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Case report

Pathology of hepatic peroxisomes and mitochondria in patients with peroxisomal disorders

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Summary. The morphology of hepatic peroxisomes in five patients with metabolic disorders believed to be due to inherited defects of peroxisomal function or biogenesis is described. Electron microscopy and cytochemical staining for catalase were used to identify peroxisomes in two boys with infantile Refsum's disease (IRD), a girl with autopsy confirmed neonatal adrenoleukodystrophy (NALD), and two boys with pseudo-Zellweger syndrome (PZS). In the patients with IRD and NALD hepatic peroxisomes were significantly reduced in size and number and contained electron dense centres. In the liver of the patients with PZS the peroxisomes were enlarged. Morphologically abnormal peroxisomes were also detected in autopsy tissue from one boy with PZS using electron microscopy. Lamellar-lipid inclusions and mitochondria with crystalline inclusions and/or abnormal cristae are also described in two patients, one with IRD, the other with NALD.

Key words: Peroxisomes – Peroxisomal disorders – Neonatal adrenoleukodystrophy – Infantile Refsum's disease – Pseudo-Zellweger disease – Mitochondria

Introduction

Peroxisomes are cytoplasmic organelles which are most abundant in the liver and kidney, but are also present in many other cell types. They play an important role in many metabolic processes. This has been emphasized by the discovery of a number of inherited disorders involving a defect in the function or biogenesis of peroxisomes, which lead to severe clinical consequences for the patient (Moser and Goldfischer 1985; Schutgens et al. 1986, 1987; Kelley et al. 1986; Zellweger et al. 1987). The biochemical abnormalities (Govaerts et al. 1982; Poulos et al. 1984a; Kaiser and Kramer 1988) and the clinical features (Schutgens et al. 1986; Zellweger 1987; Wilson et al. 1988) in these disorders are numerous and varied. The relation to peroxisomes was discovered in 1973 by Goldfischer et al., when an apparent absence of peroxisomes was noted in the hepatocytes and renal proximal tubular epithelia of biopsy and autopsy material from two patients with Zellweger syndrome. Structural abnormalities in the mitochondria of the hepatocytes were also reported. Subsequently, the morphologic absence of peroxisomes in the liver of Zellweger syndrome patients has been confirmed by other authors (Pfeifer and Sandhage 1979; Monnens et al. 1980; Mooi et al. 1983; Auborg et al. 1985; Lazarow et al. 1985; Vamecq et al. 1986).

In another peroxisomal disorder, neonatal adrenoleukodystrophy (NALD), small, sparse membrane-bound structures resembling peroxisomes have been described in the liver (Goldfischer et al. 1985; Vamecq et al. 1986). Other authors, however, have reported an absence of hepatic peroxisomes in NALD patients (Partin and McAdams 1983). Recently Roels et al. (1988) described two NALD patients with enlarged hepatic peroxisomes and variable cytochemical staining for catalase. In infantile Refsum's disease, another disorder with considerable similarity to Zellweger syndrome (Poulos et al. 1984a), small catalase reactive bodies have been described in the liver tissue of some patients (Beard et al. 1986; Roels et al. 1986) and have been reported to be absent in others (Ogier et al. 1985; Budden et al. 1986; Roels et al. 1986; Poll-Thé et al. 1987).

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A patient with many of the clinical and biochemical characteristics of Zellweger syndrome but with abundant and larger than normal peroxisomes in the liver has been reported by Goldfischer et al. (1986). This patient has since been found to have a deficiency of peroxisomal 3-oxacyl-CoA thiolase and the syndrome has been termed pseudo-Zellweger syndrome (PZS) (Goldfischer et al. 1986; Schram et al. 1987).

The heterogeneity in the morphology of hepatic peroxisomes in these disorders prompted us to examine in detail the ultrastructure and the cytochemical staining of peroxisomes in the livers of five patients with differing clinical and biochemical features.

Case reports

Patient 1 (IRD)

The clinical and biochemical data on this patient has been published previously (Robertson et al. 1988). The patient is a male who presented at 19 months of age with pendular nystagmus, hepatomegaly, retinitis pigmentosa, deafness, hypotonia and ataxia. Motor and sensory nerve conduction, and sensory nerve action potential amplitudes were normal. He was treated with a low phytanic acid diet and there was a rapid response biochemically with a reduction in phytanic acid in the plasma and liver. Growth and motor skills improved, and the hepatomegaly was not so pronounced. However vision and fundoscopy changes have remained the same. The patient is now six years old.

Biochemical findings. The biochemical findings include reduced levels of dihydroxyacetone-phosphate acyltransferase (DHAP-AT) and phytanic acid oxidase in skin fibroblasts; increased plasma phytanic acid, pristanic acid, trihydroxycholestan-26-oic acid and pipecolic acid; and elevated plasma C_{26}: C_{22} and C_{24}:C_{22} fatty acid ratios.

