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ULTRASTRUCTURAL ALTERATIONS OF GOLGI APPARATUS IN THE NERVE CELLS OF CEREBRAL CORTEX IN HUMAN HYDROCEPHALUS. A QUALITATIVE STUDY USING CORTICAL BIOPSIES

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Abstract

Cortical biopsies of 17 patients with clinical diagnosis of hydrocephalus and associated pathology were examined in the transmission electron microscope. Moderately and severely edematous neurons showed relevant structural changes of the Golgi apparatus consisting of either discrete or marked dilation, fragmentation, and partial disappearance of Golgi stacked cisternae. In Arnold-Chiari malformation small Golgi complexes of vesicular type and atrophic changes were observed in severely edematous neurons. The microtubules appeared intact, suggesting differential response between Golgi complex and microtubules. Atrophic changes of the Golgi complex coexisted with degenerated presynaptic endings.

Key Words: Golgi apparatus, neurons, cerebral cortex, hydrocephalus.

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Introduction

Most light and electron microscope investigations of hydrocephalic brains have studied the choroid plexus, the ependyma, sub-ependymal white matter, neighboring gray matter, and capillary wall (McLone *et al.*, 1971; Weller *et al.*, 1971; Mori and Raimondi, 1975; Nyber-Hansen *et al.*, 1975; Gopinath *et al.*, 1979). Some papers dealing with edematous and degenerative changes of hydrocephalic cerebral cortex have been also published (Struck and Hemmer, 1964; Foncin *et al.*, 1976; Castejón, 1980, 1988; Glees and Voth, 1988; Glees *et al.*, 1989; Glees and Hasan, 1990). However, very few reports have described alterations of nerve cell organelles induced by either hydrostatic or biochemical factors involved in the pathogenesis of experimental, congenital or human hydrocephalus. Lawson and Raimondi (1973) described, very briefly, enlargement of a prominent Golgi complex in the supranuclear region of ependymal cells in a normal and a hydrocephalic murine mutant. These authors suggested that this finding was an expression of high metabolic activity. As part of a study of human hydrocephalic cerebral cortex (Castejón, 1994), we have observed significant changes in the Golgi complex membranous elements. Pathological disorders of the Golgi apparatus have been described in a variety of diseases involving altered secretory activity (albuminemia, lipoproteinemias), cell surface pathology (cancer) and viral diseases (Morre, 1991). However, to the best of our knowledge, no studies have been published dealing with alteration of Golgi complex in human hydrocephalus. This paper deals with the edematous and degenerative changes of nerve cell Golgi apparatus in human hydrocephalus, using surgical human cortical biopsies.

Material and Methods

Samples of cerebral cortex of 17 patients with clinical symptoms of hydrocephalus were used in the present study. Cortical biopsies were performed according to basic principles of the Declaration of Helsinki. Table 1 contains the clinical data and lists the cortical

