Virchows Archiv A Pathol Anat (1988) 413:275–285

Virchows Archiv A
Pathological Anatomy and Histopathology

Hepatic peroxisomes in adrenoleukodystrophy and related syndromes: Cytochemical and morphometric data

Frank Roels[1], Marina Pauwels[1], Bwee Tien Poll-Thé[2], Jacques Scotto[3], Hélène Ogier[2], Patrick Aubourg[4], and Jean-Marie Saudubray[2]*

[1] Menselijke Anatomie, Vrije Universiteit, Laarbeeklaan 103, 1090 Brussels, Belgium
[2] Clinique de Génétique Médicale, INSERM U12, Hôpital Necker, Paris, France
[3] INSERM U56, Hôpital Bicêtre, France
[4] Neuropédiatrie, Hôpital Saint-Vincent-de-Paul, Paris, France

Summary. Peroxisomes were visualized by cytochemical staining for catalase or/and electron microscopy in liver biopsies of two boys with childhood adrenoleukodystrophy (ALD), and of two girls with autopsy confirmed neonatal adrenoleukodystrophy (NALD). In a third patient previously described as NALD, unusual organelles were seen which may be large abnormal microbodies. Enlarged peroxisomes (determined by morphometry) were also present in the livers of the other two NALD patients. In the ALD patient whose clinical disease was more severe, peroxisomes were larger than in the older ALD case. Catalase staining was diminished and markedly heterogeneous. Additional unusual features such as a separate population of tubular forms, contact with fat droplets, marginal plate and invaginations containing glycogen were seen in the neonatal cases. These data are compared to the enlarged or elongated peroxisomes and heterogeneous staining in the thiolase-deficient "pseudo-Zellweger" patient (Goldfischer et al. 1986) and in 2 siblings with acylCoA oxidase deficiency (Poll-Thé et al. 1986, 1988). Enlarged peroxisomes are a common feature in this group of patients with peroxisomal deficiency disorders, suggesting that increased size and lowered metabolic capacity are associated. Nevertheless a marked morphopathological heterogeneity of peroxisomes thus exists in syndromes described as NALD including previously published cases. Most likely this heterogeneity reflects different enzymatic deficiencies, as confirmed by the biochemical data available. Clinically similar syndromes cover divergent microscopical and enzymatic peroxisomal patterns, and naming of the disease should be adapted to reflect such data. Cytochemical studies are urged in every suspected patient.

Key words: Adrenoleukodystrophy – Pseudo-Zellweger disease – Thiolase deficiency – AcylCoA oxidase deficiency – Plasmalogens – Peroxisomes – Microbodies – Catalase – Morphometry – Autosomal recessive inheritance – X-Linked inheritance

Introduction

Neonatal (NALD) and X-linked juvenile onset adrenoleukodystrophy (ALD) have been recognized as peroxisomal disorders (Chen et al. 1987). Evidence has come from serum and tissue levels of very long chain fatty acids (VLCFA) and from enzyme activities of β-oxidation and plasmalogen synthesis. Fewer detailed studies have been published about the microscopy of peroxisomes, as emphasized recently by Kelley et al. (1986).

We report morphometric data on hepatic peroxisomes in 2 patients with classical ALD, and in 3 children who have died and were described as NALD (Aubourg et al. 1986). Catalase cytochemistry to visualize peroxisomes was carried out in 3 patients. The results are compared to novel data from the pseudo-Zellweger patient of Goldfischer et al. (1986) characterized by the absence of 3-oxoacyl-coenzyme A thiolase (Schram et al. 1987) and from 2 siblings with an isolated fatty acylCoA oxidase deficiency (Poll-Thé et al. 1986, 1988).

Some results have been briefly reported (Roels et al. 1986a, b, c).

* Supported by the Belgian F.G.W.O. 3.0071.83 and 3.0034.86, and by the Research Council of the Vrije Universiteit Brussels

Offprint requests to: F. Roels

Table 1

	NALD labelled patients			ALD patients	
N°	1	2	3	4	5
Sex	f	f	f	m	m
Age at death	14 months	7 months	8 months	Living 15 years	Living 23 years

Table 2

	VLCFA (plasma)*		
	Patient n° 4	Patient n° 5	Controls ($n=30$)
C22	79.87	83.66	38.2 ±19
C24	169.72	101.32	27.4 ±16.2
C26	2.68	2.24	0.35 ± 0.19
C24/C22	2.12	1.21	0.68 ± 0.20
C26/C22	0.033	0.026	0.011 ± 0.007

* expressed in μmol/l. Determined according to Aubourg et al. 1985

Case report

Patients (Table 1). The NALD patients (numbers 1, 2 and 3) have been described in detail by Aubourg et al. (1986) (their numbers 1, 2, 4). Diagnosis was based on the clinical course, increased serum VLCFA, degeneration of white matter observed by CTscan and at autopsy, and decreased adrenocortical function and atrophy.

In ALD patient number 4 the familial history and infancy were unremarkable. At the age of 3 a slight retardation of speech was noted. At 8 difficulties at school became apparent, followed by a progressive intellectual degradation. Successively were detected: a perceptual hearing deficit, strabismus, clumsiness of movement developing into a bilateral cerebellar syndrome, with later pyramidal symptoms and hemiparesis at left and left homonymous hemianopsia. A CTscan showed hypodensity of the white matter. Serum VLCFA were increased (Table 2). Adrenocortical function: blood cortisol was very low (21 μg/l) and did not rise after ACTH administration. Basal ACTH was 140 pg/ml (controls were below 90). Aldosterone levels did respond to ACTH.

At the age of 12 the pyramidal signs became bilateral with permanent hypertonicity. The CTscan showed cerebral atrophy and zones of oedema along the ventricles. The liver biopsy was taken at that age. His mother and sister were found to be heterozygotes on basis of their serum VLCFA but were both in good health.

Patient number 5 had a familial history and childhood which were also unremarkable. At the age of 12 the first symptoms appeared: deterioration of results at school and decrease of visual acuity. Half a year later the boy had convulsions which were repeated a few months later. A CTscan showed hypodensity in the posterior cerebral lobes. Cutaneous pigmentation appeared; plasma cortisol was found to be very low (25 μg/l) and unresponsive to ACTH administration. Basal ACTH was 800 pg/ml. Mineralocorticoid secretion was also impaired, as shown by a decline in plasma sodium and unchanged aldosterone during sodium restriction (Prof. J.L. Chaussain, Paris). The neurological disease slowly progressed; more convulsions took place despite treatment; CT showed a marked diffuse cerebral atrophy. 8 years after appearance of the first symptoms he was unable to walk, with pyramidal as well as cerebellar symptoms. Speech was monosyllabic. Since then the disease did not progress further; walking with a cane became again possible. The liver biopsy was taken at the age of 19 years. Serum VLCFA were raised (Table 2).

