Neonatal adrenoleukodystrophy

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SUMMARY Nine cases of neonatal adrenoleukodystrophy are described. All patients had abnormal facial features, moderate to severe hypotonia, hepatomegaly, and retinitis pigmentosa. The clinical course was rapidly progressive in six cases and more protracted in three others. Biological signs of adrenal insufficiency were present in five cases. CT scan showed a demyelinating process in four patients. Trilamellar inclusions were found in the liver of four cases and dark and complex lipidic inclusions in three other cases. In the three necropsied patients there was severe alteration of the white matter involving particularly the cerebellum in two cases. Gyral and cytoarchitectonic disturbances were absent in all three cases. Increased plasma levels of very long chain fatty acids (8/8), phytanic acid (7/8) and bile fluid trihydroxycoprostanic acid (2/4) confirmed the deficiency of multiple peroxisomal enzymes. Clinical, histopathological and biochemical findings of these nine cases are compared to those reported in other neonatal adrenoleukodystrophy cases and to those of other neonatal peroxisomal disorders, that is cerebro-hepato-renal syndrome of Zellweger and infantile Refsum's disease.

Childhood adrenoleukodystrophy has been recognised for over half century as a neurodegenerative disorder.^{1,2} Adrenomyeloneuropathy represents probably a phenotypic adult variant of the same disease.³ Adrenoleukodystrophy and adrenomyeloneuropathy are frequently observed in the same kindred and have an X-linked mode of inheritance. Neonatal forms have also been described.⁴⁻¹³ It is obvious now that childhood adrenoleukodystrophy and neonatal adrenoleukodystrophy are two different diseases.^{14,15} Neonatal adrenoleukodystrophy appears to be autosomal recessive and has never been observed in the same kindred with childhood adrenoleukodystrophy and adrenomyeloneuropathy. Neonatal adrenoleukodystrophy differs sharply from childhood adrenoleukodystrophy with

respect to age of onset, occurrence of retinal pigmentary degeneration and liver involvement.

Benke *et al*⁶ have drawn attention to the resemblance between cerebro-hepato-renal syndrome and neonatal adrenoleukodystrophy, and Goldfischer¹⁵ postulated that both form a closely related group of peroxisomal disorders. Evidence to support this view came from the lack of hepatic peroxisomes in both disorders^{11,12,16} and increased plasma and cultured skin fibroblasts very long chain fatty acids reflecting impaired β -oxidation of those substrates.^{10,17} Recently, Poulos *et al*¹⁸ have pointed out that infantile Refsum's disease^{19,20} may also be a possible variant of Zellweger's syndrome. Those findings raise the question of whether neonatal adrenoleukodystrophy and cerebro-hepato-renal syndrome are phenotypic variants of a specific enzyme deficiency or separate disease springing from different mutations.

We report the clinical observations and biochemical findings of nine cases of neonatal adrenoleukodystrophy and the histopathological data of three of them, which may throw some light upon this still confused area of peroxisomal disorders.

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Received 26 February 1985. Accepted 22 April 1985

Table 1

General	Case 1	2	3	4
Birth weight (g)	3680	3860	2960	2600
Head circumference (cm)	38	34	35	34
Sex (M/F)	F	F	F	F
Family history	Parents consanguinous	None	Parents consanguinous	Sister of case 3
Clinical features				
Initials symptoms	At birth: Hypotonia Convulsions	At birth: Mild hypotonia Poor feeding Convulsions (2 mths)	At birth: Stridor Severe hypotonia Convulsions (day 1) Lethargic 15 day	At birth: stridor Severe hypotonia convulsions (day 9) lethargic 7 mth
Clinical signs at the first examination	1 mth	3 mth		
Head circumference	>95th percentile	>70th percentile	>80th percentile	>95th percentile
Hypotonia	++	++	+++	+++
Deep tendon reflexes	Brisk	Brisk	Decreased	Decreased
Babinski sign	+	+	+	+
Deafness	+	+	+	+
Retinal pigmentary degeneration	+, pale optic disks	+	-	+
Diffuse amyotrophy	-	+	+++	+++
Hepatomegaly	+	+	+	+
Clinical signs of adrenal insufficiency	-	-	-	-
Neurological course	By 7 mths: decreased deep tendon reflexes, convulsions less frequent No development skills. Worsening after 12 mths. Died at 14 mths	Persistent convulsions worsening after 5 mths Died at 7 mths	 Died at 1 mth	No development skills. Persistent convulsions Died at 8 mths

Hypotonia: + = mild, ++ = moderate, +++ = severe.

Babinski sign, Deafness, Retinal pigmentary degeneration, Hepatomegaly, Clinical sign of adrenal insufficiency: + = present, - = absent.

Diffuse amyotrophy: - = absent, + = mild, ++ = moderate, +++ = severe.

Fig 1 Patients 1 (A), 5 (B) and 7 (C) at 5, 2, and 8 months, respectively.

