Human neonatal hydrocephalus

An electron microscopic study of the periventricular tissue

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An infant of 43 weeks gestational age with severe congenital hydrocephalus was operated on for removal of a subependymal astrocytoma in the region of the foramen of Monro. A biopsy of periventricular tissue was taken from the lateral ventricle for examination by scanning and transmission electron microscopy. The ependyma was largely denuded, with glial cell processes forming most of the ventricular lining. Many of the attenuated ependymal cells, however, had intact junctional complexes at areas of contact with other ependymal cells. Club-shaped unipolar cells, believed to be a previously undescribed form of immature ependymal cells, were found in the ventricular lining. Cerebrospinal fluid edema was present in the neuropil up to 60 μm from the ventricular lumen, but there was no obvious axonal pathology in the tissues examined.

KEY WORDS: hydrocephalus, ultrastructural study, neonate, ependyma, lateral ventricle

HYDROCEPHALUS is a pathological dilatation of the cerebral ventricles resulting from an imbalance between the secretion and absorption of cerebrospinal fluid (CSF). Although hydrocephalus can be caused by a variety of different pathological processes, the cytological changes seen in the periventricular region are essentially the same in experimental and human hydrocephalus. Reports of the periventricular pathology in human hydrocephalus are few and details are lacking or, at best, are based on closed biopsy material which may be subject to much distortion. In general, the contemporary literature lacks information concerning congenital hydrocephalus.

In this report, we document our findings in an open biopsy specimen obtained from the periventricular region of a human neonate with congenital hydrocephalus.

Case Report

This male infant was born on February 22, 1984, at 43 weeks of dated gestation. The pregnancy had been uncomplicated, but cephalopelvic disproportion necessitated a Caesarean section. At delivery, the head was large with open sutures, but normal Apgar scores of 8 at 1 minute and 9 at 5 minutes were recorded. The child weighed 3750 gm, and his head circumference was 40 cm (greater than the 95th percentile). All fontanels were enlarged and tense, but the remainder of the neurological examination was normal. Ultrasound examination revealed moderately dilated lateral ventricles, a midline shift to the right, and an echogenic mass in the anterior left ventricle. A computerized tomography (CT) scan confirmed these findings (Fig. 1).

The child's condition remained stable, and on February 29 a left occipitoparietal craniotomy for excision of the tumor was performed (H.D.F.). Although CSF pressure was not measured, the cortical mantle bulged out, indicating elevated pressure. The incision to reach the ventricle revealed that the mantle was approximately 8 mm thick. The ventricular lining was yellowed with a brown mottling due to diffuse hemorrhage, and the choroid plexus appeared normal. Approximately 15 minutes after the ventricle had been opened a biopsy of the ependyma was taken from the lateral wall of the occipital horn, 5 cm distant from the tumor mass, for correlative transmission and scanning electron microscopy. The subependymal tumor mass obstructing the foramen of Monro was removed from the frontal lobe by ultrasonic aspiration* and was found to be a low-grade astrocytoma.

* Ultrasonic aspirator manufactured by Cavitron-Cooper Medical, 88 Hamilton Avenue, Stamford, Connecticut.
Human neonatal hydrocephalus

FIG. 1. Computerized tomography scan demonstrating gross ventricular dilatation, more pronounced on the left side, and a hyperdense mass (arrow) in the frontal horn of the left lateral ventricle.

grade astrocytoma. Postoperative recovery was uneventful and the child was discharged 4 days later. He returned for elective ventriculoperitoneal shunting 3 weeks later, and follow-up CT scans at 1 and 6 months showed no tumor mass.

Study Methods

The biopsy specimen was carefully trimmed with a razor edge to ensure that the tissue to be studied had not been directly handled by the surgeon. The 5 × 10-mm specimen was divided and fixed by immersion in 2.5% glutaraldehyde-2% paraformaldehyde in 0.12 M phosphate buffer with 0.02 mM calcium chloride for 24 hours at 4°C, followed by postfixation in buffered 2% osmium tetroxide for 2 hours. For transmission electron microscopy, a portion of the tissue was stained en bloc in a saturated aqueous solution of uranyl acetate, dehydrated in graded alcohols, and embedded in Epon 812. Ultra-thin sections were stained with lead citrate and viewed with a Philips 201 transmission electron microscope. For scanning electron microscopy, the remaining tissue was transferred to acetone following alcohol dehydration, critical-point dried in carbon dioxide, coated with gold-palladium (60:40), and viewed with a JEOL JSM-35C scanning electron microscope.