Methods

Needle biopsies were fixed in buffered formaldehyde + $CaCl_2$ and after a brief rinse, cryostat sections were stained for catalase with diaminobenzidine at pH 10.5, as reported previously (Roels et al. 1986a). After treatment with $OsO_4 + K_3Fe(CN)_6$, 5 μm sections were mounted for light microscopy, and 50 μm sections were embedded in Epon. The latter were sectioned at one-, 2 and 0.4 μm for light microscopy, and at 650 Å for electron microscopy, and counterstained with lead only.

Cytochemical controls consisted of inactivation of the enzyme by heating the sections before incubation in diaminobenzidine.

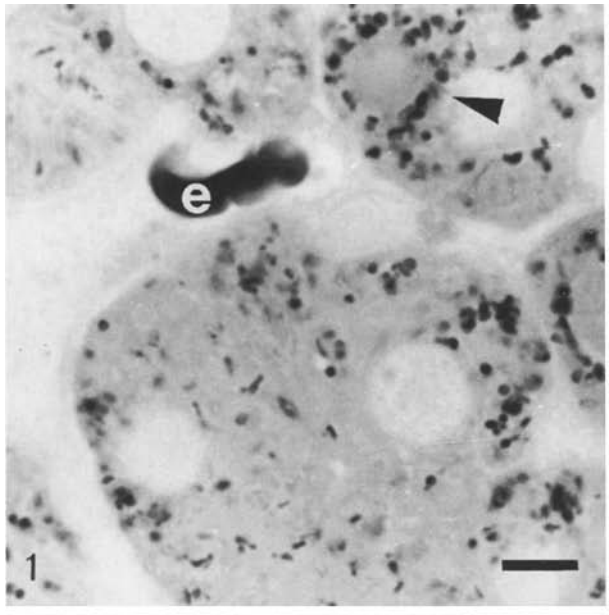
From 2 biopsies (patients 1 and 2) no cytochemical preparations could be made. They were fixed in 3% glutaraldehyde, postosmicated and embedded in Epon.

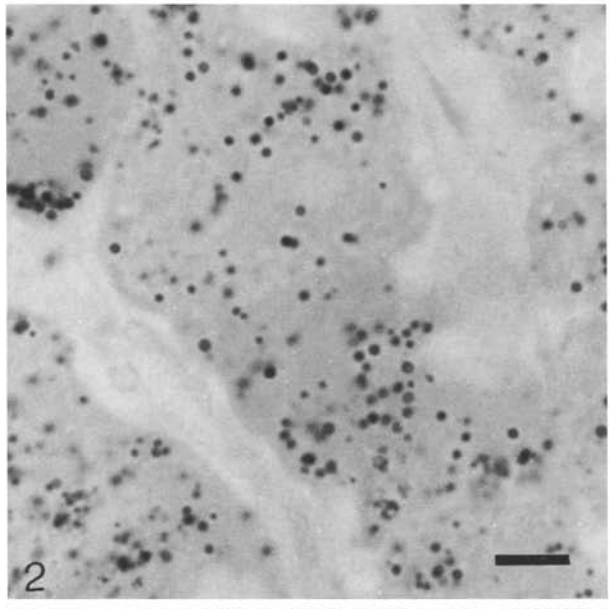
Morphometry was performed on electron micrographs at magnification of 20,000×, 63 to 214 peroxisomes per patient were measured manually by means of a digitizer tablet (Ibas I). For each organelle profile we calculated: area; diameter of the circle having the same area as the measured organelle (d-circle); ratio between shortest and longest diameter (axial ratio). The mean d-circle thus obtained is an underestimation of true peroxisomal size because it includes profiles from off-centre sections. A corrected mean was calculated according to Abe et al. (1983) assuming one size class of spherical organelles. Section thickness (according to the microtome setting) was also taken into account. As controls we measured: one adult (62 years) liver, where no pathology was seen by light microscopy, and 7 livers of children without peroxisomal disorders between 5 weeks and 1 year old.

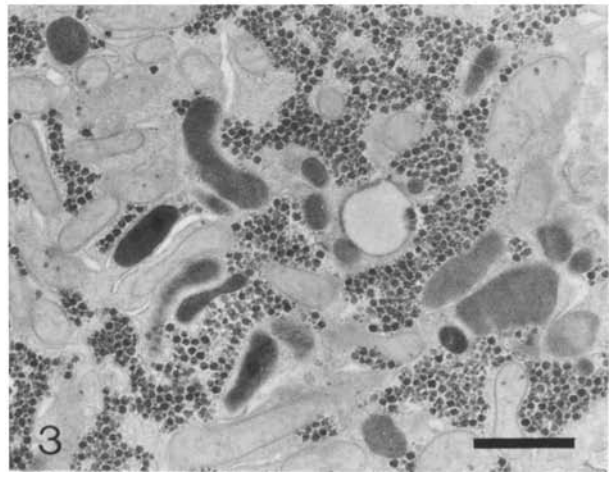
Results

By light microscopy after catalase staining (patients 3, 4, 5) typical peroxisomes were observed in the liver parenchyma of both ALD as well as of the NALD patient. Catalase staining was weakened in comparison to adult control and other children's livers; this was very marked in the ALD case number 5 where visualization of peroxisomes was further hampered by the presence of numerous large lipofuscin granules. In the NALD patient 3 some hepatic regions displayed elongated forms, while in other regions peroxisomes looked spherical as usual (Figs. 1, 2). This biopsy also contained large fat droplets and they were often in contact with peroxisomes; we have seen the same association in the livers of children with thiolase or acyl-CoA oxidase deficiencies.

After inactivation of catalase, hepatocytes from patient number 3 showed a variable number of







large, angular lipofuscin granules, which correspond to the dark bodies seen by electron microscopy (Aubourg et al. 1986).

On electron microscopy of the diaminobenzidine-stained biopsies the presence of catalase containing organelles was confirmed. In patient number 3 large rounded organelles coexisted in the same cell with oval ones, and with tubular elongated forms, the last type being much narrower that the other forms (Figs. 3, 8, 9). The ratio between short and long diameter (axial ratio) reflected the existence of different groups of peroxisomes: the histogram displayed 3 peaks (Fig. 4). A histogram of a control biopsy contained only one maximum, corresponding to nearly spherical organelles (axial ratio approaches 1) (Fig. 5). In 2 more biopsies the existence of elongated forms was reflected in the histogram: in the older case of acyl-CoA-oxidase deficiency (Fig. 6) and in the pseudo-Zellweger girl (Fig. 7). The degree of elongation indicated by the minimum axial ratio (Table 3), as well as the frequency of this type (indicated by the height of the peak) were both most extreme in patient number 3. However, spherical organelles were present simultaneously, as evidenced by the axial ratio maxima. Some of the spherical peroxisomes of patient number 3 were very large in comparison with the controls, as shown by the maximal d-circle (0.99 μm) (Table 3).

