Multiple Peroxisomal Enzymatic Deficiency Disorders

A Comparative Biochemical and Morphologic Study of Zellweger Cerebrohepatorenal Syndrome and Neonatal Adrenoleukodystrophy

JOSEPH VAMECQ, MD, JEAN-PIERRE DRAYE, PhD,
FRANÇOIS VAN HOOF, MD,
JEAN-PAUL MISSON, MD, PHILIPPE EVRARD, MD,
GASTON VERELLEN, MD,
HENDRIK J. EYSSEN, MD,
JOHAN VAN ELDERE, MD,
RUUD B. H. SCHUTGENS, MD,
RONALD J. A. WANDERS, MD, FRANK ROELS, MD,
and SIDNEY L. GOLDFISCHER, MD

From the Laboratoire de Chimie Physiologique, Institute of
Cellular and Molecular Pathology, and the Departments of
Metabolic Diseases, Pediatric Neurology, and Neonatology,
Catholic University of Louvain, Brussels, Belgium; the
Department of Microbiology, Rega Institute, Louvain, Belgium; the
Department of Pediatrics, University Hospital of Amsterdam,
Amsterdam, The Netherlands; Menselijke Anatomie, Vrije Universiteit
Brussel, Brussels, Belgium; and the Department of Pathology and the
Marion Bessin Liver Research Center, Albert Einstein College
of Medicine, The Bronx, New York

Biologic, morphologic, and biochemical investigations
performed in 2 patients demonstrate multiple peroxisomal
deficiencies in the cerebrohepatorenal syndrome of Zellweger
(CHRS) and neonatal adrenoleukodystrophy (NALD). Very long chain fatty acids, abnormal bile acids,
including bile acid precursors (di- and trihydroxycoopro-
stanolic acids), and C29-dicarboxylic acid accumulated
in plasma in both patients. Generalized hyperaminoaciduria
was also present. Peroxisomes could not be detected in
CHRS liver and kidney; however, in the NALD patient,
small and sparse cytoplasmic bodies resembling altered
peroxisomes were found in hepatocytes. Hepatocellular
and Kupfer cell lysosomes were engorged with ferritin
and contained clefts and trilaminar structures believed
to represent very long chain fatty acids. Enzymatic
deficiencies reflected the peroxisomal defects. Hepatic
glycolate oxidase and palmitoyl-CoA oxidase activities
were deficient. No particle-bound catalase was found in
cultured fibroblasts, and ether glycerolipid (plasmalogens)
biosynthesis was markedly reduced. Administration of
phenobarbital and clofibrate, an agent that induces perox-
isolomal proliferation and enzymatic activities, to the
NALD patient did not bring about any changes in plasma
metabolites, liver peroxisome population, or oxidizing
activities. (Am J Pathol 1986, 125:524–535)

THE ABSENCE of recognizable peroxisomes in liver
and kidney from patients with the cerebrohepatorenal
syndrome of Zellweger (CHRS) and the establishment
of a major role of peroxisomes in the oxidation of very
long chain fatty acids, which consequently accumu-
late in blood and tissues from patients with peroxisomal
dysfunction, have resulted in the characterization of
a new group of metabolic diseases, the peroxisomal dis-
orders. Although various discrete features of these
disorders have been described, we now report the in-
tegrated biologic, biochemical, and morphologic find-
ings in 2 patients with peroxisomal deficiency syn-
dromes. Clinically and pathologically, 1 patient with
CHRS was indistinguishable from children described
by Bowen, Lee, Zellweger, and Lindenberg. Our sec-
ond patient has had a different clinical course with
prolonged survival that is more suggestive of neonatal
adrenoleukodystrophy (NALD). Peroxisomal func-
tions were investigated in vivo by measuring plasma me-

Supported by grants of the Belgian Foundation for Scientific
Research (FNRS and NFWO), U.S. Public Health Service
(Grants AM 09235 and AM 17702), and a Laboratory Twin-
ning grant from the European Communities. J. V. and J. V. E.
are research assistants of the Belgian Foundation for Scientific
Research (FNRS and NFWO).

Accepted for publication July 22, 1986.
Address reprint requests to Dr. Sidney L. Goldfischer,
Department of Pathology (F-538), Albert Einstein College
of Medicine, 1300 Morris Park Avenue, The Bronx, NY 10461.
metabolites, including bile acids, very long chain fatty acids, and pipecolic acid and, *in vitro*, by enzymatic assays of liver samples and cultured skin fibroblasts. Four peroxisomal matrix enzymes, fatty acyl-CoA oxidase, D-amino acid oxidase, L-α-hydroxyacid oxidase, and catalase, as well as a membrane-associated enzyme that is the first step in plasmalogen synthesis, dihydroxyacetone phosphate acyltransferase (DHAP-AT), were assayed.

