

# Clinical, biochemical, neuropathological and molecular findings of the first Polish case of adenylosuccinase deficiency

Hanna Mierzewska<sup>1</sup>, Bogna Schmidt-Sidor<sup>2</sup>, Eliza Lewandowska<sup>2</sup>, Wiesława Grajkowska<sup>3</sup>,  
Katarzyna Kuśmierska<sup>4</sup>, Elżbieta Jurkiewicz<sup>5</sup>, Tomasz Stępień<sup>2</sup>, Janina Rafałowska<sup>6</sup>

<sup>1</sup>Department of Metabolic Diseases, The Children's Memorial Health Institute, and Department of Neurology, Institute of Mother and Child, <sup>2</sup>Department of Neuropathology, Institute of Psychiatry and Neurology, <sup>3</sup>Department of Pathology, <sup>4</sup>Laboratory of Metabolic Diseases, <sup>5</sup>Magnetic Resonance Unit, The Children's Memorial Health Institute, <sup>6</sup>Department of Neuropathology, Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Warsaw, Poland

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## Abstract

*Adenylosuccinase (ADSL) deficiency is an autosomal recessive disorder affecting mainly the nervous system. The disease causes psychomotor retardation, frequently with autistic features and epilepsy. ADSL deficiency may be diagnosed by detection of two abnormal metabolites in body fluids – succinyladenosine (S-Ado) and succinylaminoimidazole carboxamide riboside (SAICAr). It is assumed that the former metabolite is neurotoxic. We present clinical, biochemical and neuropathological findings of a child affected by a severe form of ADSL deficiency. She had progressive neurological symptoms that started immediately after birth and died at 2.5 months of age. Macroscopically the brain showed signs of moderate atrophy. Histological examination of all grey matter structures showed widespread damage of neurons accompanied by microspangiosis of neuropile. Cerebral white matter showed lack of myelination in the centrum semiovale and diffuse spongiosis of neuropile. Myelination appropriate for the age was visible in posterior limb of internal capsule, in striatum, thalamus and in brain stem structures but diffuse destruction of myelin sheets was seen with severe marked astroglial reaction with signs of destruction of the cells and their processes. Ultrastructural examination showed enormous destruction of all cellular elements, but astonishingly mitochondria were relatively spared. The neuropathological changes can be considered as the neurotoxic result of metabolic disturbances connected with adenylosuccinase deficiency.*

**Key words:** adenylosuccinase deficiency, microcystic encephalopathy, hypo/dysmyelination myelin destruction, newborn, neuropathology.

## Introduction

Adenylosuccinase (ADSL) deficiency is a rare autosomal recessive disorder in purine biosynthesis

affecting mainly the nervous system [8]. The disease was first described in 1984 and is caused by a mutation in the *ADSL* gene on 22q13 [1,12]. Expression study of

## Communicating author:

Hanna Mierzewska, Klinika Chorób Metabolicznych IP CZD, Al. Dzieci Polskich 20, 04-730 Warszawa, Poland, tel.: +48 22 815 74 90, fax: +48 22 815 74 89, Email: h.mierzewska@gmail.com

abnormal proteins showed that severity of clinical features correlated with residual activity of the enzyme [2,5,12]. Affected children presented encephalopathy with brain atrophy and hypomyelination detected in MRI/CT examination [5,7,10,11]. Variability in the phenotypes was observed in affected children even in the same family [5,8].

Until now there are no full data of neuropathology of the disease. Only recently Mouchegh et al. briefly mentioned that one of their patients had microcystic encephalopathy [9].

Due to ADSL deficiency, high amounts of two metabolites are detected in the cerebrospinal fluid (CSF) and in urine – succinyladenosine (S-Ado) and succinylaminoimidazole carboxamide riboside (SAICAr) [1,8,12]. It is assumed that the former metabolite is neurotoxic, because higher levels of it were detected in the most severe type of the disease [1,12]. Deficiency or lack of adenosine nucleotides seemed to be not significant, but such possibility cannot be excluded in the cases especially with antenatal onset [2].

According to the ratios of S-Ado and SAICAr ~1 – type I (severe) and ~2-4 – type II (mild) of the disease were distinguished [12].

We present clinical, biochemical and neuropathological findings of a child affected by severe form of ADSL deficiency. To the best of our knowledge this is the first full report of an autopsy examination of the disease.

## Case report

The girl was a second child of unrelated parents. Her older sister was healthy. She was born after an uneventful pregnancy and delivery, in good condition (Apgar score 10 points) with weight 3550 g, occipitofrontal circumference 34 cm. From the beginning she was slightly hypotonic, with poor suction. On her 7<sup>th</sup> day of life tonic-clonic seizures started and she was admitted to the hospital. During subsequent days she presented with repeated episodes of apnoea and epileptic state. The EEG showed burst-suppression pattern. MRI of the brain at the age of 1 month showed symmetrical lack of myelination of the cerebral white matter as well as enlargement of lateral ventricles. Fluid collections over frontoparietal and frontotemporal areas and open Sylvian fissure were visible. Abnormal signals of dentate nucleus were also seen.

During the next period the baby was unconscious and progressively hypotonic. She remained on artificial ventilation till her death at 2.5 months.

The second brain MRI examination at the age of 2 months showed progressive cortico-subcortical atrophy and progressive hypo/dysmyelination of cerebral white matter mainly in occipitoparietal areas [4].