Light microscopy. The normal hepatic architecture is disturbed with loss of central vein: portal tract relationship due to moderate fibrosis. The cytoplasm of portal histiocytes of Kupffer cells contains PAS positive, diastase resistant, spiculate material which is birefringent in H&E sections. Intranuclear glycogen is observed in isolated hepatocytes.

Patient 2 (IRD)

The patient is a male who presented at age 18 months with global developmental delay, hypotonia, arreflexia and hearing loss. Plasma phytanic acid and C_{26}:C_{22} fatty acid ratios were elevated and he commenced a low phytanic acid diet which led to a dramatic decrease in plasma phytanic acid. He has a cousin, also male, who has peripheral neuropathy and is thought to have Refsum disease (Case number 2 in Poulos et al. 1984b). The cousin has normal plasma phytanic acid and elevated C_{26}:C_{22} fatty acid ratio but a reduced activity of phytanic acid oxidase (Poulos et al. 1986).

Biochemical findings. Plasma C_{26}:C_{22} fatty acid ratios and phytanic acid are elevated. Fibroblast phytanic acid oxidase activity is decreased by approximately 50% and DHAP-AT activity is normal.

Patient 3 (NALD)

Patient 3 was a female born by emergency caesarean at 36 weeks, and was noted to be mildly dysmorphic and small for gestational age (1.49 kg). A brief seizure was noted in the first month but was controlled with phenobarbitone. No further seizures occurred and drug treatment was discontinued at 11 months. Progress was poor with marked failure to thrive, gross developmental delay, and hepatomegaly. The patient's weight never exceeded 5 kg despite an adequate calorie intake. A retinal pallor was observed in the neonatal period and progressed to severe retinitis pigmentosa. Hearing was normal but deteriorated in the last three months of life. Gradual loss of motor and developmental milestones occurred just before death at three years. The diagnosis of neonatal ALD was based on the clinical, ultrastructural, post-mortem and biochemical findings (Kelley et al. 1986).

Biochemical findings. Reduced DHAP-AT and phytanic acid oxidase activities were found in fibroblasts. The levels of plasma pipecolic acid, phytanic acid, pristanic acid and trihydroxycholestan-26-oic acid were increased. Plasma C_{26}:C_{22} fatty acid ratios were also elevated.

Light microscopy. Clusters of cells within the lobules, possibly Kupffer cells, and in portal triads contain PAS-positive, diastase resistant, spiculate material which is non-birefringent in H&E sections.

Autopsy findings. The liver was enlarged and showed moderate diffuse fatty change. The adrenals weighed 0.5 g and 0.4 g and histology showed a thickened and fibrotic capsule. The spinal cord showed widespread degeneration of long myelinated tracts. The tissue affected shows numerous foamy macrophages, gemistocytic astrocytes and areas of cystic degeneration. The cerebral hemispheres and the cerebellum showed extensive demyelination. There was no significant ocular or renal pathology and there were no renal cortical cysts. The lungs showed severe and extensive bronchopneumonia with features indicative of a recent onset.

Patient 4 (PZS)

The parents are first cousin Lebanese. Their first child had a diaphragmatic hernia and is developmentally delayed. High voltage electrophoresis of urine was normal. Their second child (Patient 4) was born at forty two weeks gestation, had respiratory distress from birth due to mild pulmonary hypoplasia and required mechanical ventilation for ten days. He had a number of dysmorphic features including a prominent nose and premaxilla and contractures of the large joints, generalized muscle weakness and marked hypotonia. He also had sparse, abnormal looking hair which was microscopically normal. Seizures developed on day one which were treated with phenobarbitone and phenytoin. His eyes were normal. His physical and developmental progress was poor and he remained hypotonic and inactive. He died at four months of respiratory failure. Post-mortem examination was refused.

Investigations undertaken at the age of two and a half months showed a mild anemia (98 g/l) with target cells on blood smear and normal blood clotting studies. Gamma-glutamyl transferase level in serum were increased (276 units/l-normal range 0–37). Urinary high voltage electrophoresis demonstrated raised levels of threonine, glycine and serine. Urinary organic acids measured by gas liquid chromatography were normal. A skeletal survey showed no punctate changes or calcification of epiphyses. A short Synacthen test was normal.

Biochemical findings. Plasma and fibroblast $C_{26}:C_{22}$ and $C_{24}:C_{22}$ fatty acid ratios were increased but pipecolic acid and phytanic acid levels were normal. DHAP-AT and phytanic acid oxidase levels were normal. The diagnosis of pseudo-Zellweger syndrome was based on clinical, biochemical and ultrastructural findings.

Patient 5 (PZS)

This child is the brother of patient 4. The parents received extensive counselling and requested prenatal diagnosis by amniocentesis with their next pregnancy. The test predicted that the fetus would be affected but the parents elected to continue with the pregnancy. He was born at term by lower segment caesarean section because of fetal distress. Apgar scores were 6 and 9 and 1 in 5 minutes respectively. Birth weight was 2880 grams and he was noted to be floppy at birth. He developed seizures within the first few days of life. These were characterised by multiple episodes of generalised and focal attacks which were partially responsive to phenobarbitone and clonazepam. There was no major facial dysmorphism. He required frequent oropharangeal suction and nasogastric gavage feeds.

