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A New Peroxisomal Disorder with Enlarged Peroxisomes and a Specific Deficiency of Acyl-CoA Oxidase (Pseudo–Neonatal Adrenoleukodystrophy)

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Summary

In the present paper two siblings are presented with clinical manifestations very similar to those of patients affected by neonatal adrenoleukodystrophy. In contrast to neonatal adrenoleukodystrophy patients, hepatic peroxisomes in these siblings were enlarged in size and not decreased in number. Accumulation of very-long-chain fatty acids (VLCFA) was associated with an isolated deficiency of the fatty acyl-CoA oxidase, the enzyme that catalyzes the first step of the peroxisomal β-oxidation. Plasma levels of di- and trihydroxycoprostanoic acid, phytanic acid, and pipecolic acid were normal; furthermore, acyl-CoA:dihydroxyacetone phosphate acyltransferase activity in cultured fibroblasts was also found to be normal. The clinical, biochemical, and cytochemical features found in these two siblings are compared with those seen in two other disorders characterized by the absence of a decreased number of hepatic peroxisomes and the presence of VLCFA: (1) pseudo–Zellweger syndrome (deficiency of peroxisomal thiolase activity) and (2) X-linked childhood adrenoleukodystrophy (deficiency of activation of lignoceric acid). Review of the different biochemical defects possible in very-long-chain fatty-acid oxidation reveals different clinical pictures of varying severity, depending on the level at which the biochemical defect occurs.

Introduction

Neonatal adrenoleukodystrophy (NALD) is a genetic disorder that differs from childhood or X-linked childhood adrenoleukodystrophy (ALD) by (1) its clinical onset at birth or in infancy and (2) autosomal recessive inheritance. In both disorders there is cerebral white-matter demyelination, involvement of the adrenal cortex, and accumulation of very-long-chain fatty acids (VLCFA) in serum and tissues (Brown et al. 1982).

NALD is now considered to belong to the group of inherited peroxisomal disorders with multiple peroxisomal dysfunction (Schutgens et al. 1986). The two other principal conditions in this group are the cerebrohepatorenal syndrome of Zellweger (ZS) and infantile Refsum disease (IRD). Although there is a clinical heterogeneity between the three conditions, they share some features, including diffuse encephalopathy, chorioretinopathy and/or optic-nerve dysplasia, often facial dysmorphism, multiple biochemical abnormalities, and an absence or markedly decreased number of morphologically recognizable peroxisomes in the liver (Poll-The et al. 1987*a*).

In the present report two siblings are described

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who have clinical features resembling NALD but who differ from NALD patients by the presence of enlarged peroxisomes in the liver and by an isolated deficiency of the peroxisomal enzyme acyl-CoA oxidase.

Case Reports

Patient 1

The older sibling, a girl, was born at term and weighed 3.4 kg. The parents were first cousins. Despite an uncomplicated delivery she was severely hypotonic and showed clonic movements of the right leg 15 min after birth. She had no dysmorphic features and no hepatomegaly. Neonatal reflexes were absent. No underlying cause could be demonstrated. Results of cranial computerized tomography (CT), electroencephalogram (EEG), and cerebral spinal fluid (CSF) examinations were normal during the neonatal period.

During the first year of life she has been hospitalized several times for stridor, apneic spells, attacks of upward deviation of the eyes that lasts for 1–2 min, and short tonic seizures. From the age of 3.5 years she had almost continuous epileptic seizures, consisting of blinking of the eyes, distortion of the mouth, and shallow respiration. Her EEG became progressively abnormal with almost continuous epileptiform discharges.

A hearing deficit was noted at age 2 years, whereas her brain-stem auditory-evoked responses had been normal during the first months of life. At age 2.6 years she developed horizontal nystagmus and had normal fundi and pupillary light reflexes. At that time her electroretinogram (ERG) was flattened and the flash-evoked visual responses (VER) were almost entirely absent. At age 4 years pupillary light responses were absent and bilateral optic atrophy was evident.

Generalized muscular hypotonia associated with normal deep-tendon reflexes was present up to 2.6 years of age, at which time the muscle tone in the extremities became hypertonic and pyramidal tract signs as well as attacks of dystonia of the arms were noted. Motor and sensory-nerve conduction velocities were normal. Her psychomotor development was severely retarded. Nevertheless, she could crawl, walk with support, and manipulate objects bimanually at age 2 years; but she had no language abilities.

Neurological deterioration began at age 2.5 years, progressing to a vegetative state at 4 years. A CT scan without contrast infusion at age 2.8 years showed minimal white-matter changes. However, a CT scan after contrast infusion at age 4 years revealed extensive white-matter hypodensities and abnormal contrast enhancement, especially bilaterally at the level of the centrum semiovale (fig. 1). At that time she had a CSF protein value of 0.82 g/liter and slightly elevated values of serum transaminases and cholesterol. She had a low morning serum cortisol value of 30 ng/ml ($N = 90 \pm 30$) and an increased plasma adrenocorticotropin hormone (ACTH) value of 200 pg/ml ($N < 80$). Serum values of lactate and pyruvate were normal, as was the profile of organic acids in the urine. She died at home at age 5 years. An autopsy was not performed.

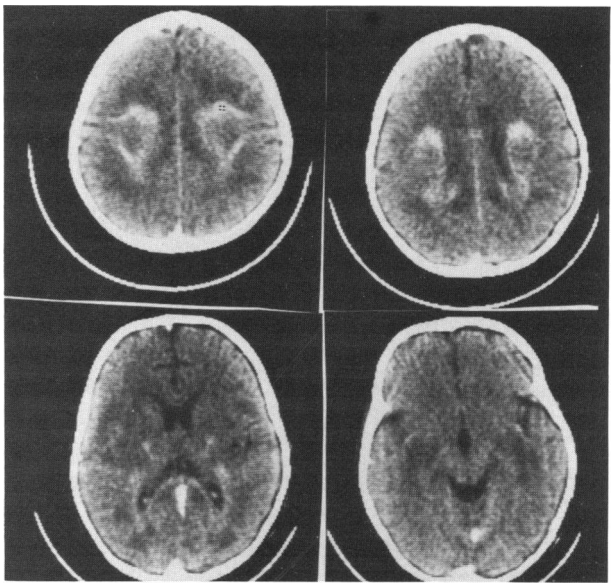

Figure 1 CT scan of patient 1 after contrast infusion at age 4 years. Note the abnormal contrast enhancement, especially bilaterally at the level of the centrum semiovale, and extensive white-matter hypodensities.

Patient 2

The younger brother of patient 1 was born at term and weighed 3.7 kg. Extreme muscular hypotonia and absence of neonatal reflexes were noted after birth. He had no craniofacial dysmorphism. Seizures, consisting of short tonic-clonic attacks accompanied by apneic spells, began at 7 days of age. His EEG showed epileptiform discharges. Mild hepatomegaly was noted at 7 days of age. Ophthalmological examination at 3 wk revealed marked myopia with normal

pupillary light responses and normal fundi. The ERG was extinguished, and the VER were severely altered. Subsequently a convergent strabismus and bilateral nystagmus were noted.