The HPLC (high pressure liquid chromatography) analysis of CSF revealed elevated level of S-Ado and SAICAr with the ratio 0.92, which confirmed the diagnosis of type I ADSL deficiency [8].

Molecular analysis performed in Prague by S. Knoch and M. Zikanova revealed compound heterozygous mutations Y114H/ T242J in both alleles of the *ADSL* gene.

## Material and Methods

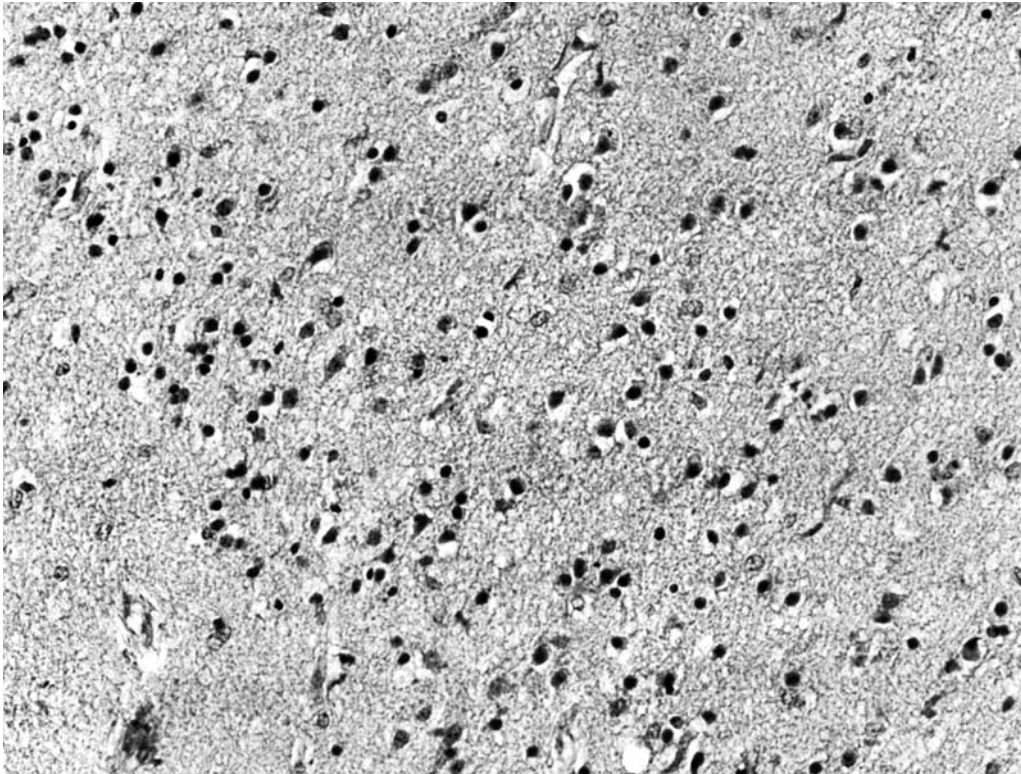
The brain was fixed in formalin. The specimens from cerebral hemispheres, brain stem and cerebellum were taken and embedded in paraffin. The sections were stained with haematoxylin-eosin (H-E), Kluver-Barrera, and Bielschovsky method; GFAP immunostaining was also done. For electron microscope evaluation, small fragments of cerebral cortex and white matter were taken from paraffin blocks. After deparaffinizing and washing several hours in water the material was processed routinely for ultrastructural examination.

## Neuropathological data

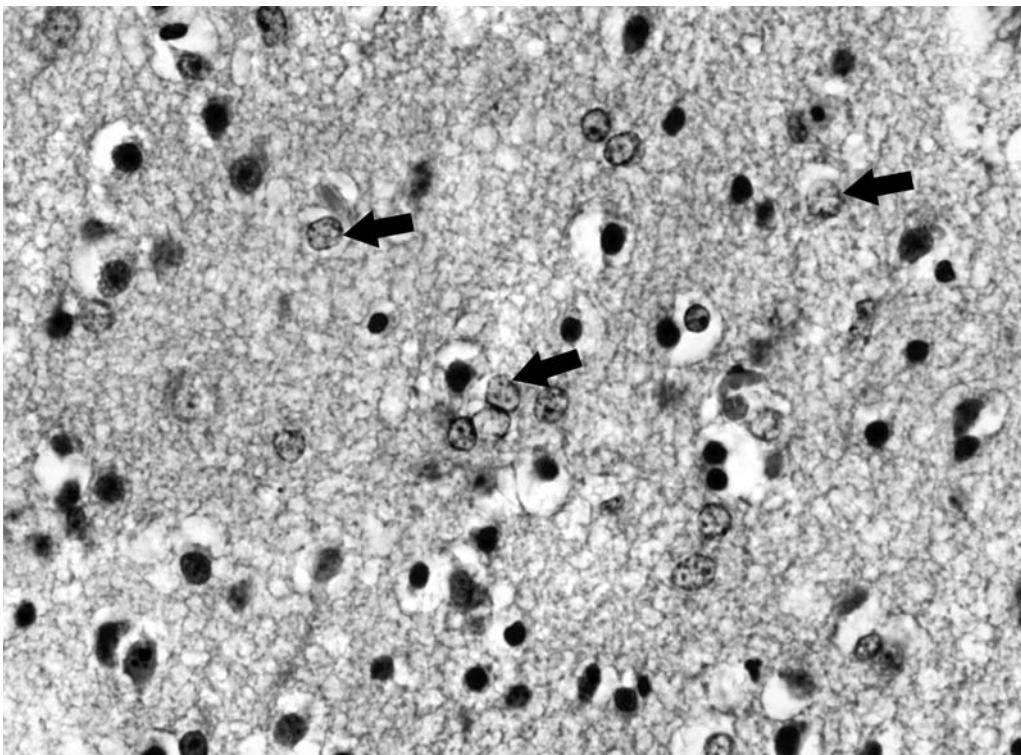
Macroscopically the gyral pattern of cerebral hemispheres was normal, but there was widening of Sylvian fissures due to failed operculisation of insula. On transverse sections moderate atrophy of the brain with widening of sulci, decrease of the white matter volume, thinning of corpus callosum and enlargement of lateral ventricles were seen. White matter was mildly greyish, especially in posterior parts of cerebral hemispheres. The cerebral cortex was not clearly delineated.