Three more peculiar features were noted in biopsy number 3:

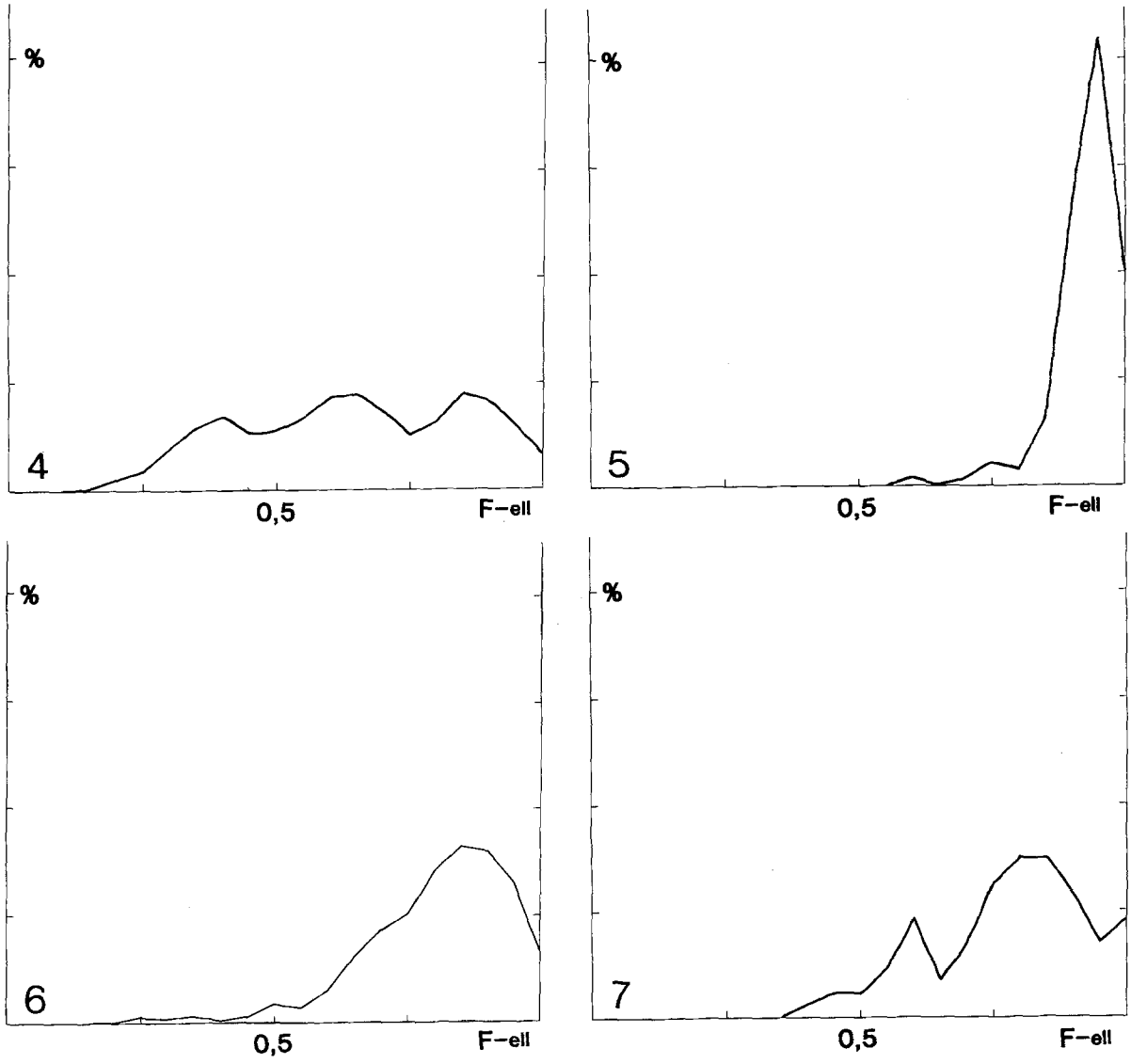
a) The direct contact between elongated peroxisomes and fat droplets (Fig. 8). We have observed an identical association in the acylCoA oxidase defect described by Poll-Thé et al. (1988).

b) Glycogen particles inside peroxisomes but surrounded by a membrane, representing a finger-like invagination (Fig. 9). We have seen this configuration also in the children with the oxidase defect.

[Bar equals 1 μm unless stated otherwise.]

Figs. 1, 2. Light microscopy of hepatic peroxisomes visualized by their catalase activity. Erythrocytes (e) also stain. 2 μm plastic section. ×2050. *Bar* = 5 μm. (**1**) patient number 3: several parenchymal cells contain elongated peroxisomes which are very unusual in human liver; in adjacent cells peroxisomes are round and large. Compare to Figs. 2 and 3. *Arrowhead:* large fat droplet surrounded by peroxisomes. (**2**) patient number 4: numerous round peroxisomes. This image resembles that in control livers except for a weaker catalase reaction

Fig. 3. Electron micrograph after catalase staining shows elongated peroxisomes typical for patient 3. The amount of enzyme reaction product markedly differs between organelles. Mitochondria appear normal, glycogen rosettes are numerous. ×13200, *Bar* = 1 μm



Figs. 4–7. Frequency histograms of the ratio between smallest and largest diameter (axial ratio or "F–ell" used by the Ibas program). **(4)** patient number 3 who possesses the elongated organelles. The histogram shows three peaks corresponding to resp. tubular forms, oval organelles and round ones. Examples of round profiles in this patient are shown in Fig. 8. **(5)** control biopsy (boy) showing one maximum with a value near 1, reflecting the preponderance of round forms. **(6), (7)** the presence of tubular peroxisomes is indicated by the tail to the left in a 4-year old girl with acylCoA oxidase deficiency (Poll-Thé et al. 1986, 1988). **(6)** and in the thiolase deficient baby described by Goldfischer et al. (1986) **(7)**. Both elongation and frequency are less pronounced than in patient number 3: compare to Fig. 4

c) Membranous lamellae with irregular spacing in parenchymal cells, at the rim of fat droplets or in the cytoplasm (Fig. 10a); these are similar to the loose lamellae observed in the oxidase deficient children (Fig. 10b). In both patients, the rim of the fat droplets is birefringent (Fig. 11a and b). In contrast to the polarizing inclusions of macrophages in Zellweger's and infantile Refsum's disease (Roels et al. 1986a), birefringence was lost after clearing in xylene or Epon embedding.

In patients number 4 and 5 with late onset ALD the peroxisomes were essentially of the rounded type (Fig. 12). Their sizes did differ however: in the childhood ALD (patient number 4) they were significantly larger than in the juvenile ALD patient (number 5), the latter falling in the range of the control biopsies (Table 3).

