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Histology and Histopathology

Encephalopathy with astrocitic residual bodies. Report of a case and review of the literature

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Summary. Biopsy and autopsy findings in a girl who died at 7% months after having suffered from progressive axial hypotonia, myoclonus, EEG changes and retarded psychomotor development. Inclusions consisting of lamellar profiles, situated in membrane-bound cytosomes were found mainly in astrocytes, but also in neurones and in axons of peripheral nerves. Lipofuscin bodies were also increased in number.

The patient belongs in the same category as cases studied by Towfighi et al. (1975) and Martin et al. (1977). Etiology and pathogenesis of this syndrome remain unknown. It is suggested, however, that the pathological changes observed might have been caused by the administration soon after birth of anti-epileptic dmgs (diphenylhydantoin, clonazepam and nitrazepan).

Key words: Progressive axial hypotonia - Myoclonus - Astrocytic lamellar cytosomes - Antiepileptic drug treatment - Amphiphilic dmgs

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Introduction

Towfighi et al. (1975) described two siblings with mental retardation, hypotonia and generalized seizures. Diagnosis of encephalopathy with astrocytic "residual" bodies was established microscopically. Non-specific alterations in peripheral nerves were also present. Martin and collaborators later described in more detail another case with peripheral nerve pathology, leading us to suggest a pathogenesis of the lesions different from that suggested by the previous authors.

Materials and methods

Case *history*

The patient weighed 2980 g at birth to parents which were not consanguinous. No other members of the family were known to suffer from neurological or storage diseases. She was born by cesarean section after an unremarkable 7½ month pregnancy. At birth she had muscular hypotonia and diffuse erratic myoclonic seizures. The EEG showed alternating periods of marked suppression interrupted by bursts of high voltage and an asynchronous sharp activity including spikes and slow waves.

Further neurologic examinations (at 1 and 2 months of age) revealed a severe delay in psychomotor development, an axial hypotonus and peripheral hypertonus. The EEG pattern remained unchanged. The optic fundi appeared normal. A general physical examination revealed moderate hepatomegaly. Laboratory determinations were all normal except for a slight elevation in GOT and GPT. When the patient was 6% months old a muscle and nerve biopsy were taken and dermal fibroblasts were cultured.

One month later she developed bronchopneumonia and died. Treatment started soon after birth included sodium valproate, clonazepan, nitrazepan, phenobarbital, dexamethasone, ACTH and pyridoxin.

Light microscopy

The brain and viscera, as well as skin and lymph nodes, were fixed in formalin, processed for light microscopy and stained with HE, PAS, and Masson's trichrome. The brain sections were also embedded in celloidin and stained with the Klüver-Barrera, Holzer, and Nissl procedures. Frozen sections were stained with Ziehl-Nielsen and Oil-Red-O methods.

Histochemistry and Electron microscopy

One segment of the muscle biopsy was frozen, cut in a cryostat and stained with HE, PAS, Gomori's trichrome, myofibrillar ATPase, DPNH, SDH, acid phosphatase and phosphorilase. The other segment of muscle and nerve biopsies, as well as autopsy samples of cerebral cortex, basal ganglia, liver, spleen, lymph nodes and adrenal glands were fixed in glutaraldehyde for electron microscopy. Some sections of the cortex were stained with the periodic acid thiocarbohydrazide-silver proteinate procedure of Thiery (1967).

Results

Light microscopy

Lung: terminal bronchioli contained numerous polymorphonuclears and necrotic cellular debris. The interalveolar walls were thickened and infiltrated with mononuclear cells. The alveolar cavities contained edema fluid.

Liver: fibrosis with collagen bundles bridging portal spaces. Moderate proliferation of biliary ducts and Meyenburg's complexes were also noted.

In the *lymphatic system* there was a generalized hyperplasia. Kidneys and adrenals showed no pathology.

The fixed *brain,* 1300 g, had an irregular appearance with macro and microgyria. Some convolutions appeared to be cleaved by furrows into 2-3 longitudinal bands. In coronal sections we observed a symmetrical dilatation of the ventricular system. The consistency of the white matter was slightly increased. Histologically we found scattered clusters of macrophages and astrocyte-like cells (Fig. 1), the cytoplasm of which stained with Oil-Red-O and PAS and was not acid fast in the Ziehl-Nielsen procedure. These nodules or clusters were mostly found in the gray matter. Topographically they were mostly concentrated in the caudate nucleus, putamen and periventricular areas. They were less abundant in the thalamus, Ammon's horn, subthalamic nuclei and hypothalamus and were scarce in the cortex, corpus callosum and cerebellum. There was no obvious decrease in the number of cortical and basal ganglia neurons. No changes were noted in the brain stem and spinal cord. Some neurons exhibited marked accumulation of lipofuscin.