With age it became obvious that he was severely developmentally retarded. He was not responsive to most stimuli, and had only occasional weak spontaneous movements.

He was admitted to hospital at 7 months of age because of uncontrolled epilepsy and it was noted at that time that he was not fixing or following and that he had horizontal nystagmus. His marked hypotonia had persisted. There was very little spontaneous movement and his cry was weak. Hepatomegaly was not present.

At 8 months of age he developed gastroenteritis and died during an apnoeic episode. A limited post mortem was allowed.

Biochemical findings. Plasma and skin taken in the newborn period confirmed the elevated levels of very long chain fatty acids seen at amniocentesis.

Autopsy findings. Autopsy was confined to examination of the abdominal organs, as requested by the parents. The kidneys were normal with no cortical cysts. The liver showed mild haemosiderosis of the periportal hepatocytes. The adrenals weighed 0.4 g and 0.8 g and histology showed extensive atrophy with focal residual clusters of cortical cells and moderate numbers of macrophages with cytoplasm containing linear clefts. The spinal cord showed moderate vacuolation of the white matter. There was no conspicuous gliosis, macrophage infiltration or decrease in myelination. The corticospinal tracts were relatively poorly myelinated, but probably normal in view of the age. Ultrastructure showed membrane-bound organelles, probably lysosomal, with trilaminar profiles in many macrophages and some cortical cells in the adrenal, occasional Kupffer cells and hepatocytes, and a few interstitial cells in the testis. In the spinal cord, only a very rare axon showed degeneration associated with macrophages containing similar lysosomes.

Control liver biopsies

Control liver biopsies were obtained from 3 patients aged 4 months, 11 and 18 years, who were undergoing liver biopsy for other investigations. Control liver was also obtained at autopsy from patients without a peroxisomal disorder.

Methods

Electron microscopy. Liver was obtained at needle biopsy or at autopsy and fixed immediately by immersion in Karnovsky's fixative consisting of 4% formaldehyde and 1% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.3. After fixation for 2 h at room temperature, the tissue was washed in 0.1 M sodium cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Spurr's low-viscosity epoxy resin. Polymerisation was carried out at 70° C under vacuum for at least 12 h. Silver-gold thin sections were cut on a Reichert Ultracut microtome, stained with uranyl acetate and lead citrate, and examined in a Jeol 100C electron microscope.

Cytochemistry. The cytochemical localisation of catalase was carried out as soon as possible after fixation in Karnovsky's fixative (as described above) in order to prevent the diffusion of catalase out of the peroxisomes (Fahimi 1973). Small pieces of the tissue were incubated in a medium containing 3′, 3 diaminobenzidine (DAB) and hydrogen peroxide at pH 9.7 for 1 h at 37° C (Novikoff et al. 1973). Negative controls were performed by adding the catalase inhibitor 3-amino-1,2,4-triazole to the incubation medium, and positive controls were carried out by incubating rat liver in the same incubation medium. The tissue was then washed in sodium cacodylate buffer, osmicated, dehydrated, embedded, sectioned, stained and examined similarly as described above.

