LYSOSOME–INDUCED DAMAGE IN THE HUMAN EDEMATOUS CEREBRAL CORTEX. AN ELECTRON MICROSCOPIC STUDY.

Orlando J. Castejón

Instituto de Investigaciones Biológicas "Dres. Orlando J. Castejón y Haydee Viloria de Castejón. Apartado 526. Facultad de Medicina, Universidad del Zulia. Maracaibo – Edo. Zulia.Venezuela.
Tel. 58-261-7414270. E-mail: ocastejo@cantv.net

Abstract
The lysosome-induced alterations on cortical nerve cells were examined in the anoxic-ischemic brain parenchyma of thirty one patients with congenital hydrocephalus, complicated brain traumatic injuries, brain tumours and vascular anomalies. Cortical biopsies of frontal, parietal and temporal cortex taken during neurosurgical treatment were processed for conventional transmission electron microscopy. The lysosomes showed fragmentation of limiting membrane and an associated dense granulation. Areas of cytoplasmic focal necrosis were observed surrounding the lysosomes, suggesting release of lysosomal enzymes. Lipofucsin granules were observed in infant patients with congenital hydrocephalus suggesting that lipofucsin formation is a lifespan process. An increased amount of lipofucsin granules was observed in young and adult patients with brain trauma, brain tumours and vascular anomalies. The brain trauma induced activation of oligodendroglial and astrocytes cells, which showed the overall spectrum of an altered endosomal-lysosomal system and phagocytic activity. The role of oxidative stress is emphasized in the damage of lysosomal limiting membrane and the role of activated lysosomal enzymes is discussed in relation with the fragmentation of plasma membrane, and focal cytoplasmatic necrosis of nerve cells.
Keywords: lysosomes, nerve cells, brain trauma, brain tumors, congenital hydrocephalus, electron microscopy.

Resumen
Se examinaron las alteraciones de las células nerviosas corticales inducidas por lisosomas en el parénquima anóxic-ischémico de 31 pacientes con malformaciones congénitas, traumas craneoencefálicos complicados, tumores cerebrales y malformaciones vasculares. Se procesaron biopsias corticales de la corteza frontal, parietal y temporal para microscopía electrónica de transmisión convencional. Los lisosomas mostraron fragmentación de la membrana limitante y una granulación densa asociada. Se observaron áreas de necrosis focal citoplasmática rodeando los lisosomas, lo cual sugiere liberación de enzimas lisosomales. Se observaron gránulos de lipofucsina en infantes con hidrocefalia congénita, lo cual indica que la lipofucsinogénesis es un proceso que ocurre durante toda la vida. Así mismo, se observó un depósito anormal de gránulos de lipofucsina en paciente jóvenes y adultos con traumas, tumores y malformaciones cerebrales. Los traumas cerebrales produjeron activación de astrocitos y oligodendrocitos, los cuales mostraron actividad fagocítica y completo repertorio endosomal-lisosomal. Se discute el rol del estrés oxidativo en el daño de la membrana limitante de los lisosomas y el papel degradativo de las enzimas lisosomales en la génesis de la fragmentación de la membrana plasmática y la necrosis focal citoplasmática.

Introduction

Primary or secondary disturbance of lysosomal function is considered one of the predominant factors in the development of brain edema in malignant neoplasia and traumatic brain injuries [1-4], cronic hypertensive conditions [5], and in several age related neuroglial disorders [6]. Breakage or increased permeability of lysosomal membrane lead to argumentation of specially neural proteases in the cytoplasm, inducing autolitic areas of edematous brain parenchyma [3], and cystic formation as well as diffuse degeneration of the white matter [5]. Dysregulation of the lysosomal system is also accompanied by the accumulation of lipofucsin or age pigment. The abnormal accumulation of undigested lipid and proteins within dysfunctional endosomal lysosomal vesicle population may serve as triggers of apoptotic cell death and neurodegeneration [6]. In the present paper we analyze the alterations induced by abnormal lysosomes and the accumulation of lipofucsin pigments in the cerebral cortex of patients with brain trauma, brain tumours, congenital malformations, and vascular anomalies, using cortical biopsies processed for

transmission electron microscopy. We have also examined the endosomal/lysosomal system in every case under study to observe disturbance of endocytosis related with edema resolution. Neurons and glial cells could endocytose proteinaceous edema fluid from the extracellular space to be transferred and digested for lysosomes [4], thus contributing to edema resolution. To test this hypothesis we traced endocytic vesicles from the plasma membrane to the lysosomes and Golgi complex area. Besides, we have study the presence of residual bodies or lipofucsin granules in order to observe if such secondary lysomes are related to the lysosomal disturbance.

