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Case of Dementia and Myoclonia in an Adult Associated with Anomalies in Polyunsaturated Fatty Acids in Leukocytes and Peripheral Nerve[1]

An Ultrastructural Study of Peripheral Nerve

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Abstract. We report a case of a 66-year-old patient presenting with abnormal movements and associated dementia. Death occurred 4 years after the onset of symptoms. In spite of the lack of autopsy results, the picture was one of late onset neuronal ceroid lipofuscinosis (Kufs' disease). Ultrastructural study of a peripheral nerve biopsy sample indicated a process of demyelination associated with unusual inclusions in Schwann cell cytoplasm. Biochemical analysis of the same sample and leukocytes showed considerable alterations in polyunsaturated fatty acid levels. These findings are discussed in the light of work on cases of infantile neuronal ceroid lipofuscinosis.

Introduction

Infantile neuronal ceroid lipofuscinosis (NCL) is characterized by abnormalities in polyunsaturated fatty acids in the brain [2, 14], in serum phospholipids [1] or leukocytes [13]. The diagnosis of this particular type of lysosomal disorder is readily confirmed from the ultrastructural study of a peripheral nerve sample. We are not aware of any published results on biochemical analysis of peripheral nerve samples in this disorder.

The adult form of the disorder (Kufs' disease) is less well known. We here report a case of an adult woman probably suffering from this disorder. We carried out an ultrastructural study of a peripheral nerve biopsy and a biochemical analysis of leukocytes and

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part of the biopsy sample. We found abnormalities in long-chain polyunsaturated fatty acids.

Materials and Methods

Case Report

The patient was a 66-year-old woman (case L.) admitted to the Neurology Department at the University Hospital in Limoges in April 1979. She was investigated for a slowly deteriorating neurological symptomatology which started in 1977.

Loss of recent memory was one of the first signs, followed by general psychomotor retardation. This was associated with a progressive extrapyramidal typical 'flapping tremor' accompanied by spontaneous myoclonic jerks. Tendon reflexes were brisk with no sign of a Babinski response.

This symptomatology gradually deteriorated and the patient became bedridden. Death ensued 4 years after onset of the first symptoms. The family did not agree to an autopsy.

This patient had no previous history of neurological disease and no nutritional deficiency. There was no family history and no evidence of consanguinity.

No abnormalities were detected in routine laboratory tests nor in the following analyses: venous and arterial ammonia, plasma copper and ceruloplasmin, bismuth, serum electrophoresis, lipids and plasma free fatty acids, plasma cortisol and Synacthen test, CSF.

Several fundoscopies during the course of the disease did not reveal any retinal lesions. Laparoscopy with liver biopsy only showed a slight steatosis. Cerebral scintigraphy and isotopic cisternography did not reveal any abnormalities.

Several EEG studies showed increasing abnormality over the course of the disease. Towards the end, the tracing was quite abnormal in a diffuse way with general slowing of cerebral rhythms.

A biopsy of the superficial personeal nerve of the left leg was carried out in 1979.

Histological Studies of the Peripheral Nerve

Light Microscopy. Standard techniques were used on paraffin sections. Semithin sections were stained with toluidine blue. A part of the sample was deep-frozen immediately after biopsy for later fluorescence microscopy.

Electron Microscopy. Small pieces of nerve were fixed immediately in 5% glutaraldehyde for 1 h and then washed in disodium phosphate for 12 h. Postfixation was done in 1% osmium tetroxide. After dehydration in ethanol, the blocks in Epon sections were stained with lead citrate and uranyl acetate. They were examined with Zeiss EM 9 and Hitachi 300 electron microscopes.

Biochemical Analyses

Peripheral Nerve. A part of the nerve was carefully dissected, lyophilized and weighed. Lipids were extracted in 2:1 chloroform methanol [2, 12]. The fatty acid methyl esters were formed by methylation in methanol/boron trifluoride. They were analyzed by gas chromatography using either a capillary column (Carbowax 20 M, length 80 m, diam. 0.25) or the usual SE 30 column. Identification was by comparison of retention times with known standards. An integrator (ICAP 10) determined the relative amounts of each component.