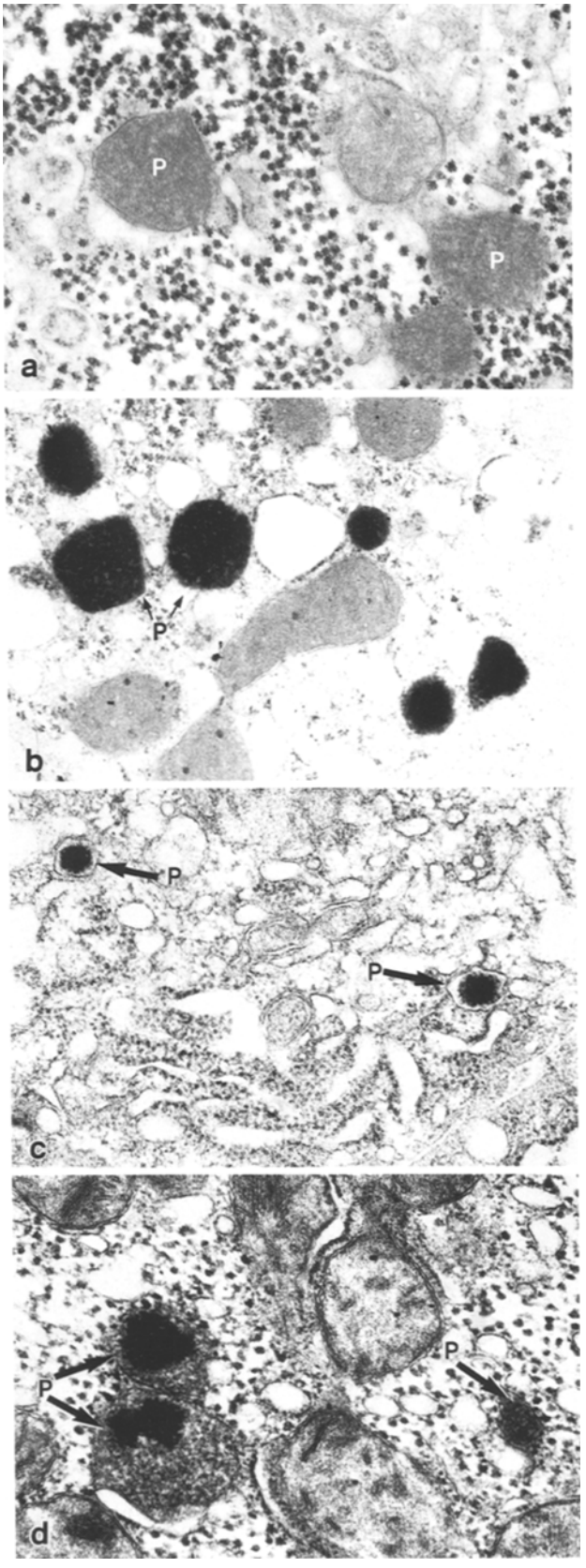
Quantitation. Twenty or more random electron micrographs of each liver at a magnification of at least 48000 times were selected for quantitation. The area of the peroxisomes was measured using a Hewlett-Packard 9847A digitizer linked to a Model 9816 Microcomputer. Statistics were carried out by pooling the results from the control liver biopsies and comparing these to the patient liver biopsies using a Student's *t* test.

Results

Peroxisomes

In the control liver biopsies peroxisomes could be readily identified in the hepatocytes as single membrane-bound organelles with a finely granular matrix (Fig. 1 a). The peroxisomes were distinguished from the mitochondria by their membrane, the lack of cristae, and the density of the matrix. Dense or crystalline cores, as described in rat liver, were not seen. The peroxisomes showed considerable heterogeneity in size and shape and often occurred in clusters. After incubation in the DAB medium, peroxisomes became markedly electron dense and were more easily identified (Fig. 1 b).

In the liver biopsy from patient 1 with infantile Refsum's disease taken at 6 years of age, very small peroxisomes were observed (Fig. 1 c). These organelles were much smaller in size (see Table 1) and also fewer in number than peroxisomes in the control liver. The peroxisomes were surrounded by a single membrane and contained electron dense cores which often filled the entire organelle except for a clear area just beneath the membrane. Larger peroxisomes which approximated the size of normal peroxisomes were present in some cells. These peroxisomes had an electron dense core and some



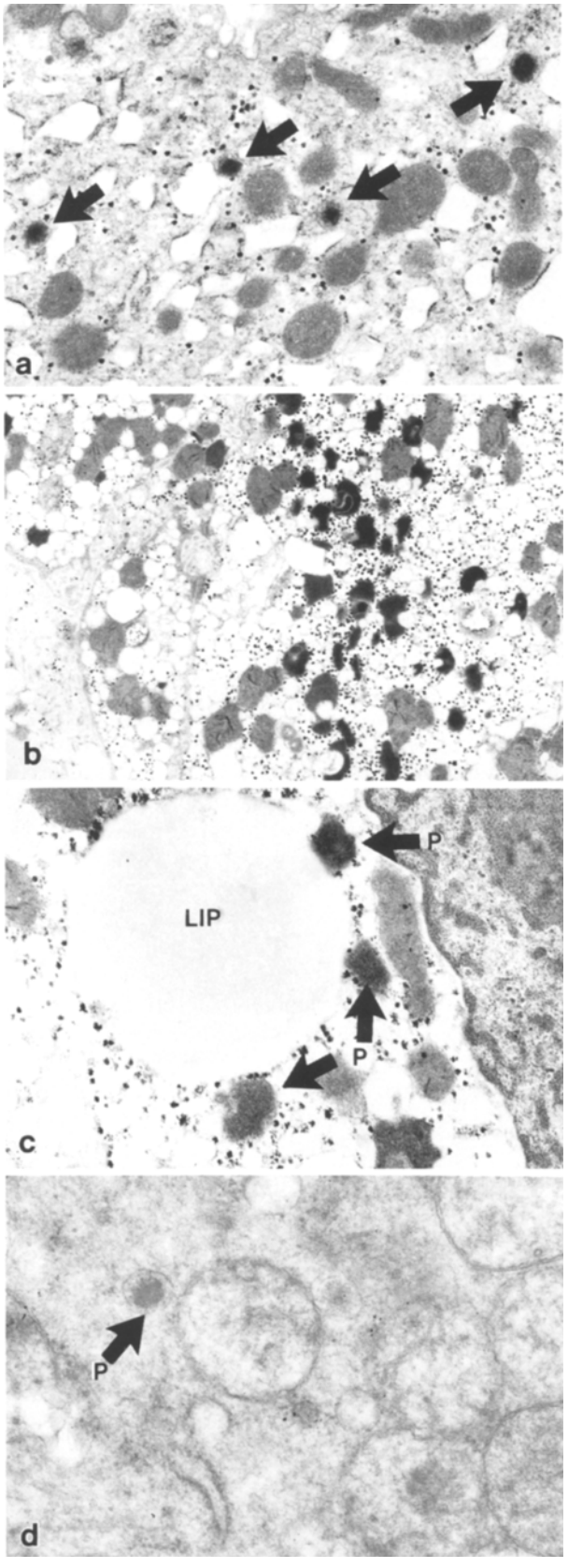
finely granular matrix. The number and size of these peroxisomes varied between hepatocytes with some cells containing no peroxisomes and others with clusters similar to normal liver. After cytochemical staining for catalase the matrix of the peroxisomes was increased in electron density (Fig. 1d), and the electron dense cores remained the same.