Material and Methods

Cortical biopsies of 31 patients with clinical diagnosis of congenital hydrocephalus, vascular anomalies, brain tumours and brain trauma were examined with the transmission electron microscope. The Table No. 1 contains the clinical data and lists the cortical regions from which the cortical biopsy was taken during neurosurgical treatment. The neurosurgical study was performed and the cortical biopsies were taken according to basic principles of Helsinki declaration. The clinical study has been published elsewhere [7].

Table I. Neurosurgical study

Sample Identification	Age And Sex	Clinical Data	Diagnosis	Cortical Biopsy
HLCS H 1	1 m, F	Increase of cephalic circumference.	Uncompensated communicant hydrocephalus.	Right parietal cortex
LMBC H3	1 m,F	Increased cephalic circumference. Hypertensive fontanelles.	Congenital communicant hydrocephalus	Right frontal cortex.
DEMI H4	6 m,M	Increased cephalic circumference. Left peridural abscess.	Congenital hydrocephalus.	Right tem-poroparietal cortex.
CMV CCH19	2 m,F	Increased cranial volumen, hypertensive fon-tanelles, deviation of gaze to the right, external rotations of both legs and increased tendinous re-flexes after treatment of meningomyelocele.	Arnold-Chiari malformation. Hydrocephalus. Parieto-occipital intraparenchymatous abscess.	Right parietal cortex.
UN CCH23	12 d,F	Increased cephalic cir-cumference after treat-ment of lumbar me-ningomyelocele.	Congenital hydroce-phalus. Meningocele.	Right parietal cortex.
IATF	3 m,M	Febril syndrome. Meningitis.	Postmeningitis hydrocephalus.	Right frontal cortex.
NSM	8 m,F	Increased cranial volume since three months.	Communicant hydrocephalus.	Right parietal cortex.
GAAPG	3 m,M	Meningeal syndrome. Tonic-clonic convulsions, increased cephalic circumference.	Postmeningitis hydrocephalus.	Frontal cortex.
RGG CCH26	4 m,F	Increased cephalic circumference, hyper-tensive fontanelles.	Congenital hydrocephalus.	Right frontal cortex.
HR CCH45	2 y, F	Increased cranial volume since 4 months of age.	Communicant hydrocephalus.	Right frontal cortex.
ISS CCH55	7 m,M	Increased cranial volume. Diagnosis of subarachnoid haemorrhage after axial computer tomography.	Communicant hydrocephalus.	Right frontal cortex.
JM CCH63	21 y,M	Sudden increase of cephalic circumference. Tonic convulsions.	Pinealome of third	Posterior