Electron microscopy

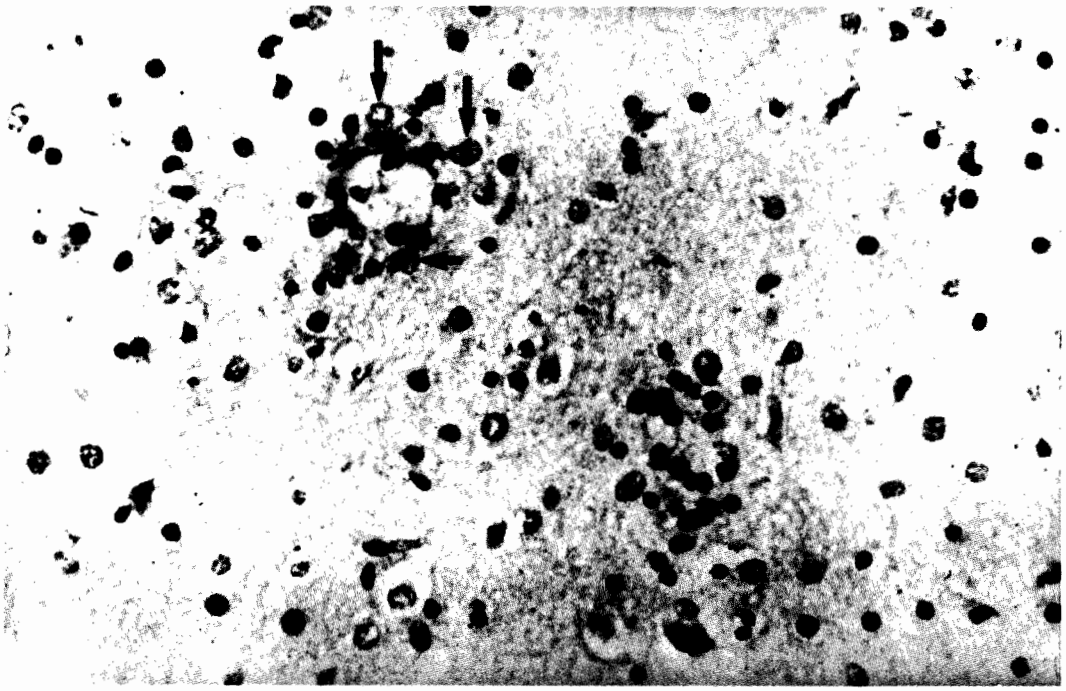
Astrocytes in the basal ganglia, cortex and white matter were swollen and contained different types of osmiophilic inclusions which were also located in their processes. Membrane-bound cytosomes were the most frequently found inclusions (Fig. 2). Their diameter varied between 2 and 5 micrometers; they were irrregular and filled with straight, or slightly curved, lamellar profiles which were composed of two parallel electron dense zones separated by an electron lucid zone. These zones measured 4, 3, 4 nm respectively. The lamellar profiles were embedded in a finely granular matrix. Other cytosomes contained, in addition to the lamellar profiles, multilamellar curvilinear inclusions, fat droplets and polygonal osmiophilic inclusions which had the structure of lipofuscin. We also found empty vesicular structures and structures containing granular or flocculent contents. These dilated structures had ribosomes on the external surface of their membrane and obviously belonged to the rough endoplasmic reticulum. Cytosomes, particularly those rich in lamellar profiles, stained positively with the Thiery technique, exhibiting granular silver salt deposits on the profiles. In many astrocytes, balloon-like dilatations of the outer nuclear membrane were observed; they were connected with the perinuclear space by a narrow bridge. Cytosomes were seldom observed in neurons, but lipofuscin accumulation were frequent.