At age 3 wks the CSF protein value was 0.83 g/liter and the total number of cells was seven. A morning plasma ACTH value was 206 pg/ml. Serum lactate and urinary organic acids were normal. A CT scan showed minimal white-matter hypodensities. Results of skeletal X-ray examination and echography of the kidneys were normal.

At 3 mo this infant's height, weight, and head circumference were appropriate and social contact was present. Motor development remained severely delayed. Severe muscle hypotonia with normal tendon reflexes was still observed. A mild splenomegaly was noted at this age. He continued to have clonic seizures. At age 2 years he started to deteriorate. Social contact diminished, and pyramidal tract signs became evident. At this age his fundi showed a tapetoretinal degeneration and sensorineural hearing loss was noted.

Material and Methods

Liver Biopsy

Liver tissue was sampled by means of needle biopsy at age 2.5 years in patient 1 and at ages 3 wk and 2.3 years in patient 2.

Microscopic visualization of peroxisomes by means of catalase cytochemistry was performed according to the method of Roels and Goldfischer (1979) as modified by Roels et al. (1986), and morphometry of peroxisomes was performed with an Ibas I (Kontron) on electron micrographs at a magnification of 20,000 ×.

A portion of the liver biopsy specimens was immediately frozen and kept at −70C until biochemical analysis.

Muscle Biopsy

For morphological studies, a deltoidus muscle biopsy was performed under local anesthetic at age 2.5 years in patient 1.

Biochemical Studies

VLCFA ($\geq C_{22}$), bile acids, phytanic acid, phytanic acid oxidase activity, pipecolic acid, and plasmalogen studies in body fluids and/or in cultured skin fibroblasts were determined as described elsewhere (Poll-The et al. 1986).

In fibroblast homogenates the activity of [1-^{14}C] palmitoyl-CoA β-oxidation in peroxisomes was measured as described by Wanders et al. (1986*b*).

The rate of β-oxidation of [1-^{14}C] lignoceric acid ($C_{24:0}$) and [1-^{14}C] cerotic acid ($C_{26:0}$) in intact fibroblasts was determined as described by Wanders et al. (1987*a*).

Immunoblotting studies on fibroblasts were performed according to the method of Tager et al. (1985).

A portion of the liver biopsy tissues was used to measure the activity of several H_2O_2-generating oxidases—including the peroxisomal palmitoyl-CoA and hexadecanedioyl-CoA oxidases—according to the fluorimetric method described by Vamecq and van Hoof (1984), with slight modifications.

Results

Morphological Studies in Liver

Light- and electron-microscopy studies showed the presence of peroxisomes in both patients.

Patient 1.—Peroxisomal size was very heterogeneous. There were very large organelles next to small ones (fig. 2). The number of peroxisomes was not increased. Triangular or elongated peroxisomes were found, which is very unusual in human liver. The catalase reaction product was very different from one organelle to another (fig. 3). In many hepatocytes fat droplets were present, mitochondria had a clear matrix, and the smooth endoplasmic reticulum was moderately dilated. Lipofuscin pigments could be observed near some bile canaliculi whose lumen was not patently dilated. Mesenchymal cells with inclusions containing trilaminar leaflets were not found.

Patient 2.—At age 3 wk, peroxisomes were abundant, all of which were increased in size. Their catalase reaction product was weak and differed from one organelle to another. This could already be observed by means of light microscopy. In the hepatocytes the steatosis was mild, mitochondria had a normal configuration, and various dense bodies, either heterogeneous or homogeneous, were located near bile canaliculi. Most of the bile canaliculi were moderately dilated, and their lumens at times contained loose pseudomembranous material. Mesenchymal cells with storage material were not observed.

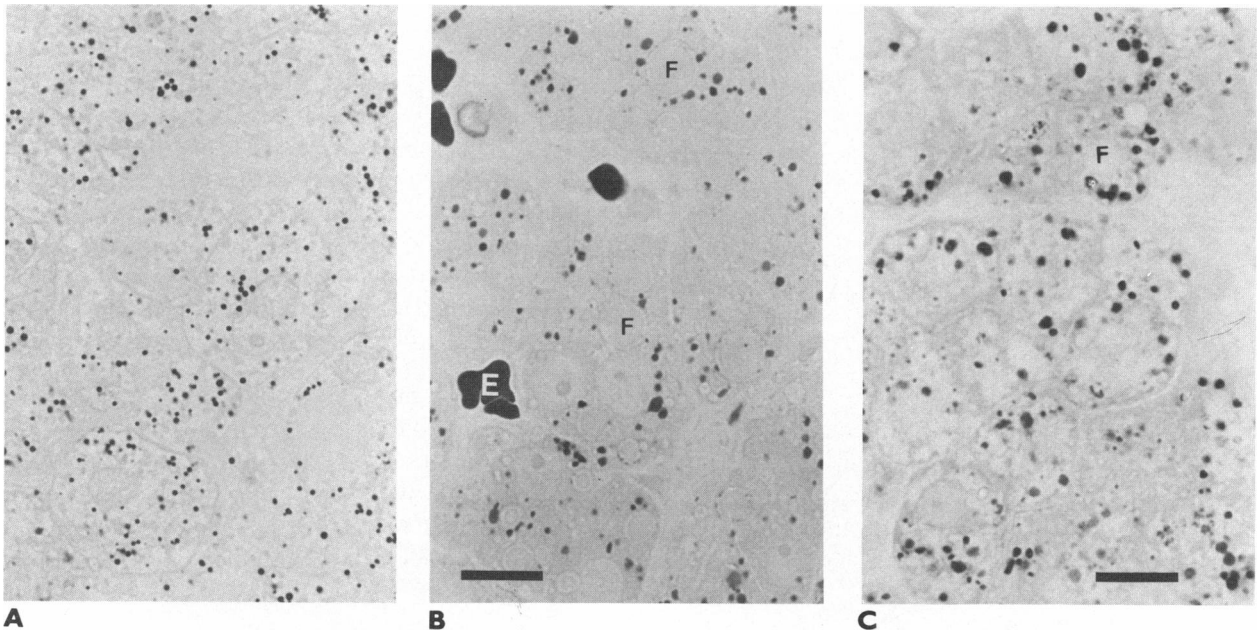

Figure 2 Hepatic peroxisomes as shown by light microscopy (1 μm Epon sections after staining for catalase). *A*, Control adult liver with a normal image. *B*, patient 1. *C*, patient 2 at age 2.3 years; peroxisomal size is very heterogeneous, and very large organelles are to be seen next to a small one. Large fat droplets (F) exist in many hepatocytes. E = Erythrocyte; bar = 10 μm.

