PROGRESSIVE POLIODYSTROPHY

ASSOCIATION WITH DISTURBANCES IN PYRUVATE METABOLISM

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The studies presented in this thesis were performed in the Department of Child Neurology, the Department of Clinical Neuropathology, and the Laboratory of Clinical Chemistry, Institute of Neurology; the Laboratory of Biochemistry, Institute of Pediatrics; the Department of Neuropathology, Institute of Pathology; the Department of Submicroscopic Morphology; and the Institute of Biochemistry; University of Nijmegen.
Parts of these studies were performed in the Department of Child Neurology and the Department of Neuropathology, State University Groningen; and the Department of Biochemistry (B.C.P. Jansen Institute), University of Amsterdam.

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GENERAL INTRODUCTION

Neurologic differential diagnosis is principally based on localisatory or anatomic criteria. The result of clinical neurologic investigation is generally a localisatory diagnosis, and a putative causal diagnosis. In many cases diagnosis is verified during a patient's lifetime by the course of the illness, by neuroradiologic and electrophysiologic investigations, and by biochemical studies. In several neurologic disorders the clinical diagnosis can be verified definitely only at autopsy. For instance, to date the diagnosis of "Progressive Infantile Poliodystrophy (Alpers' disease)" can only be confirmed at autopsy.

In recent years definite diagnosis of several disorders has become possible by means of new methods of investigation in biochemical research. For instance, the biochemical defect of several so-called inborn errors of metabolism has become known. When demonstrating a biochemical or enzymatic defect a new criterium of differential diagnosis is introduced.

Biochemical studies on enzymatic defects are indicated in disorders where the pathogenesis is still unknown, and especially in those disorders in which an autosomal recessive inheritance has been demonstrated.

Specified enzymatic studies must be indicated from clinical, radiologic, electrophysiologic, and above all from routine biochemical studies. Investigations of serum, urine and cerebrospinal fluid (CSF) may suggest a defect in specific enzymatic systems.

Elevated levels of lactate and pyruvate in serum and in CSF may indicate a disturbance of the pyruvate metabolism, namely pyruvate oxidation, the citric acid cycle and the respiratory chain. These enzymatic systems are involved in the cellular energy metabolism.

The Department of Child Neurology and the Department of Clinical Neurochemistry of the Institute of Neurology, and the Laboratory of Clinical Biochemistry of the Institute of Pediatrics of the Radboud University Hospital, Nijmegen, have had fairly lengthy experience of the study and interpretation of normal and elevated levels of lactate and pyruvate in serum and CSF of children with various disorders.

This study contains a report of the clinical, morphologic and biochemical investigations in children with a progressive disorder of the cerebral gray matter, with increased levels of lactate and pyruvate in serum, urine and/or CSF. Histopathologic studies of brain tissue in these children revealed the characteristics of progressive infantile poliodystrophy.

Chapter 1 summarizes the literature on progressive poliodystrophy. Special attention is given to the data from literature referring to the pathogenesis of the disorder.

Chapter 2 gives a survey of the cerebral energy metabolism, namely pyruvate oxidation, the citric acid cycle and the respiratory chain. Some neurologic diseases are mentioned, in which disturbances of the pyruvate metabolism have already been demonstrated. Concluding the first two chapters, a working hypothesis is formulated with respect to the pathogenesis of progressive poliodystrophy.

In chapters 3-6 the case reports of five patients are presented. In these patients with demonstrated progressive poliodystrophy, deficiencies were demonstrated in pyruvate oxidation, in the citric acid cycle and in the respiratory chain. The case reports have been published previously in several neurologic journals.

Chapter 7 summarizes the literature data and the patient studies. Considering the clinical, morphologic and biochemical findings a final conclusion is formulated, including a hypothesis on the pathogenesis of progressive poliodystrophy. Moreover, the consequences of these findings are discussed, with respect to various aspects of the disease.

This study does not pretend to be a presentation of new clinical, morphologic or biochemical investigations, although it does aim to make a fundamental contribution to the knowledge of progressive poliodystrophy and to neurologic differential diagnosis, through the use of recent methods of clinical, morphologic and biochemical investigations, and by correlating the results of these methods of investigation.

CHAPTER 1

LITERATURE ON PROGRESSIVE POLIODYSTROPHY

INTRODUCTION

This chapter gives a survey of the literature on progressive poliodystrophy, with specific emphasis on the pathophysiologic concept of this disorder. Special attention will therefore be paid to the relation of the following aspects of this disorder: clinical signs, genetic studies, pathologic findings, and biochemical investigations.

During this study of progressive poliodystrophy the cellular energy metabolism appeared to be of great importance. We will therefore look particularly at those studies in the literature which report on biochemical aspects of carbohydrate metabolism or on morphologic investigations of mitochondria.

SURVEY OF CASE HISTORIES IN THE LITERATURE

ALPERS' FIRST DESCRIPTION

Alpers[2] in 1931 described for the first time the clinical entity of poliodystrophy, reporting the case history of a 3-month-old girl. The infant was admitted to the hospital because of generalized seizures. The girl's prenatal, natal and postnatal history was unremarkable. The family history showed no abnormalities.

The illness was rapidly progressive, with increasing loss of consciousness, irregular respiration, recurrent convulsions, attacks of general rigidity of the musculature, blindness and strabismus. The girl died at the age of four months.

Alpers gave an extensive description of pathologic findings in the central nervous system, which can be summarized as follows. Gross examination of the brain showed no abnormalities. Histopathologic studies of the brain, however, revealed some remarkable findings. Focal areas with a necrotic aspect were seen diffusely spread throughout the cerebral cortex, the basal ganglia and the brainstem nuclei. These areas presented with severe loss of nerve cells and a degenerative aspect of the still persisting neurons, with an irregular, moth-eaten cytoplasm in which no Nissl substance could be made out. Moreover, an extensive astrocytosis, and increase and dilatation of capillaries was observed.

Alpers noted another, in his opinion equally important, abnormality: he described the presence of subependymal germinal foci associated with migrating neuronal elements in the cerebral white matter.

Alpers presented his report as a description of a new clinical entity, under the

title "diffuse progressive degeneration of the gray matter of the cerebrum". In his comment Alpers distinguished structural changes and developmental changes. He stressed the strict confinement of the structural pathologic features to the gray matter of the cortex and basal ganglia. In accordance with Jakob[27] Alpers described this degenerative process as an incomplete necrosis due to vascular or toxic injury. Alpers interpreted the latter abnormalities in the subependymal regio and in the cerebral white matter as a sign of a developmental defect.

OTHER CASE REPORTS IN THE LITERATURE

Before Alpers' description a similar clinical and pathologic entity was reported by Freedom[20] and by Rusk and Nixon.[44] Subsequently approximately 90 cases of the disorder have been reported (table 1).
The literature on progressive poliodystrophy was reviewed respectively by Ford et al,[19] Alpers,[3] Greenhouse and Neubuerger,[21] Jellinger and Seitelberger,[30] Huttenlocher et al[26] and Partouche.[40]
Studies involving mitochondrial dysfunction in progressive poliodystrophy were published by Suzuki and Rapin,[50] Sandbank and Lerman,[45] Shapira et al,[46] Hart et al[23] and Partouche;[40] the last case was also reported by David et al[14] and by Tommasi et al.[51]. In this study special attention will be paid to those reports which deal in detail with mitochondrial dysfunction.

DEFINITION, CLASSIFICATION AND NOMENCLATURE

In the literature no precise definition of the disorder was proposed. Like Alpers[2] most authors mentioned the cortical abnormalities as the most distinctive features of the disorder. However, neither the pathologic findings nor the clinical signs are specific to this disorder alone. Progressive poliodystrophy may be suspected when clinical signs point to a progressive disorder of cerebral gray matter; deficiencies, infectious diseases, intoxications, vascular pathology and inborn errors of metabolism must be excluded. Suspected, progressive poliodystrophy can only be proved by extensive histopathologic study of the total brain. The disorder is characterized by neuronal degeneration, astrocytic proliferation, dilatation and proliferation of capillaries in cerebral gray matter. In general, the diagnosis can be confirmed at autopsy.
About 90 cases of progressive poliodystrophy have been reported as a separate entity. Nevertheless, in many neuropathologic and neurologic handbooks and textbooks this condition is described in several ways. In most handbooks and textbooks the disease is reported in the group of degenerative

Table 1. Patients with progressive poliodystrophy previously reported in the literature.

Case	Author	Sex	Age at onset (yrs, mos)	Age at death (yrs, mos)	Duration of illness (yrs)
1	Freedom[20] 1927	F	3	19	16
2	Rusk and Nixon[44] 1927	F	2	3	1
3	Rusk and Nixon[44] 1927	M	0.10	1.6	< 1
4	Jakob[28] 1930 (quoting Somoza)	M	0	0.9	< 1
5	Alpers[2] 1931	F	0.3	0.5	< 1
6	Walthard[56] 1931	F	3	3	< 1
7	Walthard[56] 1931	F	13	13	< 1
8	Wohlwill[59] 1931	F	2	2	< 1
9	Rösch[43] 1932	F	1.3	1.3	< 1
10	Diamond[15] 1934	M	0,3½	0,3½	< 1
11	Levin[35] 1936	F	0	0.9	< 1
12	Christensen and Krabbe[10] 1949	M	0.6	3	2
13	Fattovich[18] 1950	M	0	10	10
14	Ford et al[19] 1951	F	0.6	1.3	< 1
15	Kramer[33] 1953	F	0.4	4	3
16	Palinsky et al[39] 1954	M	0.3	1.3	1
17	Wolf and Cowen[60] '54	F	0.3	2.9	2
18	Wolf and Cowen[60] '54	F	0.5	1.5	1
19	Wolf and Cowen[60] '54	M	3	4.9	1
20	Wolf and Cowen[60] '54	F	1.11	2.1	< 1
21	Wolf and Cowen[60] '54	F	0	3.3	3
22	Wolf and Cowen[60] '54	M	1	1.2	< 1
23	Wolf and Cowen[60] '54	M	1.6	27	25
24	Wolf and Cowen[60] '54	M	6	7	1
25	Smulders[48] 1956	F	14	16	2
26	Jervis[31] 1956	F	1.3	5.5	4
27	Mattyus[37] 1957	F	3	3.3	< 1
28	Liu and Sylvester[36] '60	M	0	3.2	3
29	Liu and Sylvester[36] '60	M	0	1.4	1
30	Couville[12] 1960	M	0	2	2
31	Couville[12] 1960	M	0.5	3	2
32	Couville[12] 1960	M	0	0.11	< 1
33	Courville[12] 1960	F	0	0.4	< 1
34	Courville[12] 1960	M	0	1.4	1
35	Bebin[5] 1962	F	12	14	2
36	Verhaart[54] 1962	F	0.9	0.9	< 1

Case	Author	Sex	Age at onset (yrs, mos)	Age at death (yrs, mos)	Duration of illness (yrs)
37	Verhaart[54] 1962	M	0	0	< 1
38	Blackwood[6] 1963	M	0.8	3.6	2
39	Blackwood[6] 1963	F	1	?	?
40	Blackwood[6] 1963	F	0.11	1.8	< 1
41	Blackwood[6] 1963	M	0.8	0.10	< 1
42	Blackwood[6] 1963	F	0.2	1	< 1
43	Dreifuss and Netsky[16] 1964	M	3	3.6	< 1
44	Greenhouse and Neubuerger[21] 1964	F	4	4.6	< 1
45	Christensen and Højgaard[11] 1964	F	0.6	1	< 1
46	Christensen and Højgaard[11] 1964	M	0.2	0.6	< 1
47	Christensen and Højgaard[11] 1964	M	0.2	0.5	< 1
48	Christensen and Højgaard[11] 1964	M	1.7	3	1
49	Christensen and Højgaard[11] 1964	F	1	4	3
50	Christensen and Højgaard[11] 1964	F	0.7	6	5
51	Christensen and Højgaard[11] 1964	M	0.5	2.9	2
52	Ebels[17] 1964	F	0.9	1.9	1
53	Alberca-Serrano et al[1] '65	F	<2	2.6	1
54	Ulrich and Cunz[52] '66	F	0.3	3.5	3
55	Wefring and Lamvik[58] 1967	F	1	1.7	< 1
56	Wefring and Lamvik[58] 1967	M	1.3	?	?
57	Crompton[13] 1968	M	0.5	3.5	3
58	Laurence and Cavanagh[34] 1968	F	0.2	0.10	< 1
59	Laurence and Cavanagh[34] 1968	F	0.6	0.8	< 1
60	Laurence and Cavanagh[34] 1968	M	0	0.3	< 1
61	Laurence and Cavanagh[34] 1968	M	0.3	1.9	1

Case	Author	Sex	Age at onset (yrs, mos)	Age at death (yrs, mos)	Duration of illness (yrs)
62	Klein and Dichgans[31] 1969	F	5	6	1
63	Klein and Dichgans[31] 1969	F	13	13	< 1
64	Suzuki and Rapin[50] '69	F	0.3	3.6	< 3
65	Jellinger and Seitelberger[30] 1970	F	0.6	7.6	7
66	Jellinger and Seitelberger[30] 1970	F	1	6.3	5
67	Jellinger and Seitelberger[30] 1970	M	2.4	2.10	< 1
68	Bruno[9] 1971	F	9	14	5
69	Sandbank and Lerman[45] 1972	F	0.1	1.3	1
70	Sandbank and Lerman[45] 1972	M	0.1	0.6	< 1
71	Sandbank and Lerman[45] 1972	M	0.1	1.6	1
72	Hopkins and Turner[25] 1973	F	1	5.1	4
73	Guidugli et al[22] '73	M	8	9	1
74	Skullerud et al[47] '73	F	0	0.10	< 1
75	Janota[29] 1974	M	0.9	1.4	< 1
76	Janota[29] 1974	M	0.1	1.3	1
77	Janota[29] 1974	M	2.11	3.5	< 1
78	Shapira et al[46] 1975	F	4	16	12
79	Shapira et al[46] 1975	M	3	10	7
80	Huttenlocher et al[26] 1976	F	2.6	3	< 1
81	Huttenlocher et al[26] 1976	M	2	2.1	< 1
82	Huttenlocher et al[26] 1976	F	2	2.3	< 1
83	Tommasi et al[51]/ Partouche[40] 1977	M	0.2	3.6	3
84	Tommasi et al[51]/ Partouche[40] 1977	F	0.3	3.7	3
85	Hart et al[23] 1977	F	7	18	11
86	Hart et al[23] 1977	M	9	?	?

disorders of the central nervous system;[7,24,41,55,8,57] progressive poliodystrophy is classified as a degeneration of cerebral gray matter of unknown etiology and without specific cause. In the Handbook of Clinical Neurology[53] the disease is not presented as a separate entity, but is considered as part of the entity of "diffuse cerebral degeneration".
The ambiguity which exists in the classification of this disorder is likewise found in the nomenclature. Most often a disorder of the cerebral gray matter is suggested: "diffuse degeneration of cerebral gray matter",[2,3,19] "diffuse cortical sclerosis",[20] "poliodystrophia cerebri progressiva infantilis".[10] In other publications a more general nomenclature was chosen: "spongy glioneuronal dystrophy",[30,32] "diffuse cerebral degeneration in infancy",[6,26] "progressive cerebral poliodystrophy".[21]

THE CLINICAL MANIFESTATIONS OF PROGRESSIVE POLIODYSTROPHY

CLINICAL SIGNS

The clinical manifestations of progressive poliodystrophy are not highly specific. The clinical signs are comparable to those in most of the disorders of cerebral gray matter.
The prominent signs are psychomotor retardation, epileptic manifestations, myoclonus and severe motor disability. Ophthalmologic abnormalities, deafness and neurovegetative disorders are also frequently observed.
In general, the prenatal, natal and postnatal histories are described as unremarkable. In many cases the first manifestation of the disorder occurred rather suddenly in early life. Epileptic manifestations were frequently the first clinical sign. Major epilepsy was observed, as were partial seizures in various appearances. Myoclonus seems to be a separate main sympton of the disease. Myoclonic jerks frequently occurred independent of the epileptic manifestations. Progressive mental deterioration was observed in all cases. Sometimes the initial development of the children was quite normal. However, in most children developmental retardation in early life was characteristic. Most children had severe motor disability. Spasticity and hypotonia were present. In many cases exaggeration of the deep reflexes was observed, the plantar responses often became extensor. Less frequently ataxia and extrapyramidal symptoms were noted. Various other abnormalities were also seen: ophthalmologic manifestations (i.e. optic atrophy, blindness, retinitis pigmentosa, impairment of ocular movement, and sometimes hemianopia), deafness, and defects of neurovegetative regulation (e.g. gastro-intestinal problems such as vomiting, diarrhoea, constipation).

Many authors[6,11,13,16,26,29,30,32,34,39,44,45,46,48,60] indicated that the first symptoms of the disease were seen after periods of psychic or physical stress (infections, exertion, etc.). Such situations also seemed to provoke relapses of the disorder. Klein and Dichgans[32] formed the hypothesis that a genetic predisposition towards the disorder would be manifest after some illnesses. Although this phenomenon appears to be an important feature in progressive poliodystrophy, deterioration after stressful situations was mentioned in only some of the cases. However, because such a feature is not always requested in a general anamnesis, presumably not all investigators noticed this phenomenon. Provocation of the symptoms of progressive poliodystrophy after stress was mentioned several times in the studies dealing with aspects of mitochondrial dysfunction in the disorder, namely three times after a respiratory infection,[45,46] and in two cases after exercise.[46]

HEREDITY

Progressive poliodystrophy is generally inherited as an autosomal recessive trait. In McKusick's "Mendelian Inheritance in Man"[38] the disorder is mentioned as *20370.

In the literature 43 cases have a positive family history, in the other 43 cases no family data were reported. In 12 cases only one single sib of a family was described; in these descriptions there was merely a reference to other patients within the same family. The other 31 patients with positive family-history belonged to 15 families, in which every time two or three cases were reported. In one case, twins (two boys; whether they were identical twins was not stated) suffered from the disorder.[11] No family was described with patients in more than one generation.

It is not possible to quote the number of normal sibs in the families with more than one case; in general only the affected sibs were mentioned. In the group of patients the distribution of sexes is almost equal: 47 girls and 39 boys.

These data on the family histories of patients, and on the distribution of sexes are in accordance with an autosomal recessive inheritance pattern.

AGE DISTRIBUTION

Within the group of patients there is a marked variation in the age distribution of the children. Ford[19] was the first to draw attention to the age distribution of the children suffering from progressive poliodystrophy. Describing a group of 8 patients he distinguished between an infantile and a juvenile expression. According to Ford the infantile form manifests in early

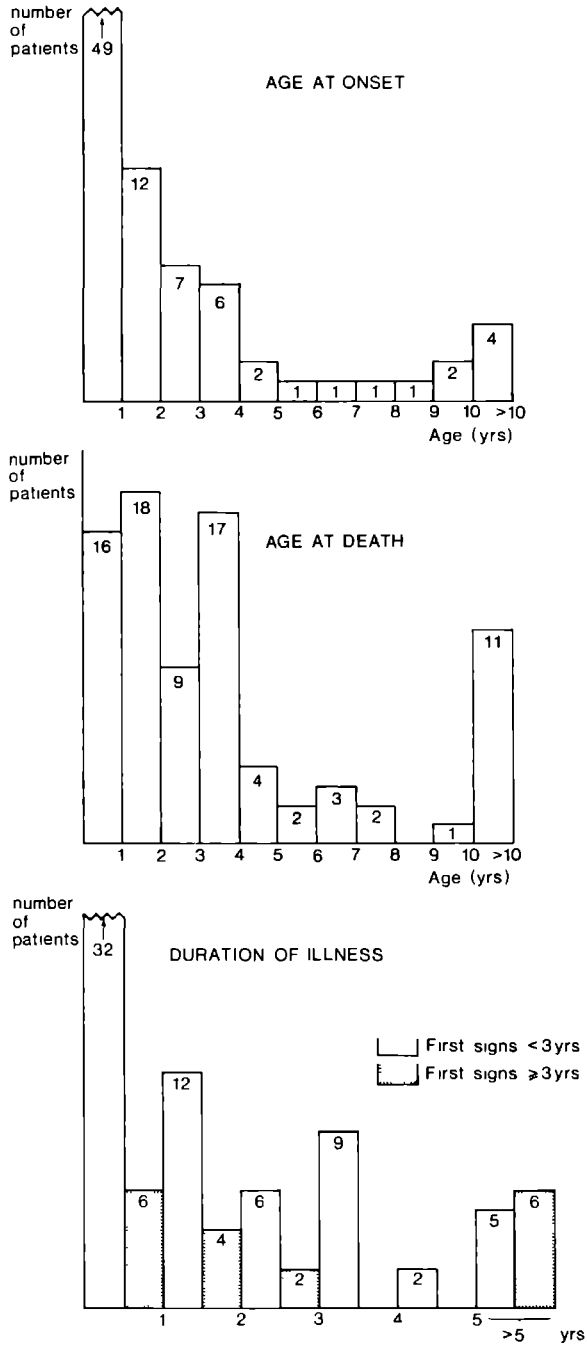

Figure 1. Age at onset, age at death, and duration of illness in patients with progressive pollodystrophy in the literature.

childhood and has a very rapidly progressive course. Most of these children die within the first year of life, but some children survive for two or three years. The juvenile form is characterized by a manifestation of the first symptoms at about five years; in the patients described by Ford himself the age varied between three and six years. This juvenile form of the disease has a less progressive course; the children mentioned by Ford died at between 6 and 19 years of age.

These two groups can also be distinguished in the literature on progressive poliodystrophy (table 1). However, the separation between the two is not as distinct as in the patients reported by Ford. In 68 of the 86 children described in the literature the first symptoms of the disease were reported before 3 years of age. 43 of them expired before the age of 3 years, 18 between 3 and 6 years, and 5 of them were over 6 years old when they died. In two cases no age of death was mentioned. From these data the large variation in the duration of the disease is also apparent; in 16 children of the infantile group the duration of the disease was more than 3 years (figure 1). In the remaining 18 of 86 children, the first symptoms occurred between 3 and 14 years of age. The duration of the disease in this group was less than one year in 6 of the children, between 1 and 3 years in another 6, and over 3 years (up to more than 10 years) in still another 6 children (figure 1).

HISTOPATHOLOGIC STUDIES

CENTRAL NERVOUS SYSTEM

Macroscopic studies. Macroscopic studies of the central nervous system in progressive poliodystrophy showed in general slight or moderate alterations. Most conspicuous findings were disseminated foci of cerebral cortical atrophy (e.g.[19,30,60]). Occasionally a slight to severe micrencephaly was reported (e.g.[34,44,47,52]). Frequently dilatation of cerebral ventricles was observed (e.g.[11,17,36]). A number of authors mentioned the spongy aspect of the cerebral cortex (e.g.[60]). In several case reports the hippocampal regio was explicitly described as normal.[11,16,23,30,45,46,47,50]

Cerebral white matter showed a fairly normal aspect in most cases reported; a firm consistency was mentioned several times (e.g.[6]). The cerebellum often revealed a picture identical to that of the cerebral hemispheres. Abnormalities in brain stem structures and spinal cord were seldom recorded.

Microscopic studies. The characteristic signs of progressive poliodystrophy

are seen on microscopic examination. Most abnormalities are localized in cerebral gray matter. In most cases severe neuronal loss was found throughout the cerebral cortex, together with increase of astrocytes. In many cases the neuronal loss was most prominent in the third to fifth cortical layers. In several patients loss of the usual cortical cyto-architecture was mentioned (most authors mentioned focal or generalized destruction of cortical structures, e.g.[11,19,45,47]). Early changes reported were of a spongy loosening of the neuropil with large perineuronal vacuoles and hydropic swelling of the astrocytes.[30,32] Often dilatation and proliferation of capillaries was described. Occasionally neuronal degeneration was observed, with neuronal swelling, chromatolysis, and loss of Nissl substance.[2,35] In later stages loss of neurons with an increase in the number of astrocytes was observed. Finally, a so-called "spongy state" (status spongiosus) was seen, ensuing from an almost complete disappearance of neuronal elements from the residual glial network.[19,29,46] Inflammatory lesions were not reported in any of the descriptions. Inclusion bodies were lacking.