Catalase reaction product was remarkably different between individual peroxisomes in patients number 3, 4 and 5 (Figs. 3, 8, 12). Heterogeneity of staining was studied in a large number of biopsies; in normal adult liver it is usually inconspicu-

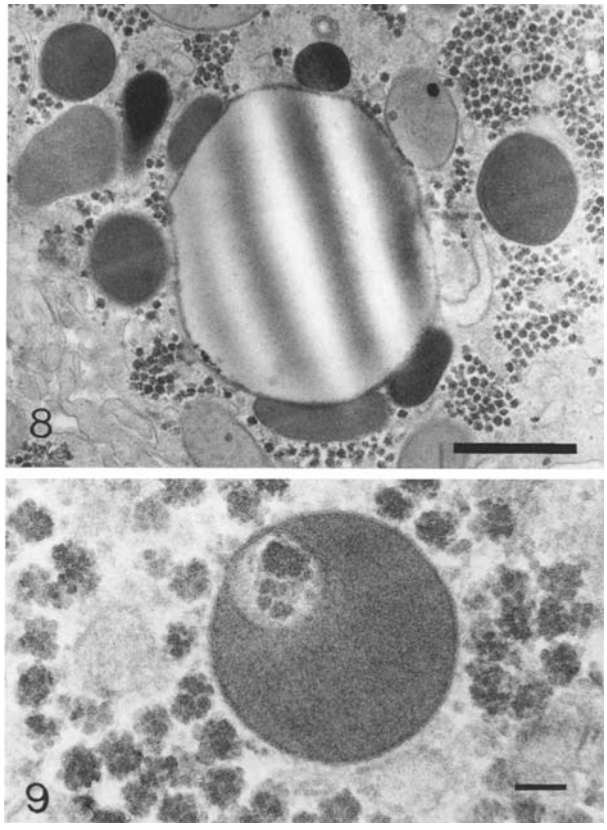


Fig. 8. Intimate contact between peroxisomes and fat droplets in patient number 3. Mark the coexistence of tubular and round forms; the latter are not transverse sections through elongated ones because their diameter is larger. Catalase staining is heterogeneous between individual organelles. ×16600, *Bar*= 1 μm

Fig. 9. A peroxisome containing glycogen which is surrounded by a membrane; this configuration probably represents an invagination of cytoplasm. Patient number 3. ×72000, *Bar*= 0.1 μm

ous but its diagnostic significance remains unclear (Roels and Cornelis 1988). In the pseudo-Zellweger and oxidase deficient livers, heterogeneity was very striking (Goldfischer et al. 1986; Poll-Thé et al. 1988).

In ultrastructural examination without cytochemical staining of the liver of NALD patient number 2, microbodies were regularly observed which were morphologically identical to normal human peroxisomes (Fig. 13). Their form and size (as documented by morphometry) were in the range of the controls except for a few large specimens (Table 3: max. d-circle). In rare microbodies a marginal plate was seen (Fig. 13, inset). NALD patient number 1 did not display normal microbodies. However an organelle was regularly seen which is not found in normal livers (Fig. 14). It was bound by a single membrane but was readily distinguished from lysosomes by:

(I) a homogeneous matrix (lysosomes often possess heterogeneous contents); (II) no electron lucent space separating membrane and matrix (lysosomes always show a halo); (III) the random distribution in the parenchymal cell (lysosomes are preferentially localized near the bile canaliculus); (IV) a border of cisternae of smooth endoplasmic reticulum (Fig. 14, inset), a feature typical of peroxisomes. These organelles were round or slightly oval and their matrix was flocculent with low density. From their size they were comparable to large peroxisomes (Table 2), the largest still staying below those of the 3 biopsies with the acylCoA oxidase defect: maximum d-circle 1.11 μm versus 1.34, 1.28 and 1.47 μm (Poll-Thé et al. 1988). In the pseudo-Zellweger patient the maximal d-circle was 1.24 μm.

Table 3. Morphometry of peroxisomes

Patient	Diameter (d-circle)[a]					Axial ratio[b]			*N*
	mean	SE	corr. mean	max.	min.	median	max.	min.	
1	703	21	1067	1105	294	0.864	0.973	0.506	68
2	529	17	807	937	239	0.881	0.993	0.526	80
3	506	12	788	992	170	0.621	0.980	0.178	214
4	587	14	867	795	254	0.831	0.992	0.567	63
5	471	10	713	814	180	0.848	0.977	0.378	154
control adult 62 years	534	14	803	820	287	0.853	0.989	0.554	73
control girl 5 weeks	478	14	727	796	244	0.892	0.986	0.399	97
control boy 4 months	481	9	723	758	242	0.916	0.995	0.590	141
pseudo-Zellweger	607	27	967	1240	184	0.765	0.975	0.371	87
oxidase deficient girl	670	14	1037	1338	197	0.807	0.981	0.244	112

[a] d-circle expressed in 10^{-3} μm. Corr. mean: corrected according to Abe et al. 1983, formula (9), taking into account section thickness; see Methods