At age 2.3 years, the number of peroxisomes was decreased in comparison with those seen at the first biopsy, but their mean size was still enlarged (figs. 2*C*, 4). The histology of the liver biopsy at this age resembled that of patient 1, with the exception that the peroxisomes were round. At the periphery of the fat droplets membranous lamellae were frequently found that were reminiscent of those in the liver of the pseudo-ZS patient reported by Goldfischer et al. (1986). The other ultrastructural abnormalities were similar, except for a marked widespread steatosis.

The morphometric analysis of peroxisomes showed a significant increase of their size in both patients (table 1). The diameters of the largest organelles were >1 μm and identical in patients 1 and 2. With respect to the form, elongated profiles were conspicuous in patient 1, in contrast to the situation in patient 2; nearly circular profiles were present in both patients.

Morphological Studies in Muscle Biopsy: (Patient 1)

Hematoxylin-eosin and modified trichome stains showed a variability of fiber diameter without an endomysial abnormality. The ATPase reaction showed a normal checkerboard distribution of fibers with type II fiber atrophy and microvacuolation of the fibers.

Fat droplets were present in all fibers. Staining for succinate dehydrogenase showed a diminished reaction in all fibers. The glycogen content was normal.

Biochemical Studies

As shown in table 2, neither abnormal amounts of di- and trihydroxycoprostanoic acid nor the accumulation of phytanic acid or pipecolic acid could be found in body fluids.

In fibroblasts, the activity of acyl CoA:dihydroxyacetone phosphate acyltransferase was in the low normal range in patient 1 and completely normal in patient 2. The de novo plasmalogen biosynthesis was slightly impaired in patient 1. Phytanic acid oxidase activity in fibroblasts was normal in both patients.

In contrast, VLCFA were markedly increased, as reflected in an elevated C_{26}:C_{22} ratio in plasma and fibroblasts. To investigate whether the accumulation of VLCFA resulted from an impaired degradation of these substrates, fatty-acid β-oxidation was studied in cultured skin fibroblasts and in liver biopsies of both patients (table 3). The rates of oxidation of lignoceric acid ($C_{24:0}$) and cerotic acid ($C_{26:0}$) measured

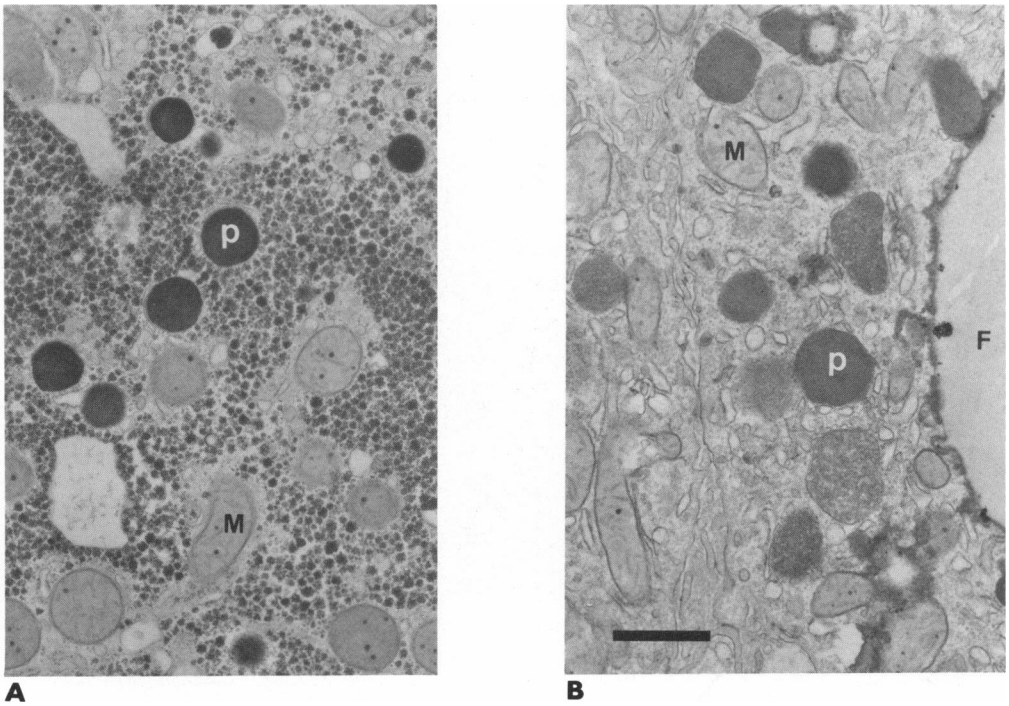

Figure 3 Electron micrographs of (*A*) a control hepatocyte from a baby not affected by a metabolic disorder and (*B*) patient 1; both hepatocytes have been stained for catalase activity. *A*, Peroxisomes (P) are round and smaller than mitochondria (M). Cytoplasmic glycogen is prominent. *B*, Peroxisomes are numerous, and some are very large. Triangular or elongated peroxisomes are typical for patient 1; such forms are very unusual in human liver. The catalase reaction product is very different from one organelle to another. Mitochondria are normal. F = Large fat droplet; bar = 1 μm.

in intact fibroblasts from the two patients was severely decreased. In fibroblast homogenates from both patients, the activity of peroxisomal [1-^{14}C] palmitoyl-CoA β-oxidation was reduced by >95%. Since VLCFA are primarily oxidized in peroxisomes rather than in mitochondria (Singh et al. 1984), we investigated whether the deficiency in peroxisomal fatty-acid β-oxidation was caused by a deficiency of acyl-CoA oxidase, bifunctional protein, or 3-oxoacyl-CoA thiolase; we did this by performing immunoblotting experiments on liver tissues from the patients, using antibodies directed against the individual β-oxidation enzyme proteins.

The results in figure 5 show that bifunctional protein and 3-oxoacyl-CoA thiolase were present. However, no immunologically cross-reactive material was found to be reacting with anti–acyl-CoA oxidase antibodies. Furthermore, enzyme activity measurements in liver biopsies revealed that acyl-CoA oxidase was highly deficient (table 3).

Discussion

These two siblings had a number of manifestations remarkably similar to those seen in NALD. As in the case of most NALD patients in the literature (Kelley et al. 1986), our patients began the neonatal period with severe muscle hypotonia and seizures. Although psychomotor development was severely delayed, they showed some progress in the first 2 years of life, after which they showed a progressive neurological regression. Both patients had a sensorineural hearing deficit and an abnormal ERG. CT-scan examinations showed progressive cerebral white-matter demyelination and no signs of cortical malformation. No dysmorphic features were identified. In both siblings the diagnosis of NALD was suspected after demonstration of both elevated basal ACTH levels and increased VLCFA values in plasma. However, in striking contrast to reported cases of NALD (Farrell et al. 1983; Partin and McAdams 1983; Goldfischer et al.