Table 1. Human hydrocephalus. Clinical Data

	Sample Identification	Age and Sex	Clinical Data	Diagnosis	Cortical Biopsy
1.	HLCS H 1	1 month Female	Increase of cephalic circumference.	Uncompensated communicant hydrocephalus.	Right parietal cerebral cortex.
2.	EEAT H2	1 month Female	Increase of cephalic circumference.	Uncompensated congenital hydrocephalus.	Right temporo-parietal cortex.
3.	LMBC H3	1 month Female	Increased cephalic circumference. Hypertensive fontanelles.	Congenital communicant hydrocephalus.	Right frontal cortex.
4.	DEMY H4	6 months Male	Increased cephalic circumference. Left peridural abscess.	Congenital hydrocephalus.	Right temporo-parietal cortex.
5.	FJRA CCH1 5	10 days Male	Bulging of fontanelles and increased cephalic circumference after surgical correction of lumbar meningomyelocele.	Arnold-Chiari syndrome. Communicant hydrocephalus.	Frontal cortex.
6.	CMV CCH1 5	2 months Female	Increased cranial volume, hypertensive fontanelles, deviation of gaze to the right, external rotations of both legs and increased tendinous reflexes after treatment of meningomyelocele.	Arnold-Chiari malformation. Hydrocephalus. Parieto-occipital intraparenchymatous abscess.	Right parietal cortex.
7.	NU CCH23	12 days Female	Increased cephalic circumference after treatment of lumbar meningomyelocele.	Congenital hydrocephalus. Meningocele.	Right parietal cortex.
8.	IATF	3 months Male	Febril syndrome. Meningitis.	Postmeningitis hydrocephalus.	Right frontal cortex.
9.	NSM	8 months Female	Increased cranial volume since three months.	Communicant hydrocephalus.	Right parietal cortex.
10.	GAPG	3 months Male	Meningeal syndrome. Tonic-clonic convulsions, increased cephalic circumference.	Postmeningitis hydrocephalus.	Frontal cerebral cortex.
11.	RGG CCH26	4 months Female	Increased cephalic circumference, hypertensive fontanelles.	Congenital hydrocephalus.	Right frontal cerebral cortex.
12.	JLAR CCH47	3 months Male	Increased cephalic circumference observed 1 month after birth.	Communicant hydrocephalus.	Right frontal cortex.
13.	HR CCH45	2 years Female	Increased cranial volume since 4 months of age.	Communicant hydrocephalus.	Right frontal cortex.
14.	ISS CCH55	7 months Male	Increased cranial volume. Diagnosis of subarachnoid hemorrhage after axial computer tomography.	Communicant hydrocephalus.	Right frontal cortex.
15.	JM CCH63	21 years Male	Sudden increase of cephalic circumference. Tonic convulsions.	Pinealome of third ventricle.	Posterior parietal cortex.
16.	CC H9	10 years Male	Increased cranial volume.	Communicant hydrocephalus.	Parietal cerebral cortex.
17.	EV H10	5 years Female	Increased cephalic circumference.	Communicant hydrocephalus.	Parietal cerebral cortex.

region from which the biopsy was taken. According to the clinical data most samples examined were high pressure cerebro-spinal fluid hydrocephalus. Two to five mm thick cortical biopsies were immediately fixed in the surgical room in 4% glutaraldehyde - 0.1 M phosphate or cacodylate buffer, pH 7.4, at 4°C. After 2 hours glutaraldehyde-fixation period, the cortical biopsies were divided into approximately 1 mm fragments and observed under a stereoscopic microscope to check the quality of fixation of the sample and the brownish coloration of the surface and deep cortical regions characteristic of good glutaraldehyde fixation by immersion technique. The cortical slabs were also done to assure optimal diffusion rates of glutaraldehyde and osmium tetroxide fixatives. Immersion in fresh glutaraldehyde solution of 1 mm slices was done for 2 hours. Secondary fixation in 1% osmium tetroxide - 0.1 M phosphate buffer, pH 7.4, was carried out for 1-2 hours at 4°C. Black staining of the cortical slices was also observed under a stereoscopic microscope to check osmium tetroxide diffusion rate and quality of secondary fixation. They were then rinsed for 5 to 10 minutes in phosphate or cacodylate buffer of similar composition to that used in the fixative solution, dehydrated in increasing concentrations of ethanol, and embedded in Araldite or Epon. For proper orientation during the electron microscope study and observation of cortical layers, approximately 0.1 to 1 μm thick sections were stained with toluidine blue and examined with a Zeiss photomicroscope. Studies of neurons, glial cells, and blood-brain barrier were performed. Ultrathin sections, obtained with a Porter-Blum and LKB ultramicrotomes, were stained with uranyl acetate and lead citrate and observed in a JEOL 100B transmission electron microscope (TEM) at magnifications ranging from 30,000 to 60,000 X. For each case, approximately 50 electron micrographs were studied.

Results

The pathological alterations of the Golgi complex were studied in moderately and severely edematous non-pyramidal neurons, which were observed in all samples collected (see Table 1). The Golgi complex showed enlargement of the stacked Golgi cisternae or smooth membrane-endoplasmic sacs arranged in parallel. This enlargement was also simultaneously seen in the perinuclear and rough endoplasmic reticulum cisternae (Fig. 1). In moderately edematous neurons partial disappearance of the stacks of Golgi cisternae and enlargement of Golgi vesicles were observed (Fig. 2), whereas the rough membrane system formed by the nuclear envelope and rough endoplasmic reticulum cisternae appeared slightly dilated, thus suggesting a differential response of both membrane system in hydrocephalic neurons. In severely edematous neurons, as illustrated in Figure 3, a vacuolization of the Golgi apparatus was observed coexisting with lacunar dilatation of rough endoplasmic reticulum canaliculi. In a case of postmeningitis hydrocephalus, a remarkable edematous change was observed mainly at the ends of the Golgi cisternae, whereas the central region of the cisternae appeared undilated. In moderately edematous neurons, a disruption of the Golgi elements was observed with displacement of intact or fragmented Golgi endoplasmatic sacs, presence of small, medium and large Golgi vesicles, and enlarged Golgi vacuoles (Fig. 4). Such images suggested fragmentation of the stacked Golgi cisternae, hypertrophy of the trans-Golgi compartment and an increased number of vesicles.