Microscopic examination showed normal for age cytoarchitecture of the cerebral cortex.

There was diffuse damage of the grey and white matter of the whole central nervous system (CNS). The cerebral cortex showed severe changes with damage and loss of neurons with different degree of spongiotic changes in neuropile (Fig. 1A) with scanty astrocytic reaction mainly of Alzheimer type II cells (Fig. 1B).



**Fig. 1A.** Cerebral cortex with widespread damage and loss of neurons as well as spongiosis of neuropile. HE,  $\times 200$



**Fig. 1B.** Alzheimer type II cell (arrows),  $\times 400$

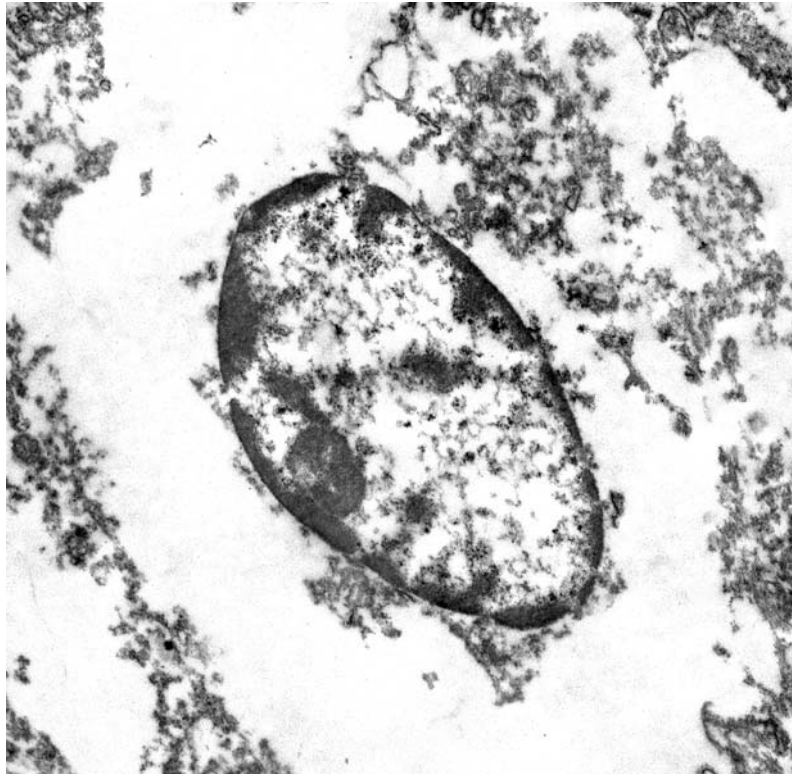


Fig. 1C. Damaged astrocyte exhibiting degeneration of organelles in empty cytoplasm. Orig. magn.  $\times 4400$

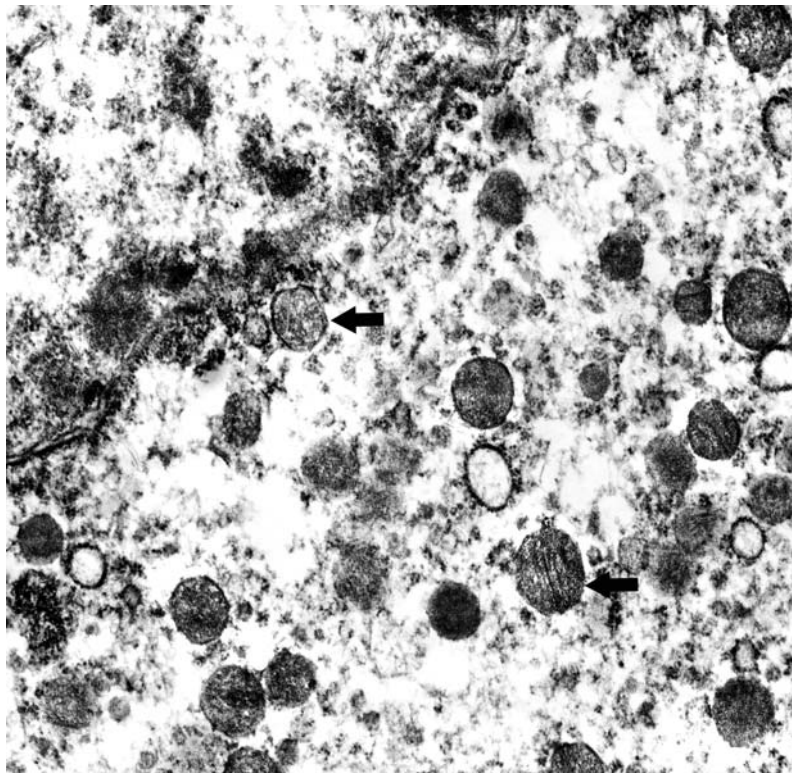


Fig. 1D. Numerous well preserved mitochondria (arrows) in neurons. Orig. magn.  $\times 12\ 000$

At ultrastructural level Alzheimer type II exhibited loss of organelles and empty areas of cytoplasm (Fig. 1C). There was a correlation between the degree of loss of neurons and spongiotic changes of neuropile. In pericarya of the neurons relatively well preserved mitochondria and swollen cisternae of granular endoplasmic reticulum were found (Fig. 1D).