The above mentioned changes were described as most pronounced in the cerebral cortex, they were also common in the cerebellum, thalamus and striatum. It was also repeatedly stressed that none or only a few abnormalities were seen in the hippocampus. In the cerebellum there was frequently loss of Purkinje cells, and a marked diminution of the granular cell layer, with a proliferation of the glia (Golgi astrocytes, Bergmann glia).[6,30,32] In some cases alterations identical to those in the cortex were observed in the globus pallidus and in the brain stem nuclei. Studies of the spinal cord were rarely reported; no major abnormalities were indicated.[40,47]

The cerebral white matter mostly showed no obvious abnormalities. In some descriptions a spongy state was recognized in the subcortical and subependymal areas.[19,30,44]

Electron microscopic studies. Electron microscopic studies on central nervous system tissue from patients with progressive poliodystrophy were reported by Suzuki and Rapin[50] and by Sandbank and Lerman.[45] Suzuki and Rapin[50] described enlarged mitochondria recognizable in the neuronal perikarya and axons. Sandbank and Lerman[45] found large disorganized perinuclear mitochondria in the neurons of cerebral tissue obtained by biopsy. Two types of abnormal mitochondria were described in this report. The first type had a variable diameter up to 3 μm and showed very short cristae with intact outer and inner membranes. The second type was of normal size and contained irregular electron-dense particles within its matrix, adhering to the inner membrane or to the cristae.

LIVER

Abnormalities in liver tissue were reported in a relatively large number of children with progressive poliodystrophy. Huttenlocher et al[26] described four patients with a progressive poliodystrophy associated with liver tissue abnormalities. They also mentioned four cases recorded earlier by Blackwood et al[6] and by Wefring and Lamvik.[58] Studies of liver tissue in these patients showed fairly similar abnormalities: in all the children accumulation of fat in parenchymal cells with vacuolation of these cells was observed. Moreover, signs of cirrhosis or of subacute hepatitis were seen. In all these cases hepatic disease became manifest only late in the course of the disease, and had a subacute or chronic rather than an acute course. The clinical and laboratory findings in these children were inconsistent with any of the recognized illnesses that affect both liver and brain. In all cases the cerebral abnormalities were consistent with progressive poliodystrophy.

In the cases published after 1976 no abnormalities in liver tissue were described, except in the patient reported by Partouche.[40] At autopsy steatosis was found in this child. It is noteworthy, however, that in this case a liver tissue specimen obtained by biopsy at the age of 12 months showed a normal aspect.

SKELETAL MUSCLE

Histopathologic studies of skeletal muscle tissue were reported only by a few authors. Janota[29] described abnormalities similar to those seen in a neurogenic muscle atrophy, specifically in the distal muscles of the limbs. An extensive histopathologic report of muscle tissue was made by Shapira et al[46] and by Hart et al.[23] In both muscle biopsies a "ragged red" appearance of the muscle fibers was seen. Electron microscopic studies showed aggregates of mitochondria of variable size in the subsarcolemmal areas. Some large mitochondria contained paracrystalline bodies.

The studies of Suzuki and Rapin,[50] of Sandbank and Lerman[45] and of Partouche,[40] describing some other children with progressive poliodystrophy associated with mitochondrial abnormalities, did not report histopathologic studies on muscle tissue.

ROENTGENOLOGIC STUDIES

The results of cerebral arteriography and of pneumoencephalography are of particular relevance to progressive poliodystrophy. These studies were only rarely mentioned in the literature. Cerebral arteriography revealed no major abnormalities. Central and cortical atrophy demonstrated by pneumoencephalography was reported in several studies (e.g.[11,34,40,46]). Hart et al[23] demonstrated by computed tomography (CT) scanning a cortical atrophy with ventricular dilatation in one patient.

ELECTROPHYSIOLOGIC STUDIES

Nearly all authors presented electroencephalographic studies. In most cases the EEG was abnormal. These abnormalities can be divided in two groups:

a. In the EEG's of many children slow activity, especially delta waves, was present to a greater degree than normal. The pathologic delta-waves were diffusely spread,[9,25] or with some preferential localization.[29,32,58]

b. In the EEG's of many children focal[46,50] or generalized irritative activity was observed; typical epileptic phenomena were also frequently seen.[6,34,46] The epileptic manifestations had in some cases the aspect of a hypsarythmia.[26,30,40,46]

Electromyographic and electroneurographic studies were reported in only a few cases; no abnormal findings were described. In the literature on progressive poliodystrophy, evoked potentials were not recorded in any of the studies.

BIOCHEMICAL STUDIES

LABORATORY INVESTIGATIONS

A survey of the literature on progressive poliodystrophy with respect to the routine laboratory investigations revealed no obvious abnormalities.
Some specific laboratory investigations however are worth mentioning sepa-

rately. Studies on the metabolism of amino acids, organic acids or fatty acids, on endocrinopathies, on chronic infectious diseases, on intoxications or on vitamin deficiencies showed no consistent abnormalities. In several reports liver function tests were abnormal; these deviant values probably reflected the histopathologic abnormalities in liver tissue described in the literature.[26] In several cases studies on cerebrospinal fluid (CSF) presented aspecific abnormalities, such as a slightly or moderately elevated total protein content (up to 5000 mg/1),[6,26,29] and in some cases an increased cell count.[11,29,32]

STUDIES OF CARBOHYDRATE METABOLISM

Only five publications mentioned values of lactate and of pyruvate in serum, urine and/or in CSF in patients with progressive poliodystrophy, but the registered abnormalities were quite remarkable. In general, the levels of lactate and pyruvate provide information on the enzymatic processes in which pyruvate is converted into CO_2 and H_2O by the citric acid cycle and respiratory chain (electron transport chain).

Elevated levels of lactate and pyruvate in serum and/or in CSF were reported by Sandbank and Lerman,[45] by Shapira et al,[46] by Hart et al[23] and by Partouche.[40] Hopkins and Turner[25] found normal values of lactate and pyruvate in the serum of a patient with progressive poliodystrophy. Suzuki and Rapin,[50] reporting on morphologically abnormal mitochondria in brain tissue, did not mention values of lactate and pyruvate in serum or in CSF. Shapira et al[46] reported increased levels of lactate and pyruvate in a 24-hour urine specimen.

Partouche[40] performed an intravenous lactate load on his patient; this test showed an increased rise of lactate and a delayed fall of the lactate levels, compared with controls.

ENZYMATIC STUDIES

With the exception of Partouche's study,[40] no other literature reports mentioned enzymatic studies in tissues of patients with progressive poliodystrophy. Tommasi et al [51] and Partouche[40] reported on a boy with a progressive neurologic disorder from his 8th week of life until his death at 42 weeks. Necropsy showed the characteristics of progressive poliodystrophy. Laboratory studies revealed normal values of citric acid cycle intermediates in serum and of amino acids in urine. Serum levels of lactate and pyruvate however, were increased. Enzymatic studies which were performed only on liver tissue, obtained at autopsy approximately 20 minutes after death, showed a decrea-

sed activity of pyruvate carboxylase due to a decreased affinity of the enzyme for pyruvate.
Another study in the literature which is worth mentioning is that of Strömme et al,[49] reporting a child with a deficiency of the pyruvate dehydrogenase complex (PDHC). At autopsy a marked communicating hydrocephalus was found with grossly reduced gray and white matter, indicating local but extensive destruction of brain tissue. These findings are possibly compatible with progressive poliodystrophy.

DISCUSSION OF LITERATURE DATA

PROGRESSIVE POLIODYSTROPHY, A NOSOLOGIC ENTITY?

There appears to be no unanimity about progressive poliodystrophy and its pathogenetic mechanism. The clinical signs which appear in progressive poliodystrophy are also seen in other progressive neurologic disorders of childhood. The cellular aberrations observed in progressive poliodystrophy are also frequently seen after periods of cerebral anoxia. The "Spongy Degeneration of the Central Nervous System" (Van Bogaert and Bertrand type; Canavan's Disease) is also characterized by a spongy state of the cerebral cortex. Van Bogaert, describing the "Spongy Degeneration of the Brain" in the Handbook of Clinical Neurology[53] referred to "Progressive Poliodystrophy" as a rarely occurring special form of the "Spongy Degeneration of the Brain".
The specific question whether progressive poliodystrophy is due to cerebral anoxia or must be regarded as a nosologic entity regularly recurs in discussions on this disorder.
In a survey, Alpers[3] stated that the presence of the well known pathologic features is required as a criterion for correct diagnosis. In the patients described by Alpers[3] as authentic cases no cerebral anoxia was mentioned in the case histories.
Greenhouse and Neubuerger[21] reviewed about forty cases of "degeneration of cerebral gray matter" of unknown etiology. They stressed that the variety of signs and symptoms makes it impossible to delineate a clear clinical picture of the disorder. Moreover, they draw attention to the similarity of pathologic features in cerebral anoxia and in the patients described as suffering from progressive poliodystrophy. They therefore argued that the disorder may well be a response by cerebral gray matter to various noxious influences, and that the occurrence of the etiologic factors of the disorder would be extre-

mely variable. They pointed to cerebral anoxia as the major etiologic factor, appearing as a result of perinatal complications, of epileptic manifestations, or of other stressful events. In their survey, Greenhouse and Neubuerger stated that in the majority of cases a period of cerebral anoxia can be demonstrated, and that, if this is not reported, the case history may be incomplete. It is therefore their conviction that progressive poliodystrophy is always due to cerebral anoxia and that the disorder is not a nosologic entity in the true sense.

A middle course in this discussion was adopted by Jellinger and Seitelberger.[30] They agree with Greenhouse and Neubuerger that in a considerable number of cases progressive poliodystrophy is the result of various pathologic situations, chiefly of cerebral anoxia. These authors argued that in two-third of the cases the disease is a symptomatic manifestation; in the history of these cases there was sufficient evidence for several etiologic factors, e.g. perinatal anoxia, repeated epileptic seizures, or infectious diseases. However, in the remaining one-third of the cases they found no principal etiologic factor. Jellinger and Seitelberger therefore suggested that these cases are an "idiopathic form" of progressive poliodystrophy. Another important argument put forward by Jellinger and Seitelberger was that in a number of cases several patients were reported within one family. This familial occurrence of progressive poliodystrophy suggests that there is a hereditary predisposition, as was stressed earlier by Klein and Dichgans.[32] Regarding the nature and distribution of the abnormalities in cerebral tissue Jellinger and Seitelberger proposed that the neuronal degeneration in cerebral gray matter could be due to a hereditary metabolic dysfunction.

The frequently described familial occurrence of progressive poliodystrophy lends particular support to Jellinger and Seitelberger's argument that there is an "idiopathic form" of progressive poliodystrophy, this disorder being a nosologic entity.

SUMMARY OF LITERATURE DATA

Progressive poliodystrophy is a neurologic disorder of childhood with an autosomal recessive inheritance. The major characteristics are mental retardation or deterioration, epileptic manifestations, myoclonic jerks, and motor disability. Visual and auditive dysfunctions were also frequently observed; moreover, neurovegetative signs were described several times. In a considerable number of patients the first symptoms appeared in a period of exertion. These clinical signs do not point to a specific etiologic factor, but broadly indicate a dysfunction of the cerebral gray matter.

With respect to the age distribution of patients described in the literature a

distinction must be made between an infantile and a juvenile form of progressive poliodystrophy, as was suggested by Ford.[19] In the first group the disorder becomes manifest in very early life, the children dying before the age of 4 years. In the juvenile form the first symptoms generally occur at the age of about 5 years or later; the disease in this group has a less rapidly progressive course, the children dying after a variable interval, sometimes in adolescence or young adulthood.

The definite diagnosis of progressive poliodystrophy can only be made by microscopic study of brain tissue, at autopsy or in a brain biopsy specimen. The disorder is characterized morphologically by degeneration and loss of neurons, proliferation of glial elements and dilatation and proliferation of capillaries in cerebral gray matter. Similar abnormalities were frequently seen in the cerebellum and in the basal nuclei. The cerebral white matter had a normal aspect; in some cases hypomyelination was observed.

Neuroradiologic investigations revealed uncharacteristic pictures. Electroencephalographic studies frequently showed a disturbed cerebral function. Two different features were reported from the EEG: diffuse slowing (delta waves) of the background rhythm, and focal or generalized irritative or epileptic activity. It might be expected that evoked potentials (particularly the cortical responses) would also be disturbed; however, in the literature on progressive poliodystrophy there was no report of evoked potentials.

Routine laboratory studies and studies on inborn errors of metabolism showed no remarkable abnormalities. In some patients abnormal liver function tests were seen.

In several patients specific studies of lactate and pyruvate were performed in serum and in CSF. In most of these cases increased levels of lactate and pyruvate were measured.

Microscopic studies of liver tissue revealed slight to moderate signs of parenchymal fatty degeneration. However, there is no other resemblance to Reye's syndrome, a hepatocerebral degeneration with much more severe abnormalities in liver tissue.[4,42]

Histochemical and submicroscopic studies of skeletal muscle tissue in patients with progressive poliodystrophy showed abnormalities in mitochondria in several cases.

The findings of elevated levels of lactate and pyruvate in serum and in CSF on the one hand, and the morphologic abnormalities in muscle tissue mitochondria and incidentally in neuronal mitochondria on the other, may point to a defect in the intramitochondrial pyruvate metabolism. In the literature such a possibility was suggested by Shapira et al[46] and by Hart et al.[23] Partouche[40] presented a patient with progressive poliodystrophy in combination with a pyruvate carboxylase deficiency in liver tissue obtained at autopsy.

These findings may indicate that the association of progressive poliodystrophy and a defective pyruvate metabolism is more than a mere coincidence. Therefore, when searching for pathogenetic factors in progressive poliodystrophy it seems worthwhile to direct attention to abnormalities in pyruvate metabolism. In chapter 2 a summary of cerebral carbohydrate metabolism will be presented together with an introduction to our studies.

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CHAPTER 2

MAIN CHARACTERISTICS OF THE CEREBRAL CARBOHYDRATE METABOLISM

In chapter 1 it was concluded that it would be worthwhile investigating pyruvate metabolism in children suspected of progressive poliodystrophy. Chapter 2 will present an outline of the cerebral carbohydrate metabolism and a survey of neurologic disorders associated with disturbances of pyruvate metabolism. In conclusion, chapters 3-6 will be introduced in a short paragraph; in these chapters our patient studies will be presented.
The carbohydrate metabolism is particularly important for a properly functioning central nervous system. Under normal conditions, the quantitatively most important substrate of cerebral energy metabolism is glucose. A transient decline in the oxidative metabolism of glucose may lead to an abrupt disruption of brain function.
When glucose falls below need, primarily glycogen is degraded to glucose in glycogenolysis; thus glycogen acts as a carbohydrate reserve. Glucose can also be formed in gluconeogenesis. Glycogenolysis and gluconeogenesis do not occur in brain tissue. Substrates for cerebral energy metabolism in circumstances of glucose-shortage are the ketone bodies (β-hydroxybutyrate and acetoacetate). It is possible that lactate can also be used as a substrate in cerebral energy metabolism.[10,15,28,33]

SOME BIOCHEMICAL ASPECTS OF CARBOHYDRATE METABOLISM

Pyruvate has a central position in carbohydrate metabolism. Carbohydrate metabolism can be divided into a number of main pathways: glycogen synthesis and glycogenolysis, glycolysis (Embden-Meyerhof pathway) and gluconeogenesis. Figure 1 gives a general survey of the main metabolic pathways of carbohydrate metabolism.
The main site of glycogen synthesis and glycogenolysis is the liver. Gluconeogenesis occurs predominantly in the liver and the kidney. Glycolysis is the only pathway of carbohydrate metabolism occurring in brain. In this pathway a molecule of glucose is broken down into two molecules of pyruvate or lactate.
In the citric acid cycle (Krebs cycle) acetyl-CoA, derived from pyruvate, fatty acids or amino acids, as well as citric acid cycle intermediates derived from amino acids, can be oxidized to CO_2 (figure 2). Reducing equivalents are made available for ATP synthesis in the electron transport chain (respiratory chain) (figure 3).
Pyruvate oxidation occurs in the mitochondria via the pyruvate dehydrogenase complex. The acetyl-CoA formed is further oxidized in the citric acid cycle and the respiratory chain.

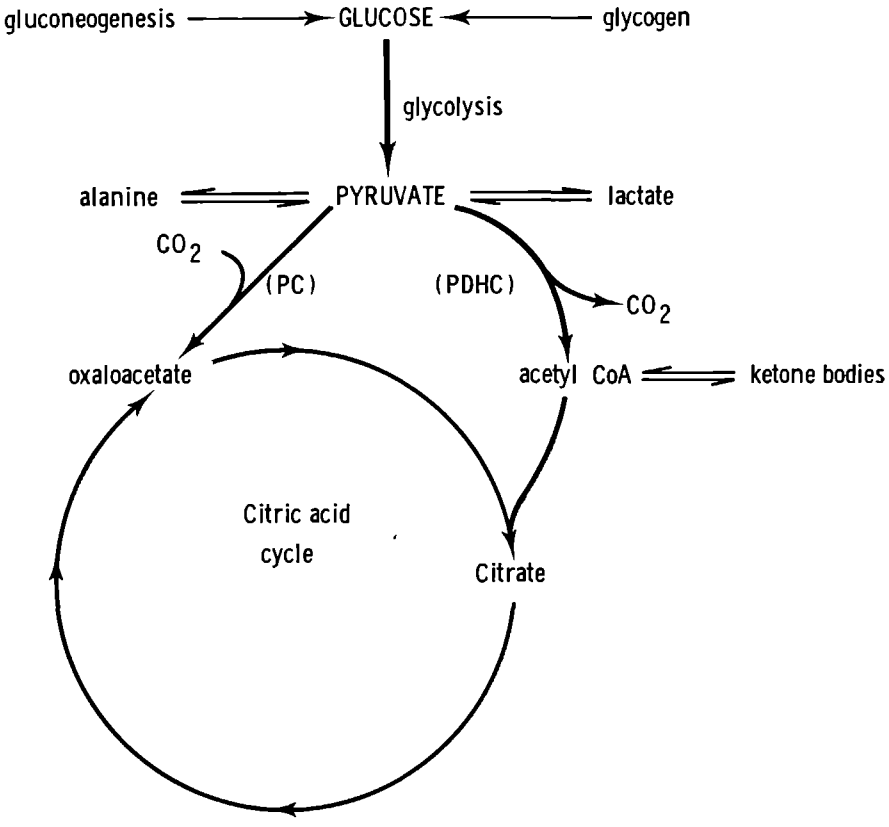

Figure 1. The main metabolic pathways of carbohydrate metabolism.

Two important intramitochondrial enzyme systems in pyruvate metabolism are the pyruvate dehydrogenase complex (PDHC) and pyruvate carboxylase (PC). PDHC catalyzes in the presence of lipoic acid, thiamine pyrophosphate (TPP) and Mg^{2+} the reaction:

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

According to Blass[9] this reaction is normally the rate-limiting step for entry of pyruvate from glycolysis into the Krebs cycle. The PDHC consists of three catalytic enzymes: pyruvate dehydrogenase (E_1), dihydrolipoate transacetylase (E_2) and dihydrolipoate dehydrogenase (E_3), and of two regulating enzymes: pyruvate dehydrogenate phosphatase and pyruvate dehydrogenate kinase.

Pyruvate carboxylase is a biotin-dependent enzyme, catalyzing the reaction:

$$\text{pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i$$

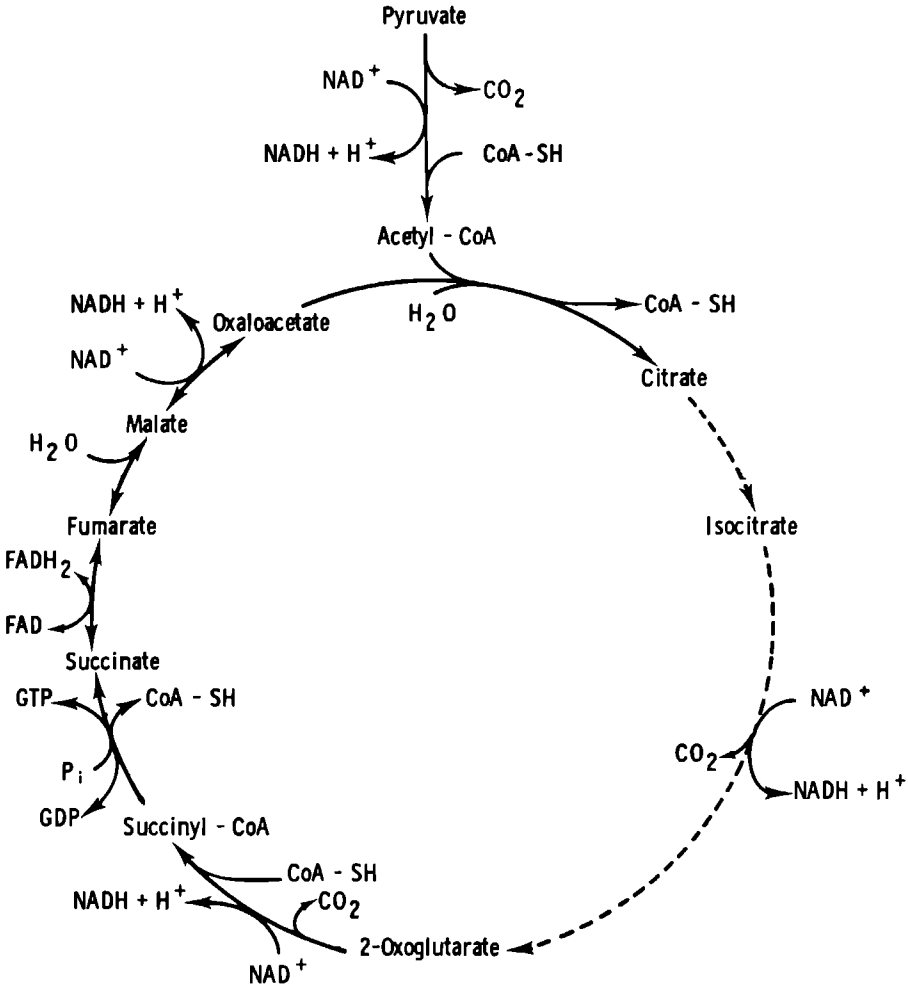

Figure 2. Citric acid cycle.

According to Blass[9] this reaction is probably the rate-limiting step in gluconeogenesis.

The PDHC and PC are of great importance in directing the pyruvate flow. In general, metabolic conditions which stimulate pyruvate carboxylation (e.g. oxidation of fatty acids) inhibit the oxidation of pyruvate. Disturbances in citric acid cycle activity may result in a decrease of the pyruvate oxidation rate, due to increase of the acetyl-CoA/CoA ratio, which inhibits the PDHC. Likewise, disturbances in the electron transport chain may impair or decrease pyruvate oxidation rate or citric acid cycle activity by feedback inhibition due to an increased $NADH/NAD^+$ ratio.[9,33]

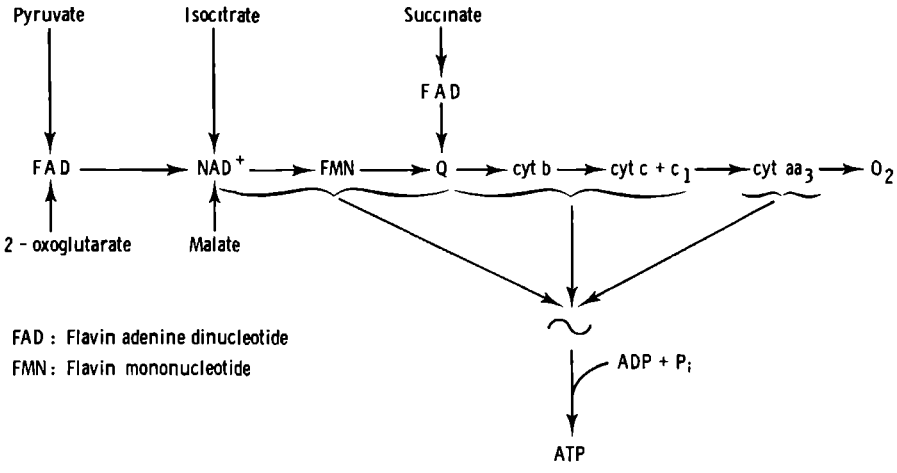

Figure 3. Electron transport chain.