In some severely edematous non-pyramidal neurons observed mainly in hydrocephalus associated with Arnold-Chiari malformation (Fig. 5), small vesicular Golgi complexes and apparently atrophic ones were observed, characterized by partial or total disappearance of Golgi cisternae and presence of numerous Golgi vesicles. In such cases, damage of the plasma membrane and swollen mitochondria were also found.

In most cases of congenital hydrocephalus, the primary lysosomes appeared intact, despite being present in severely edematous neurons. In most cases studied, the morphology of microtubules, in longitudinal and tangential sections, exhibited an apparently unaltered structure.

In addition, at the level of the neuropile, we found the presence of degenerated presynaptic endings exhibiting few synaptic vesicles and altered synaptic membrane complexes in the vicinity of degenerated neurons with atrophic Golgi complexes (Fig. 6), supporting the impression of impaired structures due to the existence of atrophic Golgi complexes.

Discussion

In the present paper we have observed enlargement, fragmentation and partial disappearance of the Golgi stacked cisternae and vacuolization, disruption and atrophic changes of the Golgi complex. Such pathological alterations (coexisting with moderately and severely edematous changes of the rough endoplasmatic reticulum and nuclear envelope) reveal alterations of the neuronal membrane system. Apparently, the primary extracellular hydrocephalic edema imposed mechanical stress and caused biochemical alterations in the plasma membranes, cytomembranes and cell organelles leading initially to cytotoxic neuronal edema and, subsequently, to degeneration. In this way, the ventricular enlargement exhibited by the patient leads to a multifactorial brain edema, in which extracellular, vasogenic and cytotoxic factors are involved. Thus, the alteration reported here in the Golgi