			ventricle.	parietal cortex.
CC H9	10 y, M	Increased cranial volume.	Comunicant hydrocephalus.	Parietal cortex.
EV H10	5 y,F	Increased cephalic circumference.	Comunicant hydrocephalus.	Parietal cortex.
NCRG (CCH12)	10 y, F	Tremor in upper and lower extremities, incoherente speech, difficulty in walking, headache, visual hallucinations, cloudy sensorium and stupor.	Cerebellar syndrome with involvement of anterior vermis.	Right parietal cortex.
LGH (CCH15)	10 m, F	Fracture of skull in left parietal region.	Brain trauma and left parietal extradural haematoma.	Right parietal cortex.
HRF (CCH17)	18 y, F	Severe frontal contusion in road accident, loss of consciusness, convulsive crisis.	Severe frontal contusion.	Left frontal cortex.
AMG (CCH18)	39 y, M	Loss of consciousness after fall from a truck. Headache. Left hemi-paresis, papilledema.	Brain trauma. Right parieto-temporal hematoma.	Right parietal cortex.
JAAS (CCH20)	17 m, M	Diminution of visual acuity, bilateral paleness of papilla, enlargement of sella turcica.	Cystic craniopharyn-gioma.	Right fronto-temporal cortex.
AASCR (CCH 22)	26 y, M	Skull trauma in right temporo-parietal region, tonic-clonic convulsions. Disorder of behaviour.	Brain trauma. Right parieto-temporal haematoma.	Right tempo-roparietal cortex.
HMB (CCH24)	37 y, M	Loss of conciousness. Tonic-clonic convulsions.	Anomaly of anterior cerebral artery. Arachnoiditis.	Right posterior parietal cortex.
PMD (CCH27)	21 y, M	Patient suffered a fall showing coma and bi-lateral papilledema.	Brain trauma. Right epidural hematoma.	Right temporal cortex.
JP (CCH29)	14 y, M	Contusion and fracture of frontal region, transitory loss of consciousness.	Contusion and fracture of frontal region.	Left frontal cortex.
OP (CCH30)	60 y, F	Head injury in traffic accident, fracture of both legs, state of coma, abolition of reflexes. Left midriasis. After recovery showed disorders of behaviour (Post-traumatic confusional syndrome).	Brain trauma. Subdural hygroma.	Right parietal cortex.
JRCR (CCH31)	80 y, M	After suffering fall, chronic alcoholic patient showed headache, diminution of muscle strength of lower extremities and right arm, temporary loss of consciousness, dysarthria, anisocoria.	Brain trauma. Left frontoparietal-occipital subdural hematoma.	Left parietal cortex.
JMV (CCH63)	21 y, M	Increased volume of cephalic circumference, headache,	Pinealome, secondary hydrocephalus.	Right parietal cortex.

		vomits.		
LCS (CCH64)	20 y, F	Frontal headache.	Brain trauma. Left frontal hematoma.	Left frontal cortex.
LJGS (CCH65)	25 y, M	Headache, loss of consciousness after alcohol ingestion.	Frontal hemangioma.	Left frontal cortex.
NJCZ (CCH84)	34 y, M	Headache, vomits, dizziness, blurred vision.	Ependymoma.	Occipital cortex.
EMP (CCH59)	5 y, M	Difficulty in walking, tremor in lower extremities, headache, vomits.	Tumor of posterior fossa. Obstructive hydrocephalus.	Right frontal cortex.
MR (CCH57)	67 y, F	Brain trauma after fall.	Brain trauma. Meningioma. Hypertension.	Right frontal cortex.

Two to five mm thick cortical biopsies were immediately fixed in the surgical room in 4% glutaraldehyde-0.1M phosphate or cacodylate buffer, pH 7.4, at 4° C. After 2 hours glutaraldehyde-fixation period, the cortical biopsies were divided into approximately 1mm fragments and observed under a stereoscopic microscope to check the quality of fixation of the sample, the glutaraldehyde diffusion rate, and the brownish coloration of the surface and deeper cortical regions indicative of good glutaraldehyde fixation by immersion technique. The cortical slabs were also performed to assure optimal diffusion rate 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-0.1M 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 primary 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 (solution) and examined with a Zeiss photomicroscope. Light microscope study of neurons, glial cells, and blood-brain barriers was performed [7], ultrathin sections, obtained with Porter-Blum and LKB ultramicrotomes were stained with uranyl acetate and lead citrate and observed in a JEOL 100B transmission electron microscope at magnifications ranging from 24,000 to 60,000X. For each case, approximately 50 electron micrographs were studied. Digitalized images were Photoshop treated.

Results and Discussion

In congenital hydrocephalus and Arnold-Chiari malformations we found fragmentation of lysosomal and multivesicular body limiting membranes (Fig. 1) in edematous pyramidal and nonpyramidal neurons.

Some lysosomes exhibited an associated coarse dense granulation, interpreted as abnormal protein aggregation of released lysosomal enzymes and adsorbed cytosolic proteins. Areas of cytoplasmic focal necrosis were observed in most nerve cells (Fig. 2).