Results

Examination of the ventricular surface by scanning electron microscopy revealed that it was almost entirely denuded of ependyma with only isolated islands of intact ciliated ependyma. The cilia were often irregular (Fig. 2 left) and artifactually matted together in places, although occasionally they were organized in characteristic clusters of 10 to 15. The surface of the ventricle, denuded of normal ependyma, was covered by either large flat cells covered with microvillus protrusions or a plexiform network of interwoven flat processes that appeared to originate from stellate cells (Fig. 2 right). The appearance of this network closely resembled the human ventricular surface following enzymatic digestion of the ependymal cells by 0.2% trypsin. Club-shaped unipolar cells, each with a narrow cylindrical process extending deep into the tissue surface between the interwoven flat processes, were common (Fig. 3). The somas of these cells were 3 to 6 μm wide and up to 8 μm long, and the processes were 1.5 to 2.0 μm wide and up to 12 μm long. Microvilli and bleb-like projections covered the cells (Fig. 3 right). Transmission electron microscopy of these cells revealed the ultrastructure of the somas and processes which entered the ependymal surface in a radial direction (Fig. 4). These processes were moderately electron-dense and contained many microtubules, filaments, and mitochondria. The somatic cytoplasm contained abundant mitochondria, smooth endoplasmic reticulum, and filaments, and the surface was irregular with many smooth protrusions filled with filamentous cytoplasm.

Scanning electron microscopy showed cells with an almost spherical shape located on the ventricular lining surface. These cells had a 4- to 6-μm body with many small bleb-like projections and were very similar to the supraependymal macrophages described by Bleier, et al. Occasionally, long narrow processes (< 1.0 μm), the origin and destination of which are unknown, were seen to course over the ventricular surface. These resembled the supraependymal axons demonstrated in the human lateral ventricle by Richards, et al. Transmission electron micrographs supported these findings. The intraventricular macrophages were round to ovoid with small irregular cytoplasmic extensions, lipofuscin granules, electron-dense inclusion bodies, and vacuoles. The nuclei were large, irregular, and pale, with prominent chromatins and nucleoli. Unmyelinated axons, 0.25 to 0.4 μm in diameter with electron-lucent vesicles, were seen in close apposition to the microvillous surface.

A survey of the ventricular wall (Fig. 5 left) demonstrated the highly attenuated nature of the lining. The ventricular lining consisted of two major cell types. The first cell type, believed to be ependymal, was relatively electron-dense with large pale ovoid nuclei. Not infrequently, the cell bodies were situated deep in the neuropil with only a thin cytoplasmic process covered with microvilli extending to the surface. Higher-power observation of these thin processes revealed a densely filamentous layer in the cytoplasm subjacent to the microvilli. Abundant smooth endoplasmic reticulum and mitochondria were present in the cytoplasm further from the ventricular lumen (Fig. 5 right). Occasionally,
small bleb-like processes containing amorphous material and vesicles projected from the ependymal cell surface on a short stalk. These appeared to be extensions of microvilli. In spite of the ependymal attenuation, we observed partial continuity of the apical lateral ependymal junctions. Intercellular junctions, usually of the zonula adherens type and rarely of the zonula occludens type, were intact in most cases where two ependymal cells were in contact (Fig. 5 right).

The second major cell type lining the ventricle was believed to be astrocytic. These relatively electron-lucent cells had multiple processes of various sizes. The nuclei were large, pale, and irregular, and many mitochondria and glial filaments filled the cytoplasm. Generally, they were lacking in intercellular junctions, although the occasional desmosome was seen. The astrocytic cell layer extended to a distance of approximately 30 μm from the ventricle, and throughout it the intercellular spaces were electron-lucent and enlarged. In the depths of this layer, normal-appearing capillaries and, less frequently, small arterioles were present.

At distances greater than 30 μm from the ventricle the neuropil consisted largely of unmyelinated axons, with oligodendrocytes and astrocytes at various stages of maturity. Up to a distance of 60 μm, the intercellular spaces were greatly increased, but beyond this the spaces were normal with no obvious change in the area we examined by transmission electron microscopy (1 mm from the ventricular lumen). Myelinated axons were rarely seen, and when they were found they were usually in small groups. There was little evidence of axonal ballooning (the most common form of axonal damage described in hydrocephalus) at any distance from the ventricle. Throughout the extent of the periventricular neuropil, however, were numerous microglia, consistent with the description by Penfield and Elvidge of frequent periventricular macrophages in hydrocephalus. These cells, often located in the pericapillary spaces, contained lipofuscin granules, vacuoles, and electron-dense lamellar inclusion bodies suggestive of active phagocytosis.