The liver biopsy from patient 2 (IRD) contains numerous peroxisomes which differ from those seen in control liver biopsies in that they are significantly smaller in size (see Table 1) and have electron dense centres (Fig. 2a). Although they resemble those seen in patient 1 (IRD) in size and ultrastructural appearance, these peroxisomes are present in approximately normal numbers per cell and in some cells are increased in number. The peroxisomes react strongly with DAB in the cytochemical incubation for catalase (Fig. 2b and c). Heterogeneity between hepatocytes is apparent with some cells having few peroxisomes and others having large numbers of small peroxisomes (Fig. 2b). The peroxisomes are often seen closely apposed to lipid droplets (Fig. 2c).

Similar small, abnormal peroxisomes were detected in the liver from patient 3 (NALD) although they were extremely rare. These small peroxisomes did not appear to react with DAB. In the liver obtained at autopsy of this patient these small, very rare peroxisomes with electron dense centres could still be visualised despite some deterioration in tissue ultrastructure (Fig. 2d).

In the hepatocytes of patient 5, enlarged and abundant peroxisomes were found (see Table 1 and Fig. 3a). These peroxisomes were bounded by a single membrane and had a finely granular matrix with no cores. The shape was usually quite irregular and elongated tails were often seen (Fig. 3a). Cytochemical incubation with DAB stained these organelles only weakly for catalase (Fig. 3a). In the liver tissue from this patient taken at autopsy, unusually large organelles with electron dense centres were observed (Fig. 3b). Cytochemical staining for catalase revealed very little reac-

Fig. 1. a Control human liver with normal peroxisomes (P) bounded by a single membrane and with a finely granular matrix. ×18200. **b** Control human liver after cytochemical staining for catalase. The peroxisomes (P) are intensely stained with an electron dense stain. ×18200. **c** Liver from patient 1 (IRD) has small, morphologically abnormal peroxisomes (P) with electron dense centres. ×28000. **d** Liver from the same patient after cytochemical staining for catalase. Peroxisomes (P) vary in size, and the matrix stains for catalase. ×23800