5 3000 35 M Brother of case 3	6 2370 33 M None	7 3150 33 M None	8 3100 33 M None	9 2450 35 M Parents consanguinous
Stridor Severe hypotonia Convulsions (day 5) Deafness 2 mth	1-3 month: Mild hypotonia Failure to thrive 4 mth	2-4 month: Poor feeding Failure to thrive Impaired vision (4-6 mths) 9 mth	2-4 month: jaundice failure to thrive mild hypotonia Impaired vision 8 mth	At birth: Massive hypotonia Absence of respiratory movements lethargic 15 day
>70th percentile ++ Decreased + + +++ + -	>50th percentile + Decreased - - +, pale optic disks ++ + -	>50th percentile + Normal + + +, pale optic disks - + -	>50th percentile ++ Normal + - + cataract + + -	>90th percentile +++ Absent - - -, pale optic disks +++ + -
No development skills	Ocular pursuit at 5 mths, head control at 7 mths, sit with support at 9 mths. No convulsion. Worsening after 12 mths Died at 14 mths	Blindness at 8 mths, head control at 10 mths, convulsions at 20 mths Worsening after 12 mths Died at 26 mths	Head control at 6 mths, ocular pursuit with nystagmus at 7 mths No convulsion Alive at 13 mths	No convulsion Died at 1 mth
Died at 4 mths	Died at 14 mths	Died at 26 mths	Alive at 13 mths	Died at 1 mth

Methods

Methods used for neuropathological, adrenal gland and liver studies have been previously described.²¹ Liver biopsy samples from cases 1, 2, 6, 7, 8 and 9 were examined at 2, 3, 5, 9, 8 and 1 months respectively. Two liver biopsy samples from case 4 were studied at 20 days and 7 months. The procedure used for the determination of bile acids concentrations in fluids and for the analysis of very long chain fatty acids and phytanic acid in the plasma have been previously reported.^{22,23}

Clinical summaries

Table 1 summarises the clinical data of the nine patients. All had abnormal facial features with dolichocephaly, mongoloid slant of palpebral fissures, and anteverted nostrils. However, the dysmorphic features and the clinical course were different in the nine cases. Patients 1 and 2 (fig 1A) appeared to be like the patient described by Benke *et al.*,⁶ while patients 3, 4, 5 and 9 (fig 1B) had small triangular shaped face and small eyes. All patients appeared to be severely ill from birth. Convulsions were the most noticeable problem for the first months of life in cases 1 and 2. The four other newborns showed a peculiar lack of adipose tissue and severe amyotrophy at birth. Patients 3,

4 and 5 had dyspnoea and inspiratory stridor from birth. Slowly progressive respiratory difficulty in cases 3 and 4 required temporary tracheal intubation. Patient 9 showed no respiratory movements from birth and required full ventilatory support until his death. On the other hand, patients 6, 7 and 8 were in a less severe condition during the first months of life. Facial features of patient 7 (fig 1C) showed some similarity to those of the patient described by Brown *et al.*¹⁰ In all those three patients, psychomotor retardation became obvious only by the age of 4-7 months, and failure to thrive and/or jaundice were the most important initial clinical problems. Vision was moderately impaired up to 4-6 months. Psychomotor development stopped at 7 and 12 months respectively for patients 6 and 7, followed by a period of rapid decline.

Results

LABORATORY, RADIOLOGICAL AND NEUROPHYSIOLOGICAL STUDIES

Table 2 summarises the main data in eight patients. Liver dysfunction was present in three cases, mild adrenal insufficiency in five patients (1, 4, 5, 8, 9) and CSF protein was increased in three. Elec-

Table 2

Case no.	1	2	4	5	6	7	8	9
Transaminases	Normal	Normal	Normal	Normal	Increased (×3)	Increased (×4)	Increased (×4)	Normal
Coagulation clots	Normal	Normal	Normal	Normal	Normal	Decreased VII + X	Normal	Normal
Fasting blood cortisol	Low	Normal (3 mth)	Low (6 mth)	Normal (1 mth)	ND	Normal	Normal	Normal
ACTH (pg/ml)	60	72	379	140	ND	92	183	202
CSF protein (g/l)	0.42	0.46	0.25	ND	0.71	0.33	0.25	0.27
Extinguished Electrorétinogram	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Electroencephalogram	Slow waves 1-3 Hz right and left spikes	Slow waves 1-5 Hz sharp waves	Sharp waves 2-4 Hz bilateral spikes	Slow waves 2-3 Hz asymmetric spikes	Left spikes slow waves 3 Hz	Normal at 9 mth bilateral spikes at 20 mth	Slow waves 3 Hz	Slow waves 1- Hz right spikes
Electromyogram	Polyphasic motor unit potentials at 6 mth	Mild polyphasic motor unit potentials at 7 mth	Normal	Normal	Polyphasic motor unit potentials at 6 mth	Normal	Normal	Decreased duration and amplitude of motor unit potentials at 1 mth
Nerve conduction velocity	22 m/s (SPE nerve) 21 m/s (ulnar nerve) at 6 mth	21 m/s (SPE nerve) 28 m/s (ulnar nerve) at 7 mth	Normal	15 m/s (SPI nerve) at 2 mth	Normal at 6 mth	Normal	Normal	Normal
CT scan	Mild atrophy at 1 mth. Oedematous density of white matter at 7 mth	Mild vermis and cerebellar atrophy at 3 mth	Mild atrophy and oedematous density of white matter at 3 and 7 mth	Oedematous density of white matter at 2 mth	Normal at 4 mth	Mild atrophy at 8 mth Oedematous density of white matter at 16 mth	Normal at 6 mth	Oedematous density of white matter at 1 mth