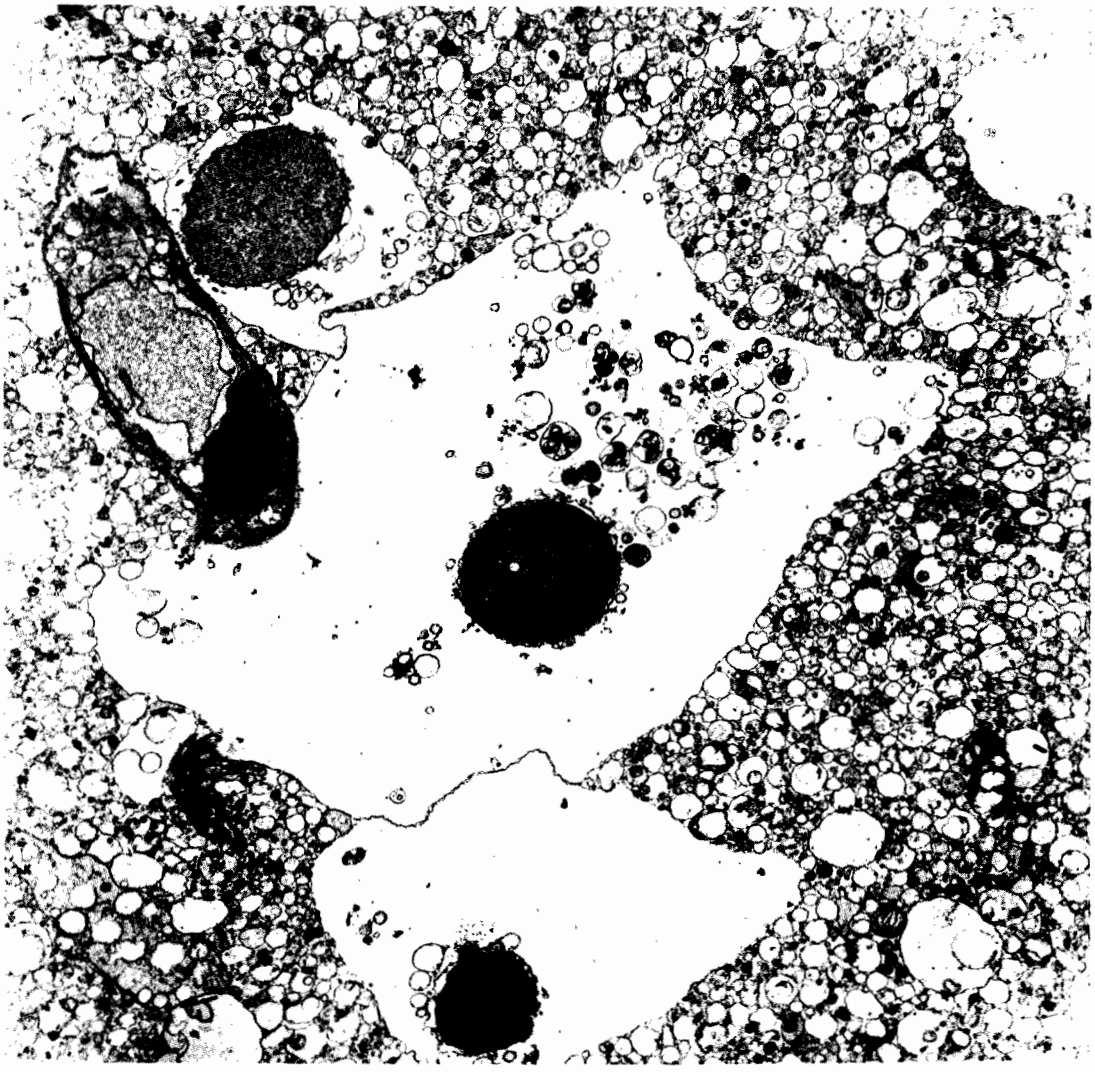
In the peripheral nerves we found lesions in Schwann cells and in the myelin. Schwann cells were swollen and occupied by membrane profiles and polygonal or concentric bodies formed by densely-packed membranes which had a myelin-like appearance.

Myelin sheaths were separated from each other and there was vacuolar degeneration, most obvious in the vicinity of the axon. Dense packing of neurofilaments gave increased electron density to the axon. There were also many organelles located in the center of the axon, such as mitochondria with disrupted crests and numerous concentric bodies similar to those seen in the CNS astrocytes were also noted. The myelin alterations observed in the peripheral nerve were not seen in myelinated fibers of the CNS. Skin, muscle and viscera showed no remarkable features. Fibroblast culture revealed no abnormalities.