Leukocytes [6]. 1 ml of a 10% gelatine solution was added to 10 ml of blood in citrate buffer. The tube was inverted once very carefully and the blood was left to sediment for about 20 min at 37 °C. The upper clearer phase was removed and centrifuged at 500 *g* for 5 min. The supernatant was rejected and the red cells in the pellet were lysed by adding 3 ml of precooled double-distilled water (0 °C) and shaking vigorously for 90 s. The solution was made isotonic by adding 1 ml of 3.6% NaCl and then centrifuged at 480 *g* for 10 min at 4 °C (Sorvall RC 2-B). The white pellet of leukocytes was washed twice with 0.9% Na Cl and then taken up in 1 ml of double-distilled water. The suspension was sonicated for 15 s in a Branson sonicator. Lipids were extracted and fatty acids analyzed as described for peripheral nerve.

A control nerve sample and leukocytes were taken from another patient who was being investigated for a collagen disorder. This 65-year-old patient had no neurological signs, and electron microscopy of the nerve sample showed no abnormalities.

Results

Histology

Light Microscopy. The following changes were seen: Moderate rarefaction especially of myelinated fibers, which was confirmed

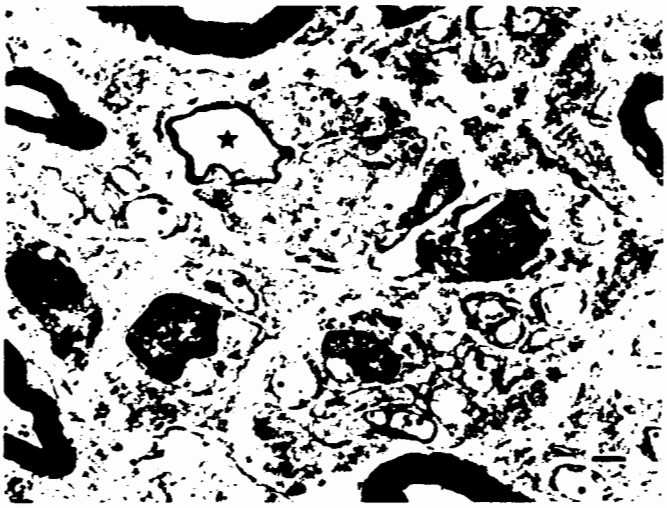

Fig. 1. Obvious thinning of myelinated fibers, one of which (*) is in the process of remyelination. Bar = 1 μm.

Fig. 2. 'Onion bulb' Schwann cell proliferation round a remyelinated fiber. Bar = 1 μm.

quantitatively (5,430/mm^2 – control 7,892/m^2). Some fluorescent granules were seen with the fluorescence microscope.

Electron Microscopy. The lesions in myelinated fibers were mainly of the demyelination/remyelination type. Many axons had myelin sheaths abnormally thin with respect to the axonal diameter (fig. 1). 'Onion bulb' formations with one or two turns of proliferating Schwann cell were clear, especially round remyelinating fibers (fig. 2). Unmyelinated fibers were unaltered both qualitatively and quantitatively.

The most unusual finding was the presence of highly abnormal inclusions in many Schwann cell cytoplasms. Although they did

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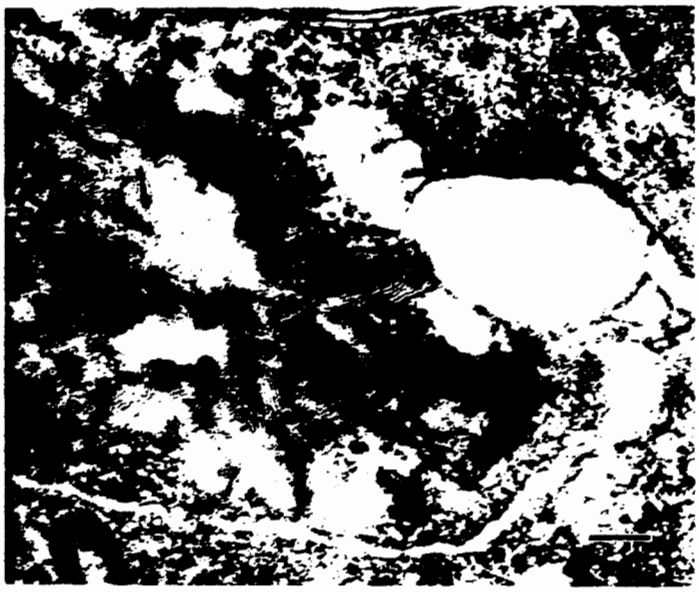

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Fig. 3-5. Patterns of lamellar inclusions in Schwann cell cytoplasms of myelinated fibers. Here they are more or less rectilinear. **3** Bar = 0.1 µm. **4** Bar = 0.2 µm. **5** Bar = 0.1 µm.

not always take the same form, they consisted of stacks of lamellae. The thickness of the dark lines was around 3.5 nm while the light lines were 2–2.5 nm thick. These structures were more or less complex in shape depending on their mutual arrangement. In some cases they were rectilinear (fig. 3–5) or curvilinear (fig. 6, 7).