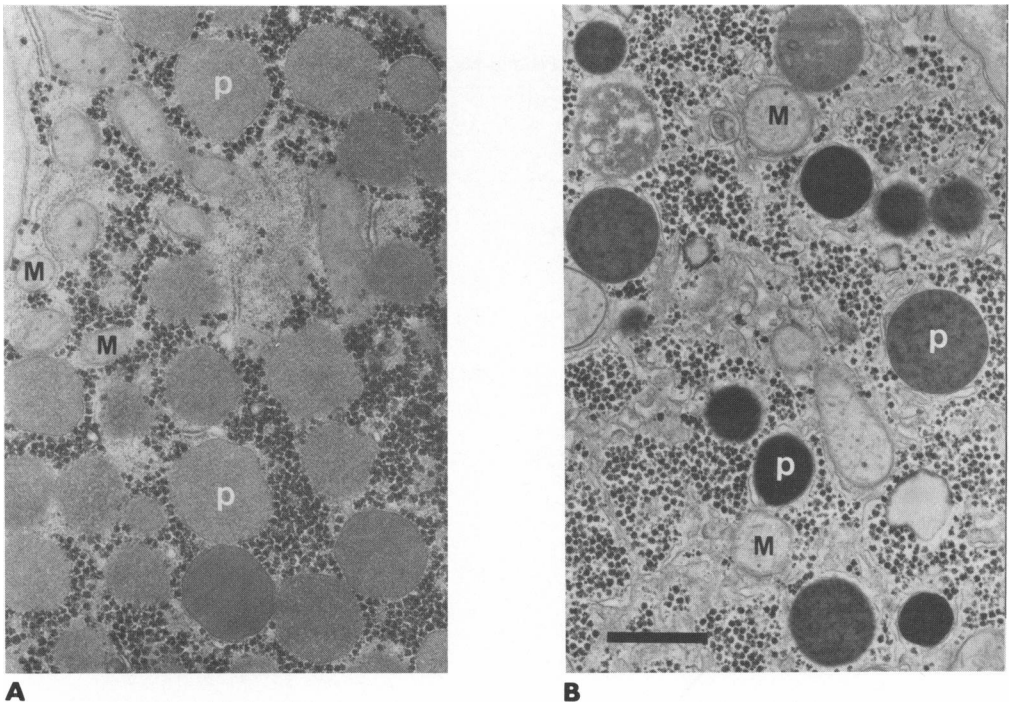

Figure 4 Electron micrographs of liver parenchyma of patient 2 at age 3 wk (*A*) and at age 2.3 years (*B*). *A*, The cytoplasm is crowded with very large and round peroxisomes (P); their catalase reaction product is weak and differs from one organelle to another. Mitochondria (M) are smaller than peroxisomes. *B*, Peroxisomes are less numerous than in the biopsy at age 3 wk, but their mean size has increased. Catalase staining is strong in some peroxisomes and not in others. The overall reaction product is difficult to compare with the earlier biopsy because fixation times were different. Glycogen rosettes are prominent in both figures. Bar = 1 μm.

1985; Aubourg et al. 1986), in both our patients peroxisomes in the liver were not decreased in number and were instead enlarged in size; in addition, a liver biopsy during infancy (patient 2) showed an increased number of peroxisomes. Further biochemical evaluation of peroxisomal functions of the two siblings revealed no accumulation of pipecolic acid or bile acid synthesis intermediates. Also, there was no marked decrease in plasmalogens, a finding that contrasts with the results for reported NALD patients (Goldfischer et al. 1985; Kelley et al. 1986; Wolff et al. 1986).

This unusual combination of enlarged peroxisomes and accumulation of VLCFA in these siblings was associated with an isolated deficiency of the peroxisomal fatty acyl-CoA oxidase. The association of the accumulation of VLCFA with no decreased peroxisomal compartment in liver has been described in

Table 1

Morphometry of Peroxisomes of the Patients and Controls

Source	Mean ± SEM (range) Size[a] (μm)	Median (range) Form[b]
Control (adult, normal liver)	0.534 ± 0.014 (0.287–0.820)	0.853 (0.554–0.989)
Control (baby)	0.478 ± 0.014 (0.244–0.796)	0.892 (0.399–0.986)
Patient 1 (2.5 years old)	0.661 ± 0.0228 (0.175–1.473)	0.807 (0.241–0.981)
Patient 2 (3 wk old)	0.667 ± 0.0177 (0.245–1.277)	0.865 (0.513–0.994)
Patient 2 (2.3 years old)	0.670 ± 0.0139 (0.199–1.338)	0.930 (0.503–0.996)

[a] Diameter of circle having the same area as measured.
[b] Ratio of shortest to longest diameter (perfect circle = 1.0).

Table 2

Biochemical Results in Two Patients with Pseudo-NALD

Parameter and Body Fluid or Tissue	Source		
	Patient 1	Patient 2	Controls
VLCFA:			
$C_{26:0}$ (μg/ml):			
Plasma	2.843		0.33 ± 0.18
$C_{26:0}$ (μg/mg protein):			
Fibroblasts	0.448	0.669	0.07 ± 0.04
$C_{26:0}/C_{22:0}$:			
Plasma	0.076	0.16	0.016 – 0.028
Fibroblasts	1.577	1.047	0.08 ± 0.03
THCA:			
(% Total bile acids):			
Plasma	nd	nd	nd
Urine	nd	nd	nd
Phytanic acid:			
(μmol/liter):			
Plasma	6.4	nd	<10
Phytanic acid oxidase:			
(pmol/h/mg protein):			
Fibroblasts	32.55	31.51	>25
Pipecolic acid:			
(μmol/liter):			
Plasma	1.66	nd	<4
(μmol/g creatinine):			
Urine	0.93	70 (1 mo)	<6 (>1 year)
(μmol/liter):			
CSF	0.07	nd	<0.12
DHAP-AT			
(nmol/2 h/mg protein):			
Fibroblasts	4.5	10.9	8.80 ± 2.10
[1-^{14}C]Hexadecanol incorporation in phospholipids:			
(% dpm in PE):			
Fibroblasts	17.2	48.8	57.3 ± 9.9
(% pPE in PE):			
Fibroblasts	74.9	74.5	91.4 ± 4.1
(% dpm in PC):			
Fibroblasts	57.7	39.6	31.9 ± 4.9
(% pPC in PC):			
Fibroblasts	3.0	8.4	20.3 ± 7.1

Note.—THCA = trihydroxycoprostanoic acid; DHAP-AT = acyl CoA: dihydroxyacetone phosphate acyltransferase; PE = total phosphatidylethanolamine; pPE = plasmalogen phosphatidylethanolamine; PC = total phosphatidylcholine; pPC = plasmalogen phosphatidylcholine; and nd = not detectable.

two other conditions—namely, (1) the pseudo-ZS, in which the symptoms are associated with an isolated peroxisomal 3-oxoacyl-CoA thiolase deficiency (Goldfischer et al. 1986; Schram et al. 1987), and (2) the X-linked childhood ALD, which is associated with a defective activity of very-long-chain (lignoceroyl-CoA) acyl-CoA ligase (Goldfischer et al. 1985; Hashimi et al. 1986; Wanders et al. 1987*b*).