Fig. 1. Putamen showing clusters of cells consisting of astrocytes (long arrows) and macrophages (short arrows). H.E. ×300

Fig. 2. Three swollen astrocytes with "empty" cytoplasm. Numerous cytosomes containing irregular lamellated structures. ×2,600





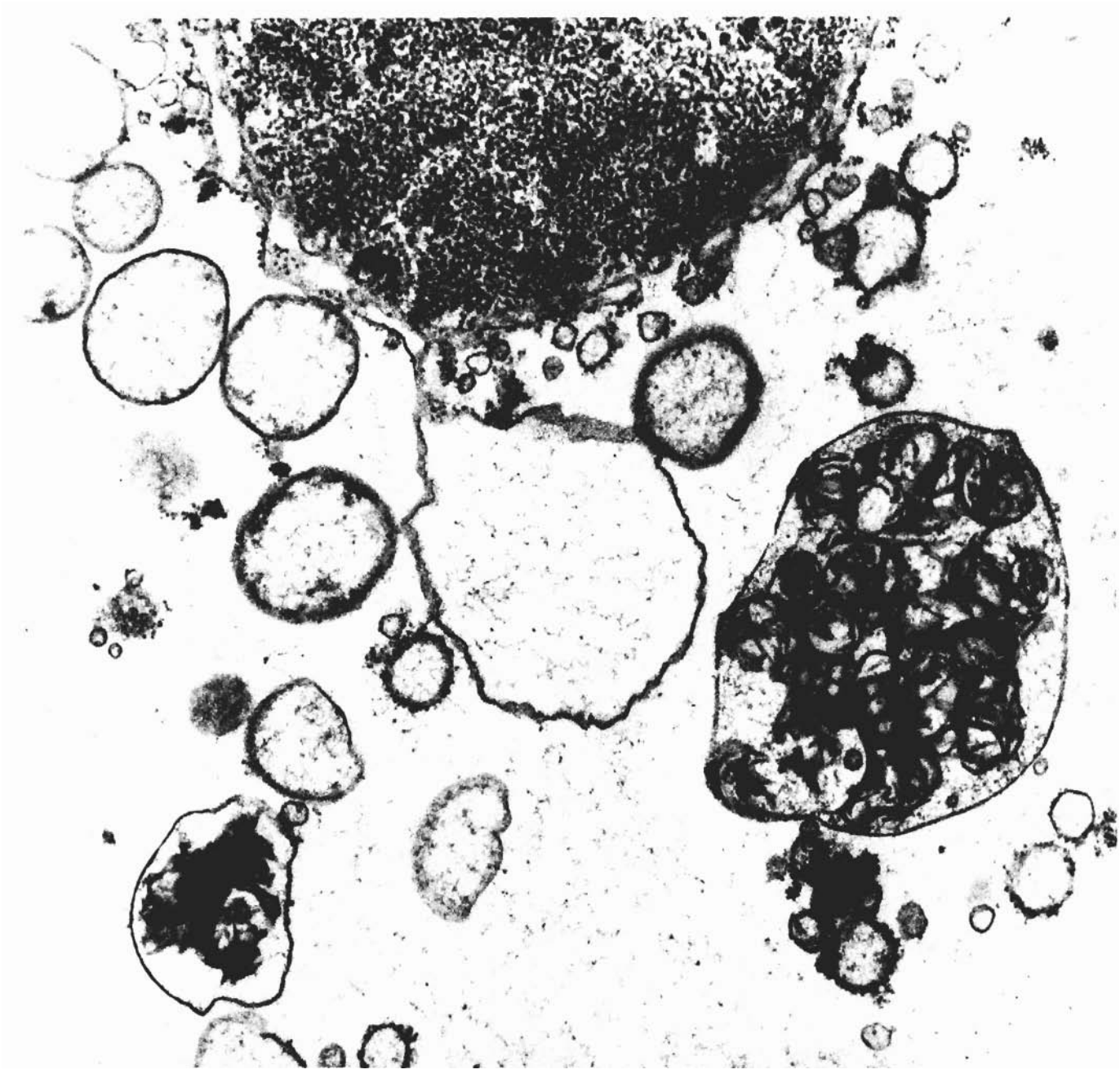


Fig. 3. Swollen astrocytic cytoplasm with cytosomes, two of which contain straight or curved lamellar profiles. x 16,200

