. This alteration consists of splitting and separation of the myelin lamellae between the major dense lines to form large spaces within the sheath.

The water content of fresh samples of the brain tissues was determined by a previously described method (21). In the subcortical white matter it was 96.8 per cent, in the cortex and in the deeper white matter it was 93.0 per cent and 94.3 per cent respectively. The localization of excess fluid in the brain in spongy degeneration, however, is different from acute cerebral edema which develops in cases of trauma, primary or metastatic tumors, etc. The light and electron
microscopic characteristics of accumulation of fluid in human and animal brain have been extensively studied (21-38). In cases of acute edema in human brain several investigators (21-26) described accumulation of excess fluid in the extracellular spaces particularly in the deep white matter, while there was a tendency to spare the adjacent subcortical white and gray matter. In addition, the occurrence of lakes of PAS-positive edema fluid in the extracellular spaces has been stressed in human brains (21) and in experimental studies (28-30). This material was probably protein and was believed to have passed from the blood vessels into the surrounding tissue. On the other hand, in triethyl tin intoxication unique histology and electron microscopic changes similar to those observed in spongy degeneration were reported (17, 39-41). In these studies, the accumulation of excess water was present within intracellular spaces, namely in the myelin lamellae and in the astrocytic cell membranes. Also, no PAS-positive fluid was demonstrated either in intracellular or in extracellular spaces. Furthermore, characteristic spongy vacuoles were more prominent in both deeper cortical laminae and subcortical white matter and the extracellular spaces were distended in the subcortical white matter due to rupture of myelin membranes (17). During the process of formation of vacuoles in triethyl tin intoxication it seemed that the intraperiod lines initiated the development of vacuoles between the major dense lines of the myelin membranes.

Megalencephaly is one of the most characteristic features of spongy degeneration. However, observations of previous studies (17) disclosed that the increasing megalencephaly and the weight of the brain in this disease did not parallel the progression of the disease. After 2 years of illness, the weight of the brain declined and approached the average weight. It was considered that this phenomenon might be related to progressive degeneration and loss of white matter. The present study illustrates that the markedly increased water content in the extracellular spaces through ruptured myelin membranes and astrocytic cell membranes might accelerate the degeneration of white matter and might thus contribute to the pathogenesis of the severe demyelination in the terminal stage.

In the ganglionic layer of the cortex what appeared to be vacuoles with the light microscope were revealed actually to be swollen and "watery" processes of astrocytes. These occurred in the perineural areas as well as around blood vessels. In spite of the marked distention of the astrocytic cytoplasm, no rupture of cell membranes occurred. Furthermore, there was no increase of the extracellular space in the cortex except in the fusiform layer. In contrast, the white matter showed many ruptured myelin lamellae and astrocytic cell membranes and displayed markedly increased extracellular spaces. The differences in severity of involvement between cortex and white matter may be related to a different histological density in each tissue. The changes at the site of rupture of the myelin lamellae, such as curling of the membranes and formation of nodules, as well as the distended extracellular spaces indicated that the ruptured membranes were not the result of artefacts of tissue processing. By light
microscopy, enlarged and pale nuclei occurred in Alzheimer type II astrocytes. Electron microscopically, the nuclei also appeared enlarged and contained sparse nucleoplasmic granules which showed a loss of chromatin and a distinctive nucleolonema. The astrocytic nuclei were surrounded by markedly swollen and "watery" cytoplasm and processes which in the fusiform layer and in the subcortical white matter also showed frequent rupture of cytoplasmic membranes.

The enormous elongation of the mitochondria with changes in the matrix and cristae seems of particular interest. To the best of our knowledge similar changes have not been previously reported, although peculiar changes in the membrane and matrix after fusion of mitochondria have been described (42). Histochemical studies revealed decreased ATPase activity in the mitochondrial matrix when compared with normal brain. The significance of these alterations is not understood, but possibly indicates a biologic dysfunction of the organelles.

The normal appearance of neurons, oligodendroglia, and blood vessels suggests that this disorder does not originate in these structures.

The nature and the characteristic distribution of the vacuoles in the subcortical white matter and the cortex still remains obscured. However, it is of interest that in a previous study (43) the distribution of water content in the normal brain was high in subcortical white matter and cortex as compared to deep white matter. It is possible, therefore, that those portions which originally contain a high water content might be more susceptible to form the vacuoles which characterize this disease.

SUMMARY

The present study describes the fine structure of cortex and white matter of a one year old Jewish boy afflicted with spongy degeneration of the brain. The most characteristic clinical and pathologic feature of this disease is megalencephaly which is due to increased intracellular water content in the brain, mainly in the subcortical white matter. The light microscopic picture was similar to that previously reported. Electron microscopically, multiple vacuoles in the subcortical white matter and deep cortical lamina (fusiform layer) were due to separation of the lamellae of myelin. The vacuoles were present between the major dense lines of the myelin membranes, and it is conjectured that the obscured intraperiod lines might be the origin of these vacuoles. The extracellular spaces in the subcortical white matter were widened due to the rupture of the myelin lamellae and astrocytic cell membranes. It is hypothesized that a secondarily increased extracellular water content in the white matter might have accelerated further degeneration in the white matter at the advanced stage. There were smaller vacuoles present in the cortex, especially in the ganglionic layer which were the result of marked enlargement of astrocytes and their processes, although no increased extracellular spaces or ruptured cell membranes were present. The large and pale astrocytic nuclei described as Alzheimer type II by light microscopy appeared ultramicroscopically also en-
larged and were seen to contain sparse nucleoplasmic granules, loss of chromatin and distinct nucleoli with preserved nucleolomema. Marked changes in size, and frequently in appearance, of mitochondria were observed in the processes of the astrocytes in the cortex, especially in the ganglionic layer. Histochimical studies showed that the activity of ATPase within these mitochondria was decreased when compared with that of normal brain tissue. The fine structure of the neurons, oligodendroglia and blood vessels was not altered.

REFERENCES

19. WACHSTEIN, M., and MEISEL, E.: Histochemistry of Hepatic Phosphatases at a


39. MARGES, P. M., STONE, H. B., AND BARNES, J. M.: The Experimental Production of