As mentioned above, under normal conditions glucose is an almost indispensable substance for good brain function. Abnormalities of carbohydrate metabolism can reasonably be expected to impair the function of the central nervous system.[5]

PYRUVATE METABOLISM AND NEUROLOGIC DISORDERS

Several defects in pyruvate metabolism were reported in association with neurologic disorders. The most frequently reported defects are those of the pyruvate dehydrogenase complex (e.g.[3,8,14,21]), of pyruvate carboxylase (e.g.[1,11,19,24]) and of the cytochromes (e.g.[13,16,18,23,29,31,32]).

In the literature disturbances of the pyruvate metabolism were reported in several neurologic disorders with autosomal recessive inheritance. The first description of a neurologic disease associated with a defective pyruvate metabolism was the report of a 9-year-old boy with intermittent ataxia, occurring after nonspecific febrile illnesses;[3,4] a deficiency of pyruvate decarboxylase was demonstrated in muscle tissue. Since this report disturbances of pyruvate metabolism have been described in patients with various neurologic disorders, especially Subacute Necrotizing Encephalomyelopathy (Leigh syndrome) (e.g.[6,12,14,22]) and Friedreich's ataxia (e.g.[2,7,20]). Disturbances of pyruvate metabolism have also been demonstrated in hereditary motor and sensory neuropathy (HMNS type I, Charcot-Marie-Tooth disease),[34] in spinocerebellar degeneration[21] and in a progressive neurologic disorder charac-

Table 1. Biochemical and morphologic findings indicating a defective energy metabolism, as reported in the literature in five children with progressive poliodystrophy.

Case no*		64	69	78	83	86
Biochemical studies						
Lactate	serum	–	↑	↑	↑	↑
	CSF	–	–	↑	–	↑
Pyruvate	serum	–	N	↑	↑	N
	CSF	–	–	N	–	N
Ratio L/P	serum	–	↑	↑	↑	↑
	CSF	–	–	↑	–	↑
Morphologic studies						
Muscle		–	–	mitochondrial myopathy	–	mitochondrial myopathy
Liver		–	–	–	lipid storage	–
Brain		giant neuronal mitochondria	giant neuronal mitochondria	–	–	–

* Cases are numbered as in chapter 1, table 1
– = not mentioned

terized by diffuse hypomyelination.[1,26] Thus, disturbances of pyruvate metabolism have been demonstrated in several so-called degenerative neurologic disorders, with pathologic abnormalities in various neuronal systems with various localizations in the nervous system.

In chapter 1 several patients with progressive poliodystrophy were mentioned, in whom biochemical or morphologic studies pointed to a disturbed carbohydrate metabolism.[17,24,25,27,30] Table 1 summarizes these cases in the literature. Partouche[24] reported a child with progressive poliodystrophy and a defect in pyruvate carboxylase activity in liver tissue. This study will be limited to disturbances in pyruvate metabolism in progressive poliodystrophy (Alpers' disease).

INTRODUCTION TO OUR STUDIES

From the literature data one may expect a disturbance in the carbohydrate metabolism in patients with progressive poliodystrophy.[17,24,25,27] Extensive metabolic studies were therefore carried out on children suspected of this disorder. Chapters 3-6 contain the case reports of five children with progressive poliodystrophy. Detailed clinical, pathologic and biochemical investigations, performed on these children, are described in these chapters. Chapter 7 summarizes these data, and gives some correlations with the literature studies in chapters 1 and 2.

The pathologic and biochemical findings are discussed in chapter 3-6 in relation to the individual case reports, and in a general discussion in chapter 7. Further, some conclusions are formulated in chapter 7, and some suggestions for further investigations are made.

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CHAPTER 3

PYRUVATE DEHYDROGENASE DEFICIENCY RESTRICTED TO BRAIN

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ABSTRACT

We studied a child with a rapidly progressive neurologic disorder, with psychomotor retardation, hypotonia, seizures, and respiratory disturbances. Laboratory studies showed elevated levels of lactate and pyruvate in cerebrospinal fluid (CSF), without notable elevated levels in serum. In liver, muscle, leukocytes, and cultured fibroblasts we found no abnormality in pyruvate oxidation; biochemical studies of a brain biopsy showed an isolated deficiency of pyruvate dehydrogenase complex in brain tissue with the morphologic picture of progressive poliodystrophy with hypomyelination.

INTRODUCTION

The first description of a deficiency in the pyruvate dehydrogenase complex (PDHC) was published 10 years ago by Blass et al[1,2], and other cases were described later[3-16]. Blass[17] has reviewed the different clinical disorders associated with abnormal pyruvate metabolism (especially deficiencies of pyruvate carboxylase and the pyruvate dehydrogenase complex). If PDHC activity is less than 15% of normal, neurologic disorder is severe in early childhood, and characterized by severe psychomotor retardation, spasticity, hypotonia, ataxia, visual disturbances, lactate acidosis, and seizures. If PDHC activity is 20 to 35% of normal, the disease is less severe with the same symptoms, but manifests later and is more slowly progressive. Ataxia is often the most prominent symptom. A mild disease may be seen with enzymatic activity of 35 to 50% of normal; hereditary ataxias dominate this category.
We now describe an infant with PDHC deficiency in brain, but not in other tissues.

CASE REPORT

This girl was the first child of healthy, nonconsanguineous parents. Pregnancy was normal and delivery occurred without complications at term. The birth weight was 3000 gm; the Apgar score was 9 after 1 minute. In the first 2 weeks of life there was no overt abnormality. In the second month, she became hypotonic, with intermittent myoclonic contractions and nystagmoid ocular movements. At 8 months, she was admitted to the hospital for myoclonus status with slight fever and Cheyne-Stokes respiration. At 18 months, she was admitted to our department.
There was severe psychomotor retardaton with microcephaly, and she did not yet walk or talk. Length and weight were normal. Cheyne-Stokes respiration was frequently seen. Absences, tonic-clonic seizures, and generalized myoclonic contractions occurred often (in periods several times a day). She was extremely hypotonic and showed severe limb ataxia. Tendon reflexes were present and plantar responses were extensor. There were nystagmoid ocular movements; visual fixation did not seem possible. Fundoscopy was normal. She seemed to hear, and sensation appeared to be intact.

Radiograms of chest, skull, and hands were normal. The electroencephalogram (EEG) showed mild generalized slowing with no epileptic pattern. Electromyography and nerve conduction velocities were normal. Somatosensory potentials could not be elicited. Carotid and vertebral artery angiography were normal. Pneumoencephalography showed symmetric and slight enlargement of the lateral ventricles, with normal third and fourth ventricles. Computed tomography (CT) scanning was not performed.

ROUTINE AND SPECIFIC LABORATORY INVESTIGATIONS

Standard blood and urinary studies, including hematologic evaluation, renal and hepatic functions, serum protein, and serum protein electrophoresis were normal. Appropriate studies excluded mercury and lead intoxication; deficiencies of thiamine, pyridoxine, cobalamine, folate, and ascorbate; abnormalities of T_3, T_4, cortisone-rhythm, calcium, phosphorus, and oral glucose tolerance; and tuberculosis, toxoplasmosis, or syphilis.

Other normal studies included arterial blood pH, serum lipids, amino acids in plasma and urine, plasma short-chain fatty acids and organic acids, lysosomal enzymes, creatine phosphokinase, ammonia, magnesium, serum copper, ceruloplasmin, fructose, and galactose. Serum levels of lactate and pyruvate were in the upper-normal range or were slightly elevated (normal = 600 to 1200 μmol per liter and 75 to 120 μmol per liter, respectively; elevated = 800 to 1350 μmol per liter and 100 to 130 μmol per liter, respectively); 24-hour urinary excretion of lactate and alanine was normal.

Cerebrospinal fluid (CSF) cell count, protein content, protein electrophoresis and immune-electrophoresis, minerals, acetoacetate, and β-hydroxybutyrate were normal. Lactate and pyruvate levels in CSF were elevated proportionally several times (table 1). Therefore, the laboratory data showed an isolated increase of lactate and pyruvate in CSF with a normal ratio.

Suspecting a primary defect in pyruvate metabolism (figure 1), we had to

Table 1. Glucose, lactate, and pyruvate levels in cerebrospinal fluid (CSF) of patient and controls.

	Glucose (μmol/l)	Lactate (μmol/l)	Pyruvate (μmol/l)	Ratio Lactate/Pyruvate
Patient	3.5–3.7	2500–3000	191–204	13.1–14.7
Controls	3.5–4.5	1200–1600	90–130	11.0–14.5

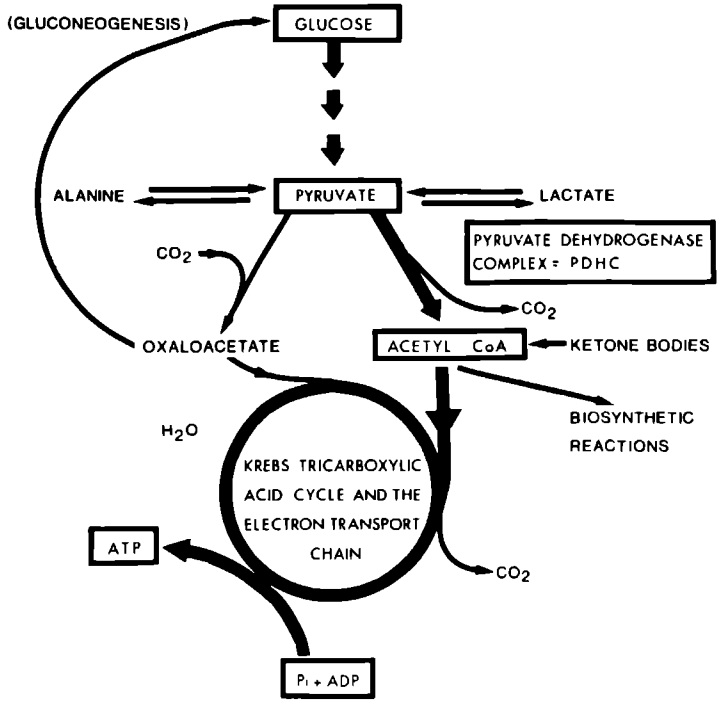

Figure 1. Cerebral oxidative metabolism.

distinguish between deficiencies of oxidation and carboxylation, and between the quantitatively severe and milder defects.

TISSUE STUDIES

HISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES

Histochemical and ultrastructural studies were performed on liver and muscle, both obtained by biopsy. No morphologic abnormalities were found.

BIOCHEMICAL STUDIES IN TISSUES

Enzymatic studies were performed in liver and muscle homogenates, whole white blood cells, and intact cultured fibroblasts. Pyruvate oxidation rate and

Table 2. $^{14}CO_2$ production from [1–^{14}C] pyruvate (PDHC activity) and from [2–^{14}C] pyruvate (activity of the citric acid cycle) in cultured fibroblasts, in leukocytes and in liver tissue of patient and controls.

	Substrate	Patient	Controls	
Fibroblasts	[1–^{14}C] pyruvate	66 7*	41 0 ± 14	(n=22)
	[2–^{14}C] pyruvate	21 0	20 7 ± 7	(n=14)
Leukocytes	[1–^{14}C] pyruvate	6 0*	2 8 ± 1 2	(n=17)
	[2–^{14}C] pyruvate	4 4	2 4 ± 1.0	(n=14)
Liver tissue	[1–^{14}C] pyruvate	42 0*	45 – 205	(n= 9)

* nmol/hr/mg protein
\+ nmol/hr/10^6 leukocytes

citric acid cycle activity were evaluated in intact fibroblasts and leukocytes by measuring $^{14}CO_2$-production from [1-^{14}C]pyruvate and [2-^{14}C]pyruvate, respectively, using a modification of the method of Blass et al[1] (see Willems[18]). Pyruvate oxidation rate in a liver tissue homogenate was studied by measuring $^{14}CO_2$-production from [1-^{14}C]pyruvate.[1,18] Pyruvate carboxylase activity was assayed in the same liver homogenate by the method of Utter et al[19]. Cytochrome *c* oxidase activity was measured in liver and muscle homogenate.[18 20]

Pyruvate oxidation rate and citric acid cycle activity were normal in leukocytes and cultured fibroblasts. In liver, pyruvate oxidation rate was slightly decreased, but within normal limits (table 2). Pyruvate carboxylase activity in liver was normal. Cytochrome *c* oxidase activity was normal in liver and muscle.

CEREBRAL BIOPSY

The biopsy was taken from the second convolution of the right frontal lobe. The specimen for biochemical studies was preserved in ice, and biochemical analysis was started immediately.

MORPHOLOGIC STUDIES

Microscopic, histochemical, and ultrastructural studies were performed in brain tissue.

In gray matter, microscopy showed foci of local degeneration and loss of neurons, most pronounced in the deeper cortical layers. Neurons showed loss

Figure 2. Horseshoe-shaped area around the base of a sulcus with degeneration and loss of neurons, with maximal intensity in the deeper parts of the gray matter. There is a sharp limit where gray matter passes into white matter. Hematoxylin and eosin, × 55.

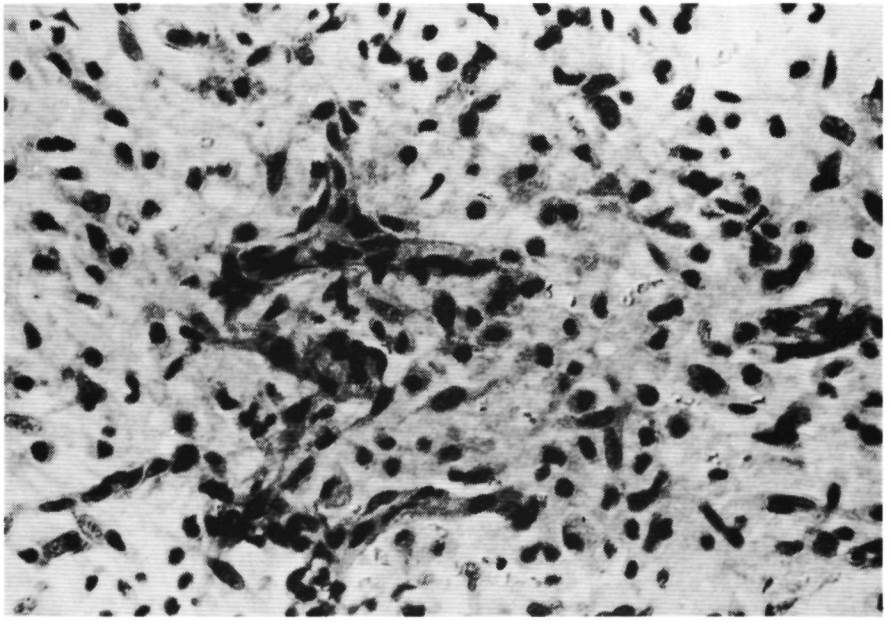

Figure 3. Vascular proliferation and reactive activity of astroglia and microglia in the area with degeneration and loss of neurons. Hematoxylin and eosin, × 550.

Table 3. $^{14}CO_2$ production (nmol/hr/mg protein) from [1–^{14}C] pyruvate in brain tissue, without malate (PDHC activity) and with malate (activity of the citric acid cycle), in our patient and eleven other patients undergoing a cerebral biopsy.

	Age (yrs, mos)	[1–^{14}C] pyruvate		[1–^{14}C] pyruvate + malate	
		Gray matter	White matter	Gray matter	White matter
Patient	1 6	7	6	12	7
J J	1 4	144	126	536	502
J P	1 5	75	68	85	86
M S	1 5	120	68	387	151
M P	1 8	201	103	725	–
A D	1 9	188	174	517	454
W A	2 0	153	118	415	297
W L	2 3	42	30	131	86
G G	5 6	54	31	–	–
E R	6 11	133	63	468	206
G Q	10 9	110	46	285	126
M H	15 9	220	54	568	156
Mean values		131 ± 59 (n=11)	80 ± 45 (n=11)	412 ± 199 (n=10)	229 ± 155 (n=9)

of tigroid substance, microvacuolization, and some lipid storage. There was a proliferation of astroglia, microglia, and blood vessels. In white matter, myelin and axons appeared qualitatively intact, although there were signs of hypomyelination. No active demyelination was seen. The process was classified as progressive infantile poliodystrophy (Alpers disease), associated with hypomyelination (figures 2 and 3).

BIOCHEMICAL STUDIES

Pyruvate oxidation in brain was measured in a homogenate by measuring $^{14}CO_2$ production, as above.[1 18] $^{14}CO_2$ production was measured with and without malate. Stimulating $^{14}CO_2$ production from [1-^{14}C]pyruvate with malate raises the activity of the citric acid cycle, but has no effect if PDHC-activity is impaired. Cerebral ketooxidation was measured by measuring the β-hydroxybutyrate production. Cytochrome *c* oxidase activity was measured in gray and white matter, as before.[18 20]

$^{14}CO_2$ production from [1-^{14}C] pyruvate with or without malate was severely decreased in both gray and white matter (table 3). Cerebral ketooxidation and cytochrome *c* oxidase activity were normal in both gray and white matter; the controls were children undergoing a cerebral biopsy with several neurologic disorders, as severe progressive oligophrenia, infantile Batten

Table 4. Effect of therapy with ketonemic diet, thiamine, lipoic acid, and aspartic acid on lactate and pyruvate in cerebrospinal fluid (CSF) of patient.

Therapy	Lactate (μmol/l)	Pyruvate (μmol/l)
Without therapy	2500–3000	191–204
Ketonemic diet	2480	160
Thiamine 1200 mg per day Lipoic acid 120 mg per day	2600	164
Aspartic acid 3000 mg per day	3060	180
Controls	1200–1600	90–130

disease, angiomatosis meningae, leukoencephalitis, Canavan disease, and Pelizaeus-Merzbacher disease.

EFFECTS OF THERAPY

First we used a ketogenic diet,[8] followed by lipoic acid (40 mg three times daily) in combination with thiamine (300 mg three times daily),[21-24] Finally, aspartic acid (1000 mg three times daily) was given, but there was no clinical or biochemical improvement (table 4).
Six months later, she suddenly became comatose and died; no permission was given for autopsy.

DISCUSSION

This 16-month-old infant suffered from a rapidly progressive disorder manifested by psychomotor retardation, microcephaly, hypotonia, respiratory disturbances, epileptic seizures, abnormal ocular movements, ataxia, and extensor plantar responses. Somatosensory evoked potential studies gave no response at all. Pneumoencephalography showed slight central cerebral atrophy. CSF lactate and pyruvate levels were increased, but there were only slightly increased levels of lactate and pyruvate in serum and urinary excretion of lactate, and alanine was normal. Acquired disorders of pyruvate metabolism with impaired cerebral oxidative metabolism (e.g., infections and vascular disorders) were excluded (normal angiograms and CSF values). Pyruvate oxidation and the activity of the citric acid cycle were normal in

Table 5. Previously reported patients with PDHC deficiency

Group I	Group II	Group III
Severe disease (10 patients)	Less severe disease (7 patients)	Mild disease (syndromes of spinocerebellar ataxia)
First symptoms in early infancy (0–6 months)	in early childhood (0–2 years)	variable (5–15 years)
Rapidly progressive	Slightly progressive	Slowly progressive
Severe mental retardation	Mental retardation	
Microcephaly	Microcephaly	
Ataxia	Ataxia	Ataxia
Hypotonia	Hypotonia	
Spasticity	Spasticity	
Optic atrophy	Optic atrophy	
Seizures	Seizures	
Respiratory distress	Respiratory distress	
Lactic acidosis		
serum CSF	serum CSF	
lactate ↑ 9/9 —	7/7 2/2	in some cases
pyruvate ↑ 7/7 —	6/6 2/2	elevated
alanine ↑ 5/5 1/1	2/2 1/1	in serum
PDHC deficiency demonstrated in:*		
cultured fibroblasts	cultured fibroblasts	cultured fibroblasts
leukocytes	leukocytes	leukocytes
liver tissue	liver tissue	
Autopsy		
poliodystrophy 1		
hypomyelination 2		
leigh's syndrome 1		

*Group I Cultured fibroblasts (seven patients), cultured fibroblasts and leukocytes (one patient), in one patient PDHC activity was decreased in liver tissue, but normal in cultured fibroblasts and leukocytes
Group II Cultured fibroblasts (six patients), cultured fibroblasts and liver tissue (one patient)
Group III Cultured fibroblasts in most cases, leukocytes in some cases, too

cultured fibroblasts and in leukocytes. In the liver, pyruvate oxidation was in the lower range of normality. In the brain, ketooxidation and cytochrome *c* oxidase activity were normal, but $^{14}CO_2$ production from [1-^{14}C] pyruvate (with or without malate) was severely decreased in both gray and white matter, suggesting lack of PDHC activity only in cerebral tissue, which is a pattern not previously described. Blass states that there is little evidence of isoenzymes of PDHC.[25] Therefore, the activity of PDHC in tissues easily obtained by biopsy (such as fibroblasts) should reflect PDHC activity in the brain. However, our case demonstrates a selective decrease of PDHC activity in the brain.

As in other tissues, hypoxia increases lactate production and concentration in brain. When a steady state exists, there is a diffusion equilibrium between CSF and cerebral tissue fluids.[26] Elevated lactate concentration in CSF with almost normal serum lactate content was also consistent with altered mitochondrial function in brain. It is uncertain if this abnormality in brain was the primary enzymatic deficiency or secondary to some other disorder.

Table 5 reviews patients with a proven PDHC deficiency with their main clinical, biochemical, and pathologic features. In general, a deficiency in the PDHC is demonstrated in cultured fibroblasts and/or in liver tissue, sometimes in leukocytes. The clinical, biochemical, and pathologic abnormalities of the previously described case conform to the "severe" category of PDHC deficiency. Blass[17] suggested that there is only one biochemical characteristic in these disorders: increased levels of lactate and pyruvate in blood and increased levels of alanine in blood and urine. In our patient, lactate and pyruvate levels were normal in blood, but increased in CSF. In similar diseases, CSF levels of lactate and pyruvate should be studied and included in future classification. The clinical signs in our case were similar to others attributed to deficiency of PDHC,[1-16] thiamine,[21-24] the respiratory chain,[17] or pyruvate carboxylase,[27] which all concern pyruvate metabolism. At autopsy, several pathologic entities can cause similar symptoms, including subacute necrotizing encephalomyelopathy (Leigh disease),[6, 9, 12, 22, 28, 29] progressive infantile poliodystrophy (Alpers disease),[30, 31] or extensive hypomyelination.[7] Pincus stressed respiratory symptoms as a key manifestation of Leigh disease,[22] occurring in 75% of the patients.

Respiratory abnormalities include dyspnea, tachypnea, and apnea – all signs of a brainstem disorder. Our patient had many respiratory problems and died because of respiratory insufficiency. Autopsy was not permitted, so we do not know the pathology in the brainstem. However, there was a poliodystrophy with hypomyelination in the cortical biopsy.

Therefore, a severe disturbance of the cerebral PDHC may be associated with several pathologic disorders, including subacute necrotizing encephalomyelopathy (Leigh disease), progressive infantile poliodystrophy (Alpers disease), and hypomyelination. Milder disorder of the cerebral PDHC may give rise to syndromes of spinocerebellar ataxia.

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CHAPTER 4

PROGRESSIVE INFANTILE POLIODYSTROPHY

ASSOCIATION WITH DISTURBED PYRUVATE OXIDATION IN MUSCLE AND LIVER

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ABSTRACT

Progressive infantile poliodystrophy (Alpers' disease) is associated with abnormalities in pyruvate metabolism or in cell mitochondria. A 3-year-old boy had a severe and rapidly progressive neurologic disorder characterized by psychomotor retardation, tetraparesis, ataxia, and myoclonic jerks, the illness being exacerbated during periods of infection. Lactate concentration in CSF was elevated. Histopathologic studies revealed lipid storage in liver and muscle. Autopsy showed a progressive infantile poliodystrophy. Mitochondrial abnormalities were found in heart muscle. Biochemical studies of muscle and liver tissue suggested a disturbance in nicotinamide adenine dinucleotide (reduced form) oxidation.