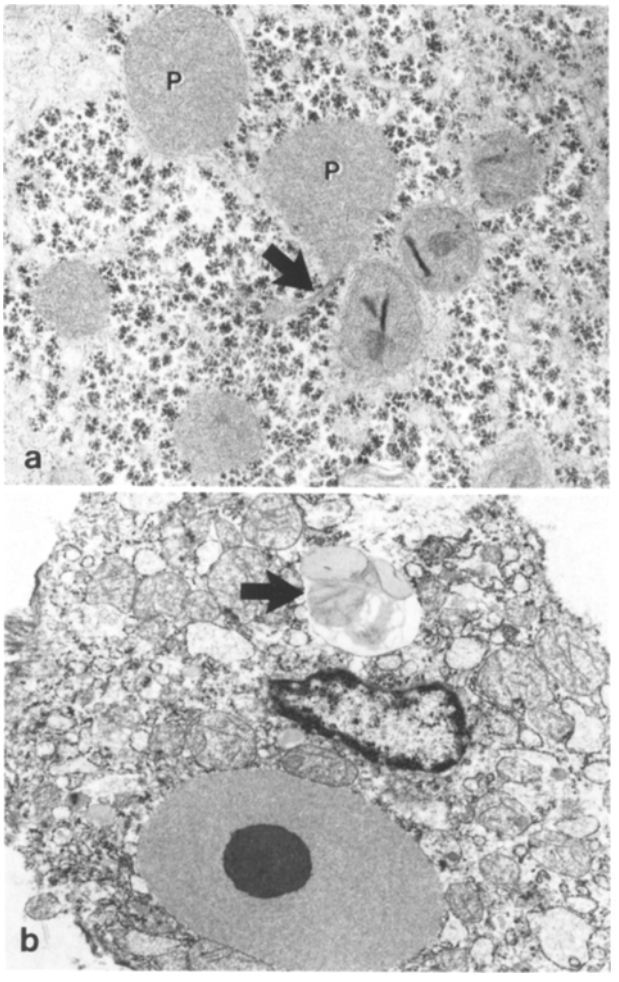
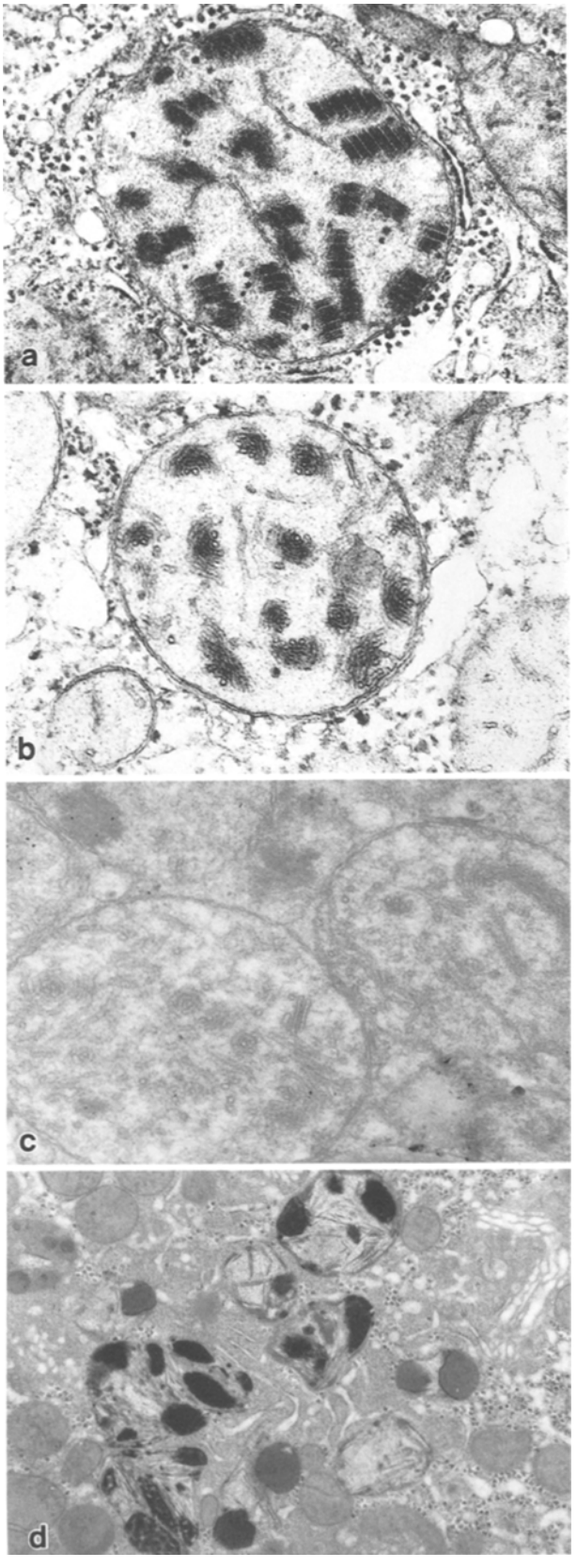


Fig. 3. a The large abundant peroxisomes (P) in the liver biopsy of patient 5 (PZS) stain only weakly for catalase. Tails (*arrow*) are commonly seen. ×18200. **b** The autopsy liver of patient 5 has large membrane-bound structures with electron dense cores. Trilaminar inclusions are also apparent (*arrow*). ×7420

Fig. 2. a Small abnormal peroxisomes (*arrows*) with electron dense centres in the liver biopsy from patient 2 (IRD). ×14000. **b** Liver from patient 2 after cytochemical staining for catalase showing an increased number of small, irregularly shaped peroxisomes. ×28000. **c** Small catalase positive peroxisomes (P) are seen closely apposed to lipid droplets (LIP) in the liver of patient 2. ×37800. **d** Small, abnormal peroxisome (P) in the autopsy liver of the NALD patient. ×28000



tion. Similarly, the liver of the brother of this patient (patient 4) contained enlarged and abundant peroxisomes (see Table 1). Cytochemistry was not carried out on the liver of this patient and autopsy was refused.

Mitochondria

In the liver biopsy of patient 1 (IRD), giant mitochondria which contained either crystalline inclusions (Fig. 4a) or "whorled" cristae (Fig. 4b) were present.

Mitochondria with whorled cristae were also observed in the autopsy tissue from the NALD patient (Fig. 4c), but were not present in the liver biopsies of the same patient.

Trilaminar inclusions

The hepatocytes of patient 1 contained lamellar-lipid inclusions as described previously (Robertson et al. 1988). Morphologically similar inclusions were visualised in patient 3. These inclusions are present in the cytoplasm of the hepatocytes and in the Kupffer cells and macrophages. The included material consists of lipid droplets of varying density, dark bodies, and trilaminar profiles (Fig. 4d).