[b] Axial ratio: short over long axis; *N*: number of peroxisomes measured

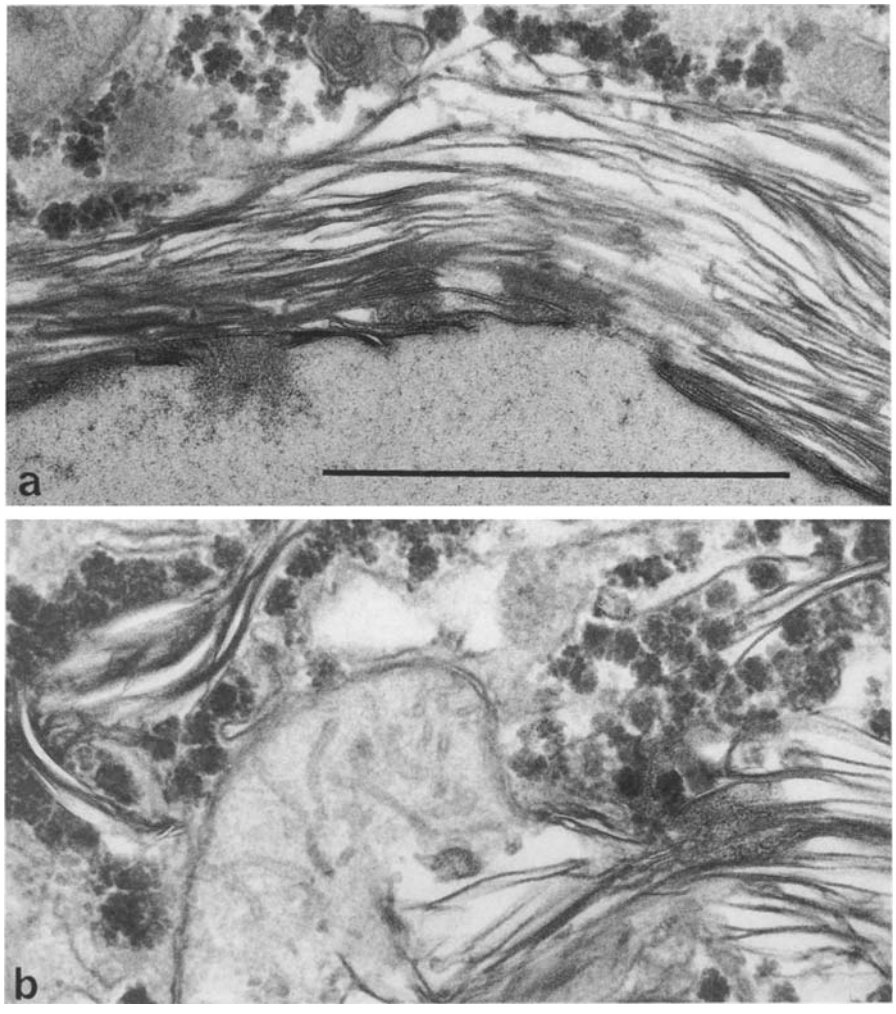


Fig. 10. Irregularly spaced membranous lamellae in hepatic parenchymal cells. **(a)** patient number 3. Compare to Fig. 2 in Goldfischer et al. (1986) describing the thiolase deficient girl. **(b)** acylCoA oxidase deficient boy reported by Poll-Thé et al. (1988). A mitochondrion is involved in the extension of the lamellar stack; although this finding was rare, it was detected also in patient number 3. ×62200, *Bar* = 1 μm

Many of these unusual organelles contained a excentrally located density (Fig. 14); in contrast to nucleoids of peroxisomes of rodents, this density did not display a periodical structure. Because ultrathin sections can be made which do not pass through this nucleoid, it is likely that the latter is actually present in most or all organelles. Ultrastructure warrants the proposal that the organelles are abnormal microbodies.

Discussion

Kelley et al. (1986) have summarized the microscopical data on peroxisomes in NALD. Peroxisomes were reported to be either normal or greatly decreased in size and number (Goldfischer et al. 1985; Vamecq et al. 1986), or absent. In 2/3 of this last group of patients, Kelley et al. estimate that diagnosis was not confirmed. Except for the paper by Goldfischer et al. (1985), detailed cytochemical or morphometric data have not documented these findings. Absence of peroxisomes was also demonstrated by digitonin titration of cultured fibroblasts (Wanders et al. 1987a), but it is unclear whether such observation may be extrapolated to liver (Arias et al. 1985; Beard et al. 1986).

In our series, patients number 2 and 3, labelled as NALD, have hepatic peroxisomes that are not decreased in size or number. In patient number 1 we are not certain about the nature of the abnormal microbodies. Sternlieb and Quintana (1977) have published micrographs of human peroxisomes with an amorphous nucleoid, or with a flocculent matrix, which are reminiscent of our findings; these peroxisomes usually were enlarged. When our results are added to those of other reports, it appears that in patients with a clinical, chemical and neuropathological picture labelled as NALD, liver peroxisomes are more often present

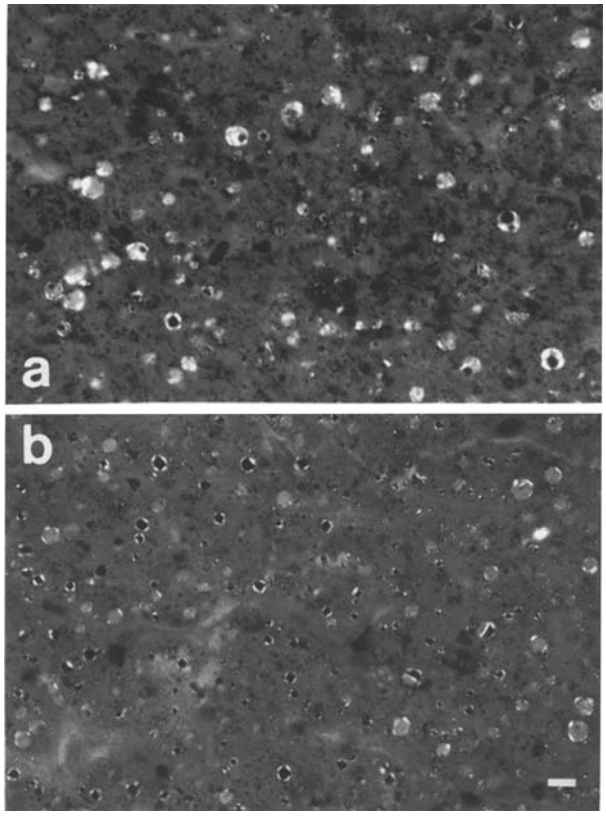


Fig. 11. Cryostat sections of formol-calcium fixed liver mounted in water soluble medium and observed in polarized light: periphery of fat droplets is birefringent. **(a)** patient number 3. **(b)** girl with acylCoA oxidase deficiency. Most, but not all fat droplets are black from postosmication indicating the presence of unsaturated fatty acids. This is similar to the thiolase-deficient patient (Goldfischer et al. 1986). In patient number 3 fat is more often unstained by osmium. When dehydrated and cleared in xylene or embedded in Epon sections lose their polarizing material. ×380, *Bar* = 10 μm

than not; their cytochemical visualization in the light microscope must be possible in many cases.

The peroxisomes which we describe here possess unusual ultrastructural features: tubular forms, invaginations containing glycogen, an entirely abnormal morphology in patient 1, marginal plates. Sternlieb and Quintana (1977) reported elongated peroxisomes in a single patient with cholangiocarcinoma. A marginal plate is characteristic in some animals and in human kidney peroxisomes but unusual in human liver; although it was reported in normal specimens, it seemed to occur more often in liver disease (Sternlieb and Quintana 1977). These results underline the necessity to perform cytochemistry and electron microscopy systematically; without catalase staining the tubular forms most probably had not been recognized.

Our results confirm and extend the rather heterogeneous picture presented by peroxisomes in patients described previously as NALD (Kelley et al. 1986). Although diagnosis was warranted on the basis of the clinical picture, neuropathology and the accumulation of VLCFA, enzymatic defects were not known at that time, nor was peroxisome morphology taken into account. Because the latter varies so much within this group of patients, it is most unlikely that enzymatic defects will be identical. This is confirmed by recent investigations on fibroblasts from patient number 3 (Poll-Thé et al. 1987): oxidation of lignoceric acid (C24:O) was severely decreased, but acylCoA oxidase was normal in immunoblotting experiments. Moreover, plasmalogen synthesis was normal. So patient number 3 can be distinguished from the two siblings with acylCoA oxidase deficiency as well as from NALD patients who have a disturbed plasmalogen metabolism (Kelley et al. 1986). Peroxisomal morphology reflects these differences. The clinical picture, on the other hand, can be very similar because the defect of any step of the β-oxidation sequence, cofactor or regulatory system, must ultimately lead to comparable accumulation of metabolites. This is well illustrated by the patient with the isolated thiolase deficiency, whose clinical course and many chemical data were almost identical to true Zellwegers syndrome (Schram et al. 1987), but who showed large and numerous peroxisomes. Some characteristics of the thiolase deficient peroxisomes are not unlike those in patient number 3 and the oxidase deficient children.