It is interesting to speculate as to what clinical similarities or differences exist in these three conditions, all of which are characterized by a defective fatty-acid β-oxidation system. After their activation by very-long-chain acyl-CoA ligase, VLCFA are degraded by peroxisomal β-oxidation (fig. 6). The peroxisomal chain shortening is catalyzed by acyl-CoA oxidase, the bifunctional protein with enoyl-

Table 3

Fatty-Acid β-Oxidation in Two Patients with Pseudo-NALD

Parameter, Tissue	Source		
	Patient 1	Patient 2	Controls (*N*)
Rate of oxidation:			
[1-^{14}C] Lignoceric acid (pmol/min/mg protein), intact fibroblasts	0.457	nd	3.1 – 8.2 (12)
[1-^{14}C] Cerotic acid (pmol/min/mg protein), intact fibroblasts	0.247	nd	2.2 – 5.9 (9)
[1-^{14}C] Palmitoyl-CoA oxidation (nmol/min/mg protein), fibroblast homogenates	0.002; 0.003	0.001; 0.002	0.088 – 0.236 (12)
Palmitoyl-CoA oxidase (units/g[a]), liver	0.032	0.038	0.158 ± 0.021 (7)
Hexadecanedioyl-CoA oxidase (units/g[a]), liver	nd	0.027	0.163 ± 0.042 (7)

Note.—nd = not done.
[a] μmoles H_2O_2 produced/min/g liver.

CoA hydratase, and 3-hydroxyacyl-CoA dehydrogenase activities and thiolase (Osmundsen et al. 1980). In X-linked childhood ALD the deficient step is at the level of the activation of lignoceric acid ($C_{24:0}$) by lignoceroyl-CoA ligase rather than an intrinsic defect in the peroxisomal β-oxidation process (Hashimi et al. 1986; Wanders et al. 1987*b*). This disease is only found in males and is hallmarked by a degenerative demyelination and variable adrenal hypofunction starting in childhood. It is important to note that (1) there is no craniofacial dysmorphism, (2) no congenital malformations are observed, and (3) the hepatic peroxisomes are normal (Goldfischer et al. 1985).

Pseudo-ZS has been recently described in one patient (Goldfischer et al. 1986). This patient had a clinical phenotype and neuromigrational abnormalities similar to those observed in classical ZS, a disorder with multiple congenital anomalies and total absence of hepatic peroxisomes (Goldfischer et al. 1973); however, in contrast to classical ZS, liver peroxisomes were present and increased in size, as in our two patients. In the pseudo-ZS there is a specific deficit in the activity of the peroxisomal enzyme thiolase (Schram et al. 1987).

The two siblings in the present report resemble the cases of X-linked ALD with respect to both the absence of dysmorphism and the presence of cerebral white-matter demyelinization and abnormal contrast enhancement on CT scan (Marler et al. 1983). However, their clinical course is much more severe and closer to that of the pseudo-ZS patient. Furthermore, their liver peroxisomes showed abnormalities somewhat similar to those observed in pseudo-ZS.

Such differences between these three conditions raise the question of the contribution of the accumulation of VLCFA in the pathogenesis of peroxisomal disorders.

In X-linked childhood ALD, microscopic abnormalities of hepatic peroxisomes have not been reported. Patients are usually completely normal for the first years of life and exhibit no errors of morphogenesis, suggesting a slow process of VLCFA accumulation as reflected by the late onset of clinical symptoms as seen in other storage disorders. Although the adrenal cortex of an ALD fetus may already show a 1,000-fold excess of $C_{26:0}$ in the cholesteryl ester fraction, during postnatal life the VLCFA accumulation in the form of cholesteryl esters is more important in NALD than in X-linked ALD (Moser et al. 1982). Indeed, it is possible that VLCFA accumulation in the form of cholesteryl esters occurs at a slower rate in X-linked childhood ALD because the VLCFA-CoA formation is first required for the acyl-CoA cholesterol acyltransferase reaction (fig. 6). If such is the case, the VLCFA accumulation in the form of cholesteryl esters might be more toxic than the nonactivated VLCFA accumulation.

In pseudo-NALD (our two patients), the defect in β-oxidation is at the level of acyl-CoA oxidase activity. Our two patients showed no evidence of errors of morphogenesis, but they displayed a more severe clinical course than that seen in X-linked childhood ALD beginning in the neonatal period. In the patient with peroxisomal thiolase deficiency, i.e., pseudo-ZS, the clinical picture displays congenital malformations and is strikingly similar to that observed in classical ZS, in which there are multiple peroxisomal dysfunc-