**Case Reports**

**Zellweger CHRS (Patient 1)**

This patient (Figure 1A) displayed severe hypotonia and peripheral neuropathy and was unresponsive to visual and auditory stimuli from birth. She had a high forehead, epicanthal folds, and an arched palate. Feeding was poor and weight gain was minimal before death at the age of 7 weeks. Seizures that were poorly responsive to phenobarbital appeared after 4 weeks. Hepatomegaly followed by icterus became evident from the fifth week of life. X-ray examination revealed typical patellar stippled calcifications. Ultrasonography showed hyperechogenicity of the subcapsular region of both kidneys, and postmortem examination showed this to correspond to multiple small cortical cysts that are characteristic of CHRS. CT scan disclosed hypodense zones at the level of cerebral white matter and abnormal gyrations, mainly in the temporal region. Abnormal laboratory data included elevated serum transaminases, lactate dehydrogenase, and alkaline phosphatase, global hyperaminoaciduria, and elevated cerebrospinal fluid protein concentrations. Significant elevations in pipecolic acid were not detected in blood or urine.

**NALD (Patient 2)**

This patient (Figure 1B) was considered normal until the age of 3 months, except for blindness. Progressive severe hypotonia, severe psychomotor retardation, and peripheral neuropathy developed. Facial abnormalities became striking only after the first year. At the age of 2 he had moderate hepatomegaly and the same blood, urine, and cerebrospinal fluid abnormalities as Patient 1. Other abnormalities included an elevated iron
binding capacity, increased pipecolic acid concentration (300 M; control, 5M) in serum and lowered plasma levels of vitamins D and E. He suffered from numerous spontaneous bone fractures. Patellar calcifications and echographic signs of renal cysts have not been detected. Computerized tomographic scan and nuclear magnetic resonance demonstrated extensive lesions in the hemispheric white matter. Ocular impairment was similar in both children, with a pale, gray fundus, and, in Patient 1, pigmentary retinopathy. In both cases, electroretinograms were flat and evoked visual potentials were highly disturbed. Patient 2 received clofibrate (25 mg/kg/day) and phenobarbital (7 mg/kg/day) for 6 months without any evident clinical or biochemical effect. He is still alive at the age of 3 years. More detailed clinical data have been given in a separate report.14

**Materials and Methods**

*Peroxisomal enzymes*, including fatty acyl-CoA oxidase, D-amino acid oxidase, and L-α-hydroxyacid oxidase, were assayed by their H2O2 production according to Vamecq and Van Hoof,15 with 0.5 mM palmitoyl-CoA, 100 mM D-proline, and 20 mM glycolate as substrates, respectively. Activity of mitochondrial monoamine oxidase was similarly measured with 20 mM tyramine as substrate. Catalase latency was assayed on fibroblasts as previously described.1617 As controls we utilized biopsy samples obtained for diagnostic purposes from children of both sexes under the age of 4. All controls were examined by electron microscopy; and hepatic peroxisomes were normal in size, number, and distribution. *De novo* plasmalogen biosynthesis ([4C-hexadecanol incorporation test], activity of acyl-CoA, DHAP-AT, and plasmalogen levels in fibroblasts were assayed as previously described.1819

**Amino acids and pipecolic acid** in plasma and urine were separated by ion-exchange chromatography with a Lichrospher III Kontron apparatus, using a Mitsubishi cation-exchange resin (Type K10F). Detection of products was achieved with ninhydrin. Pipecolic acid was quantified as described previously.20

**Bile acid and very long chain fatty acid** determinations were performed as described elsewhere.2122

*Phytic acid* concentrations were measured by extracting plasma organic acids at pH 1.0 with diethyl ether and ethyl acetate, successively. They were trimethylsilylated with bis(trimethylsilyl)trifluoroacetamide, separated and identified by gas chromatography using an Intersmat IGC-121DL gas chromatograph coupled to an Intersmat ICR-1B integrator and equipped with a capillary column 50 m long and 0.32 mm in diameter (Chrompack CP Sil 5 fused silica). The elution was obtained by raising the oven temperature from 160°C to 220°C at the rate of 2°C/min. The solid injector and the flame ionization detector were maintained at 250°C. Gas chromatography peaks were identified by comparison of their retention time with reference retention times.

For **morphologic studies**, liver and kidney samples were fixed for 1 hour with 2% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. They were rinsed with the same buffer and postfixed for 1 hour in a 1% OsO4–1% K2Fe(CN)6 mixture, dehydrated, and embedded in Spurr's plastic mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips EM-301 or Siemens 101 electron microscope. Extraction of lipids with acetone (twice 15 minutes) or n-hexane (twice 15 minutes after acetone) was performed on glutaraldehyde-fixed sections, which were subsequently osmicated.

| Table 1—Assay or Metabolites Found in Abnormal Amounts in Sera |
|---------------------------------------------------------------|
| Patient 1 (CHRS) | Patient 2 (NALD) | Patient 2 (NALD) | Controls |
| | Before therapy | During therapy | |
| Pipecolic acid (μM) | <10 | 300 | 406 | <10 |
| Very long chain fatty acids | | | |
| C16:0 ratio | 1.46 | 1.49 | 1.49 | 0.89 ± 0.18 |
| C18:0 ratio | 0.69 | 0.28 | 0.26 | 0.02 ± 0.01 |
| Bile acids (%) | | | |
| common bile acids (cholic andchenodeoxycholic acids) | 37% | 28% | 12% | 100 |
| bile acid precursors (di- and trihydroxyprostanoinoic acids) | 40% | 49% | 66% | ND |
| C16-dicarboxylic acid | 23% | 23% | 22% | ND |