ND = not done. ACTH, normal <90 pg/ml.

tromyography (EMG) showed polyphasic motor potentials in three patients (1, 2, 6) suggesting a neurogenic process but nerve conduction velocity (NCV) was decreased in two cases only (1, 2). In case 9, EMG showed decreased duration and amplitude of unit potentials suggestive of a myopathic process. CT scan showed a demyelinating process in five patients (1, 4, 5, 7, 9).

As shown in table 3, the levels of plasma C26:0 fatty acid were increased in eight patients, and C26:1 in seven. All but one patient had increased C24:0/C22:0 ratios. C22:0 levels were decreased in

four patients, whereas C24:0 levels appeared within the normal range in six. One patient (case 2) had only slightly increased levels of C26:0. Phytanic acid levels were strongly increased in two patients (6, 8) and slightly in five.

Bile acids analysis from samples of duodenal fluid aspirates were performed in two patients (6, 8) and showed increase levels of trihydroxycoprostanic acid. Small traces of dihydroxycoprostanic and varanic acids were found. In two other patients (cases 2 and 7), serum bile acids concentrations were normal.

Table 3 Very long chain fatty acids, and phytanic acid content in plasma ($\mu\text{M/l}$). Trihydroxycoprostanic acid content in bile or plasma ($\mu\text{M/l}$)

	C22:0	C24:0	C26:0	C26:1	C24/C22	C26/C22	Phytanic acid	Trihydroxy coprostanic acid
1	20.94	57.07	6.14	2.87	2.72	0.293	5.70	ND ¹
2	23.62	14.60	1.31	0.319	0.618	0.055	2.39	Absent (serum)
4	7.04	19.28	13.54	8.71	2.73	1.92	12.34	ND ¹
5	40.72	53.76	14.43	6.08	1.32	0.354	18.31	ND ¹
6	7.54	12.85	4.11	3.76	1.70	0.545	136.37	952 (bile) 17% of total bile acids
7	12.16	17.64	6.29	3.81	1.45	0.517	4.49	Absent (serum)
8	10.17	11.10	6.22	4.12	1.09	0.611	32.75	377 (bile) 13% of total bile acids
9	27.70	33.62	2.85	4.12	1.21	0.102	8.66	ND ¹
Controls ²	38.2±	27.4	0.35	0.2	0.68	0.011±	2.09	
n = 40	19.0	±16.2	±0.19	±0.1	±0.20	0.007	±1.35	

1: not done, 2: mean ±SD.

Fig 2 Case 4. First liver biopsy, parenchymal liver cells. Cluster of jet-black bodies located near a dilated bile canaliculus (uranyl acetate and lead citrate, $\times 10,000$).

Liver biopsy

Ultrastructural findings were nearly similar in cases 1, 2, and 4. The main ultrastructural change in these cases was the presence of peculiar cytoplasmic dense bodies (fig 2). These jet-black structures were found to have irregular, angular outlines and a distinct limiting membrane with a cleft-like space separating them from the surrounding cytoplasm. These spaces were probably due to technical artefacts related to the hardness of their structure. The dark bodies were generally located in the hepatocytes near bile canaliculi but were also found in some duct and mesenchymal cells. Bile canaliculi with loss of mic-

Fig 3 Case 4. Second liver biopsy. In a parenchymal hepatic cell, a lipid vacuole is divided in compartments by a ribbon like membranous material (uranyl acetate and lead citrate, $\times 10,000$).

rovilli were sometimes dilated and their lumen was filled with deposits of loose membranous material. In the second liver sample of case 4, the homogeneous bodies and the dilatation of bile canaliculi could no longer be found but the steatosis was more marked. Lipid vacuoles have an unusual pattern and were divided in compartments by a ribbon-like material of interconnecting membranes (fig 3). In hepatocytes this material appeared to result from clustering of altered smooth membranes (fig 4). No trilamellar inclusions were found in those 4 cases.