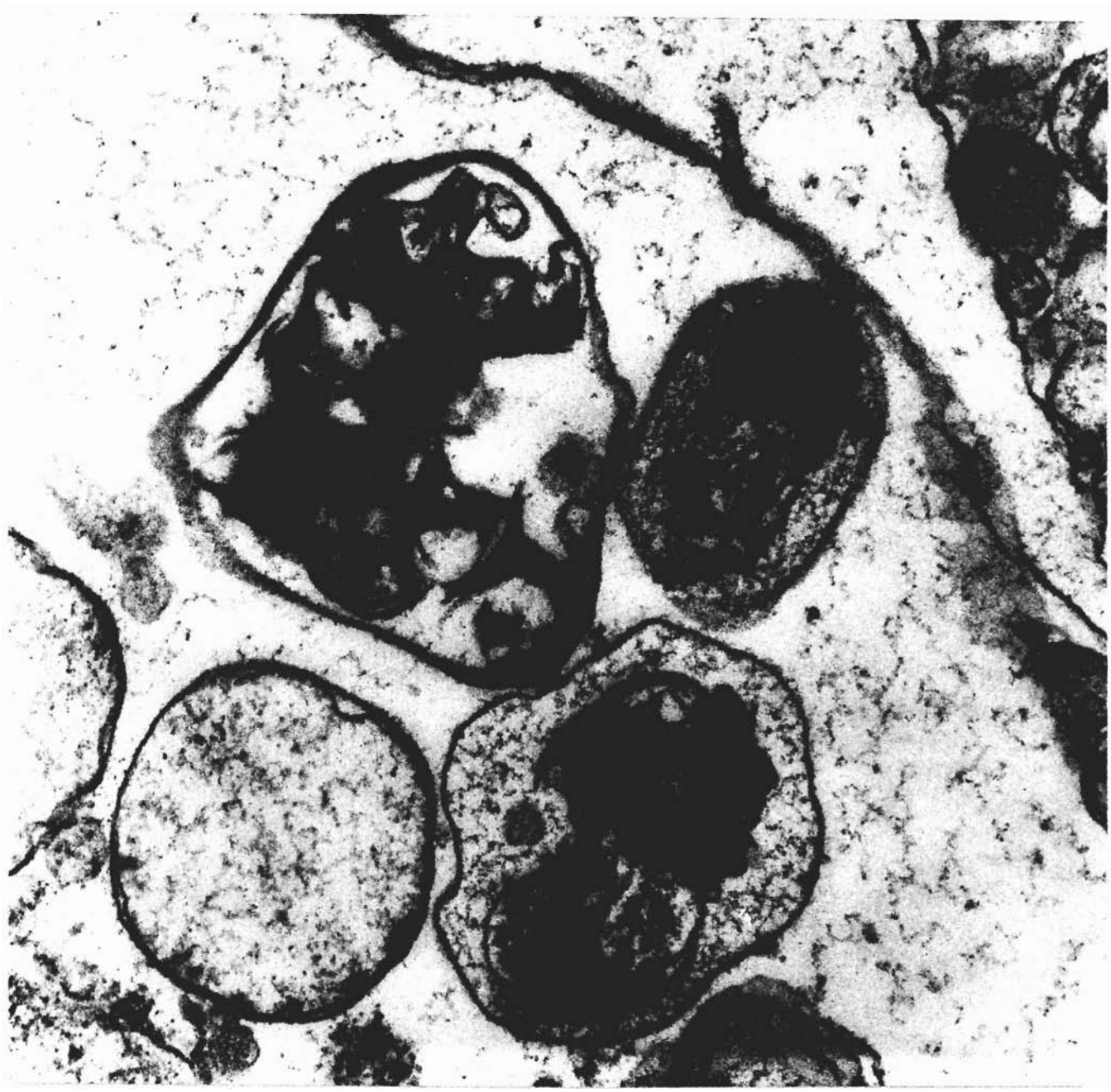


Fig. 4. Higher magnification of astrocytic cytosomes. Cytosome on the upper left contains curved profiles; the two other cytosomes contain straight and curved lamellae and ring-like structures. x 31,700