The concentration of various bile acids is expressed as the percentage of the total bile acid content. ND, not detectable.
Results

Plasma, Bile, and Urine Metabolites

Very long chain fatty acids and bile acids were assayed in plasma for estimation of the in vivo ability of peroxisomes to shorten very long chain substrates and to oxidize hydroxycoprostanolic acids. As enumerated in Table 1, all these metabolites accumulated in plasma. In both patients, the ratio between the concentration of the very long chain fatty acids, hexacosanoic acid (C26) and behenoic acid (C22) in plasma was at least 20-fold higher than normal, resulting from the large excess of hexacosanoic acid.

Bile acid analysis revealed the presence of a high concentration of the bile acid precursors, di- and trihydroxycoprostanolic acids, and the C29-dicarboxylic acid, metabolites that are not normally detected in plasma. These unusual bile acid precursors represent more than 70% of the total serum bile acid content in both patients. The bile acid patterns were also studied in urine and bile (Table 2). In CHR5 bile trihydroxycoprostanolic acid accumulations were greater than that of dihydroxy-coprostanolic acid (Table 2A). C29-dicarboxylic acid was not detected in bile, but was found in CHR5 urine (Table 2B). The total amount of bile acids in plasma was abnormally high in the CHR5 patient (mean of two samples 35 μg/ml), whereas it was normal in the NALD patient (mean of three samples, 8 μg/ml). In both patients the concentrations of common bile acids (cholic and chenodeoxycholic acids) were low, consistent with their decreased formation and the high concentrations of their precursors. In NALD, dihydroxy-coprostanolic acid was always more abundant than trihydroxycoprostanolic acid, whereas in CHR5 their ratio was reversed. In CHR5 plasma the trihydroxy-coprostanolic/dihydroxy-coprostanolic acid ratio suggests a more severe impairment in the formation of cholic acid than chenodeoxycholic acid.23

Plasma amino acids were normal in both patients except for L-pipecolic acid, which was increased in NALD. A general, but mild, hyperaminoaciduria was recorded in both cases. Plasma concentrations of phytanic acid were measured in NALD and found to be slightly increased (Table 3). In infantile Refsum’s disease (IRD), another condition in which hepato-cellular peroxisomes appear to be absent, plasma phytanic acid is increased 20-fold.24,25

Morphology

Despite an extensive search in which over 10,000 sq μm of sections were examined, no recognizable peroxiasomes could be detected in CHR5 liver and kidney samples which were obtained soon after death. In the liver, lysosomes were of normal size but more numerous than usual, especially in Kupffer cells. Most lysosomes contained ferritin; many displayed needlelike electron-lucent clefts, sometimes associated with lamellae or leaflets (Figure 2). Intralysosomal clefts and lamellae were also present in the kidney proximal tubular cells.

Neuropathologic findings were consistent with the diagnosis of Zellweger CHR5. Anatomic study of the brain (580 g) displayed zones of disturbances of gyra-
Figure 2—Intralysosomal very long chain fatty acid and ferritin deposition in hepatocytes from Patient 1 (CHRS). The presence of ferritin in lysosomes often facilitates the detection of very long chain substrates in the form of trilaminar structures (A) or clefts (B). (A, ×68,400; B, ×63,000)

Figure 3—Single membrane-bound organelles (P) in hepatocytes from Patient 2 (NALD) that are often close to mitochondria and are similar to altered peroxisomes observed in another patient with NALD,13 but staining for catalase, an important reaction for the microscopic identification of peroxisomes, was not performed on this patient. The matrix of these atypical peroxisomes generally appears dense and retracted. (A, ×36,000; B, ×34,500)
tion, mostly at the level of the parietal cortex, and partial agenesis of the corpus callosum. Light microscopy revealed an inversed cytoarchitectonic pattern of the layers of the cerebral cortex, neuronal intracortical and subcortical heterotopic fields in brain, subcortical heterotopias in cerebellum, and areas of demyelination.

Two needle biopsies of liver were examined from the NALD patient, the first before treatment and the second after treatment with clofibrate and phenobarbital for 6 months. Typical peroxisomes were not found; however, cytoplasmic bodies, often very close to mitochondria, with a moderately dense matrix shrunken away from the membrane, were observed (Figure 3). Their structure was similar to altered, catalase-positive hepatic peroxisomes previously described in a patient with NALD. The only changes induced by therapy were an apparent redistribution of mitochondrial cristae, enlarged intramitochondrial granules (Figure 4), and a somewhat greater occurrence of intralysosomal clefts. Ferritin deposition in lysosomes was not excessive. The lipid nature of the trilamellar structures present in lysosomal matrix was studied by classical lipid extraction procedures (Figure 5); however, treatment of liver slices with acetone and n-hexane prior to plastic embedding failed to dissolve these peculiar structures.

