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 retinopathy. 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. Adrenomyeloneuropathy represents probably a phenotypic adult variant of the same disease. Adrenoleukodystrophy and adrenomyeloneuropathy are frequently observed in the same kindred and have an X-linked mode of inheritance. Neonatal forms have also been described. It is obvious now that childhood adrenoleukodystrophy and neonatal adrenoleukodystrophy are two different diseases. 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 have drawn attention to the resemblance between cerebro-hepato-renal syndrome and neonatal adrenoleukodystrophy, and Goldfischer 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 and increased plasma and cultured skin fibroblasts very long chain fatty acids reflecting impaired β-oxygenation of those substrates. Recently, Poulas et al have pointed out that infantile Refsum's disease may also be a possible variant of Zellweger's syndrome. Those finding 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.
Table 1

| General Birth weight (g) Head circumference (cm) Sex (M/F) Family history | Case 1 | Case 2 | Case 3 | Case 4 |
|---|---|---|---|---|
| 3680 | 3860 | 2960 | 2600 |
| 38 | 34 | 35 | 34 |
| M | F | F | F |
| Parents consanguinous | None | None | Parents consanguinous |
| Clinical features | At birth: | At birth: | At birth: | At birth: |
| Initials symptoms | Hypotonia, Convulsions | Mild hypotonia, Poor feeding Convulsions (2 mths) | Stridor, Convulsions (day 1) | Stridor, Convulsions (day 9) |
| Clinical signs at the first examination | 1 mth | 3 mth | 2 mths | 7 mths |
| 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 | No development skills. Persistent convulsions | Died at 7 mths | Died at 1 mth | 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.
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| Stridor | Severe hypotonia | Convulsions (day 5) | Deafness | 2mth | >70th percentile | Decreased | No development skills | Died at 4mths |
|---------|-----------------|--------------------|----------|------|-----------------|-----------|----------------------|--------------|
| 1-3mth: | Mild hyptonia | Thrive | 4mth | + + + | +, pale optic disks | + | Ocular pursuit at 5mths, head control at 7mths, sit with support at 9mths. No convulsion after 12mths | Died at 14mths |
| 2-4mth: | Poor feeding | Thrive | 9mth | >50th percentile | + | + | Blindness at 8mths, head control at 10mths, convulsions at 20mths Worsening after 12mths | Died at 26mths |
| 2-4mth: | Jaundice | Impaired vision | 8mth | >50th percentile | + | + | Head control at 6mths, ocular pursuit at 7mths. Nystagmus | Alive at 13mths |
| 2-4mth: | Failure to thrive | Impaired vision | | | | | | Died at 1mths |
| At birth: | Massive hypotonia | Respiratory movements | | | | | | |
| | | Lethargic | | | | | | |

### Methods

Methods used for neuropathological, adrenal gland and liver studies have been previously described. 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.

### 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 antverted 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., while patients 3, 4, 5 and 9 (fig 1B) had small triangular 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. In all three of these 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 | Normal | Increased (x3) | Increased (x4) | Increased (x4) | Normal | Normal |
| Coagulation clots | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| Fasting blood cortisol | Low (3 mth) | Normal | Low (6 mth) | Normal | Normal | Normal | ND | Normal | Normal |
| ACTH (pg/ml) | 60 | 72 | 0-46 | 0-25 | ND | 0-71 | 0-33 | 0-25 | 0-27 |
| CSF protein (g/l) | 0-42 | 0-46 | 0-25 | ND | 0-71 | 0-33 | 0-25 | 0-27 |
| Extinguished Electrodiagnostics | 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 1-3 Hz right spikes |
| Electromyogram | Polyphasic motor unit potentials at 6 mth | Normal | Polyphasic motor unit potentials at 7 mth | Normal | Polyphasic motor unit potentials at 6 mth | Normal | Normal | Normal |
| Nerve conduction velocity | 22 m/s (SPE nerve) | 21 m/s (SPE nerve) | 15 m/s (SPE nerve) | Normal | Normal |
| (ulnar nerve) | 28 m/s (ulnar nerve) | 28 m/s (ulnar nerve) | Normal at 6 mth | Normal at 6 mth |
| 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 2 mth | Normal at 4 mth | Normal at 6 mth |
| ND = not done. ACTH, normal <90 pg/ml. |