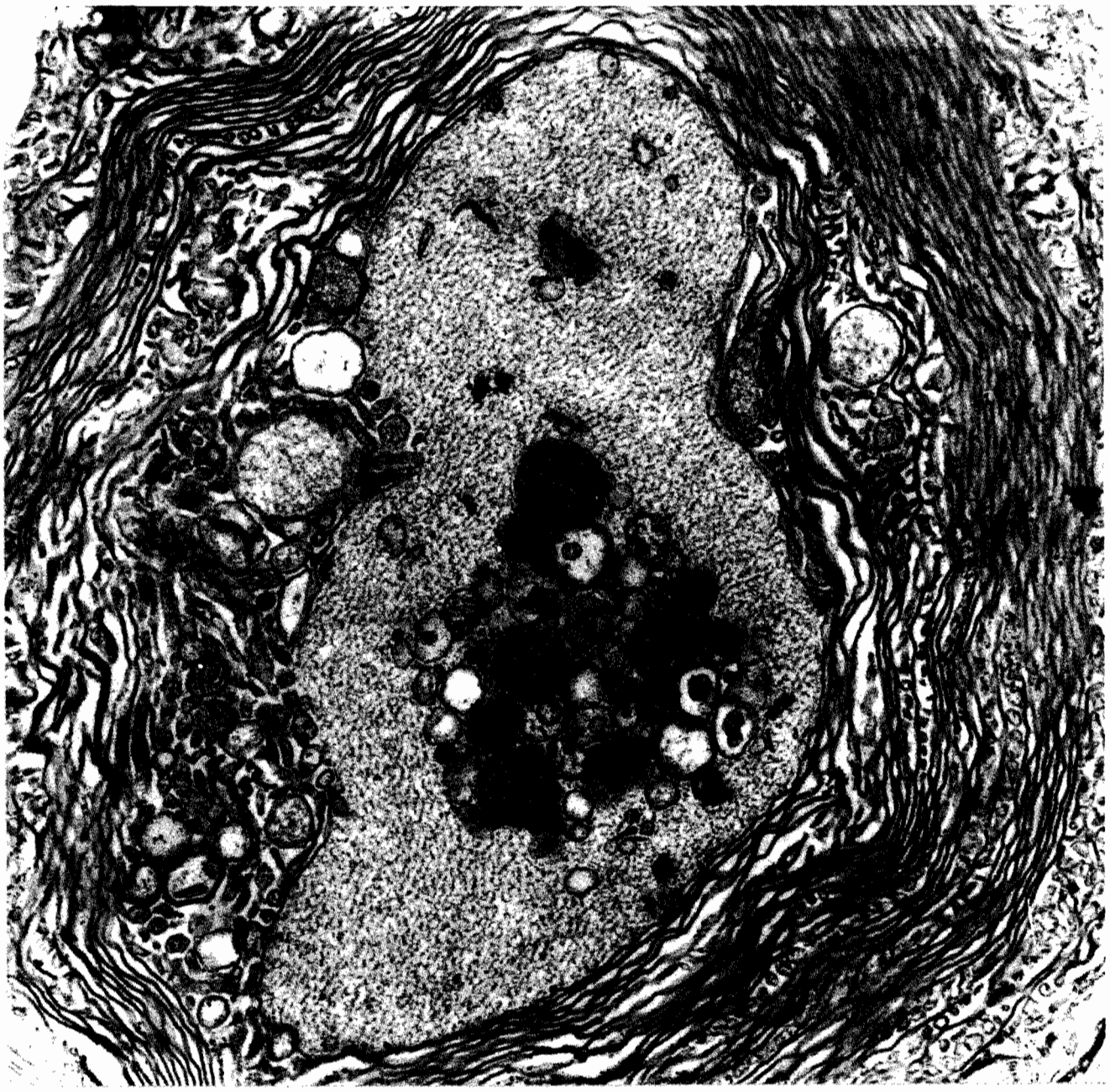


Fig. 5. Sural nerve axon containing numerous centrally located mitochondria and a few vesicular bodies. Myelin lamellae widely separated with multiple vacuoles. × 16,200