Biochemistry

Palmitoyl-CoA and glycolate oxidase activities could not be detected in either patient (Table 4). However, d-amino acid oxidase activity, measured with d-proline as substrate, was low in CHRS and higher than normal in NALD. In the latter, combined therapy with clofibrate and phenobarbital did not restore palmitoyl-CoA oxidase and glycolate oxidase activities. Tyramine oxidase, a mitochondrial H$_2$O$_2$-generating enzyme, was normally active, demonstrating good preservation of the tissue samples and the absence of artifactual interference in our assay system, which measures H$_2$O$_2$ production.

DHAP-AT activity in NALD fibroblasts was reduced to about 8% of normal (Table 5). De novo plasmalo-
gen biosynthesis was estimated by measuring the incorporation of $^{14}$C-hexadecanol into newly synthesized phospholipids in an experiment in which fibroblasts from both patients were continuously cultured in the presence of the radioactive precursor. In these conditions, alkenyl-phosphatidylethanolamine (pPE) accounted for 3.6% and 5.3% of the radioactivity incorporated in the phospholipids, as compared with 52.4% in controls. The proportion of plasmalogens among the radioactive phospholipids extracted from fibroblasts was lower than normal in both patients. In fibroblasts from the patients, radioactive hexadecanol is incorporated differently in the various classes of phospholipids (Figure 6).

Catalase activity was normal in fibroblasts from both patients, but unlike control cells, no particle-bound catalase activity could be detected in the mutant fibroblasts. This indicates that the enzyme is not linked to peroxisomal structures in sufficient quantities to be measured biochemically.

Table 4—Oxidase Activities in Human Liver

|                      | Patient 1 (CHRS) | Patient 2 (NALD) | Controls |
|----------------------|------------------|------------------|----------|
|                      | Before therapy   | During therapy   |          |
| Palmitoyl-CoA oxidase| ND               | ND               | 150 ± 40 (n = 4) |
| Glycolate oxidase    | ND               | ND               | 160 ± 20 (n = 10) |
| a-aminooxidase       | 203              | 1620             | 900 ± 280 (n = 4) |
| Tyramine oxidase     | 162              | 2442             | 860 ± 50 (n = 10) |

The enzyme activities are expressed as nanomoles of H$_2$O$_2$ produced per minute and per gram of liver ND, not detectable; NA, not assayed.

Table 5—Peroxisomal Enzyme Investigations on Human Cultured Skin Fibroblasts: $^{14}$C-Hexadecanol Incorporation Into Newly Synthesized Plasmalogens

|                      | Patient 1 (CHRS) | Patient 2 (NALD) | Zellweger patients | Controls |
|----------------------|------------------|------------------|--------------------|----------|
| Dihydroxyacetone phosphate acytransferase activity (nmol/2 hr/mg protein) | NA | 0.67 | 0.66 ± 0.50 (n = 9) | 8.80 ± 2.10 (n = 30) |

$^{14}$C-Hexadecanol incorporation test

|                      |   |   |                       |   |
|----------------------|---|---|-----------------------|---|
| % dpm in P.E.        | 13.2 | 11.5 | 14.8 ± 3.0 (n = 15) | 57.3 ± 9.9 (n = 11) |
| % pPE in P.E.        | 27.6 | 46.2 | 48.9 ± 15.8 (n = 15) | 91.4 ± 4.1 (n = 11) |
| % dpm in P.C.        | 61.6 | 57.0 | 63.1 ± 4.4 (n = 15) | 31.9 ± 4.9 (n = 11) |
| % pPC in P.C.        | 1.0 | 1.3 | 1.4 ± 0.9 (n = 15) | 20.3 ± 7.1 (n = 11) |
| Catalase : % particle bound | <5 | <5 | <5 (n = 9) | 65 ± 8 (n = 11) |

PE, total phosphatidylethanolamine; pPE, plasmalogen phosphatidylethanolamine; PC, total phosphatidylcholine; pPC, plasmalogen phosphatidylcholine; NA, not assayed.
Discussion

Common clinical features in both patients consist of severe hypotonia, visual and auditory impairment, poor sucking, facial dysmorphism (high forehead, arched palate, epicanthal folds) hepatomegaly, and failure to thrive. These are characteristic findings in children affected by neonatal peroxisomal diseases such as CHRS,\textsuperscript{1,10,26–28} and NALD.\textsuperscript{3,11–13} Clinical similarities and analogous biologic findings in both pathologic entities suggest that in these diseases essentially the same enzyme activities are affected by the basic peroxisomal defect. A remarkable but crucial feature is the absence of recognizable peroxisomes in liver and kidney in the patient with Zellweger's CHRS. In NALD we observed small cytoplasmic bodies resembling the altered peroxisomes that have been described in this disorder.\textsuperscript{13} Absence of peroxisomes has also been reported in infantile Refsum's disease (IRD).\textsuperscript{29,30} Whether the elevated, but relatively low, concentration of phytanic acid in CHRS and NALD is a reflection of a metabolic defect that is different from IRD, or a function of the younger ages of our patients, is unknown.