INTRODUCTION

Since the description of a mitochondrial myopathy by Luft et al,[1] many patients have been described as having structural or biochemical abnormalities in muscle mitochondria. Recently, Di Mauro et al[2] have stated that the definition of mitochondrial myopathies by morphologic or clinical criteria alone is inadequate. In their opinion, a rational classification should depend on the identification of the various biochemical abnormalities.

The combination of progressive infantile poliodystrophy (Alpers' disease) and a mitochondrial myopathy has been described by Shapira et al[3] and Hart et al,[4] but a biochemical defect was not identified in these cases. Tommasi et al[5] and David et al[6] reported a case of progressive infantile poliodystrophy in association with a deficiency of pyruvate carboxylase in liver.

We describe a patient with progressive infantile poliodystrophy, in whom pyruvate oxidation in muscle and liver was disturbed; a thorough biochemical and morphologic study of this case was possible.

REPORT OF A CASE

A boy, the first child of healthy, nonconsanguineous parents, was born after an uneventful pregnancy and delivery; birth weight was 2,750 g; the newborn child showed no abnormalities. There was slightly retarded motor and speech development. He became fatigued rapidly, and during infectious diseases ataxic gait was evident.

Examination at the age of 2½ years revealed a slight psychomotor retardation, a severe spastic paresis, and signs of ataxia. From the age of 3 years, febrile periods, sometimes with loss of consciousness, were increasingly frequent. Myoclonic contractions developed, and the boy's general condition gradually deteriorated. When 3½ years old, he was admitted to our department, following further deterioration of his condition.

The boy was quite pale, with extensive edema, most pronounced in face and neck. Head circumference (52 cm), height, and weight were normal. There were no cardiac or pulmonary abnormalities. He showed general hypotonia, tetraparesis, and hyperreflexia with extensor plantar responses. Mainly in the right arm and leg, myoclonic contractions were almost continuous. He failed to react to pain stimuli. Disturbances of ocular movements were not observed.

The child remained in a rather stable condition for about six weeks, with changing grades of consciousness, intermittent respiratory disturbances, and myoclonic contractions. Biopsies of liver, sural nerve, and soleus muscle were

performed in this period. Then hepatic insufficiency set in, attended by oliguria, jaundice, and Cheyne-Stokes respiration. His condition grew worse and he died, 3 years and 9 months old.

RADIOLOGIC AND ELECTROPHYSIOLOGIC EXAMINATIONS

Roentgenograms of chest, skull, and hands were normal. On cerebral computed tomography, the lateral ventricles were enlarged, whereas the fourth ventricle was normal. The EEG showed severe generalized slowing, with signs of dysfunction of the deep structures, and sometimes paroxysms with epileptic features. Somatosensory envoked potentials could not be elicited. Nerve conduction velocities and electromyograms were normal.

LABORATORY DATA

The following laboratory tests and data were normal: complete blood cell count and urinalysis, renal and hepatic function tests, serum electrolyte levels, serum protein content and electrophoretic pattern, serum lipid spectrum, serum pH, Pco_2 and bicarbonate, creatine kinase, ceruloplasmin, amino acid levels in plasma and urine, short-chain fatty acid and organic acid levels in urine, and lysosomal enzyme levels in leukocytes. Appropriate studies ruled out endocrinologic, immunologic, and chronic infectious diseases, deficiencies, and disorders caused by toxic agents.

Serum lactate and pyruvate levels were within normal ranges; 24-hour lactate excretion in urine was normal. The oral glucose tolerance test revealed a normal response of blood glucose with elevated levels of lactate (4,170 μmole/L; normal < 1,500 μmole/L) and pyruvate (200 μmole/L; normal < 95 μmole/L), both at 60 minutes, with a high lactate-pyruvate ratio (21; normal, 6 to 10). Studies on CSF showed normal WBC count, normal protein electrophoresis and immunoelectrophoresis, and normal concentrations of ketone bodies, but elevated total protein content (560 mg/L), lactate dehydrogenase level (75 units/L; normal, 10 to 25 units/L), and SGOT level (20 units/L; normal, 4 to 10 units/L). The CSF glucose and pyruvate levels were within normal ranges, but the level of lactate was elevated, ranging from 2,080 to 2,840 μmole/L (normal, 1,200 to 1,600 μmole/L), with an increased lactate-pyruvate ratio (19 to 23; normal, 11 to 15).

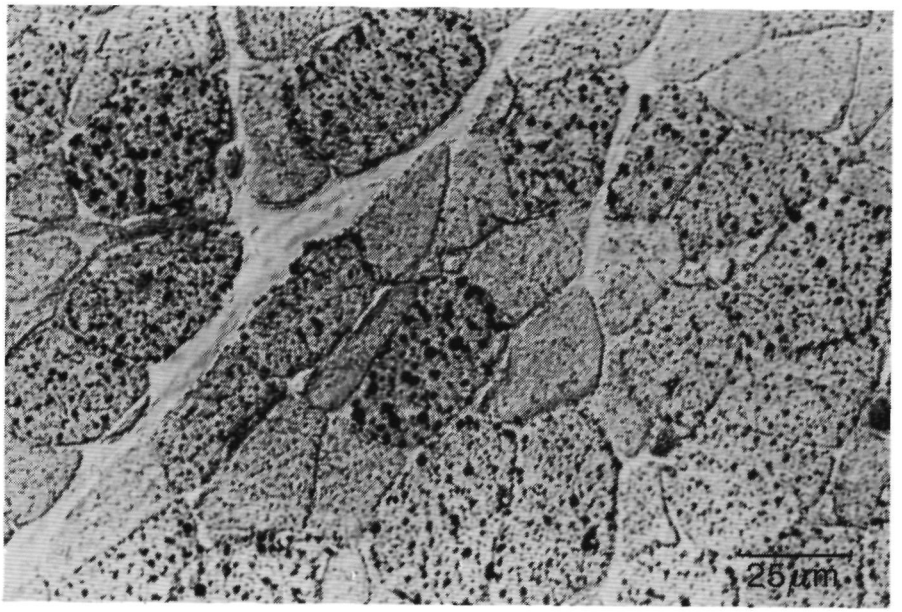

Figure 1. Transverse section of soleus muscle stained with Sudan black B. Large lipid droplets are seen in largest fibers (type I) (× 580).

HISTOPATHOLOGIC STUDIES

Necropsy disclosed cirrhosis and severe steatosis of the liver, congested kidneys, and signs of a subendocardial myocardial infarction. Specimens of skeletal muscle, peripheral nerve, and liver, obtained at biopsy, were available for histochemical and electron microscopic studies. Heart muscle and CNS tissue, obtained 12 hours post mortem, were studied by light and electron microscopy.

SKELETAL MUSCLE

Histochemical studies of the soleus muscle showed a decrease of type I fibers (47%; normal, 65%); Sudan black-positive droplets were seen, particularly in type I fibers (figure 1). In the trichromic-stained section, no ragged red fibers were seen. The nicotinamide adenine dinucleotide, reduced form (NADH) tetrazolium reductase reaction and the menadione-linked α-glycerol phosphate dehydrogenase reaction were qualitatively normal. Electron microscopy revealed a slight increase of lipid vacuoles among the myofibrils.

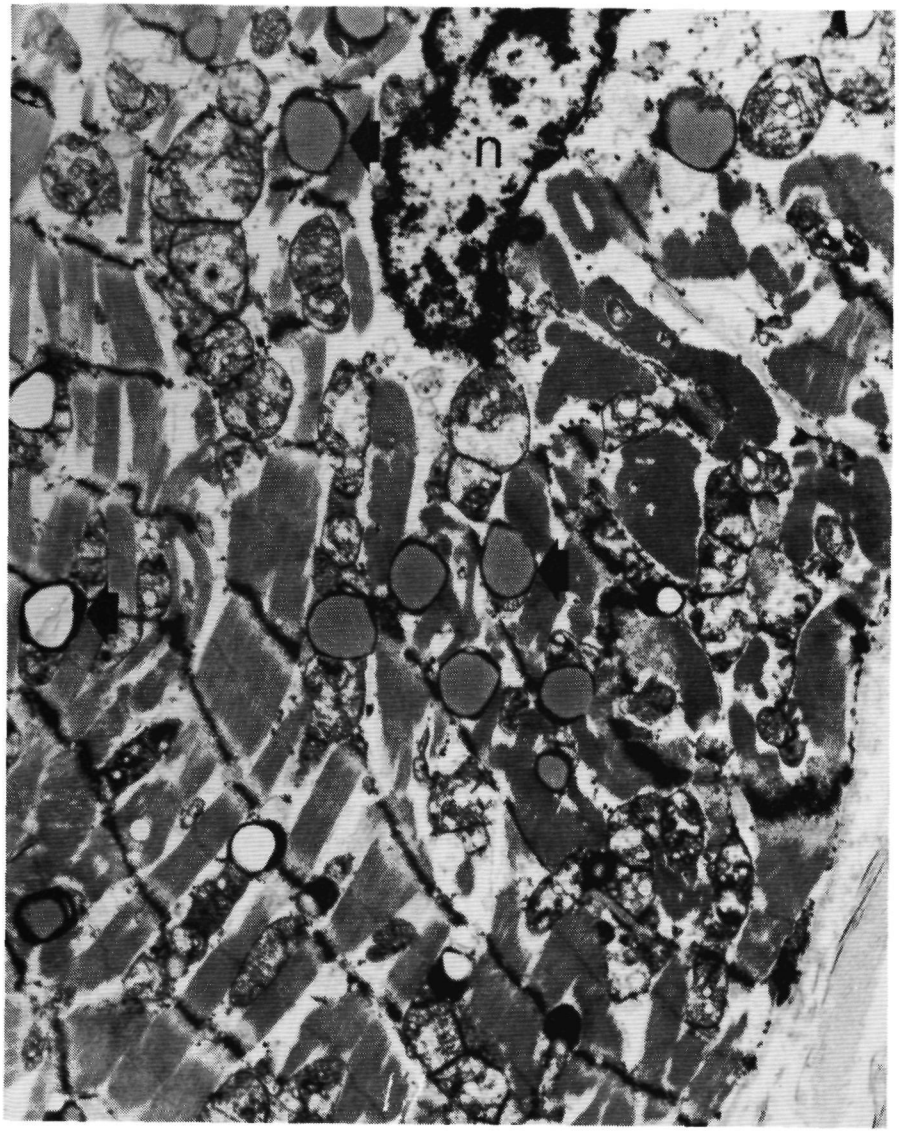

Figure 2. Electron micrograph showing part of heart muscle fibers. Note numerous lipid droplets, either electrodense or electrolucent (arrows). Most mitochondria have irregular, swollen cristae; n indicates nucleus (× 12,400).

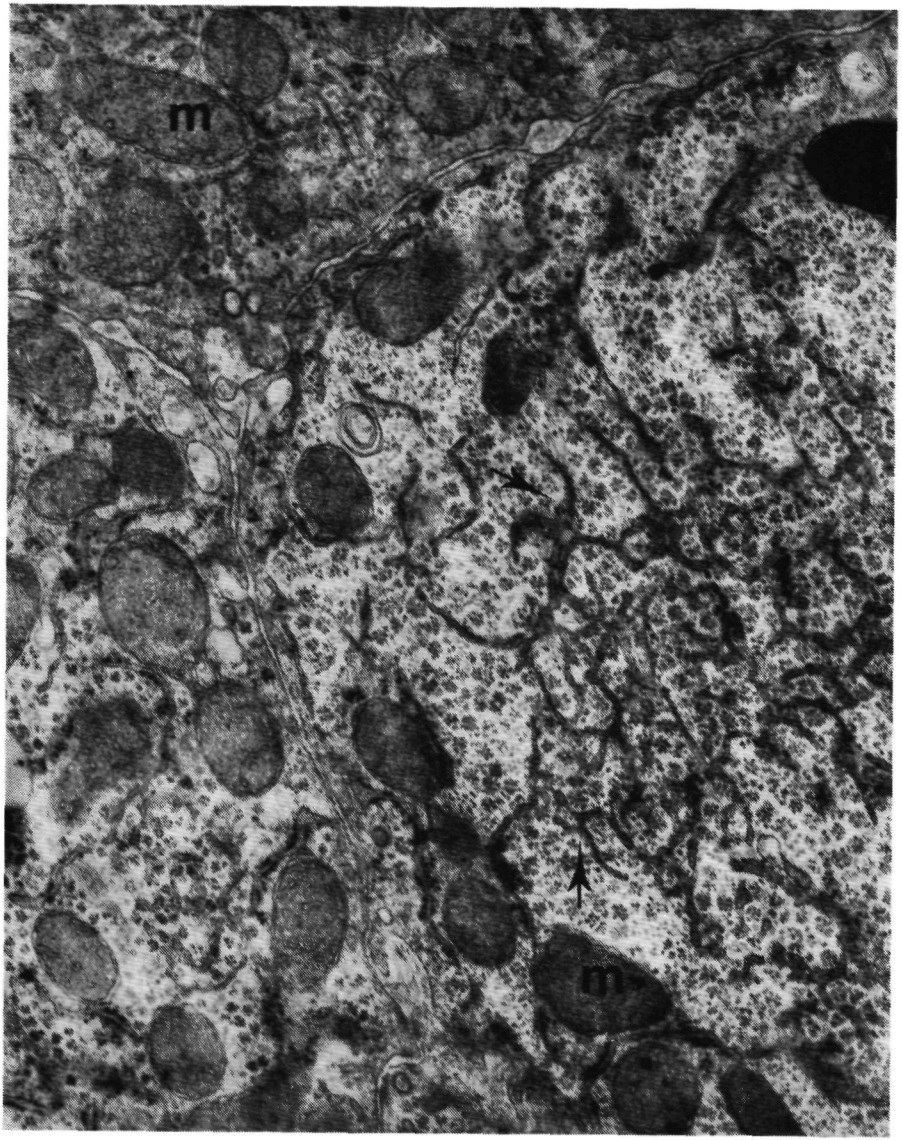

Figure 3. Electron micrograph, with three liver cells partly visible. Fair amount of smooth endoplasmatic reticulum with irregular branching aspect of lamellae (arrows); m indicates mitochondria (× 27,900).

Light microscopy showed an increase of collagen fibers without inflammatory infiltrate, suggesting interstitial fibrosis. Electron microscopy revealed a diffuse deposition of large and small lipid droplets in nearly all muscle fibers, both perinuclear and among the myofibrils. The cristae of the mitochondria did not have a tubular structure but a widened and often vesicular aspect (figure 2).

LIVER

Electron microscopy disclosed many intracytoplasmatic lipid droplets. The mitochondria showed an abnormal variation in shape and size. There was also an increase of smooth endoplasmic reticulum with an irregular ramifying lamellar network (figure 3).

PERIPHERAL NERVE

Light and electron microscopy of sural nerve tissue showed slight signs of active primary axonal degeneration (Wallerian degeneration).

CNS

External examination of the brain presented a slight atrophy of the cerebral hemispheres and a moderate atrophy of the cerebellar hemispheres. The frontal cortex appeared thinner and darker than usual. In several areas the cortex split easily, and on close inspection a rim of vacuoles could be seen in the middle layers of the cortex. The ventricles were enlarged.
On light microscopic examination, the most striking changes were a spongiosis of the cortical layers together with pericellular and perivascular edema. There was marked degeneration and loss of cortical neurons, together with considerable proliferation of astrocytes. No inflammatory signs or intranuclear inclusion bodies were seen. Cortical changes varied in different regions. Some parts of the cortex were normal or nearly so. The white matter presented a normal myelin structure; the oligodendroglial cells were normal in number and appearance. In the cerebellar cortex we found an extensive loss of Purkinje's cells and of neurons of the granular layer, as well as a slight proliferation of Bergmann's glia. There was also degeneration and loss of

neurons in the basal ganglia, pons, dentate nucleus, and inferior olive; a lumbar spinal ganglion showed extensive loss of neurons.
Electron microscopy of the cerebral cortex revealed extensive postmortem autolytic changes. No major mitochondrial abnormalities (eg, giant mitochondria) were seen in neurons or astroglia.

BIOCHEMICAL STUDIES

MATERIALS AND METHODS

Enzymatic studies were performed in intact WBCs, cultured fibroblasts, and liver and muscle homogenates, obtained at biopsy. Pyruvate oxidation rate and citric acid cycle activity in leukocytes and fibroblasts were determined by measuring the $^{14}CO_2$ production from pyruvate labeled with carbon 14 in the No. 1 position and pyruvate labeled with carbon 14 in the No. 2 position, respectively, as described by Willems et al.[7] Pyruvate oxidation rate in a liver-tissue homogenate was studied by measuring the rate of $^{14}CO_2$ production from [1-^{14}C]pyruvate (Willems et al[8]). In the same liver homogenate, pyruvate carboxylase activity was measured as described by Utter and Keech,[9] with a regenerating system for acetyl-CoA according to Henning and Seubert.[10] Pyruvate and 2-oxoglutarate oxidation rate and citric acid cycle activity in muscle tissue homogenate were studied by measuring the $^{14}CO_2$ production from [1-^{14}C]pyruvate, [1-^{14}C]2-oxoglutarate, and malate uniformly labeled with carbon 14, respectively, as described by Bookelman et al[11] for isolated muscle mitochondria. Cytochrome oxidase activity in muscle homogenate was measured according to the method of Cooperstein and Lazorow.[12] Muscle carnitine content was assayed in the same homogenate according to the method of Parvin and Pande.[13] Cytochromes in isolated muscle mitochondria from autopsy material were assayed according to methods of Bookelman et al.[14] Protein was assayed according to the method of Lowry et al.[15]

RESULTS

The $^{14}CO_2$ production rate from [1-^{14}C]pyruvate and from [2-^{14}C]pyruvate was normal in leukocytes and cultured fibroblasts (table 1). The $^{14}CO_2$ production rate from [1-^{14}C]pyruvate was decreased in liver homogenate (table 1). Pyruvate carboxylase activity in liver homogenate was normal (38.9

Table 1. Production of $^{14}CO_2$ in leukocytes, cultured fibroblasts, and liver and muscle tissue of patient and controls*.

	Substrate	Patient	Controls Range	No
Leukocytes[1]	[1-^{14}C] pyruvate	2 21	1 40–6 50	20
	[2-^{14}C] pyruvate	2 10	1 40–5 70	20
Fibroblasts[2]	[1-^{14}C] pyruvate	28 6	25 2–67 5	23
	[2-^{14}C] pyruvate	26 9	21 1–34 6	13
Liver tissue[2]	[1-^{14}C] pyruvate + malate	19 1	45–205	9
Muscle tissue[2]	[1-^{14}C] pyruvate + malate	98	243–448	7
	[1-^{14}C] pyruvate + carnitine	100	282–608	7
	Malate ^{14}C [ul] + pyruvate + malonate	25	228–546	7
	Malate ^{14}C [ul] + acetyl-carnitine + malonate	31	279–648	7
	[1-^{14}C] 2-oxoglutarate	105	261–552	4

*Production from pyruvate labeled with carbon 14 in the No 1 position and pyruvate labeled with carbon 14 in the No 2 position, also from malate uniformly labeled with carbon 14 and 2-oxoglutarate labeled with carbon 14 in the No 1 position Malonate was added to incubations with malate ^{14}C [ul] to prevent recycling of malate through the cycle

[1] *nmole* $^{14}CO_2$*/hr/*10^6 *leukocytes*
[2] nmole $^{14}CO_2$/hr/mg protein

nmole/min/mg protein; controls: 35.0 ± 15.4 nmole/min/mg, mean ± SD; n = 6). The $^{14}CO_2$ production from [1-^{14}C]pyruvate, [1-^{14}C]2-oxoglutarate, and especially from malate ^{14}C (ul) in muscle homogenate was decreased (table 1), whereas a normal cytochrome oxidase activity was found (69 nmole/min/mg protein; controls: 53.6 ± 31.3 nmole/min/mg, mean ± SD; n = 11). The total amount of carnitine (carnitine + acylcarnitines) in muscle homogenate was slightly decreased (46.2 nmole/mg protein; controls: 83 ± 22 nmole/min/mg, mean ± SD; range, 65 to 128 nmole/min/mg; n = 7). The contents of cytochrome *b* and cytochrome $c + c_1$ in muscle mitochondria were slightly decreased; the amount of cytochrome aa_3 was within normal range (table 2).

COMMENT

We have described an infant with a severe and rapidly progressive neurologic disorder, being exacerbated during infections, and characterized by psychomotor retardation, tetraparesis with ataxia, and myoclonic jerks. Clinical signs, EEG, and somatosensory evoked potentials suggested diffuse involvement of gray matter. Laboratory studies showed elevated levels of lactate in CSF. The histopathologic abnormalities found in the cerebral hemispheres

Table 2. Cytochrome *b*, cytochrome $c + c_1$, and cytochrome aa_3 in mitochondria from muscle tissue of patient and controls.

	Patient	*Controls (n = 14)*
Cytochrome *b**	156	228–424
Cytochrome $c + c_1$*	182	365–923
Cytochrome aa_3*	305	271–543

* pmole/mg protein

and cerebellum can be classified as a progressive infantile poliodystrophy (Alpers' disease).

Biochemical studies showed no abnormalities for pyruvate metabolism in leukocytes and cultured fibroblasts; in liver tissue, normal values were found for pyruvate carboxylation, but here pyruvate oxidation was decreased. In muscle tissue we found severe disturbances of pyruvate oxidation and of the citric acid cycle.

Parallel incubations of muscle tissue with [1-^{14}C]pyruvate plus either malate or carnitine, as an *acceptor* of acetyl-CoA, will demonstrate such disturbances of the pyruvate oxidation and enable us to tell whether the decreased pyruvate oxidation is the result of some defect in the pyruvate dehydrogenase complex or of some defect of the citric acid cycle. In the latter case, with carnitine a normal $^{14}CO_2$ production from [1-^{14}C]pyruvate should be found. In muscle tissue of our patient, $^{14}CO_2$ production from [1-^{14}C]pyruvate was decreased in the presence of both malate and carnitine. Hence, the faulty pyruvate oxidation is not to be attributed to a defect somewhere in the citric acid cycle.

Parallel incubations of human muscle tissue with malate ^{14}C (ul) plus either pyruvate or acetylcarnitine, as a *donor* of acetyl-CoA, will furnish proof of a decreased citric acid cycle activity and tell us whether this reduced activity is due to some defect of the citric acid cycle or to some defect in the pyruvate dehydrogenase complex. In the latter case, with acetylcarnitine a normal $^{14}CO_2$production from malate ^{14}C (ul) should be found. In our patient, $^{14}CO_2$ production from malate ^{14}C (ul) was decreased in the presence of both pyruvate and acetylcarnitine. Therefore, the decreased citric acid cycle activity is not to be attributed to some defect in the pyruvate dehydrogenase complex.

In addition, $^{14}CO_2$ production from [1-^{14}C] 2-oxoglutarate was also decreased. The foregoing findings can only be explained in terms of a disturbance of the oxidation of NADH, the common pathway in these processes.

A slight reduction of the content of the cytochrome *b* and $c + c_1$ was

observed; in our experience, this reduction cannot account for the severe disturbance of the substrate oxidation. The biochemical defect must therefore be sought in the electron transport system, especially between NADH dehydrogenase and coenzyme Q. The subnormal oxidation rate of pyruvate in liver homogenate points to the same disturbance as in muscle homogenate. The disturbance in the oxidation of NADH, demonstrated in muscle tissue, must have been located in the cell mitochondria; however, by histopathologic methods, no signs of mitochondrial myopathy were found in skeletal muscle. With regard to the mitochondrial abnormalities in heart muscle, it was difficult to differentiate between disturbances appearing during the child's terminal state and any preexistent disorders.