The cerebral white matter also showed severe pathological changes: the myelin was adequate for the child's age only in the posterior limb of the capsula interna, in the thalamus and mostly the paramedian part of the basal ganglia (Fig. 2A) as well as in the brain stem and cerebellum. There was a complete lack of myelin in the centrum semiovale with spongiotic changes, scanty astroglial reaction and reduced number of oligodendrocytes (Fig. 2B). The neuron fibres were disintegrated (Fig. 2C). In the most severely damaged white matter there was marked reaction of hypertrophic astrocytes and scanty macrophages (Fig. 2D). In myelinated structures the myelin sheets were destroyed (Fig. 3A) and a strong astroglial reaction was seen (Fig. 3B). The astroglial cells were seriously damaged: their cytoplasm was increased in volume with focal rarefaction and vacuolar changes; disintegration of their processes was also seen (Fig. 3C). Oligodendroglia in the myelinated structures were present, but in electron microscopy examination they were severely damaged – only their nuclei were relatively spared (Fig. 3D).

At ultrastructural level most axons were myelinated but myelin sheaths were thin. In some places the myelin sheath was interrupted but the lamellae of the myelin sheath were compact, without signs of splitting. The swollen cytoplasm of axons contained few filaments and mitochondria. Electron microscopic examination revealed swollen astrocytes with few organelles. In their cytoplasm well preserved mitochondria usually grouping in some places were visible. Gliofilaments were rare but regularly dispersed in enlarged cytoplasm (Fig. 3E).

The best preserved parts of the brain were the basal ganglia, thalamus and hypothalamus, but they showed similar spongiotic changes as cerebral cortex and white matter.

In the cerebellum, the external granular layer was thinner than normal. There was marked loss of Purkinje cells. The remaining cells were seriously damaged. The internal granular layer was also seriously damaged. The white matter in the axis of

cerebellar folia was very narrow with severe spongiotic changes accompanied by a strong astroglial reaction. Dentate nucleus showed marked loss of neurons and spongiosis.

Many of the meningeal and brain vessels showed a thick wall. Some of the brain vessels were surrounded by a large vacuole and disintegrated tissue.

## Discussion

So far in the literature there are described almost 63 cases of ADSL deficiency [3,4,9,12]; six of them died. In our Institute we observed 7 children [9]; five had severe form of the disease; the one presented above died.

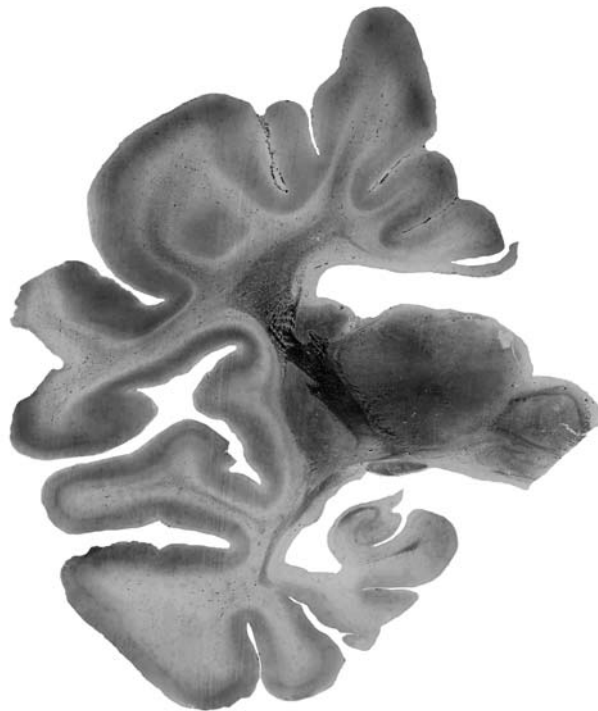
The girl was born at term in good condition, with normal birth weight and normal occipitofrontal circumference, but according to the mother, she was hypotonic with poor sucking reflex from the beginning of life. So we suppose that she had severe form of ADSL deficiency which could have started in the antenatal period. However, we could not classify the patient as prenatal fatal form described by Mouchehgh and others [9].

The MRI examination in the first month of life showed signs of mild brain atrophy and hypomyelination of cerebral white matter. During the next few weeks of life the child's condition rapidly changed for the worse and it was correlated with the brain damage observed in the second MRI, which revealed progressive hypo/dysmyelination of the cerebral white matter mainly in parieto-occipital areas.