Peroxisomal proteins are synthesized by free ribosomes and subsequently incorporated into the membrane or the matrix of preexisting peroxisomes.\textsuperscript{31} Catalase, a marker enzyme of peroxisomes, is present but not particle-bound in fibroblasts (see also Wanders et al\textsuperscript{14}). This can best be explained by a normal biosynthesis of the enzyme and a lack of its incorporation into peroxisomes. This view is supported by the finding that
in isotonic liver homogenates from Zellweger CHRS or NALD patients, unlike controls, catalase is not sedimentable.\textsuperscript{11,32,33} Other peroxisomal enzymes, including D-amino acid oxidase and L-α-hydroxyacid oxidase, are synthesized in CHRS, but not sequestered within peroxisomes.\textsuperscript{33}

Peroxisomal β-oxidation is severely affected in these patients. This is evident from 1) the in vitro deficiency of palmitoyl-CoA oxidase in liver; 2) the accumulation of very long chain fatty acids in serum; and 3) the markedly reduced production of common bile acids, which reflects a defect in the peroxisomal cleavage of the cholesterol side chain.\textsuperscript{34} The latter deficiency leads to the abnormal presence in plasma of di- and trihydroxyxycoprostanoic acids, bile acid precursors which are normally subjected to shortening in peroxisomes. Another product of sterol metabolism, the C\textsubscript{29}-dicarboxylic acid, also accumulates in plasma and urine, but not in bile.\textsuperscript{10,31} Involvement of a peroxisomal enzyme in the further metabolism of this compound has not been reported, but it is known that peroxisomes can oxidize long-, medium-, and short-chain dicarboxylic acids.\textsuperscript{15,33-37} The C\textsubscript{29}-dicarboxylic acid could be a metabolite formed from the C\textsubscript{29}-trihydroxycoprostanolic acid when peroxisomal β-oxidation is impaired.\textsuperscript{23,38,39}

Impaired catabolism of cholesterol to common bile acids as well as deficient oxidation of very long chain fatty acyl-CoA esters could give rise to increased formation of cholesteryl esters of very long chain fatty acids. Trilaminated structures and clefts within lysosomes are believed to represent free or cholesteryl very long chain fatty acids,\textsuperscript{40} but our extraction experiments do not confirm this.

Other peroxisomal H\textsubscript{2}O\textsubscript{2}-generating oxidases are affected differently. Hepatic oxidation of glycolate is impaired in the patients, but activity of D-amino acid oxidase is normal. Similar results have been reported by Wanders et al\textsuperscript{46,33} for D-amino acid oxidase. A decreased capacity for plasmalogen synthesis, a characteristic feature in CHRS patients,\textsuperscript{16-19,41} is present in both children. The first two enzymes of this pathway are associated with the peroxisomal membrane.\textsuperscript{42-44}

Activity of the first enzyme, DHAP-AT, was considerably reduced in NALD fibroblasts. Phosphoethanol lipid biosynthesis was also investigated in CHRS and NALD cultured skin fibroblasts by measuring the incorporation of \textsuperscript{14}C-hexadecanol into plasmalogens. The deficiency of this enzymatic process (6.0-11.6% of control values) is of the same order of magnitude as that of the first enzyme of plasmalogen biosynthesis (7.6% of residual activity).

Increased amounts of pipecolic acid were found in urine and plasma of the NALD patient. The normal levels of pipecolic acid in our CHRS patient do not rule out disturbances of the metabolism of the N-alkylaminoacid in this patient, as interpretation of these results in the absence of pipecolic acid loading test is hazardous.\textsuperscript{49} Relationships between peroxisomes and this imino acid rest on 1) increased serum and urine concentrations of pipecolic acid in many patients with peroxisomal disorders, 2) stimulation of pipecolic acid metabolism in animals treated by clofibrate,\textsuperscript{46} a known peroxisome proliferator in rodents, 3) the in situ oxidation of pipecolic acid by peroxisomes in histochemical preparations,\textsuperscript{47} and 4) the oxidation of pipecolic acid by peroxisome-enriched fractions.\textsuperscript{47}

Several mechanisms may be postulated by which the absence or deficiency of membrane-bounding of peroxisomal proteins can lead to multiple enzymatic deficiencies (Figure 7). The absence of a surrounding membrane could result in the exposure of active peroxisomal enzymes to rapid proteolysis and denaturation in the cytosol. Peroxisomes contain their own pool of coenzyme A.\textsuperscript{48-50} In the absence of a peroxisomal membrane, dilution of peroxisomal cofactors in the cytosol should lead to decreased efficiency of related enzymatic reactions. Peroxisomes also contain their pool of flavin cofactors in the form of flavorproteins, including D-amino acid oxidase, glycolate oxidase, and fatty acyl-CoA oxidase. One molecule of FAD (or of FMN) is associated with one protein subunit.\textsuperscript{51} In peroxisomes, the equilibrium favors the holoenzyme form, since flavins are confined in a limited space. Dilution of the holoenzyme and flavins in the cytosol could increase the proportion of the labile apoenzyme.

