

# Progressive cerebral poliodystrophy – Alpers' disease

## Disorganized giant neuronal mitochondria on electron microscopy

U. SANDBANK AND P. LERMAN

*From the J. Casper Institute of Pathology, Laboratory of Neuropathology,  
and the EEG Laboratory and Department of Pediatrics, Beilinson Hospital  
and the Tel Aviv University Medical School, Tel Aviv, Israel*

**SUMMARY** Three siblings who suffered from progressive mental retardation, seizures, and rigidity showed degeneration of the cerebral cortex. This was manifested by severe to complete neuronal loss with astrogliosis and microgliosis. In one child a brain biopsy was performed at the age of 3 months. The only lesion found was large disorganized perinuclear mitochondria in the neurones. The possibility that the cerebral poliodystrophy is due to an inherited mitochondrial disorder is discussed.

Since the description by Alpers (1931) of a case of 'diffuse progressive degeneration of the gray matter of the cerebrum', a few similar cases have been reported (Christensen and Krabbe, 1949; Ford, Livingston, and Pryles, 1951; Norman, 1958; Alpers, 1960; Blackwood, Buxton, Cummings, Robertson and Tucker, 1963; Greenhouse and Neubuerger, 1964; Laurence and Cavanagh, 1968). The nosology of this entity remains, however, unsolved. Whereas some authors regard this entity as a heredodegenerative disease (Alpers, 1931, 1960; Christensen, 1949; Ford, 1951), others attribute the lesions to birth anoxia and post-epileptic encephalopathy (Norman, 1958) or an inflammatory process (Dreifuss and Netsky, 1964).

The present study is the report of a family with three affected siblings, in one of which a brain biopsy was taken and studied by electron microscopy.

### CASE REPORT

The patient was a 3 month old baby girl. Her mother was 26 years of age and the father was 29 years of age. Both parents were in good health, of Iraqi origin, with no consanguinity. There were no known neurological disorders in the family. The mother had five pregnancies:

First pregnancy: abortion in second month.

Second pregnancy: premature delivery of 600 g stillborn infant.

Third pregnancy: male infant, 2,500 g. At the age

of 1 month he was hospitalized because of bronchopneumonia. During the hospitalization the infant became progressively spastic and demented. He died at the age of 6 months from pulmonary infection.

Fourth pregnancy: male infant, 3,000 g. Seizures developed at the age of 1 month, with progressive

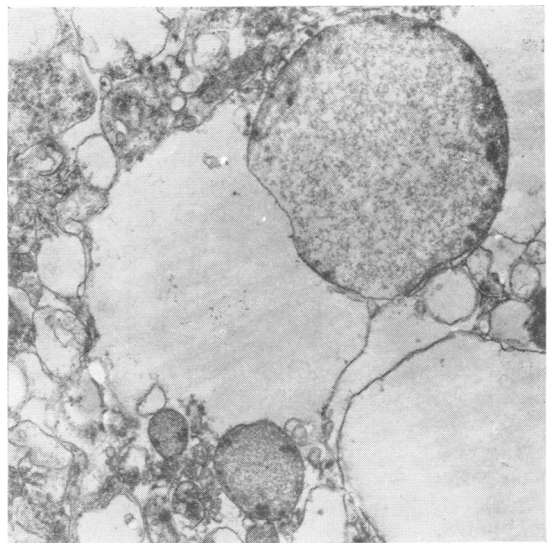


FIG. 1. Three mitochondria showing different sizes: a normal one at the lower left margin, a giant one at the right.  $\times 9,000$ .

spasticity. He died from bronchopneumonia at the age of 18 months in a decerebrate state.

Fifth pregnancy: female, birth weight 3,200 g. She developed normally up to the age of 1 month, when seizures and spasticity appeared. She was hospitalized in another hospital because of respiratory infection. At the age of 3 months she was transferred to the department of pediatrics of this hospital for further investigation.

She was a well-nourished 3 month old infant with no apparent congenital defects. Head circumference was 37.3 cm compared with chest circumference of 40.5 cm. The anterior fontanelle was flat, 3 × 3 cm. There were no pathological findings except in the nervous system. Muscular tonus was increased with brisk deep tendon reflexes. She reacted to sounds and followed objects. The fundus was normal.