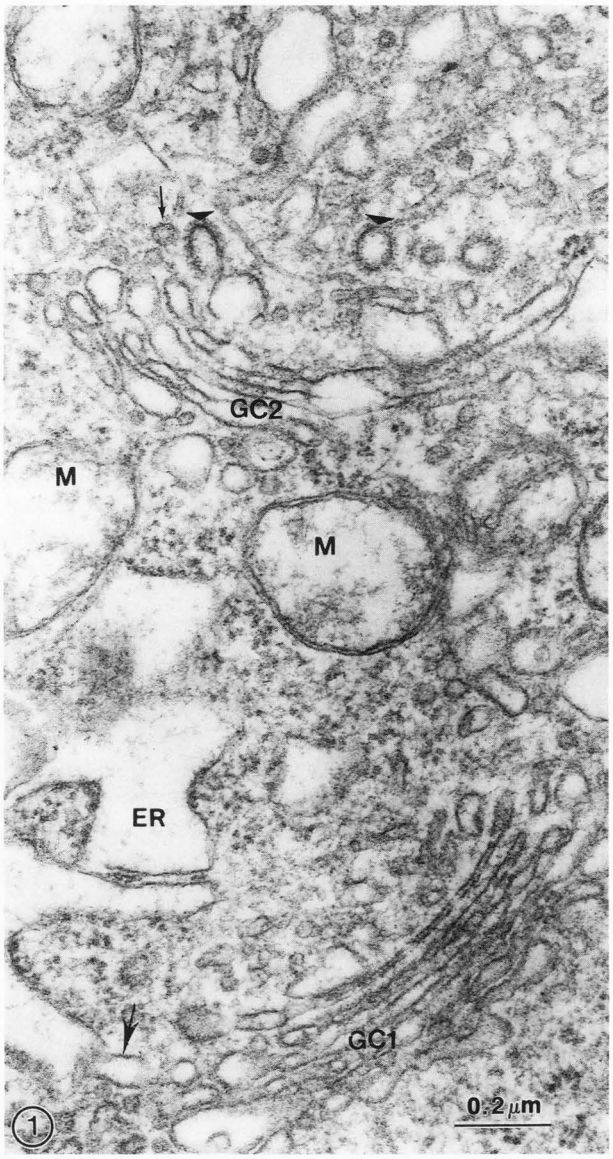

Figure 1. Severely edematous nerve cell showing two prominent Golgi complexes (GG1 and GG2) characterized by the presence of stacks of 4 to 6 moderately dilated flattened sacs. The parallel sacs in GG1 resembles the appearance of an apparently normal Golgi apparatus. GG2 exhibits dilated cisternae and vesicles. Note the remarkable edematous changes of mitochondria (M) and endoplasmic reticulum cisterns (ER). The large arrow indicates the dilated transitional zone between rough endoplasmic reticulum and the smooth membrane system of the Golgi complex. The secretory side or trans-Golgi compartment displays Golgi (small arrow) and coated vesicles (arrowheads).

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complex could be interpreted as an expression of the multifactorial hydrocephalic human brain edema.

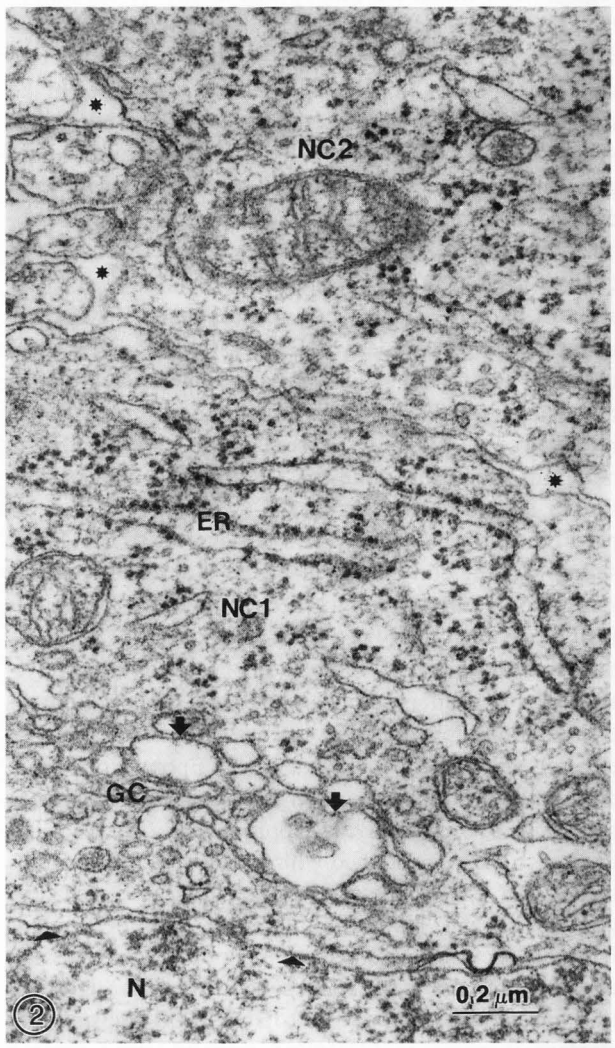

Figure 2. Moderately edematous neuron (NC1) exhibiting prominent changes of Golgi apparatus (GC) featured by partial disappearance of the stacked Golgi endoplasmic sacs and marked enlargement of Golgi vacuoles. Some dilated vacuoles may be derived from enlarged Golgi cisternae. There is discrete enlargement of the nuclear envelope (arrowheads), endoplasmic reticulum cisternae (ER) and the extracellular space (asterisks). This space separates NC1 from the neighboring nerve cell (NC2).