We have found only one report of a similar deficiency in NADH oxidation. Morgan-Hughes et al[16] reported the cases of two sisters (26 and 23 years old) with a mitochondrial myopathy, who were first observed with moderate muscle weakness and marked exercise intolerance but no signs of CNS involvement. Metabolic studies showed increased blood lactate and pyruvate levels during exercise. The CSF concentrations of lactate and pyruvate were not measured. Tissue studies gave evidence of a mitochondrial myopathy. Biochemical studies of muscle mitochondria in one of the sisters pointed to a disorder in respiratory chain activity at the level of the NADH-coenzyme Q reductase complex. The report suggests that the deficiency was restricted to muscle tissue, whereas our case report would point to a more generalized disorder.

After Alpers' description of progressive infantile poliodystrophy in 1931[17] and 1960,[18] Greenhouse and Neubuerger[19] and Jellinger and Seitelberger[20] gave a review of the literature, with the principal characteristics of this syndrome. Alpers' disease is sometimes associated with biochemical defects, suggesting a disturbance in cellular energy metabolism in neurons and/or in other tissues. Tommasi et al[5] and David et al[6] described a child having progressive infantile poliodystrophy with loss of activity of pyruvate carboxylase in liver tissue. To our knowledge, there is one report of a patient with a pyruvate carboxylase deficiency[21] and one of a patient having a pyruvate dehydrogenase complex deficiency,[22] both cases attended with extensive degeneration of cerebral tissue as seen at autopsy. Here a tentative diagnosis of progressive infantile poliodystrophy could be made, but there is not sufficient evidence on the pathologic changes in the brain to be definite. Alpers' disease has also been associated with morphologic abnormalities in cell mitochondria. Progressive infantile poliodystrophy with abnormal mitochondria (giant forms up to 8 μm) was described by Sandbank and Lerman[23] and by Suzuki and Rapin.[24] Shapira et al[3] and Hart et al[4] reported cases of progressive infantile poliodystrophy with lactic acidemia and mitochondrial abnormalities in muscle tissue.

Our case shows a combination of progressive infantile poliodystrophy and a biochemical defect in muscle and liver tissue, ie, a functional abnormality of the mitochondria. Surprisingly, no structurally abnormal mitochondria were seen in these tissues. Though we have little explanation, their association may be more than a coincidence. The elevated level of lactate in CSF, with normal serum values, might suggest a disturbed pyruvate metabolism in the CNS; appropriate studies excluded other causes of lactate elevation in CSF, eg, cerebrovascular and infectious diseases. It is our opinion that a disturbance of NADH oxidation in several organ systems, located in the cell mitochondria, was the principal factor in the present case of progressive infantile poliodystrophy.

Shapira et al[25] have proposed the concept of mitochondrial encephalomyopathies, grouping together various neuromuscular disorders with structurally and/or functionally abnormal mitochondria in brain and/or in muscle. Our case seems to support such a view. However, we must disagree with their statement that there is a specific defect in the oxidative metabolism of the various mitochondrial encephalomyopathies. It is our opinion that different pathologic patterns can be associated with any kind of biochemical abnormality in pyruvate metabolism.

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J.L. Willems, MD, and Anton J.M. Janssen performed enzymatic studies, and Urbaan J.G. van Haelst, MD, performed electron microscopic studies.

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CHAPTER 5

PROGRESSIVE INFANTILE POLIODYSTROPHY (ALPERS' DISEASE) WITH A DEFECT IN CITRIC ACID CYCLE ACTIVITY IN LIVER AND FIBROBLASTS

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ABSTRACT

We present the case history of a boy, who died at the age of 3½ years after a rapidly progressive neurologic disorder, characterized by psychomotor retardation, hypotonia, hemiparesis, seizures and myoclonic contractions. Histopathologic studies showed slight lipid storage in liver. Autopsy showed the characteristic features of progressive infantile poliodystrophy (Alpers' disease); ultrastructural examination showed an increased density of mitochondria in cerebral gray matter. Biochemical studies in leukocytes, cultured fibroblasts and liver indicated a deficiency in the citric acid cycle between succinate and fumarate; this deficiency was not present in muscle tissue.
This study supports the view that progressive infantile poliodystrophy is associated with abnormalities in pyruvate metabolism and/or in cell mitochondria.

KEY WORDS

Progressive infantile poliodystrophy - Diffuse cerebral sclerosis - Citric acid cycle - Liver - Lactates - Mitochondria

INTRODUCTION

Progressive infantile poliodystrophy (Alpers' disease) is a rapidly progressive autosomal recessive neurologic disorder in children. The clinical picture shows psychomotor retardation, epileptic manifestations, myoclonic jerks, visual disturbances, ataxia, hypotonia and paresis, which sometimes leads to spasticity. These clinical manifestations are provoked or increased by infectious diseases or other tense circumstances. Many of these children are quickly fatigued. Complaints start at 1–3 years, and usually the children die when they are between the ages of 2–6 (Alpers 1931, 1960, Greenhouse and Neubuerger 1964, Jellinger and Seitelberger 1970, Partouche 1977).

At present the definite diagnosis of Alpers' disease can only be made by histologic examination of the cerebral cortex, presenting neuronal degeneration, gliosis and spongiosis.

Enzymatic studies in various tissues are of great importance in studying diseases with unknow etiology (Di Mauro 1979). Previously three patients have been reported with progressive infantile poliodystrophy and a defect in pyruvate metabolism, in liver (Tommasi et al 1977), muscle (Prick et al 1981b) and brain (Prick et al 1981a). This study presents another case, with a defect in the citric acid cycle in liver, leukocytes and cultured fibroblasts; this defect has not yet been described in connection with Alpers' disease.

CASE REPORT

Our patient, a 3-year-old boy, was the younger child of healthy, nonconsanguineous parents. His 6-year-old brother was in good health and the family history was unremarkable. His prenatal, natal and postnatal histories were unremarkable until he had a febrile convulsion at the age of 2 years. At the age of 2½ the parents complained of a lag in the development of motor skills and speech. Physical examination showed moderate psychomotor retardation at that time. The electroencephalogram (EEG) was considered normal. When 3½ years old, the child had a generalized seizure. Three months later he was admitted to our clinic because of another seizure, persisting loss of consciousness and continuous myoclonic contractions. The boy was stuporous and slightly cyanotic, he frequently vomited and had tachycardia and irregular respiration. Head circumference was relatively small (49 cm, $P_{2,5}$), height and weight were normal. There were general hypotonia, paresis and areflexia; atypical and dysrhythmic myoclonic contractions occurred almost continuously. Fundoscopy revealed bilateral optic atrophy. Although some improvement occurred, the clinical course remained

characterized by hypotonia, myoclonic contractions, impairment of consciousness and intermittent respiratory disturbances. After two months the boy became comatose and died due to respiratory arrest.
Roentgenograms of thorax and skull, as well as carotid and vertebral artery angiograms showed no abnormalities; computed tomography of the head showed symmetric and slight enlargement of the lateral ventricles, with normal third and fourth ventricles, and no cortical atrophy. The electrocardiogram was normal. The EEG showed severe slowing of the background rhythm, with generalized paroxysmal discharges and focal sharp waves and spikes. Somatosensory potentials evoked by median nerve stimulation showed a normal peak latency of the first complex. However, the secondary complex was extremely delayed, suggesting a disorder of cerebral gray matter. Brainstem evoked potentials could not be elicited. Visual evoked responses were normal. Nerve conduction velocities and electromyograms were normal. Normal laboratory tests included: blood count, routine urine analysis, renal and hepatic functions, electrolytes, protein and electrophoretic pattern, lipid spectrum, creatine kinase, aminoacids in plasma and urine, volatile fatty acids in urine, lysosomal enzymes in leukocytes.
Fasting for 24 hours provoked no hypoglycemia and the subsequent mobilization of free fatty acids was normal. Appropriate tests ruled out the presence of endocrinologic, immunologic and chronic infectious diseases, vitamine deficiencies and poisoning. The analysis of cerebrospinal fluid (CSF) showed normal cell count, protein electrophoresis and immune-electrophoresis, but elevated total protein content (1050 to 1400 mg/l). Pyruvate levels in serum, and lactate levels in serum and urine were elevated. CSF pyruvate and lactate values were normal when the child was in a rather good condition; however, when comatose, a slight elevation of CSF pyruvate and a severe elevation of CSF lactate were observed, with increased lactate/pyruvate ratio. In urine 2-oxoglutarate and succinate levels were elevated (table 1).

HISTOPATHOLOGIC STUDIES

The light microscopic, histochemical and electron microscopic studies of a soleus muscle biopsy demonstrated no pathologic alterations. Light microscopy of a liver biopsy only showed some lipid storage in the hepatocytes. Cerebral tissue, obtained 3 hours after death, was studied by light and electron microscopy. The coronal sections of the brain showed a narrowing of the cortex with foci of spongy degeneration (figure 1). Light microscopic examination of the cerebral gray matter revealed focal degeneration in all cortical layers, characterized by marked degeneration and loss of neurons,

Table 1. Lactate and pyruvate values in serum and CSF, and lactate, 2-oxoglutarate and succinate values in 24-hour urine of patient and controls.

		Patient		Controls
		conscious	comatose	(n = 200)
Lactate[0]	serum	1850	1760	600 -1200
	CSF	1500	3880	1200 -1600
Pyruvate[0]	serum	137	140	75 - 120
	CSF	126	148	90 - 130
Ratio L/P	serum	13.5	12.6	6 - 10
	CSF	11 9	26.0	11 0- 14.5
Lacate[Ø]	urine (24 h)	650–730		< 100
2-Oxoglutarate[δ]	urine (24 h)	137		< 125
Succinate[0]	urine (24 h)	700		< 100

[0] µmol/l
Ø µmol/mmol creatinine
δ µmol/24 h

together with considerable proliferation of astrocytes, spongiosis and dilatation of capillaries, among areas with a completely normal cortical structure. The degenerative areas appeared to be distributed at random. Neuronal loss and gliosis also occurred in the globus pallidus and in some brainstem nuclei. Extensive degeneration of Purkinje cells and granular cells accompanied by extensive glial proliferation was observed in the cerebellar cortex. At electron microscopic examination of the cerebral gray matter the major part of neuronal and astrocytal mitochondria was swollen, but no specific abnormalities were seen. The cerebrai capillaries seemed quite normal, but the perivascular spaces were extremely enlarged and occupied by some striking membrane bound structures with the same size and shape as mitochondria. They mainly consisted of a very dense part, surrounding several oval or tubular structures of much lower density (figure 2).

BIOCHEMICAL STUDIES

MATERIALS AND METHODS

Enzymatic studies were performed in intact leukocytes, cultured fibroblasts, and homogenates of liver and muscle biopsies.
Pyruvate oxidation rate and citric acid cycle activity in leukocytes and fibroblasts were determined by measuring $^{14}CO_2$ production from [1-^{14}C]py-

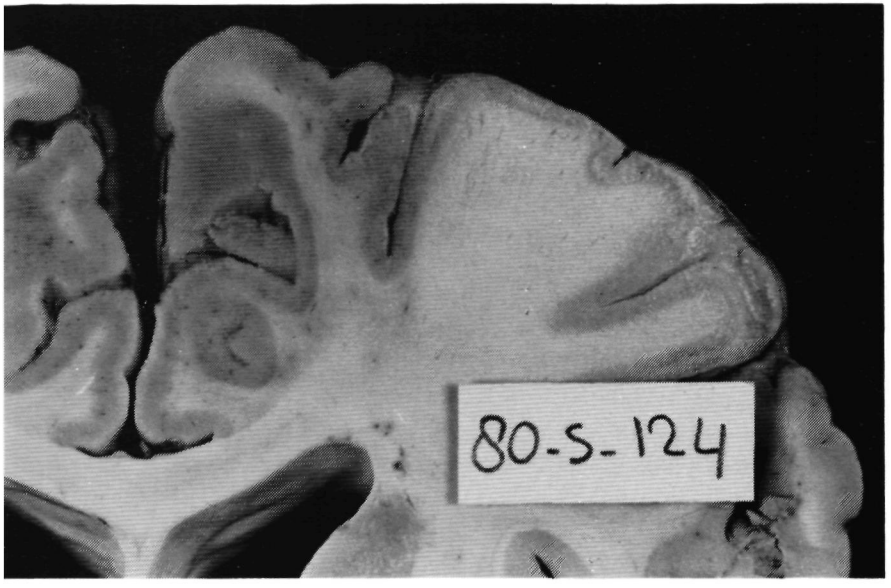

Figure 1. Part of coronal section of the brain, showing narrowing of the cortex with foci of spongy degeneration. The white matter shows a normal aspect.

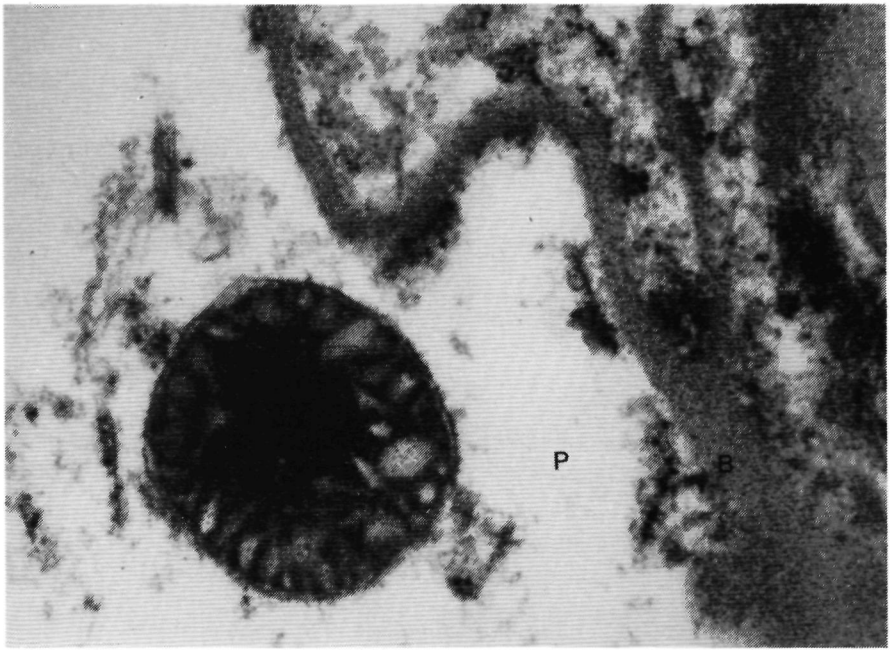

Figure 2. Electronmicrograph of an abnormal mitochondrion in cerebral gray matter. Note the dense granular matrix and the small areas of lower density. B, basal lamina; P, perivascular space (Original magnification x 68000).

ruvate and [2-^{14}C]pyruvate respectively (Willems et al 1978). In liver tissue homogenate pyruvate oxidation rate and citric acid cycle activity were studied by measuring the $^{14}CO_2$ production from [1-^{14}C]pyruvate, and [6-^{14}C]citrate, [1-^{14}C] and [5-^{14}C]2-oxoglutarate respectively (Willems et al 1977); pyruvate carboxylase activity was also determined (Utter and Keech 1963), with a regenerating system for acetyl-CoA (Henning and Seubert 1964).
Pyruvate oxidation rate and citric acid cycle activity in muscle homogenate were studied by measuring the $^{14}CO_2$ production from [1-^{14}C]pyruvate, and from [U-^{14}C]malate, [1-^{14}C] and [5-^{14}C]2-oxoglutarate respectively as described by Bookelman et al (1978b) for isolated mitochondria.
Cytochrome oxidase activity was determined in liver and muscle homogenates (Cooperstein and Lazorow 1951). Cytochromes were assayed in isolated muscle mitochondria (Bookelman et al 1978a).
Fatty acid oxidation capacity of muscle was studied with [1-^{14}C] and [U-^{14}C]palmitate in whole muscle homogenates (Van Hinsbergh et al 1980). Protein was assayed according to Lowry et al (1951).

RESULTS

In leukocytes the $^{14}CO_2$production rate from [1-^{14}C]pyruvate was normal, from [2-^{14}C]pyruvate it was slightly decreased, but the ratio of [1-^{14}C]/ [2-^{14}C]pyruvate oxidation was increased (table 2). In cultured fibroblasts $^{14}CO_2$production from [1-^{14}C]pyruvate was normal, but from [2-^{14}C]pyruvate it was reduced (table 2).
In liver homogenate $^{14}CO_2$ production from [1-^{14}C]pyruvate, [6-^{14}C]citrate and [1-^{14}C]2-oxoglutarate was normal, whereas $^{14}CO_2$ production from [5-^{14}C]2-oxoglutarate was severely decreased (table 2). Pyruvate carboxylase activity in liver homogenate was normal (20.7 nmol/min.mg protein; controls: 35.0 ± 15.4, mean ± SD, n = 6); cytochrome *c* oxidase activity was in the lower-normal range (11.2 nmol/min.mg protein; controls 37 ± 13, mean ± SN, n = 7). The contents of the cytochromes were not measured because of an insufficient amount of liver tissue. In muscle homogenate $^{14}CO_2$ production from [1-^{14}C]pyruvate, [U-^{14}C]malate, [1-^{14}C] and [5-^{14}C]2-oxoglutarate was normal (table 2), with a normal cytochrome *c* oxidase activity (53.2 nmol/min.mg protein; controls: 53.6 ± 31.3, mean ± SD, n = 11). The contents of the cytochromes in muscle mitochrondria were normal (table 3). Palmitate oxidation capacity, pro gram muscle, was in the normal range.
To summarize: we found abnormalities in leukocytes, cultured fibroblasts

Table 2. $^{14}CO_2$ production from various substrates in leukocytes, cultured fibroblasts, liver and muscle tissue of patient and controls.

	Substrate	Patient	Controls	n
Leukocytes*	[1-^{14}C] pyruvate	2 60	1 40–6 50	20
	[2-^{14}C] pyruvate	1 08	1 40–5 70	20
	Ratio [1-^{14}C]/[2-^{14}C] pyruvate	2.40	1 00–1 93	20
Fibroblasts°	[1-^{14}C] pyruvate	14 4	25.2–67 5	23
	[2-^{14}C] pyruvate	3 5	21.1–34.6	13
Liver°	[1-^{14}C] pyruvate + malate	60.1	45–205	9
	[6-^{14}C] citrate + malate	128 7	115 ; 189	2
	[1-^{14}C] 2-oxoglutarate	45.7	106	1
	[5-^{14}C] 2-oxoglutarate	4.3	46;48	2
Muscle°	[1-^{14}C] pyruvate + malate	539	243-448	7
	[1-^{14}C] pyruvate + carnitine	510	282–608	7
	[U-^{14}C] malate + pyruvate + malonate	572	228–546	7
	[U-^{14}C] malate + acetylcarnitine + malonate	494	279–648	7
	[1-^{14}C] 2-oxoglutarate	541	261–552	4
	[5-^{14}C] 2-oxoglutarate	36	30,48;49	3

* nmol $^{14}CO_2$/h 10^6 leukocytes
° nmol $^{14}CO_2$/h mg protein

Table 3. Contents° of cytochromes in mitochondria isolated from muscle tissue of patient and controls.

	Patient	Controls (n = 14)
Cytochrome *b*	400	228-424
Cytochrome $c + c_1$	639	365–923
Cytochrome aa_3	411	271–543

° pmol/mg protein

and liver. The results in fibroblasts and leukocytes pointed to a decreased citric acid cycle activity. In liver tissue $^{14}CO_2$ production from [5-^{14}C]2-oxoglutarate was severely decreased, which also pointed to a defect in the citric acid cycle.

DISCUSSION

This patient clearly displayed the clinical and histopathological characteristics of progressive infantile poliodystrophy (Alpers' disease). Progressive infantile poliodystrophy has been linked to pyruvate carboxylase deficiency in liver (Tommasi et al 1977), to a defect in NADH oxidation in muscle

(Prick et al 1981b) and to a pyruvate dehydrogenase complex (PDHC) deficiency in brain (Prick et al 1981a). Moreover, a PDHC deficiency in cultured fibroblasts (Strömme et al 1976) and a pyruvate carboxylase deficiency in liver (Atkin et al 1979) were reported in patients with a cerebral autopsy, in our view suggestive of progressive infantile poliodystrophy.

Enzymatic studies in leukocytes and cultured fibroblasts in our patient showed a normal $^{14}CO_2$ production from [1-^{14}C]pyruvate and a defective one from [2-^{14}C]pyruvate, which is indicative of a decreased citric acid cycle activity. Enzymatic studies in liver tissue showed only a severely reduced $^{14}CO_2$ production from [5-^{14}C]2-oxoglutarate with normal $^{14}CO_2$ production from [1-^{14}C]2-oxoglutarate, indicating a defect in the citric acid cycle between succinyl-CoA and malate (succinyl hydrolase, succinate dehydrogenase, fumarase). In urine lactate, 2-oxoglutarate and succinate excretion were increased, this also points to a deficiency in the citric acid cycle. Considering the elevation of 2-oxoglutarate and succinate in urine, the defect is most probably located before fumarate. In conclusion a defect in the citric acid cycle between succinyl-CoA and fumarate was proved in liver and seemed probable in leukocytes and cultured fibroblasts. This defect was not present in muscle tissue. In literature we did not find a previous report of a similar defect of the citric acid cycle.

Contrary to previous studies concerning Alpers' disease, electron microscopic studies of cerebral tissue in this patient did not reveal the presence of giant mitochondria (Suzuki and Rapin 1969), neither accumulation of mitochondria with short curved cristae and dense particles at the periphery was seen (Sandbank and Lerman 1972). The mitochondrial changes in our material consisted of swelling, probably due to post-mortem alterations, and of increased density. The latter phenomenon was described previously and was attributed to a low metabolic state of the mitochondria (Ghadially 1975). Moreover, the increased mitochondrial density might be considered as a precursor of mitochondrial pyknosis (Ghadially 1975) and subsequent autophagia, as has been described in Alpers' disease (Sandbank and Lerman 1972).

Since ultrastructural (Hart et al 1977, Sandbank and Lerman 1972, Shapira et al 1975, Suzuki and Rapin 1969) as well as biochemical mitochondrial abnormalities (Prick et al 1981a,b, Tommasi et al 1977) have been demonstrated in progressive infantile poliodystrophy, a mitochondrial pathogenesis of the disease is likely. Also in the present case there is a defect in pyruvate metabolism in liver, leukocytes and fibroblasts. We did not have the opportunity to perform biochemical studies in cerebral tissue. However, it is our opinion that a defect in pyruvate metabolism in brain is probable in this case of progressive infantile poliodystrophy.

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CHAPTER 6

PROGRESSIVE POLIODYSTROPHY (ALPERS' DISEASE) WITH A DEFECT IN CYTOCHROME aa_3 IN MUSCLE: A REPORT OF TWO UNRELATED PATIENTS

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ABSTRACT

We present two unrelated patients, a boy and a girl, with a progressive neurologic disorder, characterized by psychomotor retardation, seizures and paresis, the illness being exacerbated during stressful periods. Lactate levels in serum and cereborspinal fluid were elevated in both patients. Histopathologic studies of muscle tissue revealed mitochondrial abnormalities in the boy; in the girl, slight neuronal degeneration was observed. A cerebral biopsy in the girl showed abnormalities compatible with progressive poliodystrophy. Autopsy in the boy demonstrated progressive poliodystrophy. Biochemical studies in muscle tissue showed a defect of cytochrome aa_3 in both patients, connected with a defect of cytochrome *b* in the girl. The association of defective pyruvate metabolism and progressive poliodystrophy is discussed.

INTRODUCTION

In 1962 Luft et al showed for the first time biochemical evidence of mitochondrial abnormalities in a patient with a myopathy. They demonstrated a defect in the respiratory control of muscle mitochondria (loosely coupling state) in a patient with hypermetabolism of nonthyroid origin. Spiro et al (1970) reported a patient with a progressive neurologic disease with myopathic and neuropathic abnormalities in muscle tissue, associated with a marked reduction in the cytochrome *b* content of isolated muscle mitochondria. Di Mauro (1979) reviewed mitochondrial myopathies, including myopathies with biochemical abnormalities referring to mitochondrial dysfunction. He proposed that a rational classification of these myopathies be based on the identification of specific biochemical defects. In addition to describing Spiro's patient, Di Mauro mentioned 4 reports of a myopathy associated with a deficiency in one or two of the cytochromes. He referred to the descriptions by French et al (1972), Van Biervliet et al (1977), Morgan-Hughes et al (1977) and Willems et al (1977). Di Mauro et al (1980) and recently Heiman-Patterson et al (1982) described two other patients with a defect in the cytochromes.