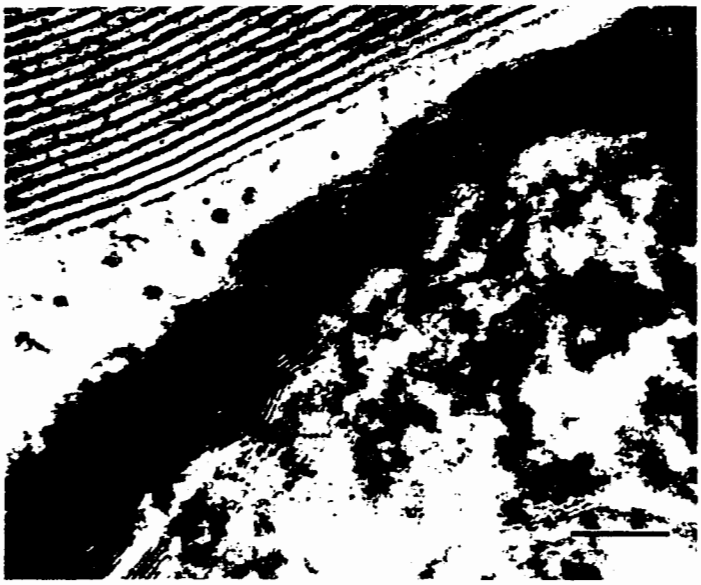

Fig. 5. For legend see p. 211.

Biochemistry

Leukocytes. There were considerable alterations in whole leukocyte fatty acids: series 6 was reduced ($C_{18:2}$, $C_{20:3}$, $C_{22:4}$). The only member of series 3 ($C_{20:3}$) detected was raised. $C_{22:6}$ was detected in trace amounts (0.2%) in the control leukocytes. It could not be detected in the sample from our patient. The ratio of saturates/polyunsaturates was elevated, due to the reduced number of total polyunsaturates (table I).

Peripheral Nerve. There were large alterations in peripheral nerve fatty acid levels, namely: The very long chain acids whether saturated, monounsaturated or polyunsaturated were lowered (very long chain = above 18 C atoms). These reductions were compensated by increases in palmitic ($C_{16:0}$) and palmitoleic ($C_{16:1}$) acids. As for leukocytes, series 6 of the polyunsaturated acids was low. However, series 3 was reduced as well. In contrast with leukocytes, 16:1 was largely increased and 18:0 reduced (table II).

Discussion

Both ultrastructural and biochemical investigations of the peripheral nerve sample as well as the biochemical analysis of leukocytes from this patient revealed unusual abnormalities. However, some of the histological and biochemical results suggested a picture of a late form of NCL (Kufs' disease).

The adult type of this disorder spans a wide range of clinical manifestations. Visual disturbances are exceedingly rare, if not entirely absent [5] and dementia is not usually a predominating feature [16]. Our patient fitted this clinical picture, and the presence of myoclonia with abnormal movements of the 'flapping tremor' type suggested a storage disorder.

A study of the peripheral nerve sample indicated several significant changes. Under UV light several autofluorescent granules were observed. In nerve samples from infantile cases, *Joosten* et al. [11] only found slight autofluorescence in 1 of their 2 cases, and

Table I. Whole leukocyte fatty acids

Chain length	Control	Case L.
14:0	1.3	1.7
16:0	25.6	31.6
16:1	3.1	3.4
18:0	15.7	20.3
18:1	22.6	25.2
18:2 (n-6)	8.5	4.4
18:3 (n-3)	Tr	Tr
20:0	0.8	0.5
20:1	7.4	4.2
20:1 (n-6)	1.1	0.4
20:4 (n-6)	10.4	4.6
20:3 (n-3)	0.6	2.0
22:0	1.4	1.6
22:4 (n-6)	1.3	0.6
22:6 (n-3)	0.2	
n-6	21.3	9.5
n-3	0.8	2.0

Fatty acid composition of whole leukocytes in patient and control. Fatty acids are abbreviated in the usual way: the number before the column represents the number of carbons in the chain and the number after the column is the number of double bond. The values are expressed as percentage of total identified unsubstituted fatty acids. Each value is the average of duplicate measurements on 3 separate samples of leukocytes obtained from the same person at different times.