The infant had repetitive seizures which gradually increased in frequency during her hospitalization. These consisted of eye-blinking, jerky movements of the eyeballs, and Moro-like extension of the upper extremities accompanied by crying. Because of the seizures there were feeding problems necessitating tube-feeding. The following drugs had hardly any effect: diazepam, nitrazepam, chloral hydrate, phenytoin. A trial with steroids was also ineffective. Muscular hypertension gradually increased. The upper extremities were kept in flexion, with clenched fists, the lower extremities being hyperextended. Gradually the tendon reflexes became clonic and the minor seizures became continuous. In spite of the feeding difficulties, the infant gained weight and at the age of 6 months she weighed 8 kg.

Radiographs of the skull and of the rest of the skeleton showed no pathological findings. An EEG (induced sleep tracing) showed excessive fast activity admixed with slow rhythms. In the posterior regions spike-like sharp waves were seen synchronously. Biopsy of the rectum showed no metachromatic material or any other abnormality. Blood count, serum proteins, urea, sodium chloride, calcium, were all normal in repeated tests, as were serum cholesterol and alkaline phosphatase. Serum transaminase was 81 units. LDH—iso-enzymes 2,3,4 were increased. Chromatography of amino acids in serum and urine was normal. PBI was 5.9  $\gamma\%$ ; Sabin-Feldman test was negative. Serum lactic acid was 40 mg/100 ml. and pyruvic acid 0.9 mg/100 ml.

In view of the family history and the progressive neurological condition, a brain biopsy was obtained. The child died of bronchopneumonia at the age of 15 months.

**PATHOLOGICAL EXAMINATION OF THE BIOPSY** On light microscopy, a 6 × 7 mm piece of cortex showed normal architectonic arrangement of the neurones

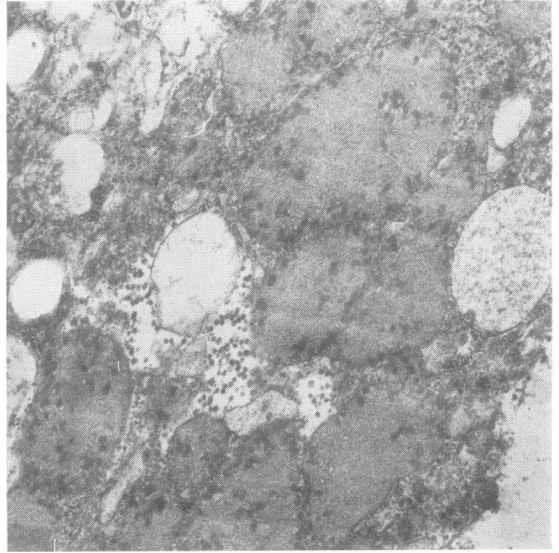


FIG. 2. A group of altered mitochondria clumped together.  $\times 9,000$ .

with haematoxylin and eosin and Nissl stain. The neurones appeared to be of normal shape and size. No signs of satellitosis, neuronophagia, or gliosis were noted. The biopsy was interpreted as normal cortex.

In the operation room, the specimen was fixed for two hours in chilled 2% glutaraldehyde in phosphate

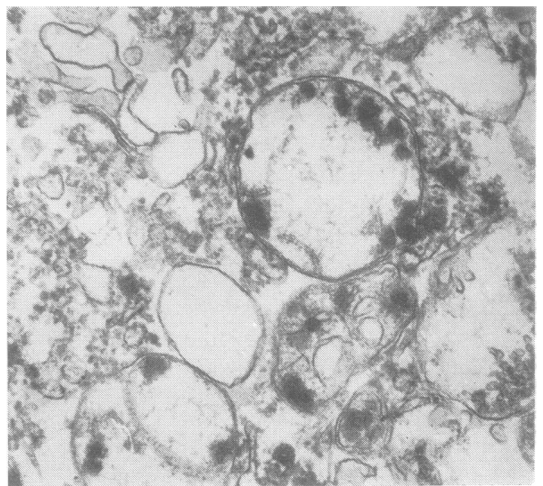


FIG. 3. Mitochondria with a few short curved cristae and a few electron-dense irregular particles.  $\times 11,000$ .