Discussion

The cytopathological features of human neonatal hydrocephalus revealed in this study are not appreciably different from changes described in animals with exper-
Human neonatal hydrocephalus

FIG. 3. Left: Scanning electron micrograph showing club-shaped unipolar cells (arrows) in the denuded ventricular lining. The bulbous somas of these cells taper to a narrow process that enters the neuropil. Note the absence of normal ciliated ependyma and the debris distributed on the surface. Bar scale = 10 μm. Right: Higher magnification of the unipolar ventricular wall cell outlined in the box at left. This cell is believed to be a form of immature ependymal cell. Note the cytoplasmic processes of various shapes. Cells to the right are damaged erythrocytes (er). Bar scale = 1 μm.

mentally induced hydrocephalus. Our observations of changes in the periventricular region correspond to those described by Page, et al., in hydrocephalic adult rabbits. In response to increasing CSF pressure they noted enlargement and elongation of the lateral cytoplasmic processes of ependymal cells, accompanied by a loss of cilia and microvilli and expansion of the extracellular space in the neuropil. With further stretching of the ventricular wall, ependymal compensation fails and, as evident from our study, the underlying glial layer is exposed to the CSF. Weller, et al., described a complete loss of ependyma and periventricular astrocytosis in a 6-week-old hydrocephalic infant, but this was based on study of a closed biopsy specimen in which significant distortion may have occurred.

Astrocytoma is the third most common type of congenital brain tumor. The most frequent and often the only presenting sign of congenital brain tumors in infants is a large head with tense fontanels. The diuresis that normally occurs in these babies during the 1st week following birth decreases the brain volume and pressure and allows compensatory increases in the CSF volume, thus masking the intensity of the clinical manifestations of hydrocephalus. A neonate's clinical findings are, therefore, not necessarily useful in determining the duration of the congenital hydrocephalus. Although it is difficult to judge the rate of ventricular expansion of the infant in this study, we may speculate that it was rather rapid. Collins proposed that the degree of ependymal damage in hydrocephalus depends on the rate of ventricular expansion. The obviously severe ependymal damage in this infant suggests that the tumor might have acutely occluded the foramen of Monro, causing a rapid ventricular dilatation. Once the ependyma and its lateral junctions are disrupted, the subependymal astroglial processes offer little resistance to the stretching force.

The possibility that some artifactual changes may have resulted from surgical manipulation and intraoperative irrigation with saline solution cannot be en-
FIG. 4. Transmission electron micrograph of the basal process of a unipolar cell (cells indicated by arrows in Fig. 3 left) projecting from the ventricular lumen (V) between two ependymal cells (E) into the neuropil. The process has a dense filamentous network (f) with microtubules (arrow). Deep to the ependymal layer there are astroglial processes (G) with prominent filaments (f'). × 15,500.

tirely excluded. The latter likely caused crenation of the erythrocytes that are adherent to the ependymal surface. Despite matting and irregularity, however, the cilial size corresponds well to the measurements of human ventricular cilia reported by Dempsey and Nielsen; the tissues otherwise appear to be well fixed, suggesting that the changes observed are not artifactual. Ependymal blebs, similar to those described in rats following 1 week of CSF drainage, were seen in this study, but it is unknown if these can form over 15 minutes in response to the opening of the ventricle.

Dooling, et al., demonstrated that fetal human ependyma is highly plastic and, under normal conditions, may undergo spontaneous desquamation until at least 35 weeks gestational age. Schimrigk reported that the posterior horns of the lateral ventricles are normally devoid of ependyma in young adults. In spite of these findings, it is unlikely that this process could entirely account for the ependymal denudation observed in this study. A 1-week-old hydrocephalic child with Arnold-Chiari malformation and only moderate ventricular dilatation has been reported to have a normal ependymal covering in a scanning electron microscopic study of autopsy specimens. Extreme degrees of ventricular dilatation may exceed the capabilities of fetal ependyma to compensate for stretching of the walls of the lateral cerebral ventricles. Weller and Shulman were able to identify ependyma in three of five hydrocephalic infants biopsied at the time of primary shunt insertion; the ependyma in those infants was considered abnormal, forming only an incomplete layer with no tight junctional complexes. However, their study was also based on closed biopsy material obtained at the time of shunting. We have demonstrated tight junctional complexes between contacting ependymal cells, suggesting that there is an attempt by the lateral apical complexes of the ependymal cells to maintain continuity.