In a cerebellar syndrome with moderate edema, hypertrophic oligodendroglial cells associated to degenerated myelinated axons also showed lysosomes with a coarse and fine dense granulations (Fig. 3).

In congenital hydrocephalus of neonate patients, the presence of lipofucsin granules also was observed, suggesting that formation of such residual bodies is a life span process. In a cerebellar syndrome with moderate

brain edema, the lysosomes showed a disrupted limiting membrane and a dense matrix containing a compact array of microcanaliculi. The fragmented limiting plasma membrane of lysosomes could be due to peroxidative stress [8-11]. One source of free radicals in ischemic cells is arachidonic acid released by membrane phospholipids under the action of Ca^{2+}-activated phospholipase A_2 [12]. Several oxygen radical species besides superoxide radicals are produced following hypoxia. Superoxide radicals have been shown to change phospholipid and protein structure. Hydroxyl radicals are the most reactive and are known to initiate lipid peroxidation and protein oxidation. Peroxidation of polyunsaturated fatty acids damages cell membranes and disrupts transmembrane ionic gradients. The products of lipid peroxidation are aldehydes, hydrocarbon gases, and other metabolites that can also cause cytotoxic and vasogenic oedema, [13-18].

In traumatic brain edema complicated with subdural hematoma, primary large and small lysosomes, and lipofucsin granules were found surrounding a swollen Golgi complex (Fig.4).

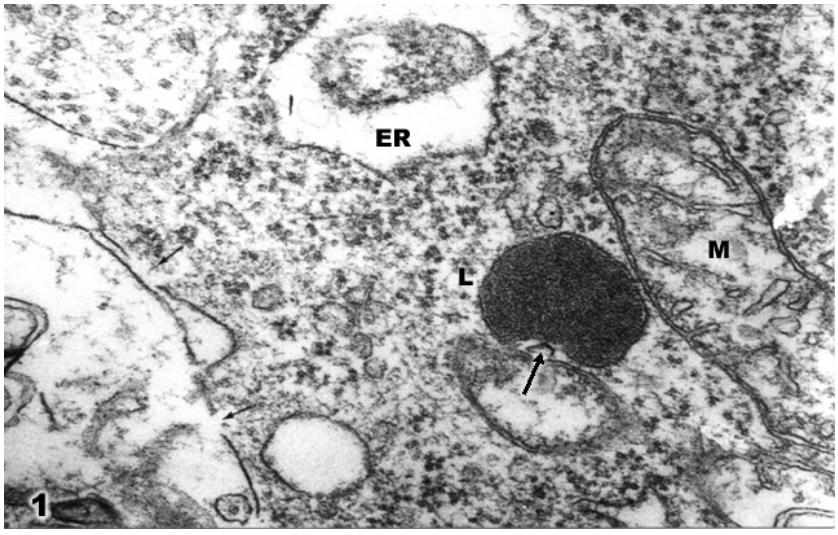

Fig. 1. Congenital hydrocephalus. Edematous non-pyramidal neuron showing a lysosome (L) with a disrupted limiting plasma membrane (long arrow). The mitochondrion (M) and the rough endoplasmic reticulum (ER) appear swollen. Note the fragmented neuronal plasma membrane (short arrows). X 60.000

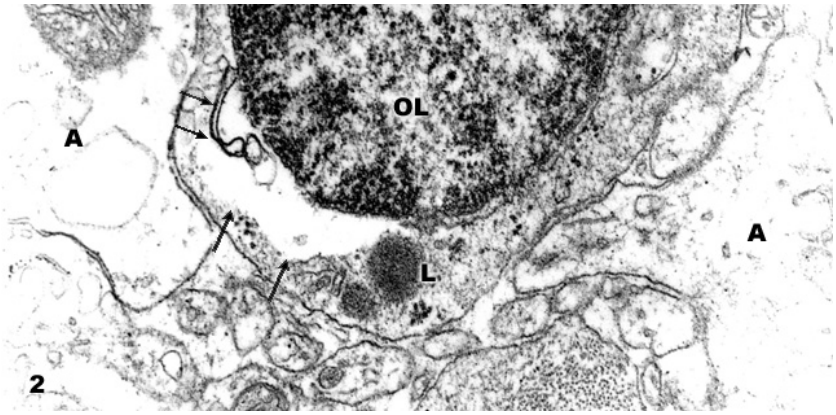

Fig. 2. Congenital hydrocephalus. Oligodendrogial cell (OL) showing a perinuclear focal cytoplasmic necrosis (arrows) in the vicinity of a lysosome (L). Note the swollen astrocytic cytoplasm (A). X 45.000