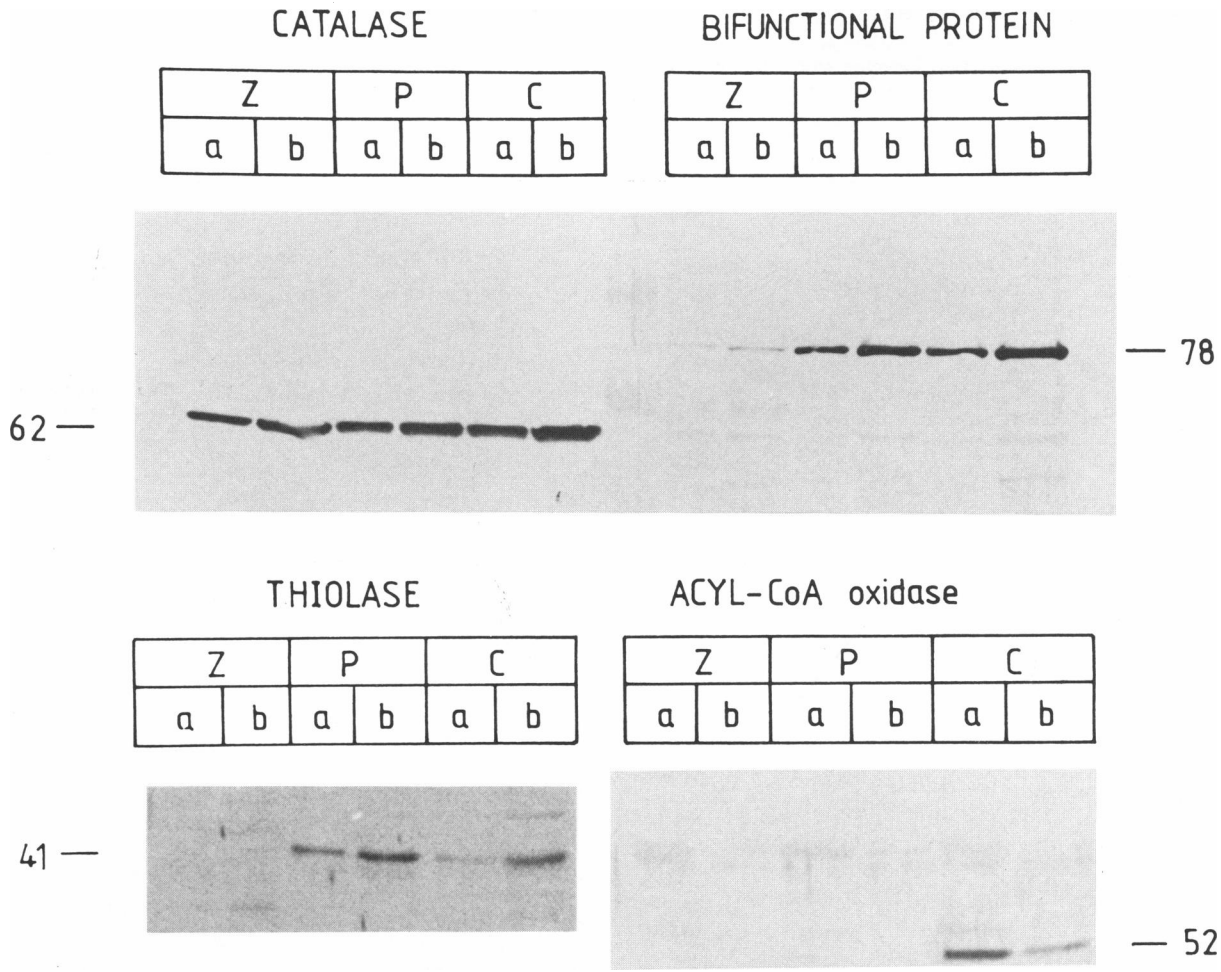

Figure 5 Immunoblotting experiments on liver tissue from a ZS patient, patient 1, and a control subject; antibodies directed against catalase, peroxisomal bifunctional protein, 3-oxoacyl-CoA thiolase, and acyl-CoA oxidase were used. The lines used were as follows: lane Z, ZS; lane P, patient 1; lane C, control. Immunologically cross-reactive material reacting with anti-catalase antibodies (62 kD) was found in all liver tissues but was not found with anti–bifunctional protein (78 kDa) and anti-thiolase (41 kD) antibodies in Zellweger syndrome or with anti–acyl-CoA oxidase antibodies (52 kD) in ZS and in patient 1.

tions, including deficient plasmalogen biosynthesis and a complete absence of hepatic peroxisomes (Schutgens et al. 1986). It is of interest that the patient with peroxisomal thiolase deficiency showed an accumulation of intermediates in the biosynthesis of bile acids.

The foregoing observations suggest that the peroxisomal fatty-acid β-oxidation defect is responsible for the majority of the clinical symptoms in classical ZS. In this regard the role of defective plasmalogen synthesis in the pathogenesis of symptoms in classical ZS is probably not predominant, since plasmalogen synthesis was not impaired in pseudo-ZS (Schram et al. 1987).

A review of the different biochemical defects possible in peroxisomal β-oxidation suggests that there are different clinical pictures of varying severity, depending on the level at which the biochemical defect occurs. On the basis of a preliminary review, one could speculate that thiolase deficiency has more consequences than does acyl-CoA oxidase deficiency, a result that would suggest that it may serve as a final common pathway for both the oxidation of VLCFA and the biosynthesis of bile acids. In two patients with NALD, a condition with an intermediate clinical severity, the bifunctional protein was found to be markedly deficient, probably owing to a rapid degradation in the cytoplasm prior to its entry into peroxi-

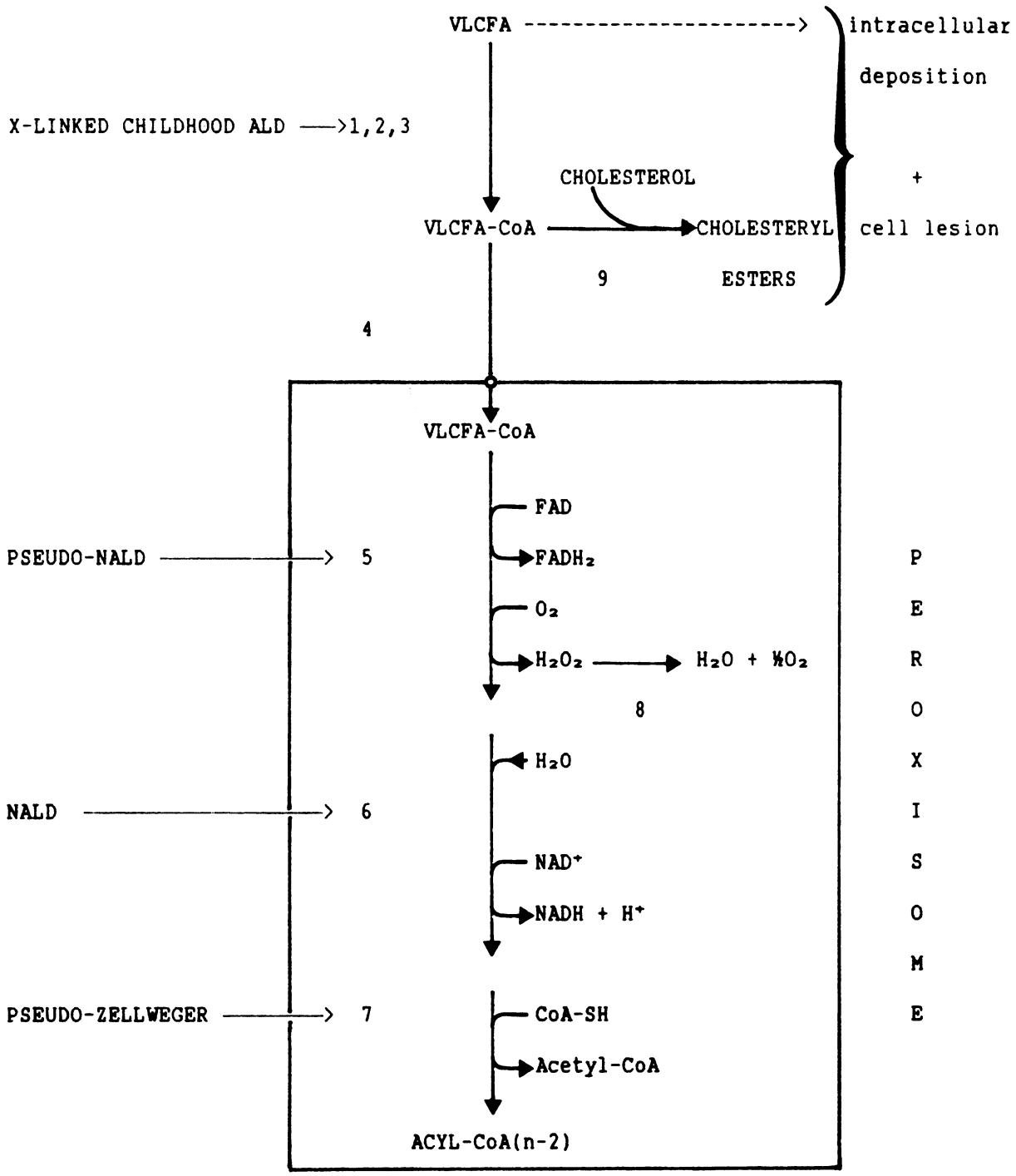

Figure 6 1, 2, 3 = Mitochondrial, microsomal, and peroxisomal fatty acyl-CoA activation; 4 = entry of acyl-CoA esters into the peroxisome; 5 = fatty acyl-CoA oxidase; 6 = bifunctional protein; 7 = 3-oxoacyl-CoA thiolase; 8 = catalase; and 9 = acyl-CoA cholesterol acyltransferase. In both ZS and IRD, all three peroxisomal β-oxidation enzymes (5, 6, and 7) are deficient.