In cases 6, 7, 8 and 9, mesenchymal cells contained ovoid or spindle-shaped inclusions with irregular outlines and some spike-like protusions. Their light or dense matrix was filled with globules of various densities and rectilinear lamellae showing a trilamellar structure (fig 5a). Hepatocytes also included heterogeneous dense bodies and trilamellar structures (fig 5b). Lipid vacuoles identical to those encountered in cases 1, 2, and 4 were scanty.

Peroxidatic activity of catalase in peroxisomes was studied in case 8: no activity could be detected. However, the hepatocytic cytoplasm of all the liver samples showed the presence of microbodies whose structure looked like peroxisomes. Their number and size were strikingly decreased (fewer than 50 microbodies per hepatocyte), and they were even absent in case 8. In all our cases, the mitochondria were morphologically normal.

Neuropathological study

Brain. The brain weight was increased in two of

Fig 4 Case 4. Second liver biopsy. Exfoliated membranes of the smooth endoplasmic reticulum appear in the cytoplasm of a hepatocyte. Isolated dark bodies, numerous in the first biopsy are still occasionally found (uranyl acetate and lead citrate, $\times 30,000$).

Fig 5 Case 6. (A). In a hepatic mesenchymal cell, inclusion with a matrix of variable density contains globules of variable density and trilamellar structures (uranyl acetate and lead citrate, $\times 30,000$). (B). Hepatocyte cell contains the same trilamellar structures and jet-black bodies which are located near a lysosome (uranyl acetate and lead citrate, $\times 30,000$).

the three examined cases (1, 4). No significant abnormalities of cortical cytoarchitecture were found except in case 2 in which there was severe cortical atrophy. In all cases, there was a severe degeneration of the white matter involving both cerebral and cerebellar hemispheres while axons were preserved. In case 4, demyelination was striking in the cerebellar white matter and in the tegmentum of the brain stem (fig 6). In case 1, demyelination was also particularly important in the cerebellar white matter with many cuffs of mononuclear cells (fig 7). Heterotopic Purkinje cells were found to be aggregated in irregular clumps in the subcortical areas of the cerebellar cortex in cases 1 and 4 (fig 8). No heterotopic neurons were observed in the corti-

cal layers or in the subcortical white matter of the cerebral hemispheres. Each olivary nucleus was found to have normal morphology.

Electron microscopic study was performed upon necropsy specimens. Ultrastructural preservation of tissue was poor. In addition to numerous lipid droplets, histiocytic cells and astrocytes contained inclusions with complex lamellar profiles and irregular electron dense bodies. Neither trilamellar inclusions nor mitochondrial abnormalities could be observed.

Sural nerve biopsy specimen (case 1) showed decreased density of myelinated sheaths with preservation of Schwann cells and axons. No trilamellar inclusions were found in the Schwann cells.

Fig 6 Case 4. Cerebellar white matter and brain stem tegmentum showing severe demyelination. Note the normal convolution of both principal inferior olivary nuclei (Loyez, $\times 2.1$).

Fig 7 Case 1. Cerebellar white matter: gliosis of the white matter and perivascular cuffs of mononuclear cells (H and E, $\times 288$).

Fig 8 Case 1. Heterotopic Purkinje cells in the subcortical white matter of the cerebellar cortex (Nissl, $\times 113$).

Adrenal gland study (case 1)

The adrenal glands were atrophic and weighed 2 g each. The main histological features were the presence of ballooned cortical cells in the zona fasciculata and reticularis, and the persistence of the fetal zone of the adrenal cortex. No striated cell was seen. Ultrastructural study was not performed.

Discussion

The criteria used for assignment of a patient to the neonatal adrenoleukodystrophy category have been the onset of disability in the neonatal period, involvement of adrenal cortex and cerebral white matter and increased very long chain fatty acids in plasma, cultured skin fibroblasts, brain and adrenal gland.^{5-10 12 13} Clinical and biological data warrant the inclusion of our nine patients in this category. All patients had in common development delay from birth, craniofacial dysmorphism, moderate to severe hypotonia, hepatomegaly and retinitis pigmentosa. Pigmentary changes in the eyes were obvi-

ous only after 4–6 months of age, but the electroretinogram was early extinguished. Elevated levels of plasma very long chain fatty acids confirmed the diagnosis. Pipecolic acid was found to be normal in the urines of three cases studied (1, 4, 8) as in some other neonatal adrenoleukodystrophy cases.¹² Increased ACTH plasma levels suggesting an abnormal adrenal function were found in four cases, and low morning serum cortisol without rise after IV ACTH (case 1) in a fourth case. Adrenal glands were studied in one case showing atrophy and ballooned cortical cells without striated cells. The apparently normal adrenal function observed in two cases with normal ACTH levels is not incompatible with this diagnosis. Powers *et al*²⁴ have shown that fetal adrenal glands from fetuses (childhood adrenoleukodystrophy) with abnormal fatty acids in cultured amniotic cells contain striations and lamellae pathognomonic of adrenoleukodystrophy. However, in this latter disorder, it is shown that signs of adrenal failure may be absent or of delayed onset.¹² A demyelinating process was observed in three necropsied cases (1, 2, 4) and was demonstrated on CT scan in three other patients 3, 7, 9. The distinction between neonatal adrenoleukodystrophy and childhood adrenoleukodystrophy is clear but the situation in regard to cerebro-hepato-renal syndrome and neonatal adrenoleukodystrophy is less well defined.