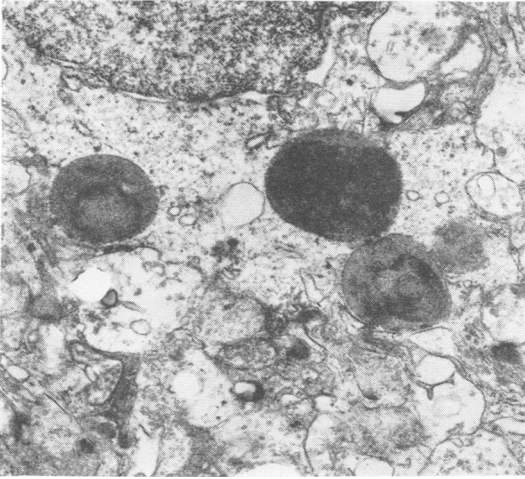


FIG. 4. Membrane bound dense bodies with concentric lamellar areas.  $\times 5,500$ .

buffer, then rinsed in phosphate buffer with 10% sucrose and post-fixed in 2% osmic acid. The tissue pieces were embedded in Epon 812. Thin sections were cut and stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope. On low magnification, the impression gained was that of normal cortex. Neurones, neuropil, and glia cells appeared to be normal. At higher magnifications, however, neuronal mitochondria were seen to be altered. These altered mitochondria

were located mainly in the neuronal perikarya but they were also found in dendrites and in a few axons. They were not found in glial or endothelial cells.

Two types of changes were observed. In the first type, some of the mitochondria were large, with the cristae being very short or entirely absent, although both outer and inner membranes were preserved. The mitochondrial matrix was composed of a granular material with a few irregular electron-dense bodies. There was a marked difference in the diameter of these mitochondria—while some appeared to be of normal size, others were gigantic, measuring up to  $3 \mu$  in diameter (Fig. 1). In a few areas these altered mitochondria were clumped into large groups (Fig. 2). In the second type, mitochondria were normal in size with short or completely absent cristae and with irregular electron-dense particles within the matrix adhering to the inner membrane or to the cristae (Fig. 3). In some mitochondria these particles were numerous and nearly filled the mitochondria. A number of these mitochondria showed various stages of disintegration. Membrane-bound dense bodies containing parallel membrane profiles (fingerprint-like in appearance) were seen and it was suggested that they represented endophagocytosis of mitochondria (Fig. 4). The number of dense bodies within the neuronal perikarya seemed to be high for the patient's age. In many astrocytes an accumulation of glycogen particles was noted. Round, membrane-bound dense bodies were seen within some endothelial cells.

POST-MORTEM EXAMINATION Bilateral confluent bronchopneumonia was the cause of death. No

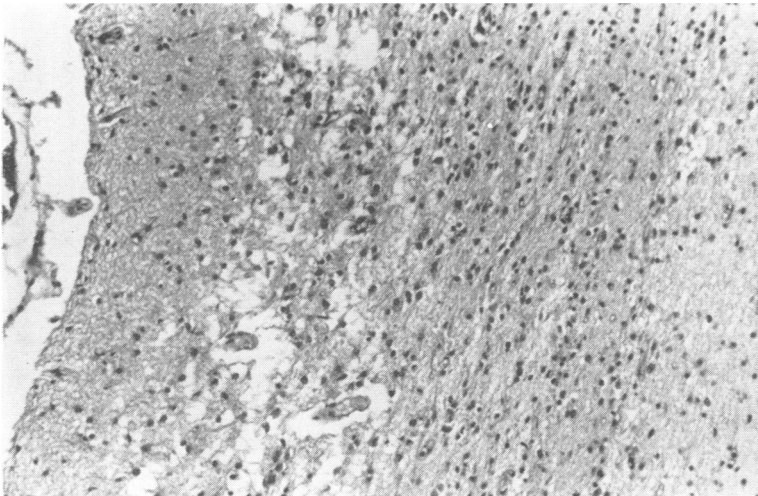


FIG. 5. Disorganized cortex, loss of neurones in layers 3-5, astrocytosis. H and E.  $\times 35$ .

other significant pathological changes were observed in any organs.

The brain was small, weighing 480 g. The cerebral hemispheres were symmetrical. The gyri were thin and the sulci widened. The leptomeninges were oedematous and excessive CSF covered the brain. The brain-stem and cerebellum were proportionally small when compared with the cerebral hemispheres. The whole brain had a firm consistency. On coronal sections the cortex was thin in all lobes. The lateral and third ventricles were extensively widened, with a

smooth ependymal lining. The white matter of the centrum semiovale was symmetrically narrowed but was of a normal white appearance. On both sides the basal ganglia were small and symmetrical.

Sections for microscopic examination were taken from the cerebral cortex of both sides and from basal ganglia, brain-stem, cerebellum, pons, medulla, and spinal cord. Paraffin-embedded sections were stained with haematoxylin-eosin, Nissl, PAS, and Luxol fast blue. Frozen sections were stained with Sudan III and Cajal gold chloride.