Apparently, the pathological events occur in the following steps: enlargement of ER cisternae also dilates the specialized portion of ER called transitional ER (adjacent to and in continuity with the Cis face of the Golgi apparatus). The stack of 4 to 6 Golgi smooth-membrane bound cisternae suffer dilation, fragmentation and finally disappear. We have used small cortical samples, which

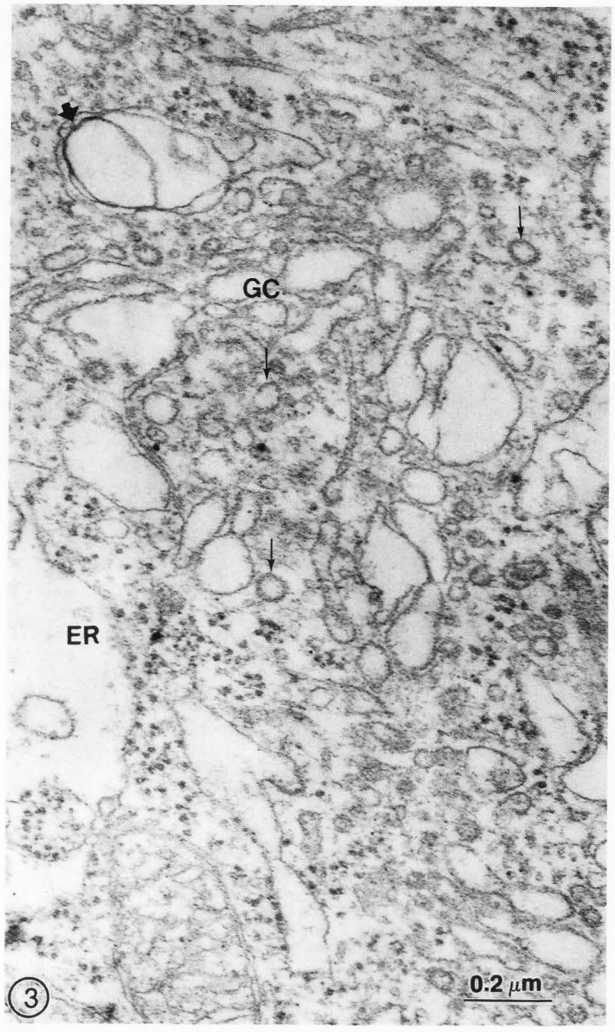

Figure 3. Severely edematous neuron showing vacuolization of Golgi complex (GC). Numerous clear, dense Golgi and coated vesicles (thin arrows) are seen originating from the trans-Golgi compartment and migrating throughout the cytoplasm. Note the extremely swollen rough endoplasmic reticulum cisternae and the presence of vacuoles with necrotic membrane profiles (thick arrow).

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have been immediately fixed in the surgical room. However, artifacts induced by glutaraldehyde fixative can not be totally discarded, but simultaneous intact preservation of swollen mitochondria and microtubules has been observed in the preparations under study.

Fixation artifacts versus real hydrocephalic pathology should be discussed here in detail. Poor brain tissue fixation with glutaraldehyde by immersion technique offers the over-all image of an edematous tissue. But normal pressure hydrocephalic edema is mainly confined