In this article we report two unrelated patients with a cytochrome aa_3 deficiency demonstrated in muscle tissue, in patient 1 associated with a moderate deficiency of cytochrome *b*. Both patients had elevated levels of lactate and pyruvate in serum and cerebrospinal fluid (CSF). Histopathologic studies on muscle tissue revealed a mitochondrial myopathy in one of them, in the other slight myopathic and neuropathic changes were seen. Histopathologic studies on brain tissue showed degeneration of cerebral gray matter, indicating a progressive poliodystrophy (Alpers' disease).

Progressive poliodystrophy is a neurologic disorder with autosomal recessive inheritance (Alpers, 1931 and 1960). A definite diagnosis can only be made by microscopic study of brain tissue, at autopsy or in a brain biopsy specimen. The histopathologic characteristics of progressive poliodystrophy are neuronal degeneration, glial proliferation, capillary dilatation and proliferation, all mainly observed in the cerebral gray matter, and to a lesser degree with a more varying pattern in other gray structures in the central nervous system (CNS).

The association of a cytochrome aa_3 deficiency in muscle tissue and progressive poliodystrophy has not been described until now.

CASE 1

Patient 1, a girl, was the first child of healthy, nonconsanguineous parents. The family history showed no abnormalities. The girl's prenatal, natal and postnatal history was unremarkable. The development of motor skills and speech was quite normal.

At approximately 2 years of age, the child was admitted to hospital because of aspecific gastro-intestinal disturbances associated with some weight-loss. She made a full recovery within some weeks. At age 2 years and 3 months, the child's condition again deteriorated. She was admitted urgently to hospital because of a myoclonus status, with loss of consciousness, irregular respiration, nystagmoid ocular movements, hypotonia, and generalized areflexia. The child received 1.5 mg clonazepam three times a day and apparently recovered. She remained at home for approximately three months without further problems. Then, she got a myoclonus status again. Her condition progressively grew worse, and at age 2 years and 9 months the girl died. During this last period a brain biopsy was performed.

RADIOLOGIC AND ELECTROPHYSIOLOGIC EXAMINATIONS

Roentgenograms of chest and skull were normal. Cerebral computed tomography at age 2 years and 3 months showed no abnormalities; at age 2 years and 9 months generalized edema of the right cerebral hemisphere was seen. The electroencephalogram (EEG) showed diffuse bilateral slowing. Moreover, paroxysmal discharges were frequently observed. Visual evoked potentials (VEP) were severely disturbed.

LABORATORY DATA

Normal laboratory tests included: blood count, routine urine analysis, renal functions, electrolytes, serum pH, P_{CO_2} and bicarbonate, serum protein and electrophoretic pattern, serum lipid spectrum, creatine kinase, serum copper and ceruloplasmin, aminoacids in plasma and urine, volatile fatty acids and organic acids in urine, lysosomal enzymes in leukocytes. Appropriate studies ruled out endocrinologic, immunologic and chronic infectious diseases, deficiencies, and disorders caused by toxic agents. Hepatic functions were intermittently moderately disturbed, subsequently a liver biopsy was perfor-

Table 1. Lactate and pyruvate values in serum and CSF of patients and controls (n=200).

			Lactate (μmol/l)	Pyruvate (μmol/l)	Ratio lactate-pyruvate
Serum	Patient 1		1100-2050	84-111	12.5-18.0
	Patient 2	normal circumstances	1100-3500	90-160	9.0-22.5
		after exertion	3300-5000	190-235	14.5-21.5
	Controls		600-1200	75-120	up to 15
CSF	Patient 1		1220-1920	70-100	18.0-20.0
	Patient 2		2550-3890	145-178	17.5-21.9
	Controls		1200-1600	90-130	up to 15

med. The analysis of CSF showed normal cell count and normal protein electrophoresis, but elevated total protein content (660–1030 mg/l).
Studies on glucose metabolism showed no major abnormalities. Lactate and pyruvate levels in serum and CSF were within normal ranges or slightly to moderately increased, with an increased lactate-pyruvate ratio (table 1).

CASE 2

Patient 2, a boy, was the third of five children of healthy, nonconsanguineous parents. An older sister had died at the age of 5 years after a short illness with generalized paralysis and dysfunction of brain, heart and skeletal muscle. A first cousin, the child of his mother's sister, had died at 16 years of age after a progressive myopathic disorder. His mother, his mother's sister and his grandmother had a severe nerve deafness, which became manifest during adulthood. Apart from this, the family history showed no abnormalities.
The boy's prenatal, natal and postnatal history was unremarkable. At 10 years of age he got a severe epileptic status. During the years following this incident, the boy suffered from some severe relapses and his condition gradually deteriorated. Several clinical observations occurred, especially during these relapses of the disease. Changing grades of consciousness were seen, as well as various central neurovegetative signs. Moreover, the boy showed a severe mental deterioration, progressive motor disability with severe paresis, ataxia, diffuse muscle wasting, and signs of a polyneuropathy with motor, sensory and sympathetic dysfunction. Later on, visual disturbances with a left homonymous hemianopia and deafness were observed. Occasional generalized fits occurred. Fundoscopy showd slight bilateral optic atrophy with moderate pigmentary degeneration. The clinical signs increased or were provoked by physical effort, infectious diseases, and other stressful situations, probably as a sign of exercise intolerance. From about 13 years of

age the boy's condition progressively grew worse, with severe mental deterioration and motor disability, especially during the periods of relapse. At age 16 years he died, while suffering a new acute relapse.

RADIOLOGIC AND ELECTROPHYSIOLOGIC EXAMINATIONS

Roentgenograms of chest and skull were normal. Pneumencephalography showed no abnormalities. The electrocardiogram (ECG) was normal. The EEG showed severe slowing of the background rhythm. Frequent generalized paroxysmal discharges were seen, with a right occipital epileptic focus. Electromyography and nerve conduction velocities showed decreased motor and sensory nerve conduction velocities, indicating a polyneuropathy (peroneal nerve: motor conduction velocity 33 m/s (normal $>$ 45 m/s), distal latency 6.0 ms (normal $<$ 5.0 ms).

LABORATORY DATA

Similar laboratory tests were performed as in case 1; no specific abnormalities were seen. The analysis of CSF showed a normal cell count, protein electrophoresis and immune-electrophoresis, but elevated total protein content (650 mg/l).
Studies on glucose metabolism in both serum and CSF showed normal to increased levels of lactate and pyruvate, with an increased lactate-pyruvate ratio, the elevation being most pronounced after exertion (table 1).

HISTOPATHOLOGIC STUDIES

Histopathologic studies were performed on liver and skeletal muscle in both patients, and on peripheral nerve in patient 2. All specimens were obtained by biopsy. In addition, histopathologic studies were performed on CNS tissue: in patient 1 a brain biopsy was performed, in patient 2 CNS tissue was studied at autopsy. Unfortunately, an autopsy was not permitted on patient 1. In patient 1 all biopsies were performed at about 2½ years of age, in patient 2 at 14 years of age. The parents of both patients gave their informed consent.

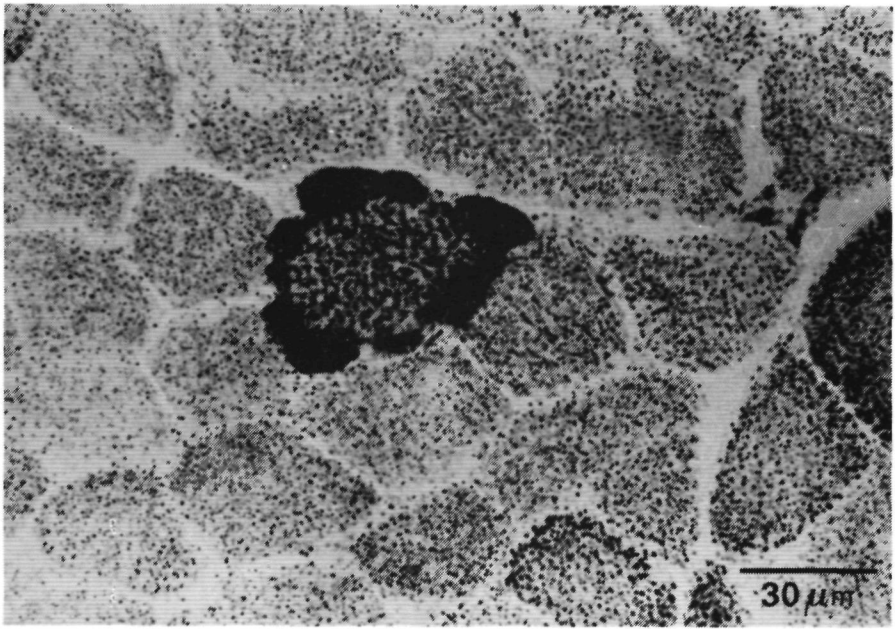

Figure 1a. Transverse section of quadricpes muscle. In Gomori's modified trichrome reaction "ragged-red fibers" are seen.

LIVER

Light microscopic studies in both patients, and electron microscopic studies in patient 2, showed no major abnormalities. In patient 1, some inflammatory infiltrates were present, indicating hepatitis. In patient 2, fatty liver degeneration was observed.

SKETETAL MUSCLE

Light microscopic, histochemical and electron microscopic studies were performed on quadriceps muscle.

Case 1. Increased variability of muscle fiber diameter was observed, without loss of the usual checker-board pattern. These observations indicate focal neuronal degeneration without clear nerve cell regeneration.

Case 2. Increased variability of muscle fiber diameter was observed, caused by some focal muscle fiber atrophy. No fiber splitting was observed. There was also some type grouping (loss of the usual checker-board pattern),

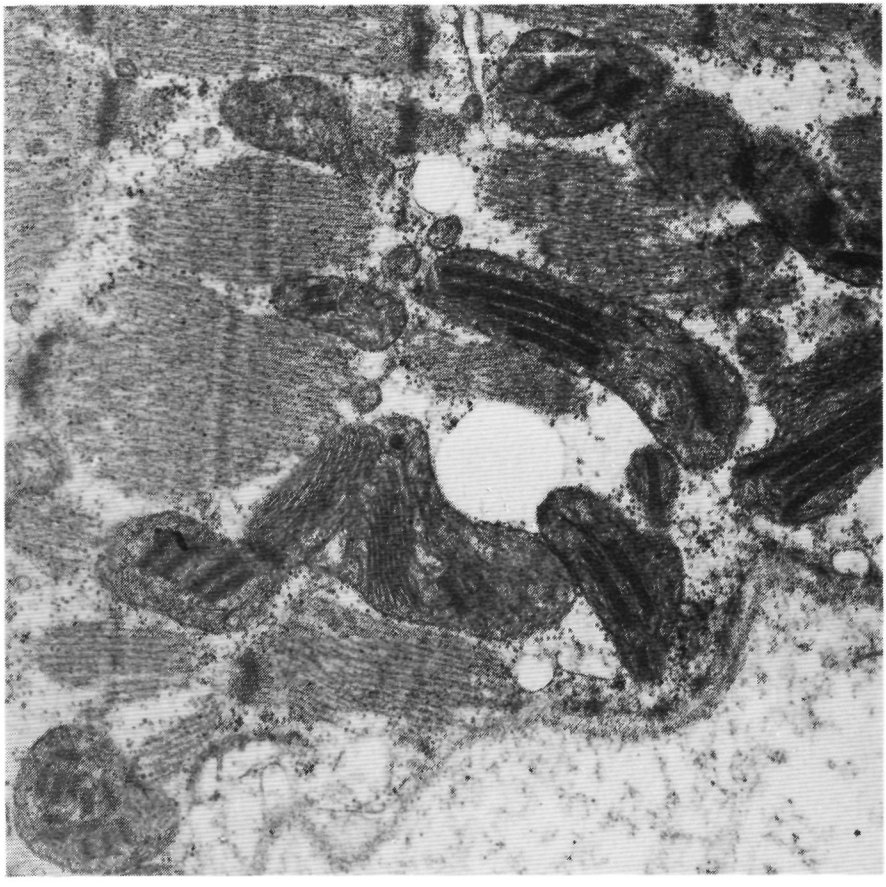

Figure 1b. Electron micrograph showing part of a quadriceps muscle fiber. Subsarcolemmal mitochondria are enlarged and show paracrystalline inclusions. A lipid droplet is present between the mitochondria (x 22,000).

indicating neuronal de- and regeneration. In Gomori's modified trichrome reaction "ragged-red fibers" were seen (figure 1a). These abnormally red-staining subsarcolemmal areas were also positive for succinic dehydrogenase, which is a mitochondrial marker. This activity indicates subsarcolemmal aggregation of mitochondria. Electron microscopic studies revealed an aggregation of mitochondria in the subsarcolemmal area. Many of these mitochondria were enlarged and contained paracrystalline inclusions, sometimes dense inclusions. In the muscle fibres too may fat droplets were seen. These abnormalities are concordant with the histopathologic characteristics of a mitochondrial myopathy (figure 1b).

PERIPHERAL NERVE

Light and electron microscopic studies of a sural nerve biopsy taken from patient 2 showed the characteristics of a slight neuropathy with axonal loss.

CENTRAL NERVOUS SYSTEM

Case 1. Light microscopic studies of brain tissue obtained by biopsy showed a distinct lipid storage, in association with slight neuronal degeneration and glial proliferation, and moderate capillary dilatation, all restricted to the cerebral gray matter. No typical spongiosis was seen. No signs of vascular pathology or infectious diseases were observed. Electron microscopic studies of the same specimen showed signs of lipid storage predominantly in glial cells, and in a lesser amount in neurons. In cerebral white matter no abnormalities were observed. In connection with the clinical signs these morphologic abnormalities are compatible with progressive poliodystrophy.

Case 2. Macroscopic observation of the brain, obtained about 24 hours post mortem, showed predominantly in the parieto-occipital region at both sides and extending from the lateral to the medial surface of the hemispheres, a pronounced cerebral atrophy with enlargement of the occipital horn of the cerebral ventricles. At section an area with a similar aspect was observed in the left cerebellar hemisphere.
Microscopic studies showed neuronal loss, most pronounced in the 4th and 5th cortical layers, with glial proliferation, and dilatation and proliferation of capillaries. In cerebral white matter some loss of myelin was observed (figure 2). Similar abnormalities were observed in a small cerebellar region. No abnormalities were observed in the mesencephalon, the pons and the medulla oblongata. The spinal cord and dorsal root ganglia were not available for histopathologic studies. The cerebral arteries showed no abnormalities. No inflammatory signs were seen in the meninges.

BIOCHEMICAL STUDIES

MATERIALS AND METHODS

Biochemical studies were performed on isolated muscle mitochondria and on muscle homogenates. Muscle tissue was obtained by a surgical biopsy in both

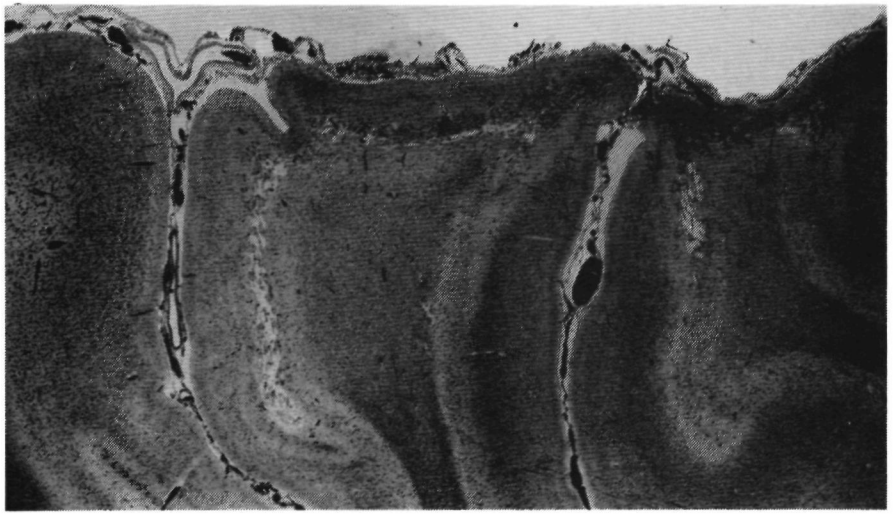

Figure 2. Part of coronal section of the brain, showing the parietal region. Pronounced cerebral atrophy with cortical degeneration predominantly in the middle cortical layers. Note the spongious aspect and the increased number of capillaries. The white matter shows a rather normal aspect.

patients. In patient 1 these studies were performed in 1980 by Trijbels and co-workers. Muscle tissue of patient 2 was studied in 1971 by Van Dam and co-workers.

Case 1. Pyruvate and 2-oxoglutarate oxidation rate and citric acid cycle activity in muscle tissue homogenate were studied by measuring the $^{14}CO_2$ production from [1–^{14}C]pyruvate, [1–^{14}C]2-oxoglutarate and [U–^{14}C]malate respectively, as described by Bookelman et al (1978b) for isolated muscle mitochondria. Cytochrome oxidase activity was measured in a muscle homogenate according to the method of Cooperstein and Lazorow (1951). Cytochromes were assayed in isolated muscle mitochondria according to methods of Bookelman et al (1978a). Protein was assayed according to the method of Lowry et al (1951).

Case 2. Mitochondria were isolated from muscle tissue using the technique described by Hülsmann et al (1969). Cytochrome oxidase activity was measured according to Schnaitmann et al (1967). Spectral analysis of cytochromes from isolated muscle mitochondria was performed with and without NADH and KCN added. Mitochondria were suspended in 250 mM of sucrose, 10 mM of NaCl and 10 mM of MOPS-buffer (pH 7.4). 1 mM of NADH and 1 mM of KCN were added. After freezing, spectral analysis was performed at the temperature of liquid nitrogen in an Aminco-Chance dual wave-length spectrophotometer.

RESULTS

Case 1. $^{14}CO_2$ production form [1–^{14}C]pyruvate, [1–^{14}C]2-oxoglutarate and [U–^{14}C] malate in muscle homogenate was decreased (table 2). Cytochrome oxidase activity was decreased (30.5 nmol/min.mg protein; controls: 73–284, n = 36). The contents of the cytochromes in muscle mitochondria were decreased, the decrease of cytochrome aa_3 being the most pronounced (table 3, figure 3).

Case 2. Cytochrome oxidase activity was extremely decreased, approximately 10% of the reference value. Spectral analysis of the cytochromes showed a decrease of cytochrome aa_3 with respect to cytochrome $c+c_1$ and b (table 3).

Table 2. $_{14}CO^2$production from various substrates in muscle homogenate of patient 1 and controls.

	Patient (nmol $^{14}CO_2$/h. mg protein)	Controls	n
[1–^{14}C]pyruvate + malate	147	343-448	7
[1–^{14}C]pyruvate + carnitine	146	282-608	7
[U–^{14}C]malate + pyruvate + malonate	139	228-546	7
[U–^{14}C]malate + acetylcarnitine + malonate	150	279-648	7
[l–^{14}C]2-oxoglutarate	163	261-552	4

DISCUSSION

We reported on two patients with a decrease in the cytochrome aa_3 content in isolated muscle mitochondria, associated with progressive poliodystrophy (Alpers' disease). Earlier reports on deficiencies in the cytochromes in muscle tissue have been made by Spiro et al (1970), French et al (1972), Van Biervliet et al (1977), Morgan-Hughes et al (1977), Willems et al (1977), Di Mauro et al (1980) and Heiman-Patterson et al (1982).

Spiro et al (1970) and Morgan-Hughes et al (1977) reported a marked reduction of cytochrome b in isolated muscle mitochondria. Histopathologic studies on brain tissue were not performed in either of these two cases. Spiro et al (1970) described a 16-year-old boy who from the age of approximately 11 years presented progressive muscular weakness associated with psychomotor retardation, epileptic manifestations, myoclonic jerks, ataxia, decreased visual acuity with chorioretinitis, and areflexia with bilateral extensor plantar responses. Lactate and pyruvate studies were not reported. A

Table 3. Contents of cytochromes in mitochondria isolated from muscle tissue of patients and controls.

	Patient 1*	Controls* (n = 14)	Patient 1†	Patient 2†	Controls † (n = 14)
Cytochrome aa_3	89	271-543	0 3	0 3	0 7
Cytochrome b	131	228-424	0 4	0 7	0 5
Cytochrome c + c_1	310	365-923	1 0	1 0	1 0

* Values are expressed as pmol/mg protein
† Values of cytochrome aa_3 and b for patient 2 are given proportionally to cytochrome $c + c_1$, for a comparison the latter values are also presented for patient 1 and controls

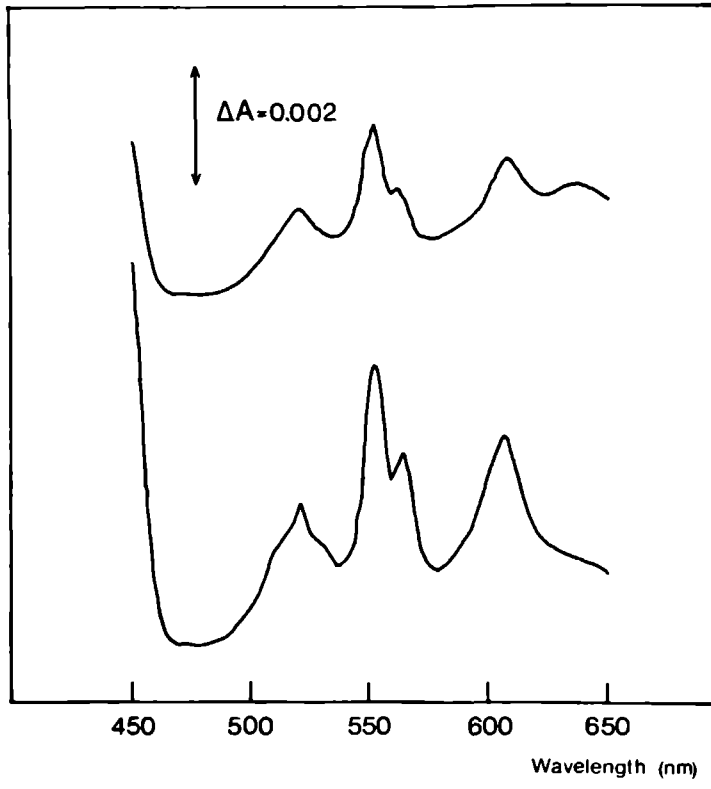

Figure 3. Difference spectra of human skeletal muscle mitochondria after reduction with succinate plus KCN according to Bookelman et al (1978a). The sample cuvette with the patient's muscle mitochondria (upper curve) contained 0.22 mg protein. The sample cuvette with the control muscle mitochondria (lower curve) contained 0.18 mg protein.

skeletal muscle biopsy showed atypical myopathic and neuropathic abnormalities. The father of this patient showed similar clinical and morphologic abnormalities. Morgan-Hughes et al (1977) described the case of a 38-year-old man with progressive muscular weakness and excessive muscular fatiguability dating back to childhood. No mental deterioration or nervous system abnormalities were mentioned. A 24-hour urine specimen contained an excess of lactate and pyruvate. Serum lactate and pyruvate levels rose sharply during exercise. Histochemical studies on muscle tissue indicated a mitochondrial myopathy.

Van Biervliet et al (1977) and Di Mauro et al (1980) reported similar cases: patients with a marked reduction of the cytochromes aa_3 and *b* in isolated muscle mitochondria, a disorder clinically resembling the DeToni-Fanconi-Debré syndrome. The report of Van Biervliet et al (1977) described a child with severe neurologic abnormalities, which became apparent during the first months of life after an uncomplicated pregnancy and delivery. The clinical signs were hypotonia, extreme muscular weakness, lethargy, and the absence of visual contact. Laboratory studies revealed renal dysfunction and high serum lactate levels. Electron microscopic studies on muscle tissue showed large and irregularly shaped mitrochondria, indicating a mitochondrial myopathy. The child died at 13 weeks of age. No autopsy findings were reported. Two sisters died at 11 weeks of age after a similar illness. The case report of Di Mauro et al (1980) showed a child dying at the age of 15 weeks after an illness very similar to that described by Van Biervliet et al (1977). Autopsy showed no cerebral abnormalities. Histochemical and electron microscopic studies on muscle tissue showed lipid storage and mitochondrial abnormalities. The enzymatic defect could also be demonstrated in kidney tissue.