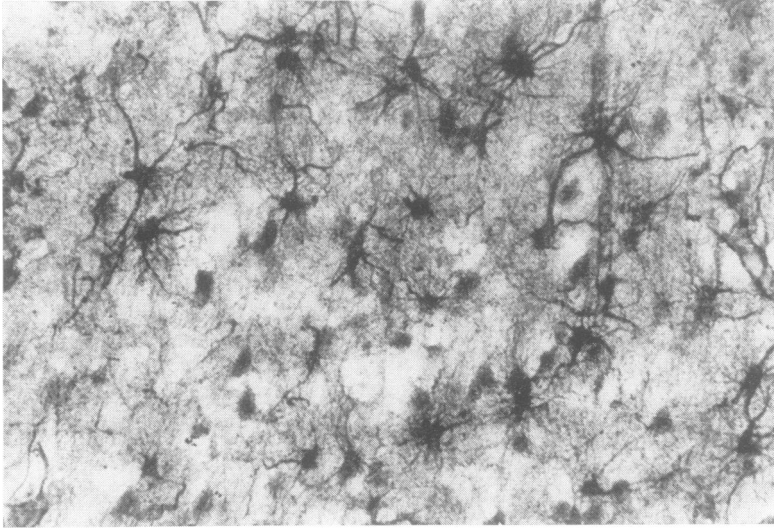


FIG. 6. *Cajal stain showing severe astrogliosis of cerebral cortex. Cajal gold chloride.  $\times 160$ .*

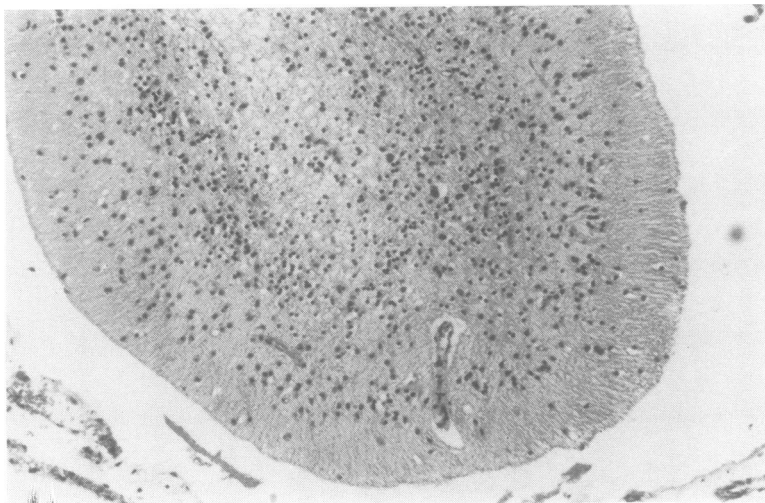


FIG. 7. *Cerebellar cortex, loss of Purkinje cells, and granular layer, gliosis of affected area. H and E.  $\times 80$ .*

The cerebral cortex was completely disorganized. This finding was bilateral and in all cerebral lobes. Layers 3–5 were the most severely affected, while layers 1 and 6 were less affected. The lesions consisted of two major findings—an absence of neurones and massive astroglial and microglial proliferation. No signs of neuronal necrosis, satellitosis, neuronophagia, or microglial nodes were found (Fig. 5). The massive astrogliosis was evident with Cajal stain for astrocytes (Fig. 6). In the hippocampus the dentate fascia and ganglionic layer showed no alterations. The thalamus and subthalamic nuclei showed extensive astrogliosis, but numerous large and small neurones of normal appearance were found. The cerebellar cortex showed a loss of Purkinje cells and a marked diminution of the granular layer cells (Fig. 7). There was hyperplasia of the Bergman glia. Luxol fast blue stain showed normally preserved myelin. A few perivascular lipid-laden macrophages were seen. No gliosis of the white matter was noted. The brain-stem, pons, medulla, and spinal cord appeared normal. The myelination of the tracts was normal.

Necropsies had also been performed in the third and fourth children. Unfortunately, only one section was available from the cerebral cortex of each child. The findings consisted of the same cortical lesions as found in the above-described case.

#### DISCUSSION

The essential problem in the present case was whether the changes in the mitochondria were the primary cause of the lesions or whether these changes were secondary to another noxious agent. Altered mitochondria have been reported in spongy degeneration of the brain (Adachi, Wallace, Schneck, and Volk, 1966; Gambetti, Mellman, and Gonatas, 1969); however, they were found not in the neurones but in astrocytes and their morphology differed from that seen in our case.