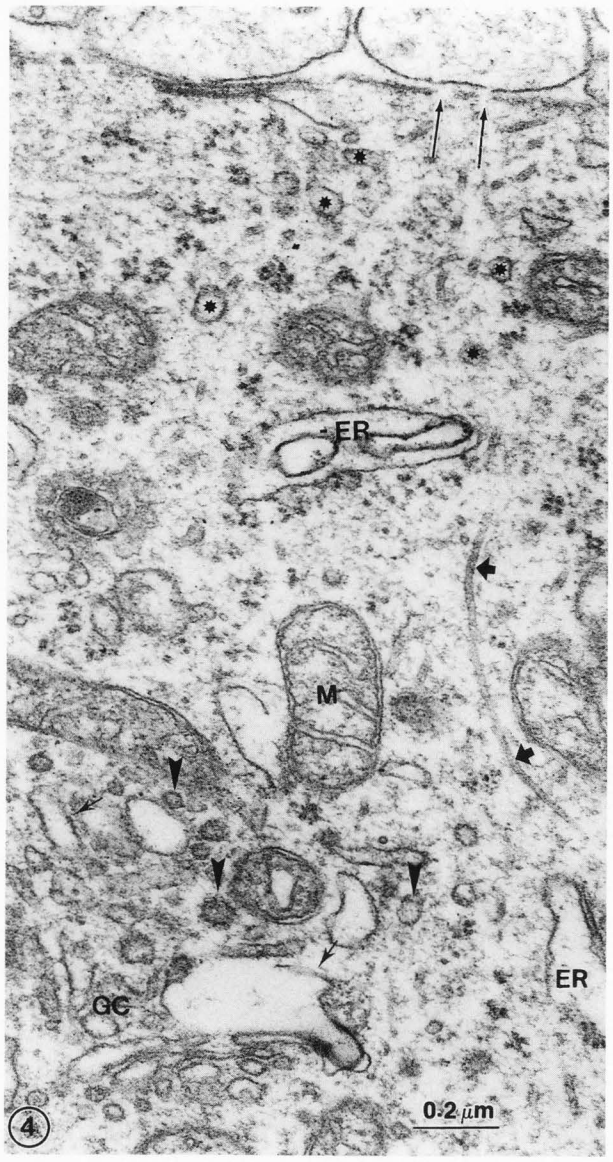

Figure 4. Moderately edematous neuron showing disruption of the Golgi complex (GC) characterized by displacement of some intact, fragmented of dilated Golgi cisternae (short arrows) and presence of numerous small, medium and large Golgi vesicles (arrowheads) in the trans-Golgi compartment. Some vesicles were observed in the peripheral cytoplasm (asterisks) in close proximity to plasma membrane discontinuities. The longitudinal section of an intact microtubule (thick arrow) is also seen. Note the swollen mitochondria (M) and the irregularly dilated endoplasmic reticulum cisternae (ER).

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to the membrane to membrane extracellular space (interstitial edema), which appears irregularly dilated. High

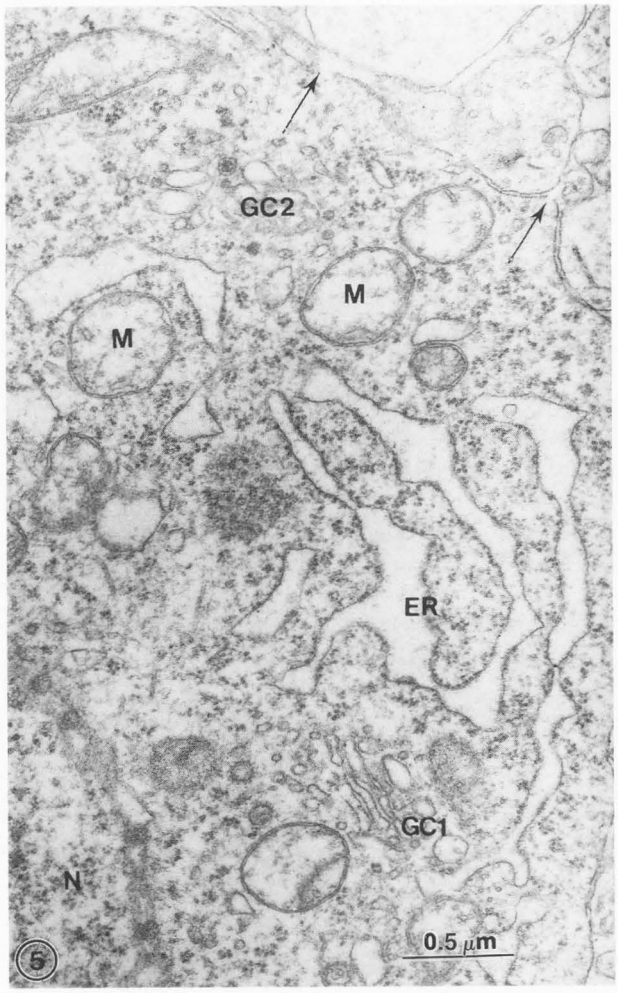

Figure 5. Arnold-Chiari malformation. Severely edematous neuron displaying atrophic perinuclear (GC1) and peripheral (GC2) Golgi complexes. Rough endoplasmic reticulum (ER) and mitochondria (M) appear notably swollen. The nucleus (N) is observed at the lower left corner of the figure. The arrows point to fragmentation of the plasma membrane.