The resemblance includes the onset of disability in the neonatal period, hypotonia with convulsions, retinal pigmentary degeneration with the presence of bileaflet inclusions and increased very long chain fatty acids in the retina,²⁵ hepatomegaly with micronodular cirrhosis and trilamellar fatty acid inclusions,^{21 26} striated adrenocortical cells^{14 21} and increased very long chain fatty acids in plasma^{17 21 27} and brain.¹⁷ However, infants with cerebro-hepato-renal syndrome have a more severe neurologic deficit and rarely survive beyond the fifth month. The strikingly dysmorphic facial features are observed in all cerebro-hepato-renal syndrome patients, which is not the case in neonatal adrenoleukodystrophy. Eventually, cerebral abnormalities observed in neonatal adrenoleukodystrophy as found in our cases are different from those encountered in cerebro-hepato-renal syndrome.^{21 28} As in some neonatal adrenoleukodystrophy reports,^{5 7 10} we found no gyral and cytoarchitectonic disturbances in the cerebral hemispheres. Some Purkinje cells were found to be present in the subcortical white matter of the cerebellum but the inferior olivary nuclei showed a normal morphology. Abnormalities of gyration with “micropolygyria” have been reported by some authors^{4 6 9} but the highly characteristic disorder of neuronal migration

which seems to be constant in all cerebro-hepato-renal syndrome patients has never been found in neonatal adrenoleukodystrophy.

The situation in regard to neonatal adrenoleukodystrophy and infantile Refsum's disease is more complex. The clinical features of the infantile Refsum's disease include retinitis pigmentosa, deafness, facial dysmorphism, growth and/or mental retardation, peripheral neuropathy (inconstant) and hepatomegaly.^{19 20 29-32} Liver biopsy shows mild to severe fibrosis and trilamellar inclusions in parenchymal and non parenchymal liver cells,^{19 20} identical to those described in adrenoleukodystrophy, neonatal adrenoleukodystrophy and cerebro-hepato-renal syndrome. The clinical course of infantile Refsum's disease is not as severe as in most patients with neonatal adrenoleukodystrophy and some patients survive beyond the tenth year. The patients described by Brown *et al*¹⁰ and Noetzel *et al*¹³ as neonatal adrenoleukodystrophy share the same clinical features as the three patients previously described by one of us,¹⁹ and seem to have followed the same protracted course, these three children being still alive at 7, 8, and 11 years. Increased levels of plasma very long chain fatty acids^{21 33} and abnormal bile metabolites^{18 32} have been documented in infantile Refsum's disease cases. Similarly, plasma phytanic acid levels are increased in infantile Refsum's disease^{19 20} as in cerebro-hepato-renal syndrome and neonatal adrenoleukodystrophy,²¹ reflecting a deficiency in phytanate oxidase^{18 20 31} which is supposed to be located in part in peroxisome.^{18 21} All those findings suggest that infantile Refsum's disease, cerebro-hepato-renal syndrome and neonatal adrenoleukodystrophy are closely related disorders involving multiple peroxisomal enzymes deficiency.

If neonatal adrenoleukodystrophy and infantile Refsum's disease are overlapping disorders, an attempt must be made to draw borderline between them. The severity of the disease in children with neonatal adrenoleukodystrophy or infantile Refsum's disease varies greatly and three different clinical pictures at least are encountered. In some patients (refs 4, 9, our cases 1 to 5 and 9), the neurological disability is as severe as in cerebro-hepato-renal syndrome. Those children have no intellectual development and survive rarely beyond the second year of life. In other patients, the clinical course is more progressive. Neurological development is delayed from birth but these children may acquire some psychomotor development. Most of them can apparently see and hold the head following the first 6 months of life. Failure to thrive, moderate developmental delay, hepatomegaly, hypotonia, decreased hearing and impaired visual acuity call for

attention between 3 and 6 months of life. For some patients, psychomotor development stops between 1 and 2 years and is followed by a rapid decline and death (refs 5-8, our cases 6 and 7). In the third group of patients, the course is more progressive. Some children can sit or walk alone by the age of five years and may be still alive after the age of ten years.^{10 13 19 32}