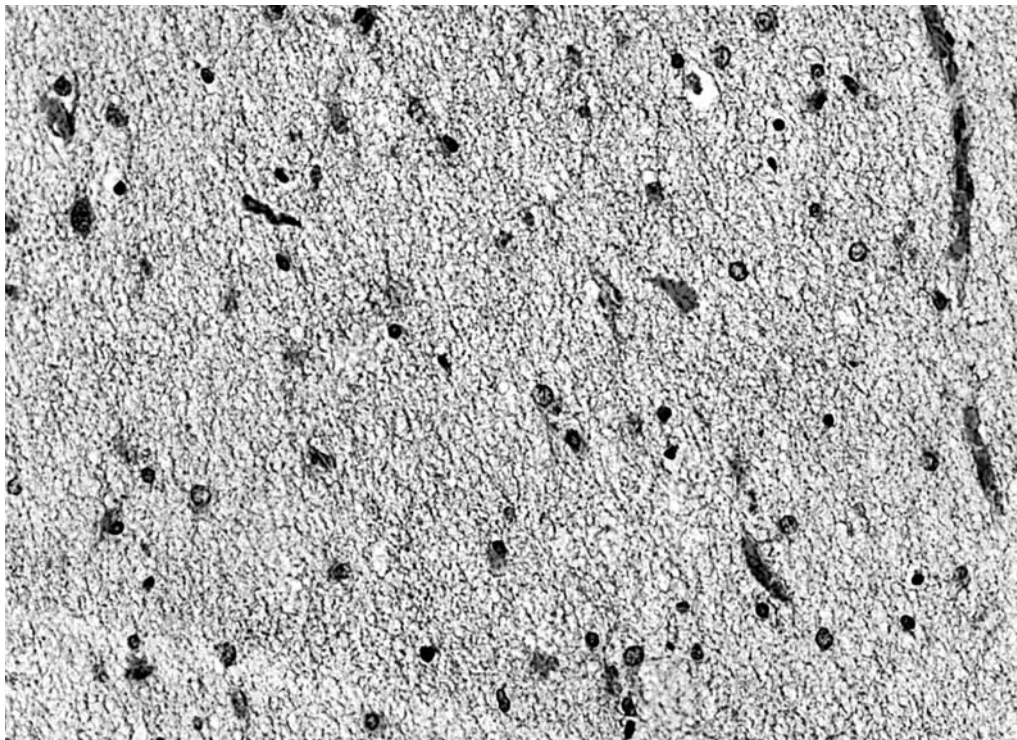
We excluded organic acidurias, aminoacidopathies as well as lysosomal diseases. Because of progressive damage of white matter observed in MRI we suspected neonatal form of vanishing white matter disease. Just the CSF examination detected S-Ado and SAICAR, confirming ADSL deficiency type I.

Development of the brain structures in our patient indicated that prenatal development of the brain was not disturbed. But the neuropathological examination showed severe microcystic encephalopathy with damage of all morphological elements of the brain (neurons, their processes, astroglial cells, oligodendroglia cells, myelin and also vessels).

It is possible that during pregnancy the maternal organism could partially compensate metabolic disturbances of an ADSL affected fetus. After birth, the metabolism of the neonate begins to be independent, so neurotoxicity of certain metabolites



**Fig. 2A.** Cerebral hemisphere. Lack of myelin in centrum semiovale; myelination adequate for the age in posterior limb of internal capsule, and paramedian part of the basal ganglia and the thalamus. Klüver-Barrera, glass magnification