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pressure hydrocephalic edema exhibits additional changes upon neurons and neuroglia cells (Castejón, 1994) due to the expansion forces induced by the cerebrospinal fluid present within the extracellular space. The damage to the gray matter results from hydrostatic and biochemical alterations induced by the extracellular pooling of cerebrospinal fluid and also by the expansion forces or stretching effect produced by the ventricular enlargement. Therefore, in high pressure hydrocephalus, the extracellular space and intracellular compartments appear dilated, suggesting associated interstitial and cytotoxic edema. The astrocytes appeared swollen and the capillary wall showed evident signs of blood-

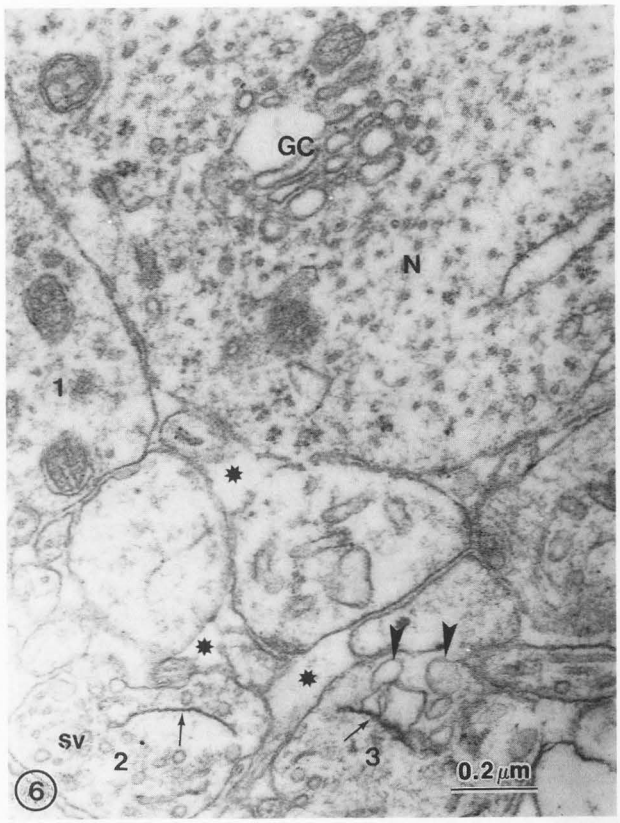

Figure 6. Degenerated neuron (N) with an atrophic Golgi complex (GC) coexisting with degenerated synaptic knobs (1,2,3) in the neighboring neuropile. Synaptic degeneration is featured by decreased number of synaptic vesicles (sv), enlarged and distorted synaptic vesicles (arrowheads) and presence of necrotic membranes (arrows). At the neuropile the extracellular space is considerably enlarged (asterisks) at the meeting point of several nerve processes.

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brain barrier dysfunction evidenced by increased endothelial and vacuolar transport, open inter-endothelial junctions and focal thickening of capillary basement membrane (Castejón, 1994). These alterations and the clinical data indicate that we are not dealing with artifactual edema due to faulty fixation but with true hydrocephalic pathology. In all the cases examined, we did not find the normal structural pattern of the Golgi complex in neither pyramidal nor non-pyramidal cells. In the present study, a careful fixation procedure of the cortical biopsies was done to avoid fixation artifacts. The cortical biopsies were placed immediately in the primary fixative solution by the neurosurgeon in the surgical room. Instrumental manipulation, pressure, and excessive handling of the brain sample were avoided. Once the cortical biopsy arrived at the laboratory, the sample was placed in a new fresh fixative solution for

longer periods ranging from 2-87 hours. This prolonged fixation appeared to be the best procedure for optimal fixation of human cortical biopsies.

Fragmentation and disappearance of the stack of Cis-Golgi cisternae occurred, whereas the secretory side or trans-Golgi compartment retained its integrity. This observation agrees with Rothman's hypothesis that the Cis- and trans- portions of the Golgi apparatus are functionally distinct subcellular organelles (Rothman, 1981). After the alteration of the Cis-Golgi region and the Golgi stacked cisternae, the trans-Golgi compartment becomes hypertrophic, possibly in an attempt to increase protein transport toward the plasma membrane in order to repair plasma membrane fragmentation, as illustrated in Figures 4 and 5. These factors demonstrate the need for immediate surgical treatment of infant hydrocephalus before the start of irreversible structural changes in nerve cells.

Marked hypertrophy, dilation, and distortion of the Golgi complex, as described in the present study, are also observed in some tumors (Ghadially, 1982), which have been related to the surface membrane changes of neoplastic cells. Atrophic changes of the Golgi apparatus have also been found in synovial cells in osteoarthritis, enucleated amoeba proteins and in hepatocytes subjected to a variety of toxic influences (for a review, see Ghadially, 1982).