Absence of hepatic peroxisomes is considered to be an essential clue for the diagnosis of cerebro-hepato-renal syndrome but has been reported also to occur in neonatal adrenoleukodystrophy.^{10 12} Peroxisomes could not be identified after histochemical reaction in one of our cases. In the other cases, microbodies whose ultrastructural appearance was similar to peroxisome were found in all the liver biopsy samples but peroxidatic activity of catalase was not studied. Microbodies were detected in the liver of our cases, but their number and size were greatly decreased as also reported by Goldfischer *et al* in neonatal adrenoleukodystrophy.³⁴

Various types of inclusions have been described in the liver of neonatal adrenoleukodystrophy and infantile Refsum's disease including especially trilamellar inclusions (refs 11, 19, 20, and four of our cases). Other types of hepatic inclusions have been reported as "arced lamellae in plump, spindle shaped cluster",⁵ "packed lamellar profiles".⁷ The dark inclusions observed in three of our cases (1, 2, and 4) seem to be peculiar on account of the homogeneous matrix, their irregular outlines and their hardness. Their ultrastructure resembled those observed in some cases of neonatal cholestasis³⁵ but signs of cholestasis were mild or absent emphasising the peculiarity of this ultrastructural pattern. These inclusions have also some similarity with those observed in cerebro-tendinous xanthomatosis,³⁶ a possible peroxisomal disorder.¹⁵ The second biopsy of case 4 shows that the ultrastructure may change in the course of time. Bile canaliculi dilatation and jet-back inclusions were less numerous in hepatocytes and were replaced by empty and lipid-filled septate vacuoles. Those complex lipidic inclusions have also been reported in lymph nodes⁵ and thymus⁹ of neonatal adrenoleukodystrophy. If trilamellar inclusions are probably related to the accumulation of very long chain fatty acids, other lipidic inclusions probably contain more complex lipids. The various ultrastructural findings may also correspond to different phases in the evolution of the disease.

Cerebral pathology has not been studied in infantile Refsum's disease, since most patients are still alive, whereas in neonatal adrenoleukodystrophy it is mainly a demyelinating process. In neonatal adrenoleukodystrophy the distribution of the lesions

is variable, and the cerebellum may be more involved than the cerebral hemispheres (refs 4, 7, and our cases). The severity of the myelin involvement is more severe than in cerebro-hepato-renal syndrome, including moderate to severe gliosis, and perivascular cuffs of mononuclear cells. This intense inflammatory cell response occurring within the demyelinated areas (as in childhood adrenoleukodystrophy) is obvious in neonatal adrenoleukodystrophy but absent in cerebro-hepato-renal syndrome.²¹ Trilamellar inclusions are also found in brain macrophages^{4,7,8,11} but other complex lipid and membrane aggregate inclusions (refs 4, 9, our case 4) may be seen. As in the liver, the aspect of the lipid storage in the brain is variable and more data are required to determine whether the difference in the distribution of the lesions and in the morphological aspects of the inclusions are significant.

Biochemical abnormalities reflecting impairment of various peroxisomal enzymes have been documented in neonatal adrenoleukodystrophy and infantile Refsum's disease as in cerebro-hepato-renal syndrome including impaired oxidation of very long chain fatty acids and phytanic acid (refs 10, 17, 18, 21, 33, and our cases), bile synthesis defect with elevated levels of certain intermediates (refs 18, 32, 37, and our cases 6 and 8) and increased plasma and urine pipelicolic acid.^{18,38} Recent studies have emphasised the role of peroxisome in the synthesis of glycerol-ether lipids³⁹ and the presence of a deficiency of dihydroxyacetone phosphate (DHAP) acyltransferase and alkyl-DHAP synthetase^{40,41} in cerebro-hepato-renal syndrome. The presence of a deficiency in plasmalogens synthesis is under investigation in our patients but it must probably be assumed that neonatal adrenoleukodystrophy and infantile Refsum's disease patients are also deficient in glycerol-ether lipids synthesis. In the absence of specific biochemical abnormalities, it must be determined whether variable deficiency in these peroxisomal enzymes or other biochemical abnormalities exist accounting for the wide clinical and histopathological spectrum encountered in these disorders.

Conclusions

Cerebro-hepato-renal syndrome, neonatal adrenoleukodystrophy and infantile Refsum's disease are peroxisomal disorders that share many clinical, histopathological and biochemical features. In the absence of specific biochemical abnormalities one should determine if the large clinical and histopathological spectrum of these diseases is related to the variable deficiencies in peroxisomal enzymes

or other biochemical disturbances. However, some major discrimination allows a temporary classification. Severe clinical outcome, and neuronal migration disturbances, are always encountered in cerebro-hepato-renal syndrome suggesting that this disease is the consequence of a specific gene mutation. At the present time, there is no definite distinction between neonatal adrenoleukodystrophy and infantile Refsum's disease but the different clinical courses observed in these diseases suggest that there may be at least three phenotypic variants of a specific enzyme deficiency or three different mutations. Liver and brain histopathological findings are to date unhelpful in establishing a nosological classification but may supply some clarification pending to a better understanding of peroxisomal function, specially outer-inner membrane transport of enzymes.