Similar angulate, trilaminar inclusions were found in the liver, kidney, adrenal, spleen and brain at autopsy in the NALD patient.

Trilaminar structures were also seen in patients 4 and 5, but with a somewhat different appearance. In patients 1 and 3 the inclusions were angulate in shape, but in patients 4 and 5 they were more round and consisted mainly of lipid material with only a few strands of trilaminar material. Similar inclusions were observed in the liver and adrenal gland of patient 5 at autopsy (Fig. 3b).

Fig. 4. a An enlarged mitochondrion in the liver of patient 1 (IRD) with crystalline inclusions. ×23800. **b** An enlarged mitochondrion from the same liver with "whorled" cristae. ×37800. **c** Mitochondria in the autopsy liver of NALD patient have similar whorled cristae. ×37800. **d** Trilaminar inclusions in the liver of patient 3 (NALD). ×14000

Table 1. Area (μm^2) of hepatic peroxisomes

	Clinical diagnosis	Mean area of peroxisomes	Standard deviation	Total area measured	Total number measured
Control 1		0.20	0.10	28.28	141
Control 2		0.19	0.11	20.08	107
Control 3		0.18	0.09	8.12	45
Patient 1	IRD	0.07	0.10	3.03	43
Patient 2	IRD	0.08	0.04	5.99	73
Patient 3	NALD	0.04	0.04	1.12	28
Patient 4	PZS	0.36	0.17	18.46	51
Patient 5	PZS	0.32	0.19	20.31	63

Student's t-test shows that the peroxisomes of patients 1, 2 and 3 are significantly smaller ($p < 0.001$) than the control group; and that the peroxisomes of patients 4 and 5 are significantly larger ($p < 0.001$) than the control group

Discussion

In Zellweger syndrome, the most severe of the peroxisomal disorders, there is an apparent absence of peroxisomes in the liver (Goldfischer et al. 1973). However in the patients with related peroxisomal disorders studied here peroxisomes of some description can be found. The NALD and IRD patients had small, morphologically atypical, hepatic peroxisomes which closely resembled those described by Goldfischer et al. (1985) in one case of NALD. These organelles have been identified as peroxisomal on the basis of their distribution in the cell and the presence of a single limiting membrane. However the electron density of the centre was unusual as this is not normally seen in human liver peroxisomes, where the matrix is homogeneous, and is of medium electron density and finely granular. The small, atypical peroxisomes observed in the patients with NALD and IRD may represent residual organelles which are unable to carry out all of the metabolic functions of normal peroxisomes.

In the NALD patient the small, atypical peroxisomes were extremely rare and showed no reaction with DAB. In the autopsy tissue, they were also very sparse but could still be visualised, despite some deterioration of the ultrastructure due to post-mortem changes.

The clinical severity of the peroxisomal dysfunction in this patient is reflected in the pathology of the liver, where very small and sparse peroxisomes and large lamellar-lipid structures representing accumulated material were found.

In most IRD patients reported in the literature, hepatic peroxisomes have been absent (Ogier et al. 1985; Budden et al. 1986; Roels et al. 1986; Goldfischer 1987). However, atypical, small peroxisomes were found in the liver of IRD patients by Roels et al. (1986), and Beard et al. (1986). Normal peroxisomes were also described in cultured skin fibroblasts from 4 out of 6 IRD patients (Beard et al. 1986). In our first patient with IRD (patient 1) small, abnormal peroxisomes were present in decreased numbers per cell. Some larger peroxisomes with normal matrix material as well as the electron dense core were also present. Heterogeneity between hepatocytes with respect to peroxisomal numbers was evident.

The findings in the liver biopsy of the second IRD patient (patient 2) have not been described previously and suggest an even greater heterogeneity of the peroxisomal disorders. Small, atypical peroxisomes were observed which were morphologically very similar to those seen in the NALD and the other IRD patient. However this patient differed from the other IRD patient in that the small peroxisomes were abundant and in some cells are increased rather than decreased in number. As in the other IRD patient there was great heterogeneity from hepatocyte to hepatocyte with respect to peroxisomal numbers. Also these peroxisomes reacted strongly to DAB indicating a high level of active catalase. Biochemically this patient differed from the typical IRD patient in that the activities of the enzymes DHAP-AT and DHAP-synthase were normal.