Table 4

Classification of Human Peroxisomal Disorders

Peroxisome Status and Disorder	Enzymatic Defect
Normal (group I):	
X-linked ALD (childhood)	Lignoceroyl-CoA ligase
Absent or markedly reduced (group II):	
Zellweger	Biogenesis? multiple?
Infantile Refsum	Biogenesis? multiple?
Neonatal ALD	Biogenesis? multiple? bifunctional protein?
Abnormal (group III):	
Rhizomelic chondrodysplasia	?
Pipecolic acidemia	?
Pseudo-Zellweger	β-Ketothiolase
Pseudo–neonatal ALD	Acyl-CoA oxidase
Unknown (group IV):	
Acatalasemia	Catalase
Hyperoxaluria type I	Alanine:glyoxylate transferase
Refsum	Phytanic acid oxidase

somes (Chen et al. 1987). However, analysis of peroxisomal β-oxidation enzymes in more NALD patients will be necessary to answer the question of whether the enzymatic defect is restricted to the bifunctional protein, since NALD is known to be associated with multiple peroxisomal dysfunction. Furthermore, the possibility of the existence of residual enzyme activity that was observed in our patients, an activity that could to some extent be efficient in vivo, remains to be studied.

Instead of the classical classification based on the extent of impaired biochemical functions, we prefer a classification based both on the morphological aspects of hepatocellular peroxisomes and on the enzymatic deficiency (table 4). According to the morphology of hepatocellular peroxisomes, the patients can be classified as normal (group I), absent or markedly reduced (group II), or abnormal (group III). It appears that the presence of peroxisomes does not exclude a peroxisomal disorder. The primary genetic defect of the disorders in group II is still unknown. This group of disorders, in which liver peroxisomes are morphologically absent or markedly reduced in number, is genetically heterogeneous as demonstrated by means of complementation analysis that uses somatic cell fusion (Wanders et al. 1986*a*; Tager et al. 1987). First, ZS and IRD belong to the same complementation group, whereas NALD represents another group. Second, although ZS and IRD belong to the same complementation group, there are differences between the two disorders, both in the severity of the disease and in survival, differences that might be due to more extensive organ involvement in ZS (Poll-The et al. 1987*a*, 1987*b*). Third, NALD might be a heterogeneous group in and of itself, as suggested by the wide variation in facial dysmorphism and neurological features observed in this syndrome (Kelley et al. 1986).

A precise diagnosis of these disorders is so much the more important because prenatal diagnosis can be performed on chorionic villi or amniotic fluid cells (Moser et al. 1984; Schutgens et al. 1985; Poulos et al. 1986; Poll-The et al. 1987*b*).

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References

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

Brown, F. R., A. J. McAdams, J. W. Cummins, R. Konkol, L. Singh, A. B. Moser, and H. W. Moser. 1982. Cerebro-hepato-renal (Zellweger) syndrome and neonatal adrenoleukodystrophy: similarities in phenotype and accumulation of very long chain fatty acids. John Hopkins Med. J. **151**:344–351.

Chen, W. W., P. A. Watkins, T. Osumi, T. Hashimoto, and H. W. Moser. 1987. Peroxisomal β-oxidation enzyme proteins in adrenoleukodystrophy: distinction between X-linked adrenoleukodystrophy and neonatal adrenoleukodystrophy. Proc. Natl. Acad. Sci. USA **84**:1425–1428.

Farrell, K., J. E. Dimmick, D. A. Applegarth, L. E. Wong, W. J. Tze, A. Q. McCormick, J. E. Jan, and H. W. Moser. 1983. Peroxisomal abnormalities in neonatal adrenoleukodystrophy. Ann. Neurol. **14**:379.

Goldfischer, S., J. Collins, I. Rapin, B. Coltoff-Schiller, C. H. Chang, M. Nigro, V. M. Black, N. B. Javit, H. W. Moser, and P. B. Lazarow. 1985. Peroxisomal defects in neonatal-onset and X-linked adrenoleukodystrophies. Science **227**:67–70.

Goldfischer, S., J. Collins, I. Rapin, P. Neumann, W. Neglia, A. J. Spiro, T. Ishii, E. Roels, J. Vamecq, and F. Van Hoof. 1986. PseudoZellweger syndrome: deficiencies in several peroxisomal activities. J. Pediatr. **108**:25–32.

Goldfischer, S., C. L. Moor, A. B. Johnson, A. F. Spiro, M. P. Valsamis, H. K. Wisniewski, R. H. Ritch, W. T. Norton, I. Rapin, and L. M. Gartner, 1973. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. Science **182**:62–64.

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

Kelley, R. I., N. S. Datta, W. B. Dobyns, A. K. Hajra, A. B. Moser, M. J. Noetzel, E. H. Zackai, and H. W. Moser. 1986. Neonatal adrenoleukodystrophy: new cases, biochemical studies, and differentiation from Zellweger and related peroxisomal polydystrophy syndromes. Am. J. Med. Genet. **23**:869–901.

Marler, J. R., B. P. O'Neill, G. S. Forbes, and H. W. Moser. 1983. Adrenoleukodystrophy (ALD): clinical and CT features of a childhood variant. Neurology **33**:1203–1205.

Moser, H. W., A. B. Moser, J. M. Powers, H. M. Nitowsky, H. H. Schaumburg, R. A. Norum, and B. R. Migeon. 1982. The prenatal diagnosis of adrenoleukodystrophy: demonstration of increased hexacosanoic acid levels in cultured amniocytes and fetal adrenal gland. Pediatr. Res. **16**:172–175.

Moser, A. E., I. Singh, F. R. Brown III, G. I. Solish, R. I. Kelley, P. J. Benke, and H. W. Moser. 1984. The cerebro-hepato-renal (Zellweger) syndrome: increased levels and impaired degradation of very-long-chain fatty acids and their use in prenatal diagnosis. N. Engl. J. Med. **310**:1141–1146.

Osmundsen, H., C. J. Neat, and B. Borreback. 1980. Fatty acid products of peroxisomal β-oxidation. Int. J. Biochem. **12**:625–630.