We thank Drs C Billard, F Despert and M Voyer for referral of their patients. We are indebted to Dr MM Ruchoux who performed liver electron microscopy studies of case 7, to Dr K Diebler who performed CT scan studies, to Dr P Plouin who performed EEG records, and to J Denni and R Wehrle for expert technical assistance.

References

- Schaumburg HH, Powers JM, Raine CS, Suzuki K, Richardson EP Jr. Adrenoleukodystrophy. A clinical and pathological study of 17 cases. *Arch Neurol* 1975;**32**:577-91.
- Aubourg P, Chaussain JL, Dulac O, Arthuis M. Adrenoleukodystrophie chez l'enfant. A propos de 20 observations. *Arch Fr Pédiatr* 1982;**39**:663-9.
- Griffin JW, Goren E, Schaumburg HH, Engel WK, Loriaux L. Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. *Neurology (Minneapolis)* 1977;**27**:1107-13.
- Ulrich J, Herschkowitz N, Heitz P, Sigrist T, Baerlocher P. Adrenoleukodystrophy. Preliminary report of a congenital case. Light- and electron microscopical immunohistochemical and biochemical findings. *Acta Neuropath (Berl)* 1978;**43**:77-83.
- Manz HJ, Schuelein M, McCullough DC, Kishimoto Y, Eiben RM. New phenotypic variant of adrenoleukodystrophy. Pathologic, ultrastructural, and biochemical study in two brothers. *J Neurol Sci* 1980;**45**:245-60.
- Benke PJ, Reyes PF, Parker JC Jr. New form of adrenoleukodystrophy. *Hum Genet* 1981;**58**:204-8.
- Haas JE, Johnson ES, Farrell DL. Neonatal-onset adrenoleukodystrophy in a girl. *Ann Neurol* 1982;**12**:449-57.
- Mobley WC, White CL, Tennekoon G, et al. Neonatal adrenoleukodystrophy. *Ann Neurol* 1982;**12**:204-5.
- Jaffe R, Crumrine P, Hashida Y, Moser HW. Neonatal adrenoleukodystrophy. Clinical, pathologic, and