Peroxisomes represent a privileged and confined region in which the concentration of the enzyme activity catalyzing the first step of sequence reactions leads to the formation in loco of products in concentrations which allow their further optimal utilization as substrates for subsequent enzymatic steps. This occurs during peroxisomal β-oxidation and in the initial steps of the phosphoethanol lipid biosynthesis. Thus, dilution in cytosol of peroxisomal enzymes would seriously affect the efficiency of peroxisomal sequence reactions.

If the transport of all peroxisomal enzymes into the organelle were blocked, it would be impossible to distinguish peroxisomes from other cytoplasmic vacuoles by morphology or enzyme cytochemistry. Many of the clinical and biochemical consequences of such a defect would be identical to those present in the disorders in which the formation of the peroxisomal membrane was inhibited or degradation of the organelle was enhanced.

The failure to find peroxisomes in electron micrographs and the absence of demonstrable latency of peroxisomal enzymatic activity must be interpreted with
Peroxisomal Enzymatic Deficiency

Figure 7—Proposed mechanisms for the pathogenesis of peroxisomal deficiency syndromes. Absence or deficiency of recognizable peroxisomes, which may result from different basic peroxisomal defects, is presented as the central event in these disorders. Similar morphologic, biochemical, biologic, and anatomic and clinical alterations may be found in different diseases.

cautions. Such results do not permit the conclusions that peroxisomes are entirely absent. Sparse and altered peroxisomes can escape detection, even in sections stained for catalase; low levels of latent activity cannot be recorded by current assay systems. The small quantity of plasmalogens present in CHRS and NALD patients is more consistent with a marked reduction of peroxisomes than their total absence. The extent to which peroxisomes are reduced in various tissues other than liver and kidney, eg, fibroblasts and intestine, may explain the differences in survival, severity of lesions and symptoms in CHRS, NALD, and related syndromes such as IRD and rhizomelic chondroplasia punctata.

The fundamental defect (or defects) in CHRS and NALD remains unknown. Whether these are different diseases reflecting distinct underlying pathogenetic phenomena or varying degrees of expression of a single entity is uncertain. Another factor that must be considered in the peroxisomal deficiency syndromes is the mitochondrial electron transfer defect in CHRS; mitochondrial respiration has not been studied in NALD. Whether this phenomenon, which has been ignored in recent studies that have focused entirely on peroxisomes, is of primary significance or secondary to the peroxisomal abnormality is unknown. The metabolic interdependence of mitochondria and peroxisomes has been defined in plants and protozoa; their functional relationship in mammalian systems is still obscure.

Although clofibrate and other hypolipidemic agents induce peroxisomal proliferation and enzymatic activities in rodents, they do not affect monkeys and humans. A recently described hypolipidemic agent, 4-[[1,3-benzodioxol]-5-yl]methyl)amino-benzoic acid (DL-040), which induces peroxisome proliferation and increased peroxisomal fatty acid oxidation in primate liver (rhesus monkey), may be of therapeutic value in human peroxisomal disorders.

References

1. Goldfischer S, Moore CL, Johnson AB, Spiro AJ, Valasnis MP, Wisniewski HK, Ritch RH, Norton WT, Rapin I, Gartner LM: Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. Science 1972, 182:62-64
2. Singh I, Moser AE, Goldfischer S, Moser HW: Lignoceric acid is oxidized in the peroxisome: Implications for the Zellweger cerebro-hepato-renal syndrome and adrenoleukodystrophy. PNAS 1984, 81:4203-4207
3. Brown FR III, McAdams AJ, Cummins JW, Konkol R, Singh I, Moser AE, Moser HW: Cerebro-hepato-renal (Zellweger) syndrome and neonatal adrenoleukodystrophy: Similarities in phenotype and accumulations of very long chain fatty acids. Johns Hopkins Med J 1982, 151:344-361
20. Parmentier GG, Janssen GA, Eggermont EA, Eyssen HJ: C27 bile acids in infants with coprostanic acidemia and occurrence of a 3a,7β,12β-trihydroxy5β-24-carboxylic bile acid as a major component in their serum. Eur J Biochem 1979, 102:173–183

21. Bakkeren JAJ, Monnens LAH, Trijbels JMF, Maas JM: Serum very long chain fatty acid pattern in Zellweger syndrome. Clin Chim Acta 1984, 138:325–331

22. Eyssen H, Eggermont EA, Van Eldere J, Jaeken P, Parmentier G, Janssen G: Bile acid abnormalities and the diagnosis of cerebro-hepato-renal syndrome (Zellweger syndrome). Acta Paediatr Scand 1985, 74:539–544

23. Scotto JM, Hachouch M, Odievre M, Laudat M-H, Saudubray J-M, Dulac O, Beucher I, Bean P: Infantile phytanic acid storage disease, a possible variant of Refsum's disease: Three cases, including ultrastructural studies of the liver. J Inher Metabol Dis 1982, 5:83–90