Table II. Whole peripheral nerve fatty acids

Chain length	Control	Case L.
14:0	0.5	1.8
16:0	14.8	21.1
16:1	8.1	14.5
18:0	4.6	1.4
18:1	50.3	53.6
18:2 (n-6)	6.6	4.1
18:3 (n-3)	0.3	0.2
20:0	1.3	0.1
20:1	1.1	0.4
20:3 (n-6)	0.3	0.1
20:4 (n-6)	1.8	0.5
20:3 (n-3)	0.2	Tr
22:0	0.5	0.1
22:1	2.0	0.4
22:4 (n-6)	0.4	0.2
22:5 (n-3)	0.9	0.4
22:6 (n-3)	0.6	0.1
23:3	1.3	0.3
24:0	2.3	0.4
24:1	2.1	0.3
n-6	9.1	4.9
n-3	2.0	0.7

Fatty acid composition of whole peripheral nerve in patient and control. The same remarks as in table I. For the patient, one nerve biopsy was used. A lipid extraction was performed and the solution divided into 3 parts which were methylated separately. The values are the average of duplicate chromatograms on the 3 separate methyl ester samples.

nerve as is often seen in lysosomal disorders affecting the CNS. The most characteristic change was the presence of abnormal inclusions in many Schwann cell cytoplasms.

In cases of NCL, various types of structure were seen with the electron microscope in both neurons and Schwann cells. For some authors these structures are specific to clinically differentiable entities of NCL, although other authors dispute this [9]. The distinction between curvilinear and rectilinear profiles would seem to be artificial. But bodies with fingerprint and other crystaloid patterns, membranous, zebra-like and granular bodies as well as mixed types are frequently encountered. *Goebel and Schulz* [8] have questioned the specificity of these patterns, as they have found abnormally structured lipofuscine granules in various cell types in different conditions not related to NCL. This suggests that

the unusual structures we observed, which have not been described previously, cannot be considered specific. However, their existence and the associated biochemical alterations are noteworthy.

Several ultrastructural studies of peripheral nerve samples of infantile cases of NCL have been published [4, 9–11, 15]. To our knowledge there have been no comparable descriptions of the adult form of the disorder (Kufs' disease). This is also true for the biochemical results.

The biochemical studies on infantile cases of NCL indicated that there were alterations in brain polyunsaturated fatty acids [2, 14], serum phospholipids [1] or leukocyte phospholipids [13]. There is an altered profile of the fatty acids of phosphatidyl-ethanolamine in the brains of these children: $C_{18:1}$ (oleic acid) and $C_{20:4,6}$ (arachidonic acid) are increased, while $C_{22:6,3}$ (docosahexadenoic acid) are low. In leukocytes the differences are less clear-cut; only $C_{22:6,3}$ is reduced, but it only represents 0.5% of total fatty acids. Recently, a reduced level of arachidonic acid has been described in blood and liver in a case of multisystem neuronal degeneration with hepatosplenomegaly and adrenocortical deficiency [7].

In our patient, the relative percentage of all the very long chain fatty acids (18 C and above), whether saturated, monounsaturated or polyunsaturated, was low. This reduction was compensated by elevations of palmitic ($C_{16:0}$) and palmitoleic ($C_{16:1}$) acids. Normal adult myelin is characterized by the presence of very long chain saturated and monounsaturated fatty acids. Reduced amounts may be a result of myelin thinning. The presence of medium chain fatty acids indicates the presence of immature myelin formed in the process of remyelination. But the fact that changes in polyunsaturated fatty acids were found in leukocytes as well suggests that the primary disorder is in the metabolism of these fatty acids. Alterations in polyunsaturated fatty acids due to nutritionally induced essential fatty acid deficiency can be excluded. 20:3 (n-9) was never detected (this acid being a marker for essential fatty acid deficiency).

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Metabolic Alterations of Endoneurial Lipids in Developing Trembler Nerve*

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