Electromyography (EMG) showed polyphasic motor potentials in three patients (1, 2, 6) suggesting a neuropathic 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 increased levels of trihydroxycoprostanic acid. Small traces of dihydroxycoprostanic and variolic 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 (μM/I). Trihydroxycoprostanic acid content in bile or plasma (μM/I)

| C22:0 | C24:0 | C26:0 | C26:1 | C24/C22 | C26/C22 | Phytanic acid | Trihydroxycoprostanic acid |
|-------|-------|-------|-------|---------|---------|---------------|--------------------------|
| 1     | 20:94 | 57:07 | 6:14  | 2:87    | 2:72    | 0:293         | 5:70                     |
| 2     | 23:62 | 14:60 | 1:31  | 0:319   | 0:618   | 0:055         | 2:39                     |
| 4     | 7:04  | 19:28 | 13:54 | 8:71    | 2:73    | 1:92          | 12:34                    |
| 5     | 40:72 | 53:76 | 14:43 | 6:08    | 1:32    | 0:354         | 18:31                    |
| 6     | 7:54  | 12:85 | 4:11  | 3:76    | 1:70    | 0:545         | 136:37                   |
| 7     | 12:16 | 17:64 | 6:29  | 3:81    | 1:45    | 0:517         | 4:49                     |
| 8     | 10:17 | 11:10 | 6:22  | 4:12    | 1:09    | 0:611         | 32:75                    |
| 9     | 27:70 | 33:62 | 2:85  | 4:12    | 1:21    | 0:102         | 8:66                     |
| Controls | 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.
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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 microvilli 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 5). 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
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 cortical 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 dark 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 a decreased density of myelinated sheaths with preservation of Schwann cells and axons. No trilamellar inclusions were found in the Schwann cells.

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**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, ×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, ×30,000).

**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, ×2.1).
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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 obvious 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 al24 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.1 2 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 cells14 21 and increased very long chain fatty acids in plasma17 21 27 and brain.17 However, infants with cerebro-hepatorenal 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,3 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 authors4 5 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. Liver biopsy shows mild to severe fibrosis and trilamellar inclusions in parenchymal and non parenchymal liver cells 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 and Noetzel et al as neonatal adrenoleukodystrophy share the same clinical features as the three patients previously described by one of us, 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 and abnormal bile metabolites have been documented in infantile Refsum's disease cases. Similarly, plasma phytanic acid levels are increased in infantile Refsum's disease as in cerebro-hepato-renal syndrome and neonatal adrenoleukodystrophy, reflecting a deficiency in phytanate oxidase which is supposed to be located in part in peroxisome. 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 Reves'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 Reves's disease varies greatly and three different clinical pictures at least are encountered. In some patients, 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.

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. 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 Goldfishe et al in neonatal adrenoleukodystrophy.

Various types of inclusions have been described in the liver of neonatal adrenoleukodystrophy and infantile Reves'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, spindleshaped cluster", "packed lamellar profiles". These dark inclusions observed in three of our cases (1, 2, and 4) seem to be peculiar on account of their homogeneous matrix, their irregular outlines and their hardness. Their ultrastructure resembled those observed in some cases of neonatal cholestatic 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 xanthomatosi, a possible peroxisomal disorder. 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 hepaticocytes and were replaced by empty and lipid-filled septate vacuoles. Those complex lipidic inclusions have also been reported in lymph nodes and thymus 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 Reves'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...
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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 macrophages7 8 11 but other complex lipid and membrane 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 phytic 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 pimelic 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 synthetase40 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.