In contrast to the other patients the hepatic peroxisomes in patients 4 and 5 were enlarged and were similar to those of a patient described by Goldfischer et al. (1986). The clinical course and biochemical features in this patient were characteristic of Zellweger syndrome, but the electron microscopy of the liver revealed peroxisomes which were abundant and somewhat larger in size than normal. Subsequent immunoblotting studies on

Table 2. Biochemical features and hepatic peroxisomes and mitochondria

Patient number	1	2	3	4	5
Clinical diagnosis and age when liver biopsy taken	IRD 6 years	IRD 1.5 years	NALD 3 years	PZS 2 months	PZS 7 months
Very long chain fatty acid levels in plasma	increased	increased	increased	increased	increased
Di-hydroxyacetone-phosphate acyltransferase level in skin fibroblasts	decreased	normal	decreased	normal	not measured
Phytanic acid level in plasma	increased	increased	increased	normal	not measured
Plasma bile acids	abnormal	not measured	abnormal	not measured	not measured
Hepatic peroxisomes	small size decreased number atypical	small size increased number atypical	small size decreased number atypical	enlarged	enlarged
Hepatic mitochondria	abnormal cristae crystalline inclusions	normal	abnormal cristae	normal	normal

liver homogenate of this patient demonstrated a specific deficiency of peroxisomal 3-oxoacyl-CoA thiolase, a peroxisomal enzyme involved in the β-oxidation of long chain fatty acids (Schram et al. 1987). Although a definitive diagnosis of pseudo-Zellweger syndrome in our patients was not possible due to the unavailability of thiolase antibody, the biochemical and ultrastructural findings would appear to be consistent with this diagnosis.

In PZS there may be an impairment of only one peroxisomal enzyme, rather than an impairment of multiple peroxisomal enzymes or of peroxisomal biogenesis as is the case in other peroxisomal disorders. The autopsy tissue of the PZS patient revealed extremely large organelles which had an electron dense core. These organelles may be residual peroxisomes and the electron dense material in the centre may be storage of unprocessed material in the peroxisome.

Cytoplasmic lamellar-lipid inclusions were observed in the hepatocytes of patients 1 and 3 and are believed to consist of very long chain fatty acids (Powers et al. 1980). These lamellar-lipid structures have been reported in the liver, brain and adrenal of patients with Zellweger syndrome (Pfeifer and Sandhage 1979; Mooi et al. 1983; Auborg et al. 1985; Roels et al. 1986), and are morphologically similar to those seen in sex-linked ALD (Schaumburg et al. 1975), NALD (Partin and McAdams 1983) and IRD (Scotto et al. 1982; Budden et al. 1986). The inclusions were also evident in the adrenal, kidney, spleen and brain of patient 3 at autopsy, suggesting a widespread involvement of the disease.

Abnormal mitochondria were also found in patient 3 at autopsy. Mitochondrial abnormalities were noted by Goldfischer et al. (1973), in the hepatocytes of his Zellweger syndrome patients. Mathis et al. (1980) also found abnormal mitochondria with a dense matrix and irregular and twisted cristae in two cases of Zellweger syndrome. Patient 1 also had mitochondrial abnormalities in the latest liver biopsy. A similarity was noted between the "whorled" cristae of large mitochondria in this patient, and those of enlarged mitochondria in the autopsy liver of the NALD patient. Intramitochondrial crystalline inclusions were also commonly seen in patient 1 and resemble those described by Mooi et al. (1983) in one of his Zellweger syndrome patients. It is possible that these mitochondrial inclusions may be a result of dietary treatment (low phytanic acid diet) as they were not seen in the earlier liver biopsies. Crystalline structures have been reported in the mitochondria of normal liver cells (Wills 1965) and, more frequently, in diseased liver (Sternlieb and Berger 1969). The significance of the inclusions is unknown but they are believed to be degenerative and associated with cells in a phase of increased metabolic activity. Optical diffraction studies of crystalline structures in mitochondria led to the conclusion that they are phospholipid micelles or large protein molecules (Sternlieb and Berger 1969). Mitochondrial abnormalities have been reported in Zellweger syndrome patients, and recently Goldfischer (1987) postulated that the primary defect in Zellweger syndrome may reside in the mitochondria rather than the peroxisomes. Mi-

tochondria with curled cristae have been described by Monnens et al. (1980) in one Zellweger syndrome patient. They suggest that a generalised mitochondrial defect may be responsible for the abnormal bile acids found in Zellweger syndrome infants. We have found similar mitochondrial cristae abnormalities in two of our patients with a related peroxisomal disorder.

Our findings support the view that the number, morphological appearance and size of hepatic peroxisomes is reflected in the clinical severity of the disorder. The NALD patient who was severely afflicted by this disorder had the least number, the smallest and the most abnormal peroxisomes. The first IRD patient, who is still living at age 6 years is less severely affected, has more peroxisomes, some of which are of normal size and appearance. The other IRD patient is only mildly affected and he has abundant although atypical hepatic peroxisomes. In the two boys with PZS, numerous abnormal peroxisomes of a different type were observed and both these patients died at a young age. The appearance of mitochondrial inclusions or cristae abnormalities may be secondary to the peroxisomal defect or may reflect a more generalised defect.

The biochemical and ultrastructural findings are summarised in Table 2.

The electron microscopic examination of liver biopsies from patients with a suspected peroxisomal disorder may reveal some information as to the scarcity and type of peroxisomes present and this may be related to the particular type of disorder. Further investigations of the liver ultrastructure are required to verify this.

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