In order to avoid confusion, future diagnosis and naming of the disease should take into account peroxisomal morphology and enzymatic data as well.

Morphological heterogeneity of liver peroxisomes has also come to light in children described as suffering from phytanic acid oxidase deficiency or infantile Refsum disease (IRD) (Roels et al. 1986a; Beard et al. 1986).

In our other patients the precise enzyme defects were not studied. As a general rule, it can be expected that if peroxisomes or any altered organelle are abundant, only one or few enzymes will be deficient. This also holds for X-linked ALD where only the activity of the VLCFA-CoA synthase (CoA-SH ligase), the very first enzyme of the β-oxidation sequence, is impaired (Hashmi et al. 1986; Wanders et al. 1987b). However, when there is a complete lack or severe reduction of peroxisomes, many enzymes are non-functional, even though their synthesis may be normal. It is not

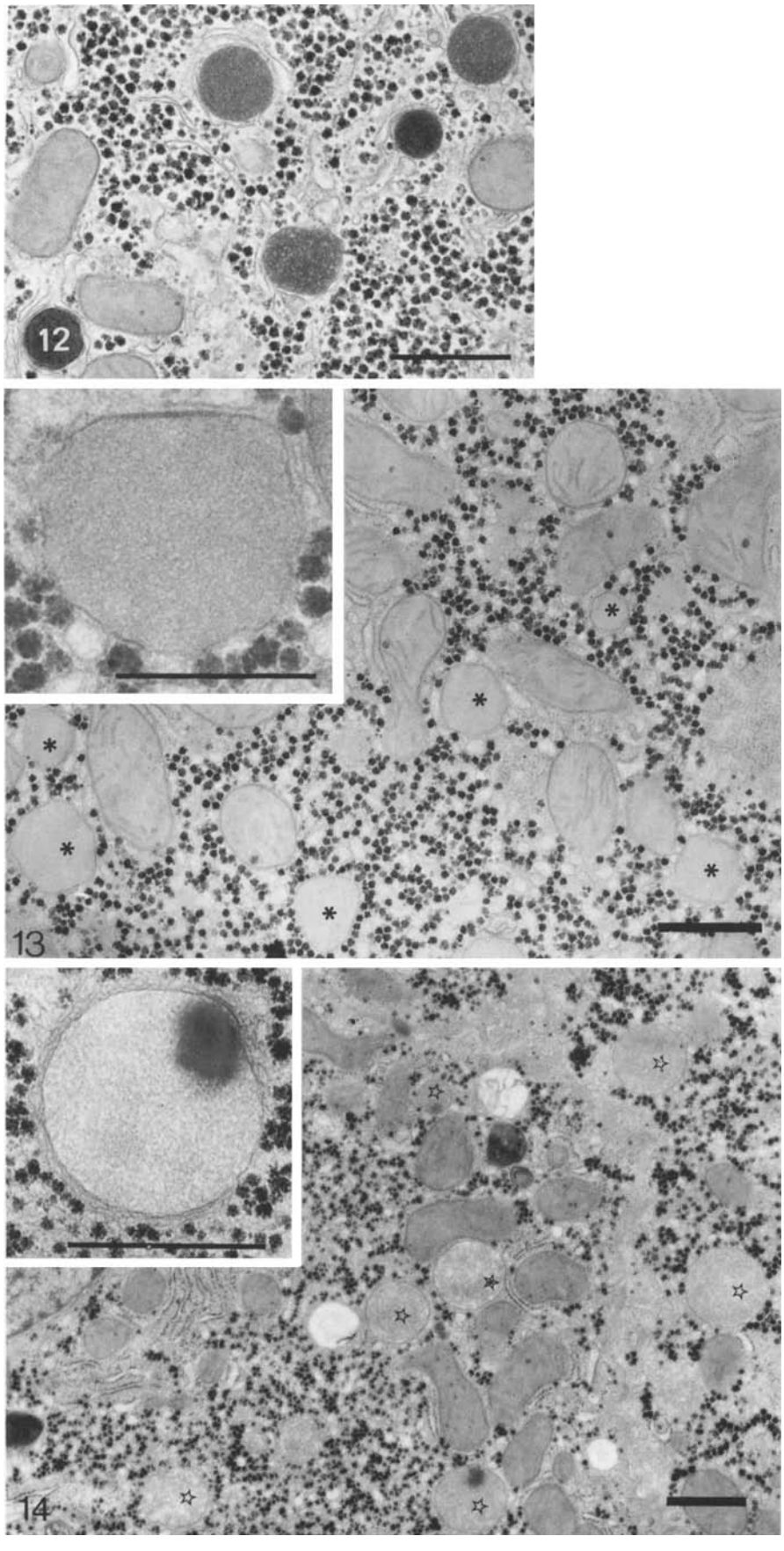


Fig. 12. Round peroxisomes in patient number 4. Catalase staining markedly differs between organelles; the larger ones tend to show a weaker reaction. Numerous glycogen rosettes. ×18300, *Bar* = 1 μm

Fig. 13. Typical human microbodies in patient number 2 (marked with *asterisk*); preparation without catalase staining. ×16000. *Inset:* microbody with marginal plate as sometimes seen in patient number 2. ×60200, *Bar* = 0.5 μm

Fig. 14. The atypical organelles in patient number 1 are marked with an *asterisk*. Their matrix has low density and is homogeneous, except for a nucleoid. ×12000. *Inset:* nucleoid is structureless. Organelle is surrounded by cisternae of SER, which is a peroxisomal characteristic. ×30000, *Bar* = 1 μm

known which deviations are pathogenetic, and which are of little consequence. The defect of plasmalogen synthesis in Zellweger's, IRD and most NALD is accompanying the malformation of peroxisomes but appears unrelated to the aetiology of neurological disease, since in thiolase deficiency (Goldfischer et al. 1986), acylCoA oxidase deficiency (Poll-Thé 1986; 1988) and in our patient number 3 this pathway was functioning while lesions of the central nervous system were severe.