Heiman-Patterson et al (1982) described a 8-week-old girl with a clinical presentation identical to that of the cases reported by Van Biervliet et al (1977) and Di Mauro et al (1980), the child died at 14 weeks of age. A skeletal muscle biopsy showed lipid storage and mitochondrial alterations at histochemical and electron microscopic studies. Biochemical studies on muscle tissue showed a decreased activity of cytochrome-*c*-oxidase, with normal levels of the other mitochondrial enzymes and of carnitine. The content of the cytochromes in isolated muscle mitochondria has not been mentioned.

Willems et al (1977) observed a child, having a marked reduction of cytochrome aa_3 content in isolated muscle mitochondria, with subacute necrotizing encephalomyelopathy (Leigh disease), proven by autopsy. The disorder was characterized by progressive muscular weakness, psychomotor deterioration, hypotonia, bilateral papillary atrophy, vomiting, respiratory disturbances, and lactic acidemia. The child died at 6 years of age. Histochemical studies on muscle tissue revealed no major abnormalities.

French et al (1972) described a patient with Menkes' disease (kinky-hair disease, trichopoliodystrophy) as having a marked reduction of cytochrome aa_3 in mitochondria isolated from muscle tissue obtained by biopsy, and in liver and brain tissue obtained at autopsy. The boy died at 21 months of age after a progressive neurologic disorder characterized by seizures, psychomotor deterioration, hypotonia, and neurovegetative disturbances. Histopathologic studies on brain tissue showed the characteristics of poliodystrophy in the cerebral gray matter, which were associated with the same abnormalities in the cerebellar cortex (Purkinje cells) as reported in other patients with Menkes' disease. Histochemical studies of muscle tissue showed occasional areas of hyaline degeneration. In these regions, electron microscopic studies showed disruption of the fine mitochondrial structures. Pathologic findings in liver tissue were not reported. In their study from 1972, French et al don't mention the values of copper or ceruloplasmin. However, in the same year, Danks et al demonstrated that Menkes' disease is due to a defective copper metabolism.

In both patients we described, a marked reduction of cytochrome aa_3 content was demonstrated in isolated muscle mitochondria. In patient 1, cytochrome *b* content was decreased to some extent too. The low activity of cytochrome oxidase, as measured in patient 1, corresponds positively with the decrease of cytochrome aa_3. The low pyruvate oxidation rate and citric acid cycle activity results from a decreased oxidation of NADH in the respiratory chain. In patient 2, the enzymatic analysis was restricted to the study of cytochrome oxidase activity and of the cytochrome spectrum. The results of that study indicate a deficiency in cytochrome aa_3. Comparing these results with the results obtained from patient 1, revealed a similar deficiency of cytochrome aa_3 in both patients.

Histopathologic studies on muscle tissue in patient 1 revealed only slight neuropathic changes, no abnormal mitochondria were seen. This finding is concordant with the findings of Spiro et al (1970), French et al (1972) and Ghatak et al (1972). Although biochemical evidence was found for a mitochondrial dysfunction, no major mitochondrial abnormalities were seen at the microscopic and submicroscopic levels. In patient 2, histopathologic studies in muscle tissue showed the characteristics of a mitochondrial myopathy. Van Biervliet et al (1977), Morgan-Hughes et al (1977), Di Mauro et al (1980) and Heiman-Patterson et al (1982) also reported concomitant morphologic and biochemical mitochondrial abnormalities in muscle tissue.

In our two patients we found the histopathologic characteristics of progressive poliodystrophy (Alpers, 1960; Jellinger et al, 1970; Partouche, 1977). Unfortunately, autopsy was not permitted on patient 1. However, a brain biopsy in this patient indicated progressive poliodystrophy. In patient 2, autopsy revealed the well-known characteristics of progressive poliodys-

trophy, with marked focal diversity in the severity of the pathologic process in the various regions of the cerebral cortex.

In general, progressive poliodystrophy is a disorder of early childhood. The clinical signs are progressive psychomotor retardation, seizures, myoclonic jerks, hypotonia, paresis, and various other neurologic dysfunctions (Greenhouse et al, 1964; Partouche, 1977). The first manifestations of the disorder occur mostly at the age of about 1-2 years, the child dies before the age of 5-6 years, after an illnes with a rapidly progressive course (Greenhouse et al, 1964; Jellinger et al, 1970; Partouche, 1977). This infantile pattern of progressive poliodystrophy was seen in patient 1. Patient 2, on the other hand, was a representative of the juvenile form of progressive poliodystrophy. This form, manifestating from the age of about 4-8 years, with a moderate course, the patients dying approximately between 12-20 years, has been reported by several authors (Freedom, 1927; Klein and Dichgans, 1969; Guidugli et al, 1973; Shapira et al, 1975; Hart et al, 1977). In patient 2, the initial signs were observed at about 10 years; progression was moderate until a rather sudden death at 16 years.

In literature, French et al (1972) report a cytochrome aa_3 deficiency in association with poliodystrophy. Their report concerns a child with Menkes' disease, a sib of the patient Menkes himself described. Poliodystrophy is a common characteristic of Alpers' disease and Menkes' disease, the latter also showing cerebellar abnormalities and specific hair abnormalities. Menkes' disease is biochemically characterized by a copper deficiency (Danks et al, 1972). In view of the similarities between Menkes' disease and progressive poliodystrophy, it is interesting to know that copper is an important co-factor in the cytochrome aa_3 complex.

In 1975, David et al reported the association of progressive infantile poliodystrophy with a pyruvate carboxylase deficiency in liver tissue. In earlier communications we reported three cases of progressive poliodystrophy associated with a pyruvate dehydrogenase complex deficiency in brain (Prick et al, 1981a), with a disturbed NADH oxidation in muscle and liver (Gabreëls et al, 1981; Prick et al, 1981b), and with a defect in citric acid cycle activity in liver and fibroblasts (Prick et al, 1982). Moreover, a pyruvate dehydrogenase complex deficiency in cultured fibroblasts was reported by Strömme et al (1976) in a patient whose brain biopsy, in our view, is suggestive of progressive poliodystrophy. In all these cases, elevated levels of lactate and pyruvate were found in CSF and/or in serum.

The combination of progressive poliodystrophy, mitochondrial myopathy and lactic acidemia has been reported by Shapira et al (1975) and Hart et al (1977). The lactic acidemia may point to a disturbed pyruvate metabolism. No enzymatic studies were reported in these cases.

The number of these reports suggests that there is not a mere casual

association, but a causal relationship between progressive poliodystrophy (Alpers' disease) and a defect in pyruvate metabolism. It is our opinion that a defective cerebral energy metabolism may be a principal etiologic factor in the neuronal degeneration of progressive poliodystrophy.

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CHAPTER 7

CONCLUSIONS AND DISCUSSION

PROGRESSIVE POLIODYSTROPHY IN THE LITERATURE

In chapter 1 the literature data on 86 cases of progressive poliodystrophy were summarized. Progressive poliodystrophy is a pathologic entity characterized by degeneration and loss of neurons, by astrocytic proliferation, and by dilatation and proliferation of capillaries. These abnormalities are restricted grossly to cerebral gray matter; they occur diffuse in some cases, but have a focal distribution in others. Definite diagnosis is only possible by pathologic study of brain tissue.[19,23]

Progressive poliodystrophy has an autosomal recessive inheritance (McKusick *20370[22]). The clinical picture is principally that of all progressive disorders of cerebral gray matter. The major symptoms of the disorder are progressive mental deterioration or retardation, epileptic manifestations, myoclonic jerks, and motor disability, manifesting as paresis, hypotonia, ataxia. These clinical signs were reported in nearly all reports of the disorder. A notable feature is that the symptoms of the disorder were often provoked by physical or psychic stress.

Progressive poliodystrophy was described as a clinical entity for the first time by Alpers in 1931.[1] The clinical signs and the pathologic findings reported in the literature by various authors show no major differences from those described by Alpers.[15,16,19,23] The literature data on progressive poliodystrophy make it clear that most of the clinical signs and pathologic features of the disorder are now well known. However, little is known about the pathogenesis of the disorder. The literature was therefore studied with specific emphasis on the pathophysiologic concept of progressive poliodystrophy.

PATHOGENETIC CONCEPTS IN THE LITERATURE

The discussion as to whether progressive poliodystrophy can be considered as a nosologic entity was summarized in chapter 1. With respect to this question several pathogenetic concepts were proposed in the literature. Some important pathogenetic concepts will be summarized and discussed here.

Most authors, with Alpers[2] as a representative of this group, avoided the discussion on the pathogenesis of progressive poliodystrophy. Distinct positions in the problem of the pathogenesis were adopted by Greenhouse and Neubuerger[16] and by Jellinger and Seitelberger.[19] Greenhouse and Neubuerger stated that cerebral anoxia is the major causal factor of progressive

poliodystrophy. In their opinion, periods of cerebral anoxia may occur during delivery, during seizures, and possibly also in other situations. Jellinger and Seitelberger discussed this proposition that cerebral anoxia could be a causal factor in progressive poliodystrophy. They collected from their own material and from literature case reports of patients who certainly did not pass through periods of cerebral anoxia in such a way that these periods could account for a progressive poliodystrophy. Moreover they pointed to the familial occurrence of the disorder. They therefore assumed the existence of an "idiopathic form" of progressive poliodystrophy. In their hypothesis a metabolic disorder would be a major causal factor of this "idiopathic form" of the disease.

DISCUSSION ON THE PATHOGENETIC CONCEPTS: CEREBRAL ANOXIA

A close scrutiny of the literature data provided some arguments relating to the discussion on the pathogenesis of progressive poliodystrophy. The arguments regarding the pathophysiologic concept of the disorder will be discussed here. On the basis of this study it is possible to draw some conclusions, which can be used to direct the clinical studies on patients. First, the theory on cerebral anoxia, as a major causal factor of progressive poliodystrophy, will be discussed from a pathologic and a clinical point of view.

a. The pathologic picture of progressive poliodystrophy, as seen generally at autopsy, is dominated by degeneration and loss of neurons. Several pathologic features are against the theory of a generalized cerebral anoxia as a major cause of the disorder.
- The degeneration of neurons is usually fairly obvious, while other gray matter structures are relatively unimpaired.[15,18,27]
- The pathologic picture is that of a progressive neuronal degeneration. Pathologic studies often revealed different stages of neuronal degeneration.[1,19] A period of cerebral anoxia generally occurs only once, and is therefore not sufficient to account for the pathogenesis of progressive poliodystrophy.
- The pathologic features are often focally distributed, and restricted to cerebral gray matter.[15,19,32] In several cases these features are not present in the hippocampal regio, which is very sensitive, especially in generalized cerebral anoxia.[9,19,26]
- A swelling and proliferation of endothelial cells in the cerebral capillaries is characteristic for cerebral anoxia. This feature was not described in progressive poliodystrophy.

b. Some aspects of the clinical picture also do not accord with the theory of a

generalized cerebral anoxia as a major cause of progressive poliodystrophy.
– The disorder is often inherited as an autosomal recessive trait.[22] The expression of such a genetic defect is usually that of a primary or secondary enzymatic deficiency.
– The clinical symptoms develop gradually, in such a way that the disorder has a progressive course, which in the juvenile form may even be slowly progressive.[15,17,27] An anoxic period would be more likely to cause an acute deterioration.
– A remarkable clinical feature in many cases is that the first symptoms of the disease manifest after physical or psychic stress, or that the disorder has an exacerbation after stress, for instance after febrille illnesses. Normally such periods are not associated with severe anoxia in the descriptions.

From the aforegoing it may be concluded that it is unlikely that cerebral anoxia is a major causal factor of progressive poliodystrophy.

DISCUSSION ON THE PATHOGENETIC CONCEPTS: METABOLIC DYSFUNCTION

This literature study provides two important arguments in support of the hypothesis of Jellinger and Seitelberger,[19] which suggests that a metabolic disorder is perhaps the main causal factor of progressive poliodystrophy.

a. In many clinical descriptions of progressive poliodystrophy it was reported that the first symptoms of the disorder or the exacerbations occurred after physical or psychic stress. This clinical feature could be an argument for the hypothesis of the metabolic pathogenesis of progressive poliodystrophy. During hospital observations it was noticed that the lactate and pyruvate levels in serum, urine and CSF were more greatly increased after exertion and after glucose or lactate load.[23,27] Physical stress normally results in a greater demand for ATP, which is produced by means of an increased glycolysis. Both in physical stress and during a glucose loading test there is an increased load of the pyruvate metabolism. A dysfunction of the pyruvate metabolism causes a shortage of ATP and an increase of lactate, the latter possibly leading to lactic acidosis. The caracteristic clinical feature that symptoms occur or exacerbate after stress may point to a marginal capacity of the pyruvate metabolism in these patients.
The observation of Greenhouse and Neubuerger[16] that a period of cerebral anoxia was often reported in patients with progressive poliodystrophy does not contradict the hypothesis of a metabolic disorder. Cerebral anoxia may possibly be such a stress to these children that the first symptoms of the disease or the exacerbations can be provoked by a period of anoxia. If the pyruvate metabolism has a marginal capacity in these patients, cerebral

anoxia may have such serious consequences. This could explain the fact that cerebral anoxia was so often reported in association with progressive poliodystrophy.

b. A second important argument for a metabolic pathogenesis of progressive poliodystrophy can be found in the outcome of biochemical and pathologic studies of some patients described in the literature. Progressive poliodystrophy was reported several times in association with a lactic acidemia.[17,23,26,27] Moreover, morphologic studies on tissues in patients with the disorder revealed abnormal mitochondric in muscle.[17,27] and in the brain.[26,29] Two authors reported on patients in whom both a lactic acidemia and a morphologically abnormal mitochondria in muscle tissue were found.[17,27]

These clinical, biochemical and morphologic findings in patients with progressive poliodystrophy indicate a metabolic disorder, namely a dysfunction of the mitochondrial pyruvate metabolism.
Enzymatic studies on tissues were performed in only one patient with progressive poliodystrophy. Partouche[23] described a child with a lactic acidemia, and the characteristics of progressive poliodystrophy at autopsy. In liver tissue, obtained at autopsy, a severely decreased activity of pyruvate carboxylase was found. It is questionable, however, whether the measurement of the labile pyruvate carboxylase in liver tissue obtained after death is sufficiently reliable. Partouche did not mention whether, as a control, he studied tissue also obtained at autopsy or at biopsy.
These findings can be an important starting-point for patient studies on the pathogenetic mechanism of progressive poliodystrophy.

THE CEREBRAL ENERGY METABOLISM

The pyruvate metabolism is particularly important for a properly functioning central nervous system (figure 1). Pyruvate –derived from glucose– is the main substrate of cerebral energy metabolism. In the citric acid cycle, acetyl-CoA –derived primarily from pyruvate– is oxidized to CO_2. Reducing equivalents are made available for ATP synthesis in the electron transport chain (respiratory chain). Cerebral pyruvate metabolism is identical to that in other tissues, but it is of special significance, since the brain depends entirely on the oxidation of pyruvate. It can also utilize ketone bodies when their concentration in the blood rises to a certain level, but this is unusual. Thus, as far as energy metabolism is concerned, the brain normally depends more than other tissues on continuous oxidative decarboxylation of pyruvate. Because of this dependency, abnormalities of pyruvate metabolism can reasonably be expected to impair the function of the central nervous system.[8,30]

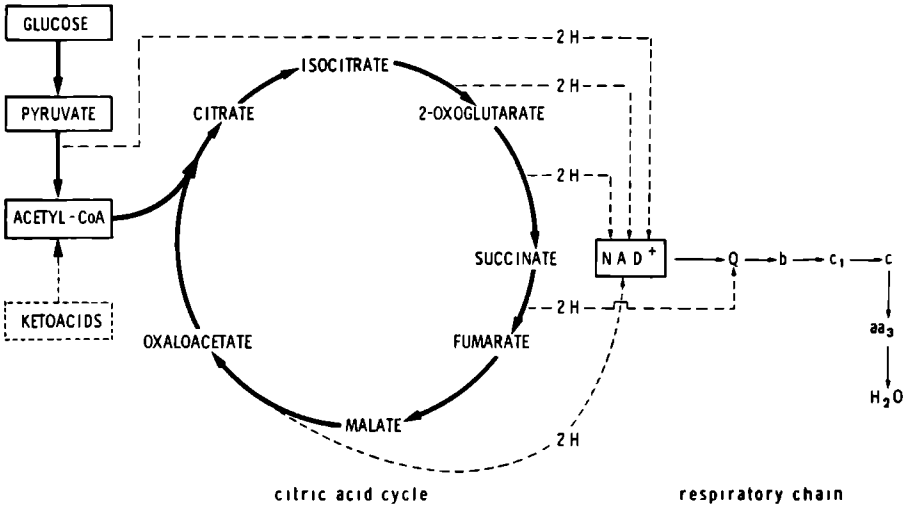

Figure 1. Pyruvate oxidation, citric acid cycle and respiratory chain.

It is noteworthy that a disturbance of pyruvate metabolism was demonstrated previously in several other neurologic disorders. The first report of a neurologic disorder with a defective pyruvate metabolism was that of a 9-year-old boy with intermittent ataxia, occurring after a nonspecific febrile illness;[5,6] a deficiency of pyruvate decarboxylase was demonstrated in muscle tissue and in fibroblasts. In the years following, disturbances of pyruvate metabolism were reported in patients with various neurologic disorders, especially in subacute necrotizing encephalomyelopathy (Leigh's disease)[14] and in Friedreich's ataxia.[20] A few patients with a disturbed pyruvate metabolism were also reported with hypomyelination,[3] with spinocerebellar degenerations without further classification,[21] and with hereditary motor and sensory neuropathy (HMNS I – Charcot-Marie-Tooth).[31] Thus, neurologic disorders with pathologic abnormalities in various systems and at various levels of the nervous system were described in association with disturbances of pyruvate metabolism.[7] There is probably a causal relationship between these morphologic and biochemical abnormalities.

To summarize: according to the literature on progressive poliodystrophy the pathogenesis of the disorder is still unknown. However, a detailed study of the literature data indicates that further investigations of the pathogenetic factors of progressive poliodystrophy must include enzymatic studies of the pyruvate metabolism and morphologic studies of cell mitochondria.

Table 1. Lactate and pyruvate values in serum and cerebrospinal fluid (CSF), and lactate, 2-oxoglutarate and succinate values in 24-hour urine of own patients and controls.

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Controls
Serum	Lactate°	800 1350	N	1850	1100-2050	1100-3500	600-1200
	Pyruvate°	100 130	N	137	84-111	90-160	75 120
	Ratio L/P	8 0-10 3	N	13 5	12 5-18 0	9 0-22 5	< 15
CSF	Lactate°	2500 3000	2080 2840	1500-3880	1220 1920	2550 3890	1200-1600
	Pyruvate°	191-204	N	126 148	70 100	145 178	90-130
	Ratio L/P	13 1 14 7	19 23	11 9 26 0	18 20	17 5 21 9	< 15
Urine (24 h)	Lactate°	N	N	650-730	–	–	< 100
	2-Oxoglutarate$^{\delta}$	–	–	137	–	–	< 125
	Succinate$^{\epsilon}$	–	–	700	–	–	< 100

° µmol/l
δ µmol/mmol creatinine
ε µmol/24 h

OWN PATIENT DATA

The conclusions from the literature studies made it probable that abnormalities of pyruvate metabolism could be found in patients with progressive poliodystrophy Based on this assumption we carried out biochemical and morphologic studies on five children suspected of progressive poliodystrophy In these children lactate and pyruvate levels were measured in serum, urine and CSF Enzymatic studies of the pyruvate metabolism and morphologic studies paying special attention to cell mitochondria were performed on tissues obtained by biopsies

Five children with progressive poliodystrophy are reported in this study (chapters 3–6)* The clinical signs and the pathologic features in the brain tissue of these five children were similar to those described in the literature. In all these children clinical manifestations were provoked or increased by exertion Routine laboratory studies showed no relevant abnormalities, but incidentally moderately disturbed liver function tests

Metabolic investigations of serum, urine and CSF revealed increased levels of lactate and pyruvate in all patients (table 1) In four patients (patients 1, 3, 4, 5) serum levels of lactate and pyruvate were elevated with a normal to slightly increased lactate/pyruvate ratio In one patient (patient 3) the excretion of lactate, 2-oxoglutarate and succinate in 24-hour urine was increased In all five patients repeated CSF examinations showed increased levels of lactate, with normal to moderately elevated pyruvate levels and increased lactate/pyruvate ratio

Table 2. Results of histopathologic and electron microscopic studies on tissues of own patients with progressive poliodystrophy.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Muscle	N	lipid myopathy	N	focal neuronal degeneration	mitochondrial myopathy
Liver	N	lipid storage	lipid storage	some inflammatory infiltrates	lipid storage
Nerve	–	axonal degeneration	axonal degeneration	–	axonal degeneration

Histochemical and electron microscopic studies of muscle tissue showed no abnormalities in two patients (patients 1, 3). In one patient (patient 2) a lipid myopathy was observed, in another patient (patient 4) signs of a neurogenic muscle atrophy were seen, in yet another patient (patient 5) a mitochondrial myopathy was demonstrated (table 2).

Histochemical and electron microscopic studies of liver tissue revealed slight to moderate signs of fatty degeneration (lipid storage) in three patients (patients 2, 3, 5). In one patient (patient 4) some inflammatory infiltrates were observed. In one patient (patient 1) no abnormalities were seen in liver tissue (table 2).

Enzymatic studies of tissues showed a disturbance of the pyruvate metabolism in all patients (table 3). A deficiency of the pyruvate dehydrogenase complex in brain was demonstrated in patient 1, a disturbance in NADH oxidation in muscle and liver in patient 2, a decreased citric acid cycle activity in liver and fibroblasts in patient 3, and a cytochrome aa_3 deficiency in muscle in patients 4 and 5 (table 4, figure 2).

It is not yet clear whether the decreased enzymatic activity is a primary defect, or is due to a disturbance of the regulation of the activity of one or more enzymes of the pyruvate metabolism.

It is remarkable that the enzymatic disturbances demonstrated in tissues, indicating a mitochondrial dysfunction, are not always associated with corresponding morphologic abnormalities of mitochondria. This phenomenon is known in the literature; Di Mauro[11,12] described it in mitochondrial myopathies. There is no satisfactory explanation of this phenomenon in the literature.

* The patients are numbered here as follows: patient 1 was described in chapter 3, patient 2 in chapter 4, patient 3 in chapter 5, patient 4 in chapter 6 as case 1, patient 5 in chapter 6 as case 2.

Table 3. $^{14}CO_2$ production from various substrates in leukocytes, cultured fibroblasts, and liver and muscle tissue of own patients.