24. Poulos A, Sharp P, Whiting M: Infantile Refsum's disease (phytanic acid storage disease): a variant of Zellweger's syndrome? Clin Genet 1984, 26:579–586

25. Vanpe JI, Adams RD: Cerebro-hepato-renal syndrome of Zellweger: An inherited disorder of neuronal migration. Acta Neuropathol 1972, 20:175–198

26. Versmold HT, Bremer HJ, Herzog V, Siegel G, Bassewitz DBV, Irle V, Voss HV, Lombeck J, Brauser B: A metabolic disorder similar to Zellweger's syndrome with hepatic acatalasia and absence of peroxisomes, altered content and redox state of cytochromes, and infantile cirrhosis with hemosiderosis. Eur J Pediatr 1977, 124:261–275

27. Goldfischer S: Peroxisomes and human metabolic diseases: The cerebrohepatoeryndal syndrome (CHR5), cerebrotendinous xanthomatisos, and Schilder's disease (adrenoleukodystrophy). Ann NY Acad Sci 1982, 386:526–529

28. Ogier H, Roels F, Cornelis A, Poll The BT, Scotto JM, Odievre M, Saudubray JM: Absence of hepatic peroxisomes in a case of infantile Refsum's disease. Scand J Clin Lab Invest 1985, 45:767–768

29. Roels F, Cornelis A, Poll The BT, Aubourg P, Ogier H, Scotto JM, Saudubray JM: Hepatic peroxisomes are deficient in infantile Refsum's disease: A cytochemical study of four cases. Am J Med Genet (In press)

30. Lazarow PB, Fujiky Y: Biogenesis of peroxisomes. Ann Rev Cell Biol 1985, 1:489–539

31. Lazarow PB, Black V, Shio H, Fujiki Y, Hajira AK: Zellweger syndrome: Biochemical and morphological studies on two patients treated with clofibrate. Pediatr Res 1985, 19:1356–1364

32. Wanders RJA, Schutgens RBH, Tager JM: Peroxisomal matrix enzymes in Zellweger syndrome: Activity and subcellular localization in liver. J Inher Metab Dis 1985, 8(Suppl 2):151–152

33. Hagey LR, Kristas SK: Degradation of cholesterol to propionic acid by rat liver peroxisomes. Biochem Biophys Res Commun 1982, 107:834–841

34. Vamecq J, de Hoffman E, Van Hoof F: Mitochondrial and peroxisomal metabolism of glutaryl-CoA. Eur J Biochem 1985, 146:663–669

35. Vamecq J, de Hoffman E, Van Hoof F: The microsomal dicarboxyl-CoA synthetase. Biochem J 1985, 230:683–693

36. Vamecq J: Peroxisomes and metabolism of dicarboxylic acids. Peroxosomes and Their Metabolites in Cellular Functions. Edited by KWA Wirtz, JM Tager. Joint UNESCO-IUB Workshop. Zeist, The Netherlands, August 22–24, 1985

37. Bjorkhem I, Kase BF, Pedersen JI: Role of peroxisomes in the biosynthesis of very long chain fatty acids. Scand J Clin Lab Invest 1985, 45(Suppl 177):23–31

38. Kase BF, Pedersen JI, Standvik G, Bjorkhem I: In vivo and in vitro studies on formation of bile acids in patients with Zellweger syndrome. Evidence that peroxisomes are
of importance in the normal biosynthesis of both cholic and chenodeoxycholic acid. J Clin Invest 1985, 76: 2393–2402.

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

41. Datta NS, Wilson GN, Hajra AK: Deficiency of enzymes catalyzing the biosynthesis of glycerol-ether lipids in Zellweger syndrome: A new category of metabolic disease involving the absence of peroxisomes. N Engl J Med 1984, 311:1080–1083.

42. Hajra AK, Bishop JE: Glycerolipid biosynthesis in peroxisomes via the acyl dihydroxyacetone phosphate pathway. Ann NY Acad Sci 1982, 386:170–182.

43. Borst P: Animal peroxisomes (microbodies), lipid biosynthesis and the Zellweger syndrome. Trends Biochem Sci 1983, 8:269–272.

44. Hajra AK: Biosynthesis of O-alkylglycerol ether lipids, Ether Lipids: Biochemical and Biomedical Aspects. Edited by HK Mangold, F Paultauf. New York, Academic Press, 1983, pp 85–106.

45. Arneson DW, Tipton RE, Ward JC: Hyperpipelicolic acidemia: Occurrence in an infant with clinical findings of the cerebrohepatic (Zellweger) syndrome. Arch Neurol 1982, 39:713–716.

46. Tijbels F, Govaerts L, Monnens L, Bakkeren J, Stadhouders A: Peroxisomal disturbances in the cerebrohepato-renal syndrome of Zellweger, 21st Annual Symposium of the Society for the Study of Inborn Errors of Metabolism. Lyon, France, September 6–9, 1983, p 67.