**Fig. 2B.** Severe damage of white matter with spongiosis of neuropile and reduced number of oligodendroglia. HE, × 200

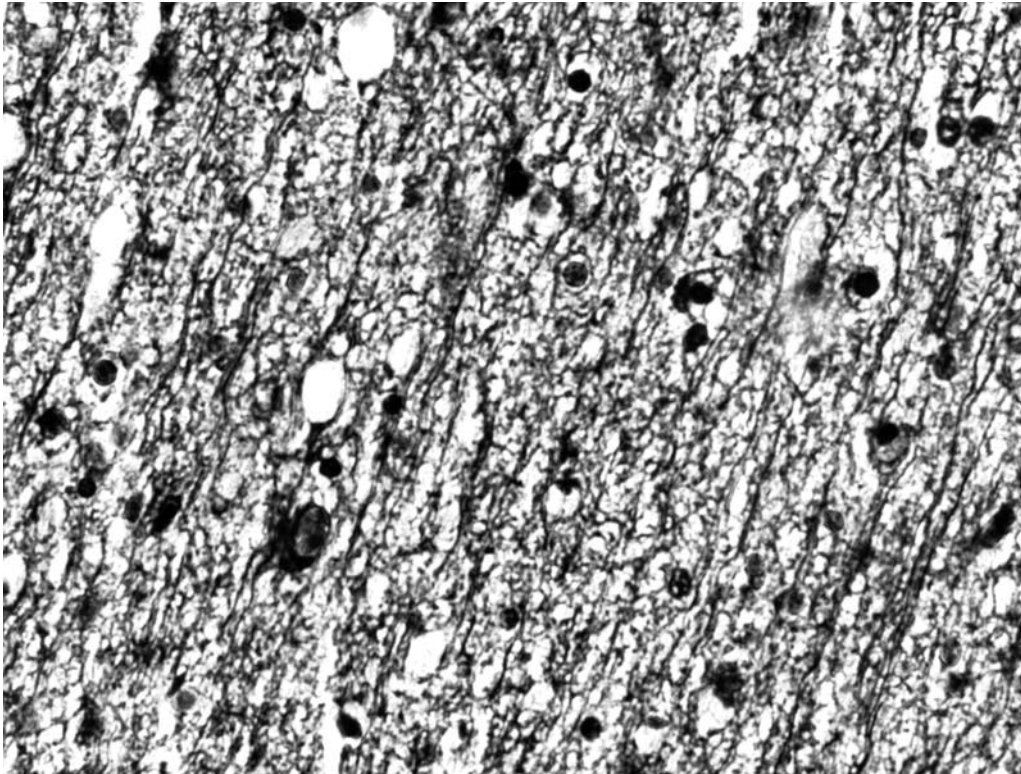


Fig. 2C. Disintegration of neuron fibres. Bielschovsky,  $\times 400$

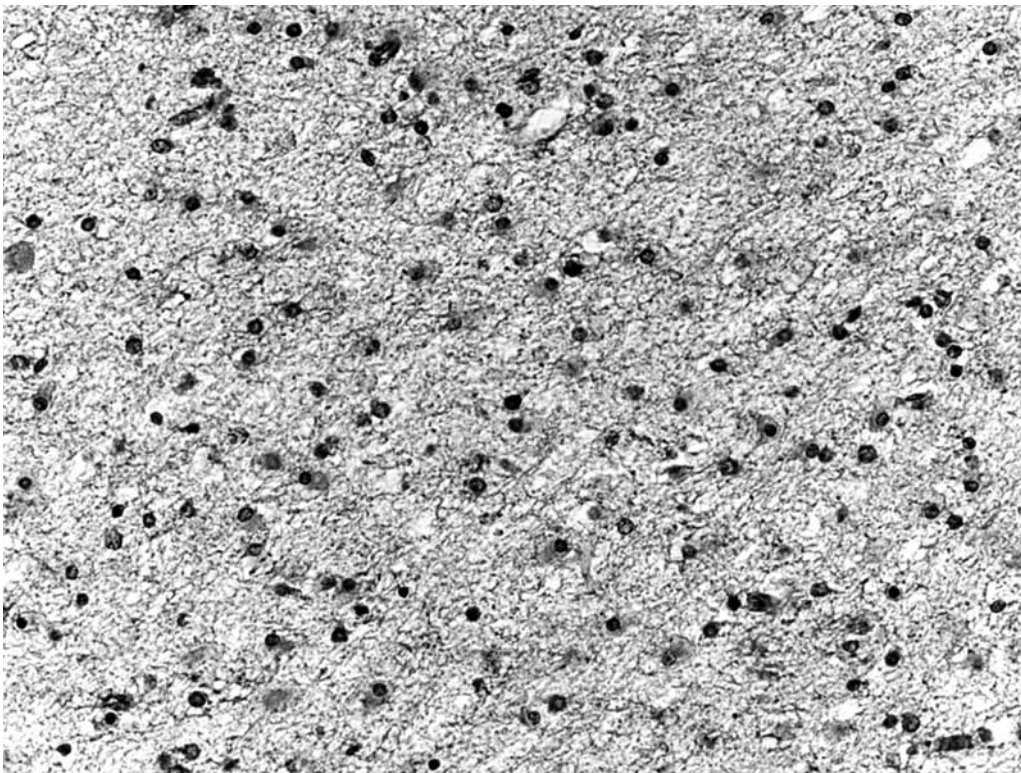


Fig. 2D. Strong reaction of hypertrophic astrocytes and scanty macrophages. HE,  $\times 200$