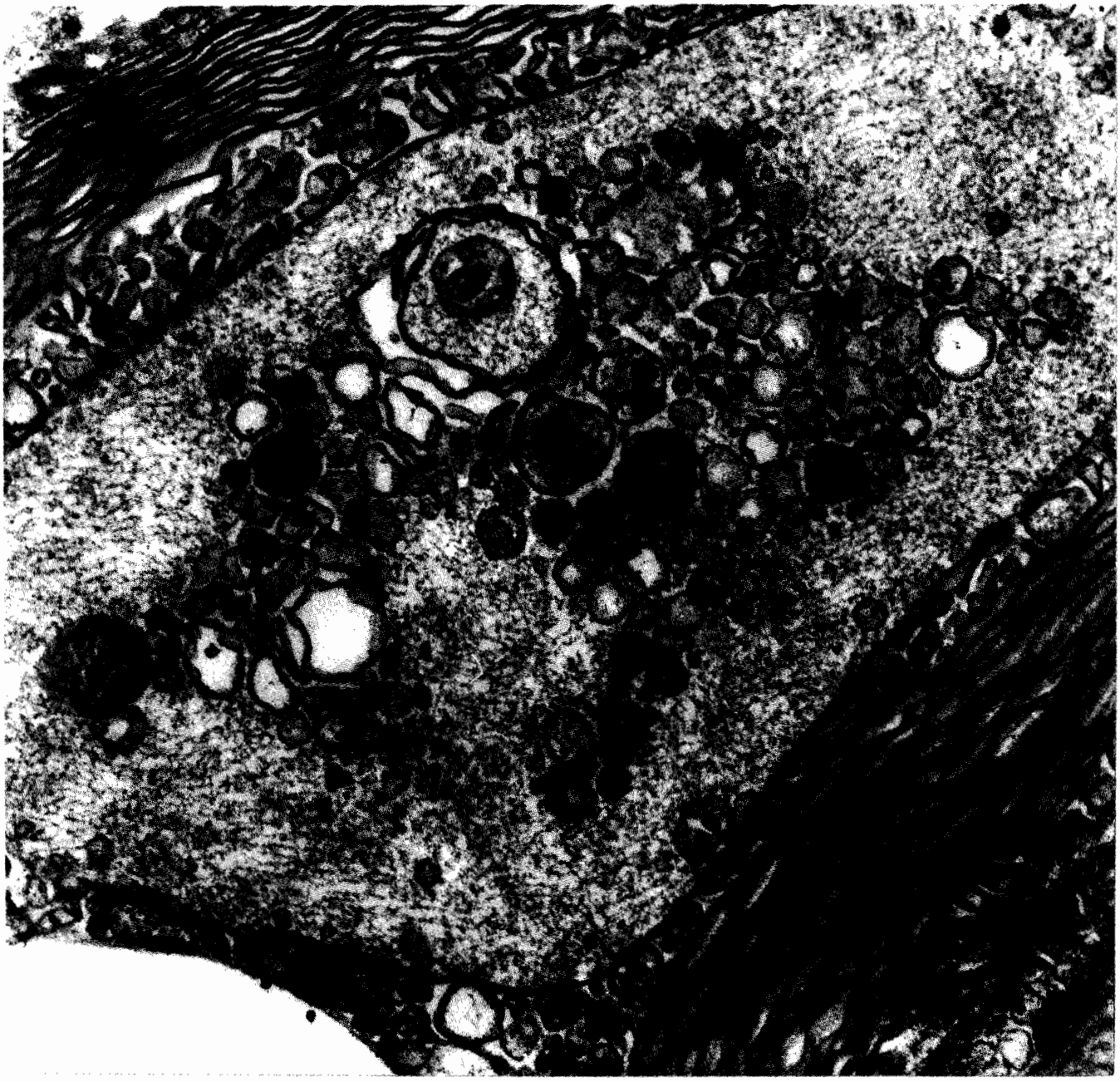


Fig. 6. Sural nerve axon with lamellar cytosomes and numerous vesicular bodies. Disorganization of myelin. × 25,200

Microscopical, electron-microscopical and histochemical study of other organs, including liver (excepting the above-described lesions) and muscle, were not contributory.

Discussion

The clinical and morphological findings indicate that this case belongs in the same nosological category as the cases of Towfighi et al. (1975) and of Martin et al. (1977). In all these cases convulsions, hypotonia and mental deficits were present since birth and lamellar residual bodies and lipid pigment granules were observed in astrocytes, macrophages and, to a lesser extent, in neurons. Changes were also noted in axons and in Schwann cells. Both groups of authors compared the cases to primary lipid storage diseases.