47. ZaaR K, Angermuller S, Voiki A, Fahimi HD: Pipelicolic acid is oxidized by renal and hepatic peroxisomes: Implications for Zellweger cerebrohepato-renal syndrome (CHRHS). Exp Cell Res 1986, 164:267–271.

48. Mannaerts GP, Debeer LJ: Beta-oxidation of fatty acids: Relative contribution of mitochondria and peroxisomes, Short-Term Regulation of Liver Metabolism. Edited by L HeU, G van de Werve. Amsterdam, Elsevier North-Holland, 1981, pp 272–290.

49. Van Broekhoven A, Peeters MC, Debeer LJ, Mannaerts GP: Subcellular distribution of coenzyme A: Evidence for a separate coenzyme A pool in peroxisomes. Biochem Biophys Res Commun 1981, 100:305–312.

50. Mannaerts GP, Debeer LJ: Mitochondrial and peroxisomal beta-oxidation of fatty acids in rat liver. Ann NY Acad Sci 1982, 386:30–38.

51. Masters C, Holmes R: Peroxisomes; New aspects of cell physiology and biochemistry. Physiol Rev 1977, 57: 815–882.

52. Farrell K, Dinnick JE, Applegarth DA, Wong LE, Tze WJ, McCormick AQ, Jan JE, Moser HW: Peroxisomal abnormalities in neonatal adrenoleukodystrophy. Ann Neurol 1983, 14:379.

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

54. Black VH, Cornacchia L III: Stereological analysis of peroxisomes and mitochondria in intestinal epithelium of patients with peroxisomal deficiency disorders; Zellweger’s syndrome and neonatal adrenoleukodystrophy. Am J Anat (In press).

55. Aubour P, Scotto J, Rocchiocchi F, Feldmann-Pautrat D, Robain O: Neonatal adrenoleukodystrophy. J Neurol Neurosurg Psychiatr 1986, 49:77–86.

56. Evrard P, Caviness VS Jr, Prats-Vinas J, Lyon G: The mechanism of arrest of neuronal migration in the Zellweger malformation: An hypothesis based upon cytoarchitectonic analysis. Acta Neuropathol 1978, 41:109–117.

57. Heymans HSA, Oortuys JWE, Nelck G, Wanders RJJA, Dingemans KP, Schutgens RBH: Peroxisomal abnormalities in rhizomelic chondrodysplasia punctata. J Inher Metab Dis 1986, 9(Suppl 2):329–331.

58. Tijbels JMF, Berden JA, Monnens LAH, Willems JL, Janssen AJM, Schutgens RBH, van den Broek-van Essen M: Biochemical studies in the liver and muscle of patients with Zellweger syndrome. Pediatr Res 1983, 17:514–517.

59. Muller-Höcker J, Walther JV, Bise K, Pongratz D, Hübner M: Mitochondrial myopathy with loosely coupled oxidative phosphorylation in a case of Zellweger syndrome. A cytochemical-ultrastructural study. Virchows Arch [Cell Pathol] 1984, 45:125–138.

60. Kelley RI: Review: The cerebrohepato-renal syndrome of Zellweger, morphologic and metabolic aspects. Am J Med Genet 1983, 16:503–517.

61. Blane GF, Pinoroli F: Fenofibrate: études de toxicologie animale en rapport avec les effets secondaires chez les malades. Nouv Presse Med 1980, 9:3737–3746.

62. Hanefeld M, Kemmer C, Kadner E: Relationship between morphological changes and lipid-lowering action of p-chlorophenoxyisobutyric acid (CPIB) on hepatic mitochondria and peroxisomes in man. Atherosclerosis 1983, 46:239–246.

63. Cohen AJ, Grasso P: Review of the hepatic response to hypolipidaemic drugs in rodents and assessment of its toxicological significance to man. Food Cosmet Toxicol 1981, 19:585–605.

64. de la Iglesia FA, Lewis JE, Buchanan RA, Marcus EL, McMohem G: Light and electron microscopy of liver in hyperlipoproteinemic patients under long term gemfibroil treatment. Atherosclerosis 1982, 43:19–37.

65. Lalwani ND, Reddy MK, Ghosh S, Barnard SD, Molelo JA, Reddy JK: Induction of fatty acid beta-oxidation and peroxisomes proliferation in the liver of rhesus monkeys by DL-040, a new hypolipidaemic agent. Biochem Pharmacol 1985, 34:3473–3482.

Acknowledgments

We are indebted to Mrs. A. Marcelis-Hersens, Miss B. Wantier, and Mrs. T. T. Muller for their skillful technical assistance. We wish to express our thanks to Ir. G. Parmentier for GLC analysis of bile acids and R. Libert, M. Thiillaye, and M. Serneels for ppecolic acid determination. Prof. G. Lyon and Dr. R. Van Coster are gratefully acknowledged for clinical and neuropathologic studies and Dr. G. Schrakamp (Utrecht) for hexadecanol incorporation studies on cultured skin fibroblasts.