The microscopy of peroxisomes in X-linked ALD was reported in only one patient (Goldfischer and Reddy 1984; Goldfischer et al. 1985). Their size was similar to that of our patient number 4, as was the age of onset of the illness (8 years). In patient 5, peroxisomes were smaller. We suggest that peroxisomal size is related to the severity of clinical disease, i.e. that larger peroxisomes are associated with earlier manifestation and faster progression of the symptoms. The clinical course clearly differed between both patients, and there is more new evidence for such a relationship. Unusually large peroxisomes were found in disorders with a deficient peroxisomal enzyme: in thiolase deficiency, in all 3 biopsies of the oxidase defective siblings, in our patients number 1, 2 and 3. Moreover, in many livers the larger peroxisomes possess lower catalase concentrations (Roels and Cornelis 1988). In order to explain this relationship, one should point out that the increased metabolic rate and raised peroxisomal β-oxidation capacity elicited by thyroid hormones is expressed in numerous but smaller hepatic peroxisomes (Fringes and Reith 1982; Just and Hartl 1983) while hypothyroidism is accompanied by macroperoxisomes (Riede et al. 1978). The reduced catalase staining in many peroxisomes of ALD patient number 4, of the pseudo-Zellweger child and in all peroxisomes of the oxidase defective siblings and patient number 5, could reflect one mechanism for lowered metabolic rate, i.e. a diminished enzymatic activity per unit volume.

Diminished catalase staining of hepatic peroxisomes is in itself not specific for a particular disorder (Roels et al. 1983), and some of the ultrastructural abnormalities, as mentioned earlier, have been reported in more than one pathological condition.

A striking configuration of peroxisomes associated with fat droplets was observed in patient 3 as well as in both children with acylCoA oxidase deficiency. As fatty acid breakdown is depressed in these patients, the intimate contact of both structures may be interpreted as some "compensatory" phenomenon. The proliferation of peroxisomes in lipodystrophy was also linked to the raised levels of fatty acids (Klar et al. 1987).

Fat droplets in liver parenchyma do not contain the trilaminar, regularly spaced, rigid structures typical of hepatic lysosomes in IRD and Zellweger, and of brain macrophages, adrenocortical cells, Leydig cells, a.o., in ALD and NALD (Ghatak et al. 1981; Haas et al. 1982; Jaffe et al. 1982; Manz et al. 1980; Schaumburg et al. 1977; Ulrich et al. 1978; Vamecq et al. 1986). These structures are believed to reflect the storage of VLCFA (Powers et al. 1980; Vamecq et al. 1986). The fat droplets in patient number 3, as well as in the thiolase deficient infant and the oxidase deficient siblings, do contain membranes with irregular spacing and their birefringence is also very similar. Membranous accumulations have also been observed after experimental in vivo inhibition of peroxisomal β-oxidation (Van den Branden et al. 1987; Vamecq et al. 1987).

We have searched for common microscopical features in a group of patients with peroxisomal disorders. The occurrence of enlarged peroxisomes was associated with the clinically more severe syndromes. Heterogeneity of staining with reduced catalase activity in many peroxisomes was seen in all cases, but is not specific for diseases caused by peroxisomal deficiencies. Elongated peroxisomes, their association with fat, invagination of cytoplasm and fat droplets with birefringent lamellae were found in several but not all syndromes with neonatal onset. In addition to common features, liver microscopy displayed important differences between clinically comparable i.e. NALD labelled patients.

In several patients it is proven that these microscopical differences correspond to separate enzyme defects. We propose such a relationship as a general working hypothesis.

Acknowledgements. The authors are greatly indebted to Dr. Olivier Dulac, Paris, who took care of the ALD patients and made available the clinical data, to Prof. J.L. Chaussain, who performed the adrenal function tests, and to Prof. Sidney Goldfischer, New York, for his critical review of the manuscript.

References

Abe K, Matsushima S, Mita M (1983) Estimation of the Mean Size of Spheres from the profiles in ulthrathin sections: a stereological method. J Electron Microsc 32:57–60

Arias JA, Moser AE, Goldfischer SL (1985) Ultrastructural and cytochemical demonstration of peroxisomes in cultured fibroblasts from patients with peroxisomal deficiency disorders. J Cell Biol 100:1789–1792

Aubourg P, Bougnères PF, Rocchiccioli F (1985a) Gas liquid

chromatographic mass spectrometric measurement of very long chain (C22 to C26) fatty acids in microliter samples of plasma. J Lipid Res 26:263–267

Aubourg P, Scotto J, Rocchiccioli F, Feldmann-Pautrat D, Robain O (1986) Neonatal adrenoleukodystrophy. J Neurol Neurosurg Psychiat 49:77–86

Beard ME, Moser AB, Sapirstein V, Holtzman E (1986) Peroxisomes in infantile phytanic acid storage disease: a cytochemical study of skin fibroblasts. J Inher Metab Dis 9:321–334

Chen WW, Watkins PA, Osumi T, Hashimoto T, Moser HW (1987) Peroxisomal β-oxidation enzyme proteins in adrenoleukodystrophy: distinction between X-linked adrenoleukodystrophy and neonatal adrenoleukodystrophy. Proc Natl Acad Sci USA 84:1425–1428

Fringes B, Reith A (1982) Time course of peroxisome biogenesis during adaptation to mild hyperthyroidism in rat liver. Lab Invest 47:19–26

Ghatak NR, Nochlin D, Peris M, Myer EC (1981) Morphology and distribution of cytoplasmic inclusions in adrenoleukodystrophy. J Neurol Sci 50:391–398

Goldfischer S, Reddy JK (1984) Peroxisomes (microbodies) in cell pathology. Int Rev Exp Path 26:45–84

Goldfischer S, Collins J, Rapin I, Coltoff-Schiller B, Chang Ch, Nigro M, Black VH, Javitt NB, Moser HW, Lazarow PB (1985) Peroxisomal defects in neonatal-onset and X-linked adrenoleukodystrophies. Science 227:67–70

Goldfischer S, Collins J, Rapin I, Neumann P, Neglia W, Spiro AJ, Ishii T, Roels F, Vamecq J, Van Hoof F (1986) Pseudo-Zellweger syndrome: Deficiencies in several peroxisomal oxidative activities. 108:25–32

Haas JE, Johnson ES, Farrell DL (1982) Neonatal-onset adrenoleukodystrophy in a girl. Ann Neurol 12:449–457

Hashmi M, Stanley W, Singh I (1986) Lignoceroyl-CoASH ligase: enzyme defect in fatty acid β-oxidation system in X-linked childhood adrenoleukodystrophy. FEBS-Lett 196:247–250

Jaffe R, Crumrine P, Hashida Y, Moser W (1982) Neonatal adrenoleukodystrophy. Clinical, pathologic and biochemical delineation of a syndrome affecting both males and females. Am J Pathol 108:100–111

Just WW, Hartl FU (1983) Rat Liver Peroxisomes, II. Stimulation of peroxisomal fatty-acid β-oxidation by thyroid hormones. Z Physiol Chem 364:1541–1547