- biochemical delineation of a syndrome affecting both males and females. *Am J Pathol* 1982;108:100-11.
- 10 Brown III FR, McAdams AJ, Cummins JW, *et al*. Cerebro-hepato-renal (Zellweger) syndrome and neonatal adrenoleukodystrophy: similarities in phenotype and accumulation of very long chain fatty acids. *John Hopkins Med J* 1982;151:344-61.
 - 11 Partin JS, McAdams AJ. Absence of hepatic peroxisomes in neonatal onset adrenoleukodystrophy. *Pediatr Res* 1983;17:294A.
 - 12 Farell K, Dimmick JE, Applegarth DA, *et al*. Peroxisomal abnormalities in neonatal adrenoleukodystrophy. *Ann Neurol* 1983;14:379A.
 - 13 Noetzel MJ, Brent Clark H, Moser HW. Neonatal adrenoleukodystrophy with prolonged survival. *Ann Neurol* 1983;14:379-80A.
 - 14 Goldfischer S, Powers JM, Johnson AB, Axe S, Brown III FR, Moser HW. Striated adrenocortical cells in cerebro-hepato-renal (Zellweger) syndrome. *Virchows Arch* 1983;401:355-61.
 - 15 Goldfischer S. Peroxisomes and human metabolic diseases. The cerebro-hepato-renal syndrome, cerebrotendinous xanthomatosis and Schilder disease. *Ann NY Acad Sci* 1982;386:526-9.
 - 16 Goldfischer S, Moore CL, Johnson AB, *et al*. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. *Science* 1973;182:62-4.
 - 17 Moser AE, Singh I, Brown III FR, *et al*. The cerebrohepatorenal (Zellweger) syndrome. Increased levels and impaired degradation of very-long-chain fatty acids and their use in prenatal diagnosis. *N Engl J Med* 1984;310:1141-6.
 - 18 Poulos A, Sharp P, Whiting M. Infantile Refsum's disease (phytanic acid storage disease): a variant of Zellweger's syndrome? *Clin Genet* 1984;26:579-86.
 - 19 Scotto JM, Hadchouel M, Odièvre M, *et al*. Infantile phytanic acid storage disease, a possible variant of Refsum's disease: three cases, including ultrastructural studies of the liver. *J Inher Metab Dis* 1982;5:83-90.
 - 20 Poulos A, Pollard AC, Mitchell JD, Wise G, Mortimer G. Patterns of Refsum's disease (phytanic acid oxidase deficiency). *Arch Dis Child* 1984;59:222-9.
 - 21 Aubourg P, Robain O, Rocchiccioli F, Dancea S, Scotto J. The cerebro-hepato-renal (Zellweger) syndrome: lamellar lipid profiles in adrenocortical, hepatic mesenchymal, astrocyte cells and increased levels of very long chain fatty acids and phytanic acid in the plasma. *J Neurol Sci* 1985;69:9-25.
 - 22 Aubourg P, Bougnères PF, Rocchiccioli F. Capillary gas chromatographic mass spectrometric measurement of very long chain (C22 to C26) fatty acids in microliter plasma samples. *J Lipid Res* 1985;26:263-7.
 - 23 Feldmann-Pautrat D. *Thèse de Doctorat ès-sciences Pharmaceutiques*, 1984, Université Paris Sud.
 - 24 Powers JM, Moser HW, Moser AE, Schaumburg HH. Fetal adrenoleukodystrophy: the significance of pathologic lesions in adrenal gland and testis. *Hum Pathol* 1982;13:1013-9.
 - 25 Cohen SMZ, Brown III FR, Martyn L, *et al*. Occular histopathologic and biochemical studies of the cerebro-hepato-renal syndrome (Zellweger's syndrome) and its relationship to neonatal adrenoleukodystrophy. *Am J Ophthalmol* 1983;96:488-501.
 - 26 Mooi WJ, Dingemans KP, van den Bergh Weerman MA, Jobsis AC. Ultrastructure of the liver in the cerebro-hepato-renal syndrome of Zellweger. *Ultrastruct Pathol* 1983;5:135-44.
 - 27 Bakkeren JA, Monnens LAH, Trijbels JMF, Maas JM. Serum very long chain fatty acid pattern in Zellweger syndrome. *Clin Chim Acta* 1984;138:325-31.
 - 28 Volpe JJ, Adams RD. Cerebro-hepato-renal syndrome of Zellweger: an inherited disorder of neuronal migration. *Acta neuropathol* 1972;20:175-98.
 - 29 Richterich R, van Mechelen P, Rossi E. Refsum's disease (heredopathia atactica polyneuritiformis). An inborn error of lipid metabolism with storage of 3, 7, 11, 15-tetramethylhexadecanoic acid. *Am J Med* 1965;39:230-6.
 - 30 Kahle W, Goerlich R, Feist D. Erhöhte phytansäurepiegel in plasma und leber bei einem kleinkind mit unklarem hirnschaden. *Klin Wschr* 1974;52:651-5.
 - 31 Boltshauser E, Spycher MA, Stelmann B, *et al*. Infantile phytanic acid storage disease: a variant of Refsum's disease. *Eur J Pediatr* 1982;139:317A.
 - 32 Stokke O, Skrede S, Ek J, Bjorkhem I. Refsum's disease, adrenoleukodystrophy and the Zellweger syndrome. *Scand J Clin Lab Invest* 1984;44:463-4.
 - 33 Poulos A, Sharp P. Plasma and skin fibroblast C26 fatty acids in infantile Refsum's disease. *Neurology (NY)* 1984;34:1606-9.
 - 34 Goldfischer S, Collins J, Biempica L, Chang CH. The cerebro-hepato-renal syndrome (CHRS) and neonatal adrenoleukodystrophy (ALD): disorders of peroxisomal biogenesis. *Hepatology* 1983;3:876A.
 - 35 Phillips J, Latham PS. Electron microscopy of human liver disease. In: Schiff L and Schiff ER eds, *Diseases of the Liver*, 5th, ed, 1982:59-92.
 - 36 Salen G, Zaki FG, Sabesin S, Boehme D, Shefer S, Mosbach EH. Intrahepatic pigment and crystal forms in patients with cerebrotendinous xanthomatosis (CTX). *Gastroenterology* 1978;74:82-9.
 - 37 Monnens L, Bakkeren J, Parmentier G, *et al*. Disturbances in bile acid metabolism of infants with the Zellweger (cerebro-hepato-renal) syndrome. *Eur J Pediatr* 1980;133:31-5.
 - 38 Danks DM, Tippet P, Adams C, Campbell P. Cerebro-hepato-renal syndrome of Zellweger. A report of eight cases with comments upon the incidence, the liver lesion, and a fault in pipecolic acid metabolism. *J Pediatr* 1975;86:382-7.
 - 39 Hajra AK, Bishop J. Glycerolipid biosynthesis in peroxisomes via the acyl dihydroxyacetone phosphate pathway. *Ann NY Acad Sci* 1982;386:170-81.
 - 40 Schutgens RBH, Romeyn GJ, Wanders RJA, van den Bosch H, Schrakamp G, Heymans HSA. Deficiency of acyl-CoA: dihydroxyacetone phosphate acyltransferase in patients with Zellweger (cerebro-hepato-renal syndrome) syndrome. *Biochem Biochem Biophys Res Commun* 1984;120:179-84.
 - 41 Datta NS, Wilson GN, Hajra AK. Deficiency of enzymes catalyzing the biosynthesis of glycerol-ether lipids in Zellweger syndrome. *N Engl J Med* 1984;311:1080-3.