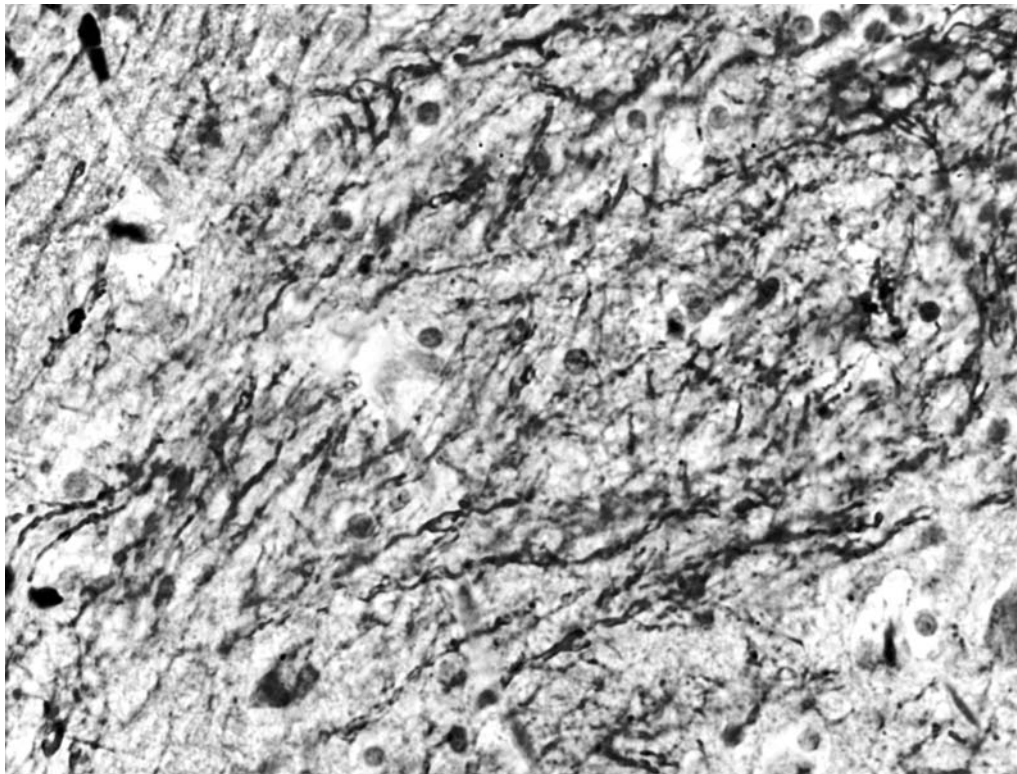
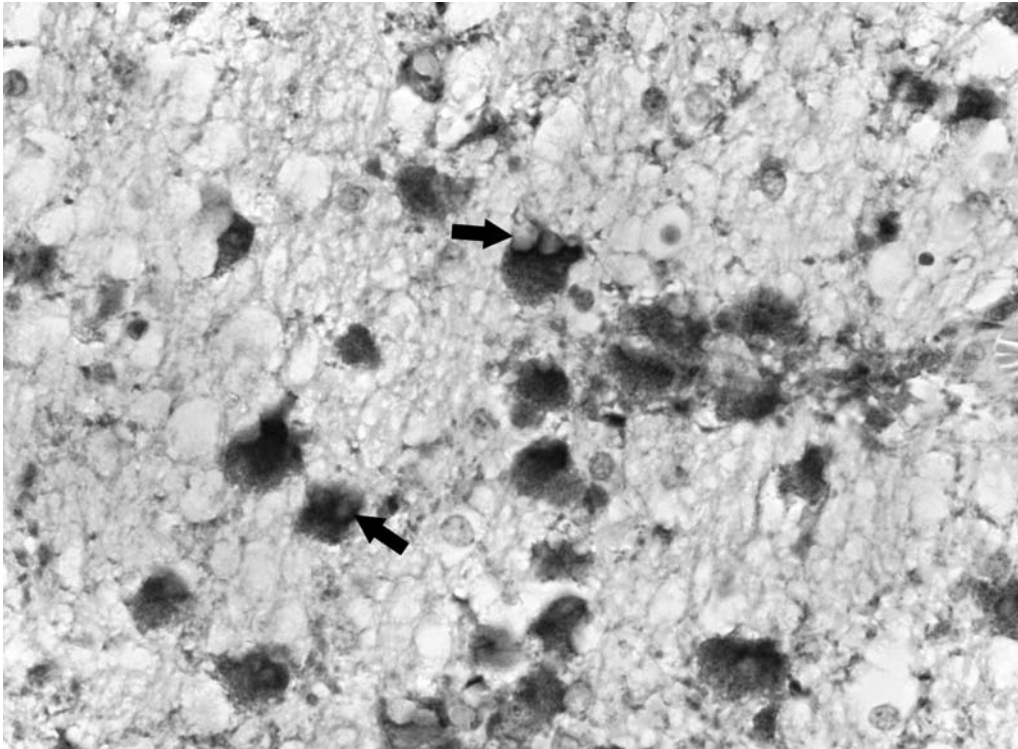


Fig. 3A. Destruction of myelin sheaths. Klüver-Barrera,  $\times 200$

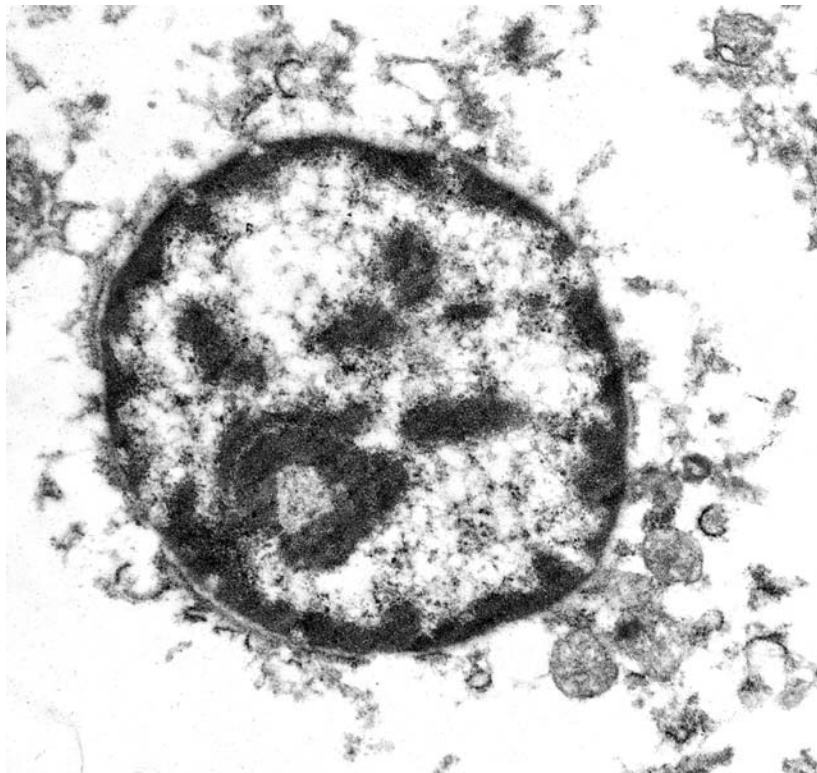


Fig. 3B. Astroglial reaction in myelinated white matter. GFAP, glass magnification





**Fig. 3C.** Damage of astroglia with rarefaction of cytoplasm, vacuolar changes (arrows) and disintegration of processes. GFAP,  $\times 400$