Suzuki and Rapin (1969) recently reported the case of a 3½ year old child who suffered from seizures, lethargy, spasticity, and microcephaly, the symptoms of which had started 10 weeks after birth. A left frontal biopsy was taken at the age of 3 months; light microscopy showed a normal cortex, while electron microscopic examination showed unusual mitochondria in most of the neuronal perikarya. The mitochondria were increased in diameter, measuring between 1.5–3.5  $\mu$  and in some cases as much as 8  $\mu$ . The cristae were deformed. On post mortem examination the brain was found to be small, with the cortex showing a band of hypocellular-

ity corresponding to layers 3–4; fibrous astrocytosis was prominent. Myelination was poor but no sudanophilic myelin breakdown material was found. In Ammon's horn, Sommer's sector was normal. Only slight changes were found in the cerebellar cortex. This case bears a few similarities to our case. In both, light microscopy of the biopsy material showed a normal cortex, while post mortem examination revealed severe cortical changes. In both, there were enlarged neuronal mitochondria and Sommer's sector was normal. In our case, however, the cerebellum was severely affected, while in Suzuki's case it was nearly unaffected. Poor myelination was found in both cases, but whereas in Suzuki's case no free fat was found, in our case perivascular macrophages contained sudanophilic droplets. Suzuki and Rapin interpreted these findings as 'arrested' or 'retarded' myelination, rather than demyelination.

Altered mitochondria have, however, been reported in various other conditions. Chronic anoxia and repeated seizures may account for enlarged neuronal mitochondria. Hager, Hirschberger, and Scholz (1960) reported the presence of enlarged neuronal mitochondria after anoxia. Webster and Ames (1965) observed changes in the size and shape of the mitochondria in the rod and bipolar cells in isolated rabbit retinae kept in an oxygen deprived media. Mitochondrial hypertrophy in neurones was also observed in association with chromatolysis due to an axonal lesion (Smith, 1961; Lentz, 1967; Mackey, Spiro, and Weiner, 1967). Lentz (1967) reported finding giant mitochondria (diameters up to 10 times the normal size) with dense granules within the mitochondrial matrix in chromatolytic neurones. Enlarged mitochondria with dense matrix have been found after x-ray irradiation of the brain (Roizin, 1964; Zeman, 1968). On the basis of these findings, we may assume that 'giant mitochondria' are a reactive form resulting from contact with various noxious agents.

Myopathies with abnormal mitochondria have recently been reported (Shy, Gonatas, and Perez, 1966). Numerous enlarged mitochondria described as megaconial and pleoconial were demonstrated on electron microscopic study of muscle. While the first such cases were sporadic, recent studies also reported familial cases (D'Agostino, Ziter, Rallison, and Bray, 1968).

Reviewing the above-mentioned communications, the impression is gained that mitochondrial changes are either reactive or a result of a degenerative familial disorder.

In the family that we studied, the preservation of Ammon's horn neurones, the history of a normal birth in all three children, the fact that all of them had a low birth weight, and the delay in the appearance of the symptoms, argue against a hypoxic origin of the lesions. The appearance of the disease in three consecutive children and the history of a previous abortion and stillbirth may be considered to be in favour of a familial disorder.

Since the clinical and pathological findings were similar to those reported by Alpers (1960), the question arose as to whether or not this family could be classified as suffering from Alpers' disease. There is still some confusion as to the nosological position of the latter disease. Recently Jellinger and Seitelberger (1970) classified the rather ill-defined group of disorders of 'progressive degeneration of the cerebral cortex', or Alpers' disease, into three groups:

*Group 1* The symptomatic form in which evidence points to a causal factor, with a history of complicated birth (Greenhouse and Neuburger, 1964) or seizures and infections (Weffring and Lamvik, 1967) preceding the nervous system lesions.

*Group 2* The idiopathic type. Jellinger and Seitelberger (1970) reviewed 30 cases in which clinicopathological data failed to disclose any evidence of a noxious factor, while a familial occurrence was found in 23 patients. The finding in these cases was of a progressive disorder not initiated by any previous brain damage. Our case, as well as that of Suzuki and Rapin (1969), may fall into this subgroup of idiopathic poliodystrophy. *Group 3* was classified as atypical unclassified and consisted of the border-line cases between poliodystrophy and spongy degeneration and the Jakob-Creutzfeldt disease (Morse, 1949; Sandbank and Chemke, 1965).

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