Partin, J. S., and A. J. McAdams. 1983. Absence of hepatic peroxisomes in neonatal onset adrenoleukodystrophy. Pediatr. Res. **17**:294A.

Poll-The, B. T., J. M. Saudubray, H. Ogier, R. B. H. Schutgens, R. J. A. Wanders, G. Schrakamp, H. van den Bosch, F. Trijbels, A. Poulos, H. W. Moser, J. van Elderen, and H. Eyssen. 1986. Infantile Refsum's disease: biochemical findings suggesting multiple peroxisomal dysfunction. J. Inherited Metab. Dis. **9**:169–174.

Poll-The, B. T., J. M. Saudubray, H. A. M. Ogier, M. Odièvre, J. M. Scotto, L. Monnens, L. C. P. Govaerts, F. Roels, A. Cornelis, R. B. H. Schutgens, R. J. A. Wanders, A. W. Schram, and J. M. Tager. 1987*a*. Infantile Refsum's disease: an inherited peroxisomal disorder; comparison with Zellweger syndrome and neonatal adrenoleukodystrophy. Eur. J. Pediatr. **146**:477–483.

Poll-The, B. T., J. M. Saudubray, F. Rocchiccioli, J. Scotto, F. Roels, J. Boue, H. Ogier, Y. Dumez, R. J. A. Wanders, R. B. H. Schutgens, A. W. Schram, and J. M. Tager. 1987*b*. Prenatal diagnosis and confirmation of infantile Refsum's disease. J. Inherited Metab. Dis. **10**(Suppl. 2): 229–232.

Poulos, A., C. van Crugten, P. Sharp, W. F. Carey, E. Robertson, D. M. O. Becroft, J. M. Saudubray, B. T. Poll-The, E. Christensen, and N. Brandt. 1986. Prenatal diagnosis of Zellweger syndrome and related disorders: impaired degradation of phytanic acid. Eur. J. Pediatr. **145**:507–510.

Roels, F., A. Cornelis, B. T. Poll-The, P. Aubourg, H. Ogier, J. M. Scotto, and J. M. Saudubray. 1986. Hepatic peroxisomes are deficient in infantile Refsum's disease: a cytochemical study of 4 cases. Am. J. Med. Genet. **25**:257–271.

Roels, F., and S. Goldfischer. 1979. Cytochemistry of human catalase: the demonstration of hepatic and renal peroxisomes by a high temperature procedure. J. Histochem. Cytochem. **27**:1471–1477.

Schram, A. W., S. Goldfischer, C. W. T. Van Roermund, E. M. Brouwer-Kelder, J. Collins, T. Hashimoto, H. S. A. Heymans, H. van den Bosch, R. B. H. Schutgens, J. M. Tager, and R. J. A. Wanders. 1987. Human peroxisomal 3-oxoacyl-coenzyme A thiolase deficiency. Proc. Natl. Acad. Sci. USA **84**:2494–2497.

Schutgens, R. B. H., H. S. A. Heymans, R. J. A. Wanders, H. van den Bosch, and J. M. Tager. 1986. Peroxisomal

disorders: a new recognized group of genetic diseases. Eur. J. Pediatr. **144**:430–440.

Schutgens, R. B. H., G. Schrakamp, R. J. A. Wanders, H. S. A. Heymans, H. W. Moser, A. E. Moser, J. M. Tager, H. V. D. Bosch, and P. Aubourg. 1985. The cerebro-hepato-renal (Zellweger) syndrome: prenatal detection based on impaired biosynthesis of plasmalogens. Prenat. Diagn. **5**:337–344.

Singh, I., A. E. Moser, S. Goldfischer, and H. W. Moser. 1984. Lignoceric acid is oxidized in the peroxisome: implications for the Zellweger cerebro-hepato-renal syndrome and adrenoleukodystrophy. Proc. Natl. Acad. Sci. USA **81**:4203–4207.

Tager, J. M., W. A. Ten Harmsen Van Der Beek, R. J. A. Wanders, T. Hashimoto, H. S. A. Heymans, H. Van Den Bosch, R. B. H. Schutgens, and A. W. Schram. 1985. Peroxisomal β-oxidation enzyme proteins in the Zellweger syndrome. Biochem. Biophys. Res. Commun. **126**:1269–1275.

Tager, J. M., A. Westerveld, A. Strijland, A. W. Schram, R. B. H. Schutgens, H. Van Den Bosch, and R. J. A. Wanders. 1987. Complementation analysis of peroxisomal diseases by somatic cell fusion. Pp. 353–357 *in* H. D. Fahimi and H. Sies, eds. Peroxisomes in biology and medicine. Springer, Berlin and Heidelberg.

Vamecq, J., and F. van Hoof. 1984. Implication of a peroxisomal enzyme in the catabolism of glutaryl-CoA. J. Biochem. **221**:203–211.

Wanders, R. J. A., D. Saelman, H. S. A. Heymans, R. B. H. Schutgens, A. Westerveld, B. T. Poll-The, J. M. Saudubray, H. van den Bosch, A. Strijland, A. W. Schram, and J. M. Tager. 1986*a*. Genetic relation between the Zellweger syndrome, infantile Refsum's disease, and rhizomelic chondrodysplasia punctata. N. Engl. J. Med. **314**:787–788.

Wanders, R. J. A., C. W. T. van Roermund, C. T. De Vries, H. van den Bosch, G. Schrakamp, J. M. Tager, A. W. Schram, and R. B. H. Schutgens. 1986*b*. Peroxisomal β-oxidation of palmitoyl-CoA in human liver homogenates and its deficiency in the cerebro-hepato-renal (Zellweger) syndrome. Clin. Chim. Acta **159**:1–10.

Wanders, R. J. A., C. W. T. van Roermund, M. J. A. van Wijland, J. Heikoop, R. B. H. Schutgens, A. W. Schram, J. M. Tager, H. van den Bosch, B. T. Poll-The, J. M. Saudubray, H. W. Moser, and A. B. Moser. 1987*a*. Peroxisomal very long-chain fatty acid β-oxidation in human skin fibroblasts: activity in Zellweger syndrome and other peroxisomal disorders. Clin. Chim. Acta **166**:255–263.

Wanders, R. J. A., C. W. T. Van Roermund, M. J. A. Van Wijland, J. Heikoop, A. Van den Put, P. Bentlage, E. Meijboom, J. M. Tager, A. W. Schram, H. Van Den Bosch, and R. B. H. Schutgens. 1987*b*. Peroxisomal fatty acid β-oxidation in human skin fibroblasts: X-linked adrenoleukodystrophy, a peroxisomal very long chain fatty acyl-CoA synthetase deficiency. J. Inherited Metab. Dis. **10**(Suppl. 2): 220–224.

Wolff, J., W. L. Nyhan, H. Powell, D. Takahashi, J. Hutzler, A. K. Hajra, N. S. Datta, I. Singh, and H. W. Moser. 1986. Myopathy in an infant with a fatal peroxisomal disorder. Pediatr. Neurol. **2**:141–146.