In certain non-pyramidal neurons, the Golgi apparatus appears disrupted and disorganized. Golgi and coated vesicles were observed in both the Cis- and trans-Golgi regions. These observations have also been described by Merisko *et al.* (1986) in acinar cells of pancreatic lobules incubated under anoxic conditions. Anoxia inhibited ATP synthesis and this slowed intracellular secretory protein transport. Oxygen metabolism is impaired in normal pressure hydrocephalus (Ishikawa *et al.*, 1989). It is therefore possible that disrupted and unpolarized Golgi complexes, mitochondrial swelling and dilated endoplasmic reticulum are pathological alterations related to altered oxygen metabolism in patients with high pressure hydrocephalus (clinical data listed in Table 1). We do not know if disorganization of the Golgi complex is a reversible process or a stage prior to reorganization, as described by Bainton *et al.* (1989) in macrophages during phagocytosis.

In non-pyramidal cortical neurons, there is a differential response between the Golgi complex and the microtubules. As illustrated in Figure 4, the latter appeared intact, despite the swelling and fragmentation of Golgi stacked cisternae. We have not found structural indications of microtubular dissembly as related to the role of microtubules in the organization of the Golgi apparatus (Sandoval *et al.*, 1984; Thyberg and Moskalewky, 1985).

Some authors have postulated a continuum between Golgi apparatus, synaptic membranes and synaptic vesicles (André, 1964) and a relationship between Golgi and synaptic vesicles (Gray, 1970). Figure 6 shows an atrophic neuronal Golgi complex coexisting with neighboring degenerated synaptic endings, suggesting a possible correlation between Golgi complex dysfunctions and degenerated presynaptic endings.

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Discussion with Reviewers

F.N. Low. The edematous changes occurring take place, for the most part among lipoprotein membranes that are normally arranged roughly in parallel with each other. In contrast, macromolecular structures not necessarily associated with parallel lipoprotein membranes remain undisturbed. Does your broad experience with edematous preparations and molecular profiles permit you to comment on these differences, particularly in terms of molecular size and arrangement?

Authors: In human hydrocephalus, the edematous changes are widely distributed in neurons, glial cells and blood brain barrier. The hydrocephalic edema is primarily confined to the extracellular space (interstitial edema), as it has been classically described since more than three decades in TEM studies of experimental hydrocephalus. In neurons, the changes occurs in cytomembranes (rough and smooth membrane system) and cell organelles, such as mitochondria and Golgi complex. We do not know if macromolecular structures are altered or not, since our studies are at low magnification TEM. However, the impaired oxygen metabolism in normal pressure hydrocephalus (Ishikawa *et al.*, 1989) allows us to think that this alteration is more accentuated in high pressure hydrocephalus, as clinically described in the present paper (see Table 1). Thus, we can speculate that according to the results of the present paper, presumably the alteration of the cis-Golgi compartment and the stacked Golgi cisternae would impair the transport of proteins elaborated in the rough endoplasmic reticulum and would alter the covalent modifications of these transported protein, including glycosylation, selective proteolysis, phosphorylation and the addition of fatty acids, inherent functions of the Golgi apparatus (Rothman, 1981).

P. Mestres: Does the size of the cortical biopsy, 2-5 mm thick, represent a handicap for a careful fixation?

Authors: The size of the cortical biopsy depends for ethical reasons of the clinical diagnosis. More often, the neurosurgeon takes a 1-2 mm thick sample after opening the meninges and before initiating the surgical treatment to avoid pressure induced artifacts on the cerebral cortex. The samples are immediately fixed in the surgical room and thereafter cut into thinner slices to improve glutaraldehyde diffusion rate. Observations of the cortical slices in the stereo microscope after fixation allowed us appreciate the typical brownish coloration produced by glutaraldehyde fixation. To us, the major handicap is the immediate swelling process that occurs in the brain cortex once the neurosurgeon cuts the meningeal coverings.

P. Mestres: In which layer of the cerebral cortex were the investigated neurons located?

Authors: The toluidine blue stained thick sections examined under the optical microscope in every case, as a preliminary step to TEM examination, allowed us to study all layers of the cerebral cortex. Pyramidal and non-pyramidal cells (layers II to V) were affected by the infant high pressure hydrocephalus. In the present paper, alterations of the Golgi complex are shown mainly in non-pyramidal cells of layers II to IV.