	Substrate	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Leukocytes	[1-^{14}C] pyruvate	N	N	N	–	–
	[2-^{14}C[pyruvate	N	N	N	–	–
	Ratio [1-^{14}C]/[2-^{14}C] pyruvate	N	N	↑	–	–
Fibroblasts	[1-^{14}C] pyruvate	N	N	↓	–	–
	[2-^{14}C] pyruvate	N	N	↓↓	–	–
Liver	[1-^{14}C] pyruvate + malate	N	↓	N	–	–
	[6-^{14}C] citrate + malate	–	–	N	–	–
	[1-^{14}C] 2-oxoglutarate	–	–	N	–	–
	[5-^{14}C] 2-oxoglutarate	–	–	↓↓	–	–
Muscle	[1-^{14}C] pyruvate + malate	–	↓↓	N	↓	–
	[1-^{14}C] pyruvate + carnitine	–	↓↓	N	↓	–
	[U-^{14}C] malate + pyruvate + malonate	–	↓↓	N	↓	–
	[U-^{14}C] malate + acetylcarnitine + malonate	–	↓↓	N	↓	–
	[1-^{14}C] 2-oxoglutarate	–	↓↓	N	↓	–
	[5-^{14}C] 2-oxoglutarate	–	–	N	–	–

↑ = increased
↓ = decreased
↓↓ = severely decreased

Table 4. Results of enzymatic studies in leukocytes, cultured fibroblasts, and liver, muscle and brain tissue of own patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Leukocytes	N	N	decreased citric acid cycle activity	–	–
Fibroblasts	N	N	decreased citric acid cycle activity	–	–
Liver	N	defective pyruvate oxidation	defective citric acid cycle activity between 2-oxoglutarate and fumarate	–	–
Muscle	N	defective NADH oxidation before CoQ	–	cytochrome aa_3 deficiency	cytochrome aa_3 deficiency
Brain	PDHC deficiency	–	–	–	–

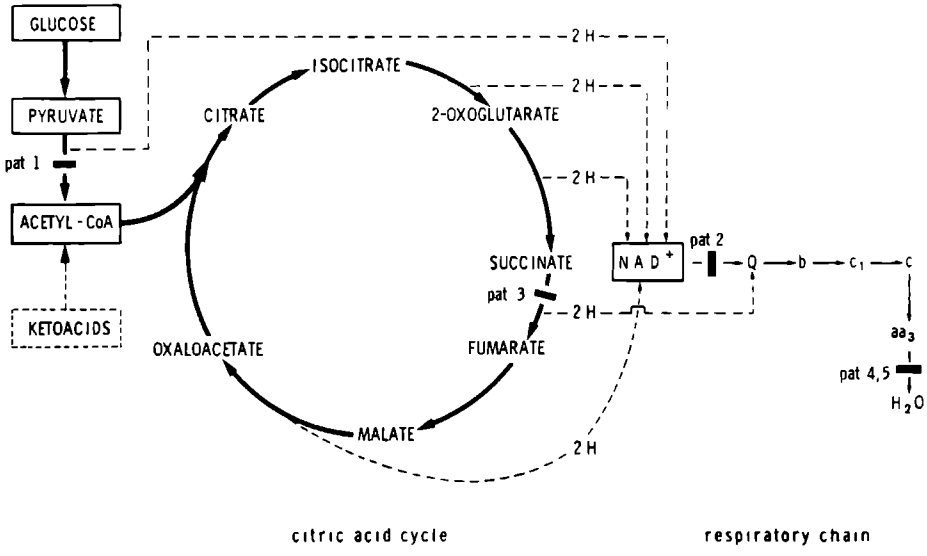

Figure 2. Disturbances in pyruvate oxidation, citric acid cycle and respiratory chain in own patient.

CONCLUSIONS

In this study five children were reported with progressive poliodystrophy in association with morphologic abnormalities in muscle and liver tissue, and with a disturbance of pyruvate metabolism demonstrated in one or more of the following tissues: brain, muscle, liver or fibroblasts.

The occurrence of these biochemical abnormalities in five patients with progressive poliodystrophy, coupled with the reports in the literature describing pathologically abnormal mitochondria or a metabolic dysfunction in this disease, suggests the existence of a causal relationship between this metabolic disorder and this neurologic disease. This association is an important argument for the existence of a nosologic entity "progressive poliodystrophy", or "Alpers' disease".

This study confirms Jellinger's hypothesis[19] of the existence of an "idiopathic form" of progressive poliodystrophy caused by a hereditary metabolic dysfunction. The aforegoing does not exclude the possibility that some cases of progressive poliodystrophy could be interpreted as a secondary, not hereditary, but symptomatic form of the disease.

The literature study and the investigations carried out on five patients lead to this final conclusion:

Progressive poliodystrophy is a neurologic disease of childhood, with a well-defined clinical picture, an autosomal recessive inheritance, and well-defined pathologic features; the disorder is most probably caused by a disturbance of the pyruvate metabolism.

DISCUSSION

This conclusion gives rise to some questions and reflections, which lead to recommendations for further investigations. The following areas will be discussed:

- the diagnostic procedures of the disease
- carrier detection and antenatal diagnosis
- possible therapeutic approaches
- the relation between morphologic and biochemical abnormalities
- progressive poliodystrophy and neurologic differential diagnosis.

DIAGNOSTIC PROCEDURES IN PROGRESSIVE POLIODYSTROPHY

Final diagnosis of progressive poliodystrophy in a child with a progressive neurologic disease still requires histopathologic study of brain tissue. In a living child a cortical biopsy would be required. To avoid such an invasive surgical intervention it would be highly desirable to have less aggressive diagnostic procedures.

On the basis of the reports in the literature and the findings in the five patients described above, we are convinced that the diagnosis of "progressive poliodystrophy" may be verified by biochemical demonstration of a deficiency in the pyruvate metabolism.

The decision to perform enzymatic studies on tissues depends very much on the results of the determination of lactate, pyruvate and citric acid cycle intermediates in serum, urine and CSF. Elevated values may point to a disturbed pyruvate metabolism. Other causes of a progressive disorder of cerebral gray matter and of elevated levels of lactate and pyruvate must be excluded. Thus, infectious diseases, vascular pathology, intoxications, vitamin deficiences, storage diseases, and inborn errors of metabolism must be specifically excluded.

The question may arise as to whether the elevated values of lactate and pyruvate, as found in progressive poliodystrophy, might be the result of or appear in connection with the epileptic manifestations occurring in the course of the disease, as the result of concurrent cerebral anoxia or acidosis. Our

own investigations* revealed that in children with epileptic manifestations normal values of lactate and pyruvate were found in serum, urine and CSF, even with frequently recurring seizures (e.g. in Lennox-Gastaut seizures). An exception is seen in the epilectic status. Therefore it would be implausible to state that the elevated lactate and pyruvate levels in progressive poliodystrophy are caused by the epileptic manifestations occurring in the disorder. Enzymatic studies of tissues must be performed if, on the basis of differential diagnostic considerations, a child is suspected of progressive poliodystrophy, and the studies of serum, urine and CSF reveal elevated levels of lactate, pyruvate, and possibly of citric acid cycle intermediates. In order to find morphologic abnormalities, especially abnormal mitochondria, in the same tissues microscopic and submicroscopic studies must also be carried out.

In children clinically suspected of a disorder of the cerebral gray matter, lactate and pyruvate levels in serum, urine and CSF constitute a central criterion in differential diagnosis. Increased values of lactate and pyruvate indicate the possibility of progressive poliodystrophy. As stated earlier in the discussion on patient 1 in chapter 3, the study of lactate and pyruvate must not be restricted to serum and urine alone, since in many cases the study of CSF levels of lactate and pyruvate will be an indispensable diagnostic criterion.

The study of lactate, pyruvate and citric acid cycle intermediates could probably be completed by an intravenous lactate[23] or pyruvate[10] loading test. The latter diagnostic procedures appear to be very useful in patients suspected of progressive poliodystrophy.

CARRIER DETECTION AND ANTENATAL DIAGNOSIS

The prevention of progressive poliodystrophy, a severe and rapidly progressive autosomal recessive disorder, requires procedures of carrier detection and antenatal diagnosis.

Carrier detection and antenatal diagnosis are generally performed on cultured fibroblasts, obtained by biopsy or amniocentesis respectively. It therefore seems to be important that the results of enzymatic studies on fibroblasts are in agreement with those of other tissues. In the present study enzymatic studies of fibroblasts were performed in three patients (patients 1, 2, 3). In two patients, although there was decreased enzymatic activity in the brain (patient 1) and in muscle and liver tissue (patient 2), normal activities of the enzymes of the pyruvate metabolism were measured in fibroblasts. In only one patient (patient 3) the enzymatic activity of pyruvate oxidation measured

* Gabreëls, personal communication.

in fibroblasts was obviously decreased. From other patient studies it was already known that there can be a discrepancy between fibroblast studies and studies of other tissues. These findings show that carrier detection and antenatal diagnosis by means of fibroblast studies alone may be insufficient at this moment. The same problem exists in the study of leukocytes.
In carrier detection investigations on muscle or even on liver tissue may also be performed, but for these studies more invasive procedures are needed.
Another problem will arise in the several tissue studies, namely that of the interpretation of the laboratory data. Biochemical studies of the patients described in this study revealed a distinct deficiency in the pyruvate metabolism in fibroblasts, leukocytes, muscle, liver or brain tissue. However, in all cases a residual activity was found. In control studies a considerable range of the values of enzymatic activity was measured. On theoretical grounds it may be virtually impossible to differentiate between patients and carriers, and between carriers and normals, because of an overlap of the values of enzymatic activity. In our opinion, this problem will arise both in carrier detection and in antenatal diagnosis.
To resolve these problems, further studies of the enzymatic activities of the pyruvate metabolism in fibroblasts must be carried out.

POSSIBLE THERAPEUTIC APPROACHES

At present progressive poliodystrophy is still an irreversible progressive neurologic disease. Therapeutic involvement in the disorder is relevant with respect to the relief of symptoms, e.g. epileptic manifestations. It seems important to search for a possible causal management of the disease.
In the literature on progressive poliodystrophy no obvious results concerning the causal therapeutic management of the disorder were described. In other diseases with similar deficiencies examples of therapeutic management are known, e.g. in PDHC deficiency.[13,24] and in pyruvate carboxylase deficiency,[4]
Therapeutic intervention in patients with a cytochrome related defect appears to be quite impossible. In disorders with a defective citric acid cycle activity, therapeutic management seems to be rather difficult; perhaps the addition of citric acid cycle intermediates may be of benefit in such cases.
With respect to PDHC deficiency Blass[7] suggested several possible therapeutic approaches. A high fat diet (ketogenic diet) may provide a substrate that bypasses a deficiency located before acetyl-CoA. Thiamine, a cofactor of the PDHC system, may favor the activity of PDHC. Dichloracetate is an activator of PDHC, and may therefore possibly be profitable in a PDHC deficiency.
It is our opinion that further studies of the therapeutic approaches to

deficiencies in pyruvate metabolism, as suggested above, must be performed in progressive poliodystrophy.

THE RELATION BETWEEN MORPHOLOGIC AND BIOCHEMICAL ABNORMALITIES

It has been noted that enzymatic disturbances demonstrated in tissues, pointing to a mitochondrial dysfunction, are not always associated with corresponding morphologic abnormalities of mitochondria. This observation is found in the literature; Di Mauro[11] described a similar phenomenon in mitochondrial myopathies. A satisfactory explanation of this discrepancy has not yet been given in the literature.

Di Mauro[11,12] emphasized that the definition of mitochondrial abnormalities by morphologic or clinical criteria alone is inadequate. He stressed that a rational classification should depend on the identification of the various biochemical defects.

It is our opinion that both biochemical investigations and morphologic studies are very important for a better understanding of these functional and structural abnormalities of mitochondria. Only combined clinical, morphologic and biochemical studies will lead to an understanding of the pathogenesis of progressive poliodystrophy and of related disorders.

PROGRESSIVE POLIODYSTROPHY AND NEUROLOGIC DIFFERENTIAL DIAGNOSIS

Until now progressive poliodystrophy was classified in neurologic differential diagnosis as a so-called degenerative disorder of the cerebral gray matter, without further specification. The addition of biochemical criteria to the well known clinical, genetic and morphologic criteria concerning the differential diagnosis of neurologic disorders may lead to a new classification of several of these "degenerative" neurologic disorders.

Disturbances of pyruvate metabolism, demonstrated by biochemical studies of tissues, may first of all be expected in those disorders, in which histochemical and electron microscopic studies revealed unexplained abnormalities in mitochondria. Disturbances in pyruvate metabolism have already been demonstrated in various disorders of the nervous system. Besides progressive poliodystrophy some other disorders which are associated with a defect in pyruvate metabolism have already been mentioned, e.g. subacute necrotizing encephalomyelopathy (Leigh's desease),[14] cerebral hypomyelination,[3] spino-

cerebellar degenerations,[21] Friedreich's ataxia[20] and hereditary motor and sensory neuropathy (Charcot-Marie-Tooth disease).[31]

Shapira[28] grouped together several neurologic and neuromuscular disorders, characterized by common morphologic and biochemical abnormalities in mitochondria. He proposed calling them "mitochondrial encephalomyopathies". Shapira suggested that these different "mitochondrial encephalomyopathies" would be characterized by morphologic abnormalities in mitochondria and by a specific defect in the oxidative metabolism.

In the five case reports in our study of progressive poliodystrophy one fairly well defined pathologic pattern is associated with various abnormalities in the pyruvate metabolism (chapters 3-6). In the literature all these abnormalities in pyruvate metabolism were also mentioned in association with other neurologic disorders.[7] The existence of a specific relation between these disorders and the several metabolic defects, as proposed by Shapira,[28] therefore seems unlikely. It seems probable that different disorders of the nervous system and neuromuscular disorders can be associated with any kind of biochemical abnormalities in pyruvate metabolism.

Modifying Shapira's hypothesis, we will propose the concept of mitochondrial encephalomyeloneuropathy, defined as a primary neuronal dysfunction anywhere in the nervous system, caused by a defect in the pyruvate metabolism and sometimes associated with morphologic abnormalities in mitochondria.

So far, there has not been an adequate explanation of the fact that these metabolic disturbances lead to a specific neurologic disorder, with localized abnormalities within the nervous system. Some studies indicate that there is a differentiated sensitivity of various cerebral areas with respect to the enzymes of pyruvate metabolism.[25] However, much more exploratory work must be done, with differentiated clinical, morphologic and biochemical studies, before we achieve a real insight in the pathogenesis of progressive poliodystrophy and other encephalomyeloneuropathies.

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SUMMARY

This study summarizes the literature on progressive poliodystrophy in a review of 86 cases, and presents the reports of five children with progressive poliodystrophy in association with a disturbed pyruvate metabolism.
Progressive poliodystrophy is a neurologic disorder of childhood with an autosomal recessive inheritance. The clinical picture is principally that of all progressive disorders of cerebral gray matter. The major symptoms are mental retardation or deterioration, epileptic seizures, myoclonic jerks, and motor disability. Visual and auditive dysfunctions are also frequently observed, as well as neurovegetative signs. The symptoms of the disorder are often provoked by physical of psychic stress. With respect to the age distribution, a distinction must be made between an infantile and a juvenile form of the disease.
The definite diagnosis of progressive poliodystrophy can only be made by histopathologic study of brain tissue. The disorder is characterized by degeneration and loss of neurons, by astrocytic profileration, and by dilatation and proliferation of capillaries. These abnormalities are restricted grossly to cerebral gray matter; they occur diffusely in some cases, but have a focal distribution in others.
Neuroradiologic, electroencephalographic and routine laboratory studies revealed no specific abnormalities. In several cases in the literature, increased levels of lactate and pyruvate in serum and in cerebrospinal fluid (CSF) were reported. Histochemical and submicroscopic studies of skeletal muscle tissue showed abnormalities in mitochondria in several cases.
The literature data on progressive poliodystrophy are summarized in chapter 1. In the literature little is known about the pathogenesis of progressive poliodystrophy. Two important pathogenetic concepts, proposed in the literature, are summarized and discussed in this study.
The findings of increased levels of lactate and pyruvate in serum and in CSF, and the morphologic abnormalities in muscle tissue mitochondria, indicated a defect in the intramitochondrial pyruvate metabolism. Some studies on the pathogenesis of the disorder supported this hypothesis. Therefore, when searching for pathogenetic factors in progressive poliodystrophy, special attention was directed to the cerebral pyruvate metabolism. Some biochemical aspects of the carbohydrate metabolism were summarized in chapter 2. The association of defects in the pyruvate metabolism with neurologic disorders (e.g. subacute necrotizing encephalomyelopathy and Friedreich's ataxia) was reported in the same chapter.
In four studies (chapters 3-6) extensive clinical, morphologic and biochemical investigations of five unrelated patients were reported. They all showed clinical symptoms indicating a progressive disorder of cerebral gray matter;

in all the children the illness exacerbated during stressful periods. Lactate levels in serum and/or CSF were elevated in all patients. Histopathologic studies of brain tissue showed the characteristics of progressive poliodystrophy in all patients. A deficiency of the pyruvate dehydrogenase complex in the brain was demonstrated in patient 1*, a disturbance in NADH oxidation in muscle and liver in patient 2, decreased citric acid cycle activity in liver and fibroblasts in patient 3, and a cytochrome aa_3 deficiency in muscle in patients 4 and 5.

The occurence of these biochemical abnormalities in five patients with progressive poliodystrophy, coupled with the reports in the literature describing pathologically abnormal mitochondria or a metabolic dysfunction in this disease, suggests the existence of a causal relationship between this metabolic disorder and this neurologic disease. The literature study and the investigations carried out on the five patients lead to this final conclusion. Progressive poliodystrophy is a neurologic disease of childhood, with a well-defined clinical picture, an autosomal recessive inheritance, and well-defined pathologic features; the disorder is most probably caused by a disturbance of the pyruvate metabolism.

In a final discussion, the diagnostic procedures of the disease, carrier detection and antenatal diagnosis, possible therapeutic approaches, the relation between morphologic and biochemical abnormalities and the relation between progressive poliodystrophy and neurologic differential diagnosis are discussed (chapter 2).

* patients are numbered as in chapter 7.

SAMENVATTING

Deze studie geeft in een overzicht van 86 gevallen een samenvatting van de literatuur over progressieve poliodystrofie, en beschrijft daarnaast 5 kinderen met progressieve poliodystrofie bij wie een stoornis in het pyruvaatmetabolisme werd aangetoond.
Progressieve poliodystrofie is een neurologische aandoening met een autosomaal recessieve erfmodus, optredend bij kinderen. Het klinisch beeld is in wezen gelijk aan dat van alle progressieve grijze stof aandoeningen. De voornaamste kenmerken zijn: mentale retardatie of deterioratie, epilepsie, myoclonieën en motorische uitvalsverschijnselen. Daarnaast worden vaak stoornissen waargenomen van het gehoor en de visus, evenals neurovegetatieve stoornissen. Lichamelijke of geestelijke stress provoceert dikwijls de verschijnselen. Onderscheid moet worden gemaakt tussen een infantiele en een juveniele vorm van de ziekte.
De diagnose "Progressieve Poliodystrofie" kan uiteindelijk alleen gesteld worden door histopathologisch onderzoek van hersenweefsel. De aandoening wordt gekenmerkt door degeneratie en verlies van neuronen, door proliferatie van astrocyten en door verwijding en proliferatie van capillairen. Deze afwijkingen zijn globaal genomen beperkt tot de cerebrale grijze stof; in sommige gevallen treden ze diffuus verspreid op, soms wordt een pleksgewijze verdeling gezien.
Neuroradiologisch, elektro-encefalografisch en routine laboratoriumonderzoek tonen geen kenmerkende afwijkingen. In de literatuur werden verschillende gevallen beschreven met verhoogde waarden voor laktaat en pyruvaat in serum en liquor. Histochemisch en submikroskopisch onderzoek van spierweefsel toonde in verschillende gevallen afwijkende mitochondria.
De gegevens uit de literatuur zijn samengevat in Hoofdstuk 1. Uit de literatuur blijkt dat er weinig bekend is over de pathogenese van de progressieve poliodystrofie. Twee belangrijke in de literatuur voorgestelde pathogenetische concepten zijn in deze studie samengevat en bediscussieerd.
Zowel de verhoogde waarden voor laktaat en pyruvaat in serum en liquor, alsook de morfologische afwijkingen in spiermitochondriën, gaven aanwijzingen voor een stoornis in het intramitochondriële pyruvaatmetabolisme. Enkele studies over de pathogenese van de aandoening gaven steun aan deze hypothese. Daarom werd in deze studie bijzondere aandacht besteed aan het cerebrale pyruvaatmetabolisme.
Enkele biochemische aspekten van het koolhydraatmetabolisme zijn samengevat in hoofdstuk 2. Tevens worden in dit hoofdstuk enkele neurologische aandoeningen vermeld (o.a. subacute necrotiserende encephalomyelopathie en de ataxie van Friedreich), waarbij stoornissen in het pyruvaat metabolisme aangetoond werden.

In de hoofdstukken 3 t/m 6 wordt uitgebreid ingegaan op klinisch, morfologisch en biochemisch onderzoek bij 5 niet verwante patientjes. Al deze patientjes toonden klinische verschijnselen die wezen op een progressieve aandoening van de cerebrale grijze stof; bij al deze kinderen trad er een verergering van de ziekte op tijdens perioden met stress. Bij deze patiëntjes werden verhoogde waarden voor laktaat gemeten in serum en/of liquor, bovendien toonde histopathologisch onderzoek van hersenweefsel de typische kenmerken van progressieve poliodystrofie. Aangetoond werden: bij patientje 1. een deficientie van het pyruvaat dehydrogenasecomplex in hersenweefsel, bij patiëntje 2. een stoornis in de NADH oxidatie in spier- en leverweefsel, bij patiëntje 3. een verminderde aktiviteit van de citroenzuurcyclus in lever en fibroblasten en bij de patiëntjes 4. en 5. een cytochroom aa_3 deficientie in spierweefsel.
Het optreden van deze biochemische stoornissen in de 5 beschreven patiëntjes, samen met de gegevens uit de literatuur die spreken over pathologo anatomisch abnormale mitochondriën, of over een metabole stoornis bij deze ziekte, geven sterke aanwijzingen voor het bestaan van een oorzakelijk verband tussen de biochemische en de morfologische afwijkingen.
De bestudering van de literatuur en het uitgevoerde onderzoek leiden tot de conclusie: Progressieve poliodystrofie is een neurologische ziekte optredend op kinderleeftijd, met een duidelijk omschreven klinisch beeld, een autosomaal recessieve erfmodus en vaste patholoog anatomische kenmerken. De aandoening wordt hoogstwaarschijnlijk veroorzaakt door een stoornis in het pyruvaatmetabolisme.
In de discussie worden de methoden van onderzoek, de carrier detectie en prenatale diagnostiek, de mogelijke therapeutische benaderingen, de relatie tussen morfologische en biochemische afwijkingen en de samenhang tussen progressieve poliodystrofie en de neurologische differentiaal diagnostiek besproken.

Dit onderzoek is verricht vanuit de Afdeling Kinderneurologie (Hoofd: Prof. Dr. F.J.M. Gabreëls) van het Instituut voor Neurologie (Hoofd: Prof. Dr. B.P.M. Schulte) van het Sint Radboudziekenhuis te Nijmegen.
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De interesse van de Afdeling Kinderneurologie in de normale en gestoorde cerebrale stofwisseling en het daarover reeds jaren lopende onderzoek, hebben deze studie mogelijk gemaakt.

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CURRICULUM VITAE

Mathé Prick werd geboren op 17 september 1946 te Nijmegen. Hij volgde daar het lager onderwijs en het Gymnasium Beta (Canisius College). Hierna begon hij aan de studie filosofie-theologie, resulterend in een vijf jarig kandidaats theologie aan de Katholieke Theologische Hogeschool Amsterdam, 1974. In 1969 begon hij met de studie geneeskunde aan de Universiteit van Amsterdam, waar hij in 1976 het artsexamen behaalde. In 1976 begon hij zijn opleiding tot neuroloog; de opleiding neurologie volgde hij aan het Radboud Ziekenhuis in Nijmegen (opleider Prof. Dr. J.J.G. Prick, later Prof. Dr. B.P.M. Schulte), de stage psychiatrie volgde hij in het Academisch Ziekenhuis van de Universiteit van Amsterdam (opleider Prof. Dr. P.C. Kuiper), de opleiding voor de aantekening klinische neurofysiologie volgde hij wederom aan het Radboud Ziekenhuis in Nijmegen (opleiders Prof. Dr. S.L.H. Notermans en Drs. P.J.H. Bernsen). De opleiding werd beëindigd op 31 augustus 1981. Sedert 1 november 1981 is hij werkzaam als neuroloog-klinisch neurofysioloog aan het Canisius-Wilhelmina Ziekenhuis in Nijmegen, in associatief verband met J.J. Prick (tot juli 1983), E.F.J. Poels, en Dr. C.W.G.M. Frenken (vanaf juli 1983). Sedert september 1977 is hij als docent neurologie/psychiatire verbonden aan de Opleiding Logopedie te Nijmegen.