**Fig. 3D.** Damaged oligodendrocyte with degenerated cytoplasm. Orig. magn.  $\times 7000$

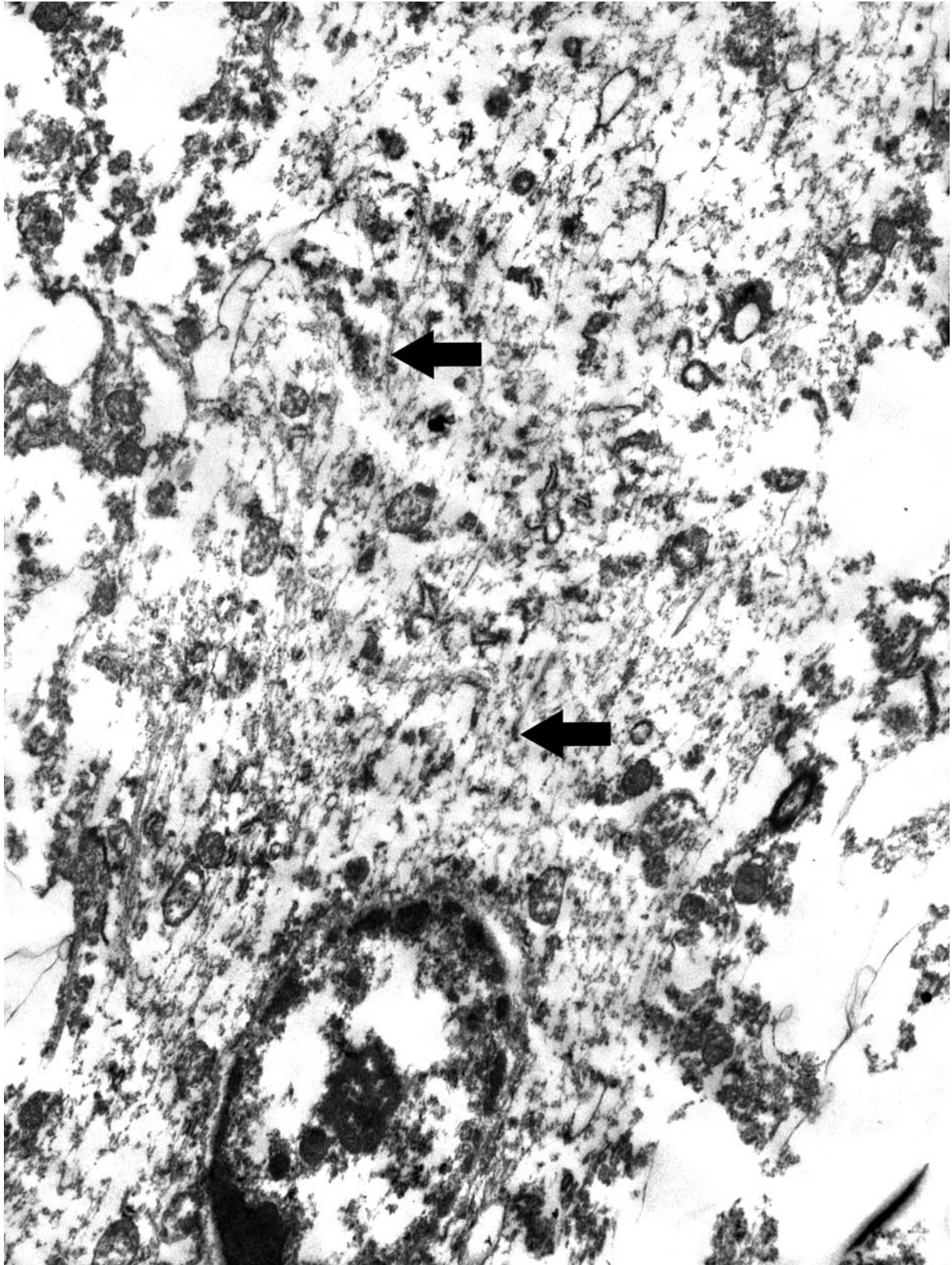


Fig. 3E. Dispersed gliofilaments (arrows) in abundant cytoplasm of astrocyte. Orig. magn.  $\times 4400$

as well as insufficiency of others may be revealed, disturbing the functioning of all the cells.

Our neuropathological findings confirmed that ADSL deficiency is an inborn metabolic disorder seriously destroying the brain. Until today only in one paper [3] do the authors briefly mention the neuropathological findings of a patient with ADSL deficiency summed up as microcystic encephalopathy. Our detailed neuropathological examination confirmed that observation.

Microcystic encephalopathy is not specific to ADSL deficiency but there was also observed in other metabolic disorders, such as Canavan disease, certain aminoacidopathies, disorders of energy metabolism, nonketotic hyperglycinaemia and others [6].

We consider that the changes might not be entirely caused by neurotoxicity of SAICAr, but are also the result of combined biochemical disorders in brain tissue. Although the deficiency of purine nucleotides in the disease is so far unproven, it seemed to us that this factor may play a role in pathology of the disease. Purine nucleotides and adenylylase are important for transduction of cellular signalling and are indispensable for activation of many enzymes. So its insufficiency, even subtle, may disturb the functioning of all the cells, especially of the brain, the organ whose postnatal development is the most intensive. Further examination is necessary to explain the problem.

According to our observation in the reported case we suppose that MRI features suggesting hypo/dysmyelination in ADSL deficiency were the result of severe devastation of all morphological elements of cerebral white matter – oligodendroglial cells as well as axons.

In conclusion, we suggest that in every case of progressive encephalopathy with hypomyelination/dysmyelination, examination for SAICAr and S-Ado in urine and CSF should be performed.

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