Lipidoses described until now mainly in humans are primary diseases caused by a genetically determined deficit in a specific enzyme activity. Deposition of lipids in cells may also be caused by acquired inactivation of an enzyme or inhibition of an enzyme activity. Such secondary lipidoses and cytosomal deposits of lamellated crystalloid bodies probably derived from damaged membrane material have been studied and described in humans and animals treated with a variety of drugs (for a review, see Lüllmann et al., 1978).

While the clinical findings in this and the two preceding reports might correspond to a well-defined primary disease of the nervous system, which might possibly be genetically determined, no definite evidence can be adduced with regard to the pathogenesis of the observed pathological changes. The early occurrence of clinical symptoms and the fact that the first report (Towfighi et al., 1975) deals with two sibs render the possibility of a genetic disease plausible. It seems probable, however, that most or all the pathological changes in the two preceding reports and in our case were due to the treatment, and the early age at which treatment was started might have played a role in the occurrence and distribution of the lesions. Our reasons for assuming that the pathological changes are related to treatment and not to the clinical syndrome are as follows.

1.— The clinical and pathological findings do not fit any of the known primary storage diseases. In fact, the diagnoses of lipofuscinosis of the Hagberg-Santavuori type (Hagberg et al., 1968; Santavuori et al., 1973), of Krabbe's disease and of adrenoleukodystrophy can be easily discarded on clinical and pathological grounds.

2.— According to Lüllmann et al. (1978), secondary lipidosis and formation of lamellated bodies occurs with amphiphilic cationic drugs. These have special affinity for lysosomes where they become trapped and may exert their inhibitory function on acid hydrolases by binding to polar lipids with resultant increase of the intralysosomal pH and stabilization of the structure of polar lipids so that they cannot be easily hydrolyzed. It has, in fact, been shown by Homewood et al. (1972) that chloroquine (a known producer of lamellated bodies in many organs) inhibits various lysosomal hydrolases and by Stern et al. (1983) that chlorocyclizine (another producer of secondary lipidosis) inhibits the activities of acid esterase and acid phospholipase.

Of the drugs used in the treatment of the infants in the three studies diphenylhydantoin, clonazepam and nitrazepam are likely candidates for producing multilamellar bodies. They are all amphiphilic as they contain both hydrophobic and polar groups but they are not cationic (for which reason Lüllmann-Rauch in a personal communication does not consider them as members of the class of drugs studied by her). Valproic acid obviously does not qualify as it is not amphiphilic (Eadie and Tyrer, 1980).

3.— In primary, genetically determined lipidoses the enzymatic defect is ubiquitous. Storage occurs in cells which are most active in the involved metabolic path and wherever no alternate pathway for metabolic disposal of the involved product exists. In lipidoses caused by external causes the enzymatic defect is present only in cells which have been reached and damaged by the drugs. Thus, drugs administered *per os* are likely to cause more changes in the liver than elsewhere, and drugs which do not pass the blood brain barrier will not produce lamellated bodies in the brain. The peculiar localization of the lesions in the three former cases under discussion might correlate better with a drug-induced than with a genetically determined lesion. In fact, the drugs used in these cases were all neurotropic, were administered to the infants from earliest infancy and after crossing the incompletely effective blood brain barrier were likely to be deposited in cells of the nervous system. With regard to the liver of our patient, in spite of her congenital fibrosis, no lamellar bodies were found at the ultrastructural level. For this reason no relation between this feature and the CNS lesions could be established.

4.— In different classes of primary storage diseases (lipidoses, glycogenoses, mucopolysaccharidoses) in which storage occurs in the nervous system, neurons are the cells which are mainly affected. Involvement of glial cells is often secondary and mostly less pronounced. In some of the secondary changes due to drugs, the nervous system is involved. Lamellated cytosomes were produced in nervous system cultures and in whole animals treated with chloroquine (Tischner, 1974, 1975), in peripheral nerve axons and Schwann cells with chlorphentermine and iprindole (Drenckhahn and Lüllmann-Rauch, 1976). The lamellated cytosomes were similar to those described in the present article. A peculiarity of our case and of the others, all treated with antiepileptic drugs from birth, was that the cytosomes occurred mostly in astrocytes. This preferential localization might have been due to the nature of the drugs, to the early age at which treatment was commenced, or to both.

It may be concluded that it is likely that the disease described by Towfighi et al., by Martin et al., and ourselves is a disease of unknown etiology and with no hitherto described morphological lesions. The changes observed in these cases in the nervous system are probably iatrogenic.

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