The cytoplasm of phagocytic astrocytes showed the presence of dense and clustered lysosomes and phagocytic vesicles containing proteinaceous edema fluid, suggesting that astrocytes can contribute to edema resolution by means of lysosomal digestion (Fig. 5)

Some phagolysosomes contained dark myelin debris associated to the protein and lipidic matrix. Lysosomes displayed bizarre shapes in glycogen rich-astrocytes. Besides, in traumatic brain edema fine granular deposits stored in clear vacuolar spaces were observed in lysosomes and lipofucsin granules, demonstrating the presence of metal or metalloid compounds (Fig.6).

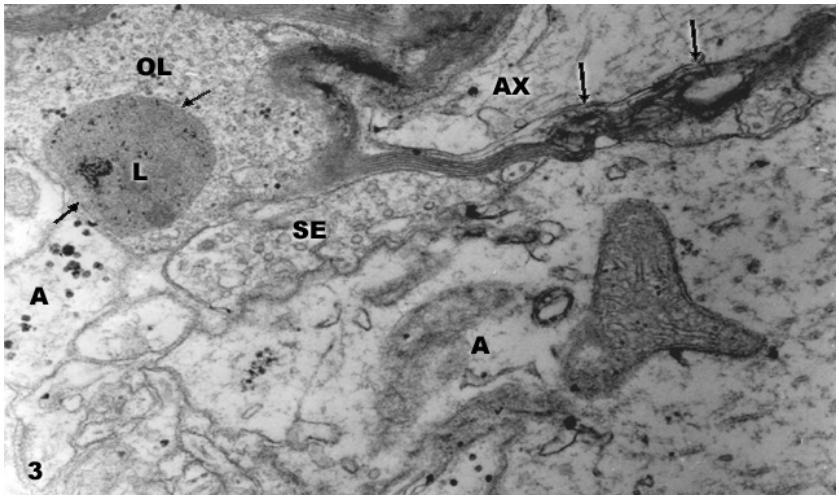

Fig. 3. Cerebellar syndrome. Periaxonal hypertrophic oligodendroglial cell (OL) showing a lysosome (L) with a fragmented limiting membrane (short arrows) and a coarse granulation. Note the degenerated myelinated axon (AX) with vacuolated myelin sheath (long arrows), and the swollen astrocytic cytoplasm (A) in the neighboring neuropil.. X 60.000