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References

1. 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.
2. Aubourg P, Chaussain JL, Dulac O, Arthuis M. Adénoleucodystrophie chez l'enfant. A propos de 20 observations. Arch Fr Pédiatr 1982;39:663–9.
3. Griffin JW, Goren E, Schaumburg HH, Engel WK, Loriaux L. Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. Neurology (Minneapolis) 1977;27:1107–13.
4. Ulrich J, Herschkowitz N, Heitz P, Sigrist T, Baerlocher P. Adrenoleukodystrophy. Preliminary report of a connal case. Light- and electron microscopical immunohistochemical and biochemical findings. Acta Neuropathol (Berl) 1978;43:77–83.
5. Manz HJ, Schulein 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.
6. Benke PJ, Reyes PF, Parker JC Jr. New form of adrenoleukodystrophy. Hum Genet 1981;58:204–8.
7. Haas JE, Johnson ES, Farrell DL. Neonatal-onset adrenoleukodystrophy in a girl. Ann Neurol 1982;12:449–57.
8. Mobley WC, White CL, Tennekoon G, et al. Neonatal adrenoleukodystrophy. Ann Neurol 1982;12:204–5.
9. Jaffe R, Crumrime 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.

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.

Partin JS, McAdams AJ. Absence of hepatic peroxisomes in neonatal onset adrenoleukodystrophy. Pediatr Res 1981;17:294A.

Farell K, Dimmick JE, Applegarth DA, et al. Peroxisomal abnormalities in neonatal adrenoleukodystrophy. Ann Neurol 1983;14:379–80A.

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.

Goldfischer S. Peroxisomes and human metabolic diseases. The cerebro-hepato-renal syndrome, cerebro-tendinous xanthomatosis and Schilder disease. Ann NY Acad Sci 1982;386:526–9.

Goldfischer S, Moore CL, Johnson AB, et al. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. Science 1973;182:62–4.

Moser AE, Singh I, Brown III FR, et al. The cerebrohepato-renal (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.

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.

Scotto JM, Hachchou M, Odière 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.

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.

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 phytic acid in the plasma. J Neurol Sci 1985;69:9–25.

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.

Feldmann-Pautrat D. Thèse de Doctorat ès-sciences Pharmaceutiques, 1984, Université Paris Sud.

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.

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.

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.

Bakkeren AJAM, Monnens LAH, Tribjels JMF, Maas JM. Serum very long chain fatty acid pattern in Zellweger syndrome. Clin Chim Acta 1984;138:325–31.

Volpe JJ, Adams RD. Cerebro-hepato-renal syndrome of Zellweger: an inherited disorder of neuronal migration. Acta neuropathol 1972;20:175–98.

Richterich R, van Mechelean P, Rossi E. Refsum’s disease (heredopathia atactica polynuertiformis). An inborn error of lipid metabolism with storage of 3, 7, 11, 15-tetramethylhexadecanoic acid. Am J Med 1965;39:230–6.

Kahlbe W, Goerlich R, Feist D. Erhöhte phytasaurespiegel in plasma und leber bei einem kind mit unklarem hirnschaden. Klin Wscr 1974;52:651–5.

Bolthauser E, Spycher MA, Stollmann B, et al. Infantile phytanic acid storage disease: a variant of Refsum’s disease. Eur J Pediatr 1982;139:317A.

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.

Poulos A, Sharp P. Plasma and skin fibroblast C26 fatty acids in infantile Refsum’s disease. Neurology (NY) 1984;34:1606–9.

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.

Phillips J, Latham PS. Electron microscopy of human liver disease. In: Schiff L and Schiff ER eds, Disease of the Liver. 5th, ed, 1982:59–92.

Salen G, Zaki FG, Sabesin S, Boehme D, Shefer S, Modabach EH. Intrahepatic pigment and crystal formation in patients with cerebrotendinous xanthomatosis (CTX). Gastroenterology 1978;74:82–9.

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.

Danks DM, Tippett 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 pipemidic acid metabolism. J Pediatr 1975;86:382–7.

Hajra AK, Bishop J. Glycerolipid biosynthesis in peroxisomes via the acyl dihydroxyacetone phosphate pathway. Ann NY Acad Sci 1982;386:170–81.

Schutgens RBH, Romeyn GJ, Wanders RJ, van den Bosch H, Schrakamp G, Heymans HSA. Deficiency of acyl-CoA: dihydroxyacetone phosphate acyltransferase in patients with Zellweger (cerebro-hepato-renal syndrome) disease. Biochim Biochr Biophys Res Commun 1984;120:179–84.

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.