To our knowledge the unipolar cells with basal processes that extend radially into the subependymal layer have not previously been described in the human lateral ventricle. The shape and orientation of these cells are reminiscent of the tanycytes and/or radial glia described in circumscribed regions of the ventricle in a variety of species, including humans. Stretching of the ventricular wall with the attendant separation of and damage to the ependymal cells may have exposed these cells and their anchoring basal processes. To assign a definite label to this cell type is not possible. In favor of a tanycytic identity, Walsh, et al., showed that tanycytes from the third ventricle of rats of various ages contain microtubules in the basal processes, and Millhouse stated that tanycytes are similar to embryonic ependyma. Tanycytes have been described only with great uncertainty in the human lateral ventricle, however, and in animals these cells have a more electron-dense cytoplasmic matrix than the cells we have described. To call these cells "radial glia" would also be incorrect. During primate fetal development, the somas of radial glial cells migrate from the ventricular region along the basal cell processes to positions deep in the neuropil, and there they differentiate into astrocytes. At birth, the remaining cells with radial fibers in the ventricular region are ependymal cells. By 20 weeks gestation, the majority of radial glia in human fetuses have already migrated to the subventricular zone. At 43 weeks gestation, the somas of radial glia would not be expected at the ventricular surface. In a recent review paper, Hajos and Basc22 pointed out the difficulty in differentiating these cell types early in their development, and Levitt and Rakic considered tanycytes to be a modified form of embryonic radial glial cell. Whether tanycytes, radial glia, and ependymal cells are distinct subclasses of neuroglia or the same cell type responding differently to unlike environments remains to be determined, so we conclude that the experimental literature is inadequate to allow us to accurately identify these club-shaped unipolar cells.

Increased extracellular space was clearly observed in
Human neonatal hydrocephalus

the periventricular axonal layer of our biopsy specimen up to a depth of 60 μm. Subsequent to ependymal disruption, CSF edema likely forms rapidly, following the path of least resistance along the glial cells, which lack tight intercellular junctions. The transition to "normal" neuropil was distinct, consistent with experimental findings that CSF edema is sharply demarcated. Between 60 and 1000 μm, we saw no obvious changes in the extracellular space. Lux et al. reported that periventricular water content in hydrocephalic cats was increased to a depth of 600 μm following intraventricular perfusion with artificial CSF at high pressure, and to 200 μm when perfusion was performed at low pressures. Because they sampled only at 200-μm intervals, the actual depth of edema may have been significantly less. Ogata et al. noted morphological changes to a depth of 200 μm in cats with experimentally induced hydrocephalus. Our morphological findings are largely consistent with these experimental findings.

Weller et al. described occasional degenerating axons in the completely unmyelinated and otherwise normal neuropil of a 6-week-old hydrocephalic infant. They found no damage in the subjacent neuronal layers and concluded, based on these and experimental observations, that tissue damage sustained in hydrocephalus is primarily axonal and is proportional to the degree of myelination. We have found no axonal ballooning that would suggest gross axonal destruction, but the abundance of lipid and lamellar inclusion laden microglia in the neuropil would indicate that some component of this region is being damaged and is subsequently undergoing phagocytosis. Possibly, the small number

FIG. 5. Left: Transmission electron micrograph of the ventricular lining. Intraventricular macrophages (M) are present in the ventricular lumen (V). Ependymal cells (E) are attenuated, with a soma located deep in the neuropil and only a narrow cytoplasmic process (solid arrow). The surface microvilli are exposed to the ventricular lumen. Interwoven glial processes (G) are exposed to the cerebrospinal fluid (open arrow), x 5700. Right: Transmission electron micrograph showing an ependymal cell junction. The apical lateral contact between “E” and “E’” has intact zonula adherens junctions (arrows). A prominent microfilamentous network (f) fills the cytoplasm immediately subjacent to the microvilli which project into the ventricular lumen (V). Below that are abundant vesicular and tubular profiles of smooth endoplasmic reticulum. x 20,300.
of myelinated axons around the occipital horn of the ventricle are being affected. Myelination in the occipital optic radiation in humans is found as early as 39 weeks gestation and is completed by 46 weeks.\textsuperscript{19} We have demonstrated ultrastructural evidence of a small number of myelinated axons in this region. As hydrocephalus is believed to cause demyelination\textsuperscript{4} and myelination is believed to be a vulnerable process in brain development,\textsuperscript{11} the possibility exists that hydrocephalus could also delay myelination. The decrease in the number of myelinated axons would explain the delayed latency periods of visual evoked potentials seen in hydrocephalic infants.\textsuperscript{21}

Although the pathophysiological effects of ventricular dilatation on the brain are poorly understood, the efficacy of shunting has been well demonstrated\textsuperscript{17} in spite of the potential complications. Even neonates with much more severe hydrocephalus than was exhibited by the one described here have been shown to develop within normal limits following shunting.\textsuperscript{25} This demonstrates a functional recovery of the brain following relief of the mechanical stress of hydrocephalus. The anatomical correlate of this recovery is not known, however; experimental studies have failed to show complete remyelination following shunting of hydrocephalic adult cats\textsuperscript{38} or after a prolonged period following chemical demyelination in weaning mice.\textsuperscript{27} We feel that the understanding of morphological changes in the hydrocephalic brain following shunting requires clarification.

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**References**


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Human neonatal hydrocephalus


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