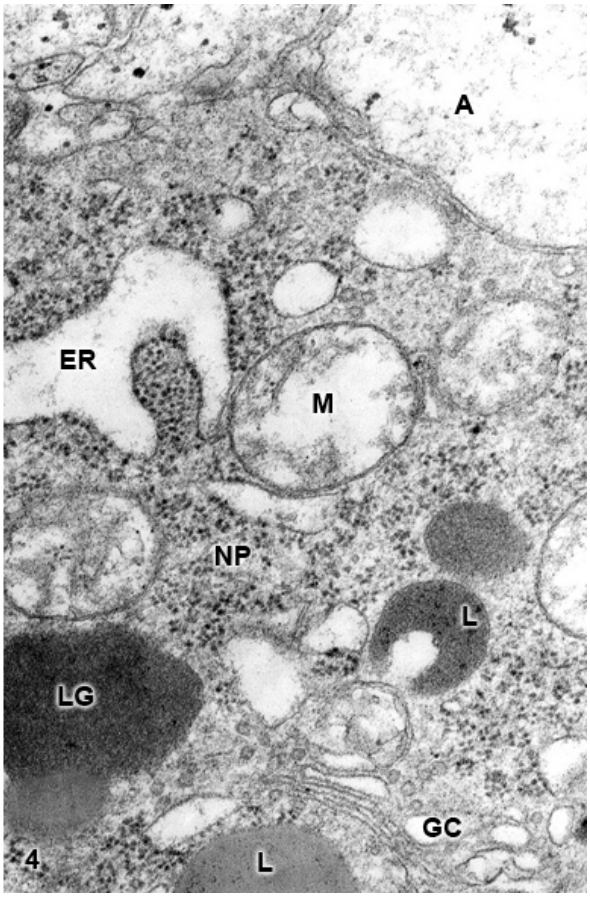

Fig.4. Traumatic brain edema. Left frontal cortex. Severely edematous non-pyramidal neuron (NP) showing clustered primary lisosomes (L), and a lipofucsin granule (LG) surrounding a swollen Golgi complex (GC). One of them appears lobulated and with a fine granular matrix. A swollen mitochondriom (M) and enlarged cisterns of endoplasmic reticulum (ER) also are distinguished. X 60.000

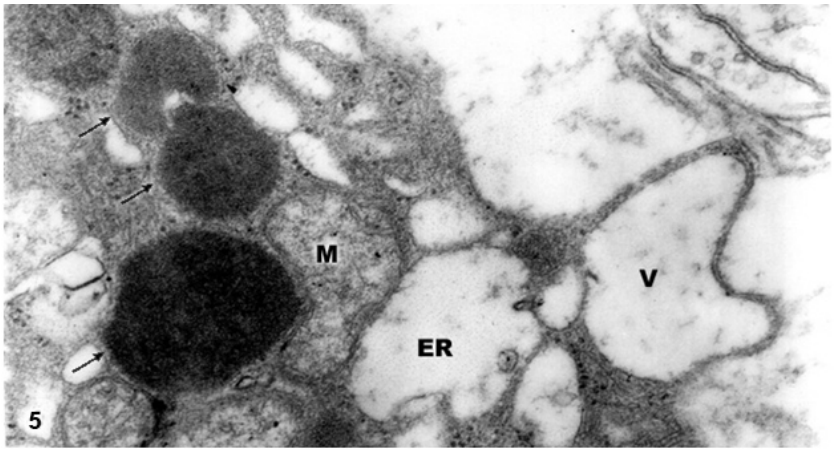

Fig.5. Traumatic brain edema. Phagocytic astrocyte containing dark lysosome (arrows). The cytoplasm exhibits a swollen mitochondrion (M), enlarged rough endoplasmic reticulum (ER), and a phagocytic vacuole (V) containing proteinaceous edema fluid, suggesting a role of astrocytes in edema resolution. X 60.000