Kelley RI, Datta NS, Dobyns WB, Hajra AK, Moser AB, Noetzel MJ, Zackai EH, Moser HW (1986) Neonatal adrenoleukodystrophy: new cases, biochemical studies, and differentiation from Zellweger and related peroxisomal polydystrophy syndromes. Amer J Med Genet 23:869–901

Klar A, Livni N, Gross-Kieselstein E, Navon P, Shalin A, Branski D (1987) Ultrastructural abnormalities of the liver in total lipodystrophy. Arch Pathol Lab Med 111:197–199

Manz HJ, Schuelein M, McCullough DC, Kishimoto Y, Eiben RM (1980) New phenotypic variant of adrenoleukodystrophy. Pathologic, ultrastructural and biochemical study in two brothers. J Neurol Sci 45:245–260

Poll-Thé BT, Ogier H, Saudubray JM, Roels F, Cornelis A, Vamecq J, Schutgens RBH, Wanders RJA, Tager JM (1986) A peculiar peroxisomal disorder. Two siblings previously diagnosed as having neonatal-onset adrenoleukodystrophy. Eur J Cell Biol 41 (Suppl 14):33

Poll-Thé BT, Bonnefont JP, Roels F, Scotto J, Fardeau M, Ogier H, Wanders RJA, Schutgens RBH, Schram AW, Saudubray JM (1987) A genetic disorder with elongated peroxisomes due to a deficiency in the peroxisomal β-oxidation system. SSIEM Meeting, Sheffield

Poll-Thé BT, Roels F, Ogier H, Scotto J, Vamecq J, Schutgens RBH, Wanders RJA, van Roermund CWT, van Wijland MJA, Schram AW, Tager JM, Saudubray JM (1988) A new peroxisomal disorder with enlarged peroxisomes and a specific deficiency of acyl-CoA oxidase (pseudo neonatal adrenoleukodystrophy). Am J Hum Genet 42:422–434

Powers JM, Schaumburg HH, Johnson AB, Raine CS (1980) A correlative study of the adrenal cortex in adrenoleukodystrophy. Evidence for a fatal intoxication with very long chain saturated fatty acids. Invest Cell Pathol 3:353–376

Powers JM, Moser HW, Moser AB, Ma CK, Elias SB, Norum RA (1987) Pathologic findings in adrenoleukodystrophy heterozygotes. Arch Pathol Lab Med 111:151–153

Roels F, Pauwels M, Cornelis A, Kerckaert I, van der Spek P, Goovaerts G, Versieck J, Goldfischer S (1983) Peroxisomes (microbodies) in human liver. Cytochemical and quantitative studies of 85 biopsies. J Histochem Cytochem 31:235–237

Roels F, Cornelis A, Poll-Thé BT, Aubourg P, Ogier H, Scotto J, Saudubray JM (1986a) Hepatic peroxisomes are deficient in infantile Refsum disease: a cytochemical study of 4 cases. Amer J Med Genet 25:257–271

Roels F, Poll-Thé BT, Aubourg P, Cornelis A, Scotto J, Van den Branden C, Saudubray JM (1986b) Cytochemistry of hepatic peroxisomes: their absence in infantile Refsum's disease (IRD) and their presence in neonatal adrenoleukodystrophy (NALD). Eur J Cell Biol 41 (Suppl 14):36

Roels F, Poll-Thé BT, Scotto J, Vamecq J, Cornelis A, Ogier H, Saudubray JM, Goldfischer S (1986c) Peroxisomes in several congenital syndromes (Infantile Refsum's disease, adrenoleukodystrophy, Menkes' disease, Batten's ceroid lipofuscinosis, GM1 gangliosidosis, a.o.). Ultramicroscopy 19:112

Roels F, Cornelis A (1988) Heterogeneity of catalase staining in human hepatocellular peroxisomes. J Histochem Cytochem (in press)

Riede UN, Riede PR, Horn R, Batthiany R, Kiefer G, Sandritter W (1978) Mechanisms of adaptation of hepatocytes to chronical hypothyroidism (a cytophotometrical and morphometrical study). Path Res Pract 162:398–419

Schaumburg HH, Powers JM, Raine CS, Suzuki K, Richardson EP (1975) Adrenoleukodystrophy. A clinical and pathological study of 17 cases. Arch Neurol 32:577–591

Schram AW, Goldfischer S, van Roermund WT, Brouwer-Kelder EM, Collins J, Hashimoto T, Heymans HSA, Van den Bosch H, Schutgens RBH, Tager JM, Wanders RJA (1987) Human peroxisomal 3-oxoacyl-coenzyme A thiolase deficiency. Proc Natl Acad Sci USA 84:2494–2496

Sternlieb I, Quintana N (1977) The peroxisomes of human hepatocytes. Lab Invest 36:140–149

Ulrich J, Herschkowitz N, Heitz Ph, Sigrist Th, Baerlocher P (1978) Adrenoleukodystrophy. Preliminary report of a connatal case. Light- and electron microscopical, immunohistochemical and biochemical findings. Acta Neuropathol (Berl) 43:77–83

Vamecq J, Draye JP, Van Hoof F, Misson JP, Evrard P, Verellen G, Eyssen HJ, Van Eldere J, Schutgens RBH, Wanders RJA, Roels F, Goldfischer SL (1986) Multiple peroxisomal enzymatic deficiency disorders. Am J Pathol 125:524–535

Vamecq J, Roels F, Van den Branden C, Draye JP (1987) Peroxisomal proliferation in heart and liver of mice receiving chlorpromazine, ethyl 2(5(4-chlorophenyl)pentyl) oxiran-2-carboxylic acid or high fat diet: a biochemical and morphometrical comparative study. Pediat Res 22:748–754

Van den Branden C, Vamecq J, Dacremont G, Premereur N, Roels F (1987) Short and long term influence of phenothiazines on liver peroxisomal fatty acid oxidation in rodents. FEBS Lett 222(1):21–26

Wanders RJA, Schutgens RBH, Schrakamp G, Tager JM, Van den Bosch H, Moser AB, Moser HW (1987a) Neonatal adrenoleukodystrophy. Impaired plasmalogen biosynthesis and peroxisomal β-oxidation due to a deficiency of catalase-containing particles (peroxisomes) in cultured skin fibroblasts. J Neurol Sci 77:331–340

Wanders RJA, van Roermund CWT, van Wijland MJA, Nijenhuis AA, Tromp A, Schutgens RBH, Brouwer-Kelder EM, Schram AW, Tager JM, van den Bosch H, Schalkwijk C (1987b) X-Linked adrenoleukodystrophy: defective peroxisomal oxidation of very long chain fatty acids but not of very long chain fatty acyl-CoA esters. Clin Chim Acta 165:321–329

Accepted April 20, 1988