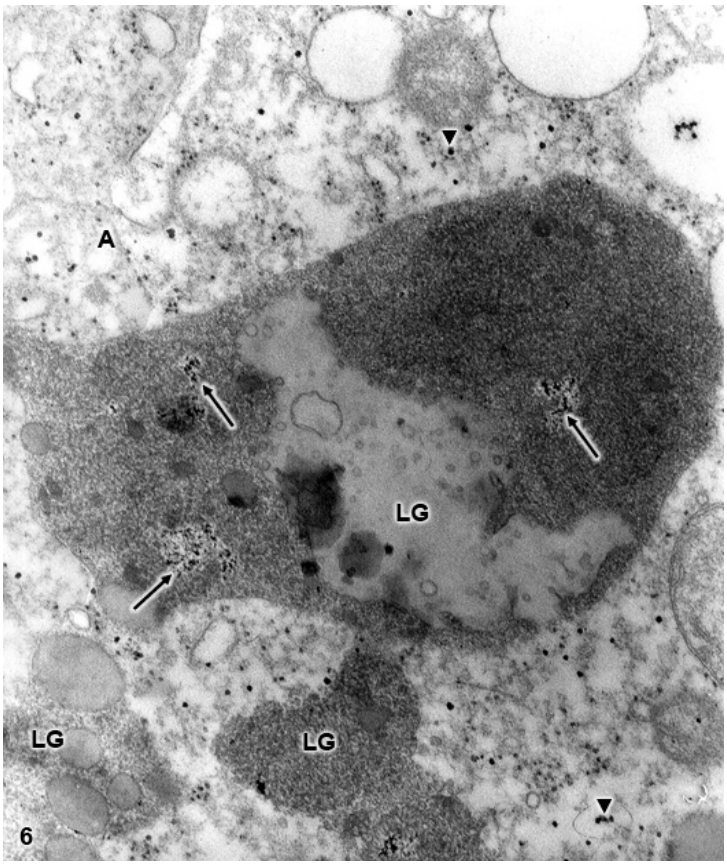

Fig. 6. Brain trauma. Right parietal cortex. Swollen glycogen-rich astrocytic cytoplasm (A) showing an aggregate of large and small lipofucsin granules (LG). Electron dense fine particles (white arrows) appear included within clear vacuoles, suggesting metal or metaloid content. Beta-type glycogen granules (arrowheads) are seen distributed throughout the astrocytic cytoplasm. X

In complicated head trauma and associated subdural hematoma, some swollen astrocytes exhibited at the cell body and perivascular end-feet an accumulation of large lipofucsin granules with vacuolated lipidic components, and clusters of dense microgranules (Fig. 7).

The brain traumatic injuries activated microglial and astrocyte cells, which displayed bizarres lysosomal shapes and phagocytosis of degenerated myelinated axons.

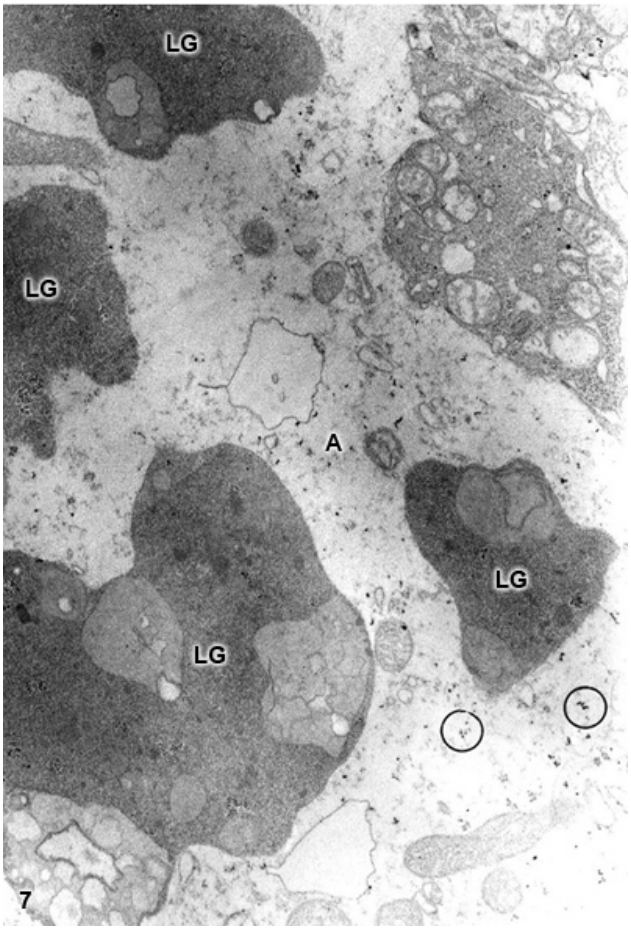

Fig.7. Brain trauma. Subdural hygroma. Right parietal cortex. Edematous glycogen-depleted astrocytic cytoplasm (A) exhibiting an accumulation of large lipofucsin granules (LG). Scarce amount of gamma particles of glycogen granules (circles) are seen. X 24.000

Conclusions

Lysosomes showed fragmentation of their limiting membrane in patients with congenital hydrocephalus, vascular anomalies, brain trauma and tumors. The neighboring nerve cell cytoplasm showed swollen mitochondria, areas of focal necrosis, and fragmentation of plasma membrane suggesting lysosomal activation and release of lysosomal enzymes. Abnormal deposit of lipofucsin granules were observed in most patients studied. The presence of lipofucsin granules in infant patients with congenital hydrocephalus suggests that lipofucsinogenesis is apparently a life span process. The serum proteins spreaded through the extracellular space in traumatic and peritumoral edema could be digested by astrocytic lysosomes contributing to edema resolution.

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