Long Chain Fatty Acids and Peroxisomal Disorders

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INITIAL DEMONSTRATION THAT PEROXISOMES OXIDIZE FATTY ACIDS

A connection between peroxisomes and fatty acid metabolism was established first by Lazarow and deDuve in 1976, who demonstrated a fatty acid coenzyme A (CoA) oxidizing system in rat liver that is enhanced by peroxisome proliferators (1). The system is most active toward saturated acyl-CoAs with chain lengths of C12-C16 and long chain unsaturated fatty acids (2,3). The enzymes involved in peroxisomal fatty acid oxidation are totally distinct from those in the mitochondria (4). The physiological contribution of peroxisomes to long chain fatty acid oxidation has been estimated to vary from 5% to 30% (5,6) depending upon physiological conditions.

PEROXISOMES AND VERY LONG CHAIN FATTY ACIDS

That peroxisomes have a major role in the oxidation of very long chain fatty acids (VLCFA) was discovered serendipitously through the study of disease states that only subsequently were shown to be peroxisomal disorders. In 1976 Igarashi et al. demonstrated abnormally high levels of saturated VLCFA, particularly hexacosanoic acid (C26:0), in the postmortem brain and adrenal glands of patients who had died of X-linked adrenoleukodystrophy (ALD) (7). Similar accumulations were also present in patients with the neonatal form of this disease (8), which resembles the Zellweger cerebro-hepato-renal syndrome (9). Because of this resemblance, VLCFA levels were measured in patients with Zellweger syndrome and found to be elevated consistently and to such an extent that this is now the most frequently used diagnostic assay (10). It had been shown previously that patients with Zellweger syndrome lack demonstrable peroxisomes (11). Singh et al. then showed that VLCFA are metabolized mainly, and possibly exclusively, in the peroxisome (12). Increased VLCFA
TABLE 1. Classification of peroxisome disorders

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormalities</td>
<td>Peroxisomes look normal; single enzyme defect</td>
<td>Peroxisomes present; more than one enzyme defective</td>
</tr>
<tr>
<td>Peroxisomes reduced or absent; multiple enzyme defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td>X-linked ALD</td>
<td>Rhizomelic</td>
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<tr>
<td>Zellweger syndrome</td>
<td>Acatalasemia</td>
<td>chondrodysplasia</td>
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<tr>
<td>Neonatal ALD</td>
<td>Hyperoxaluria type 1</td>
<td>punctata</td>
</tr>
<tr>
<td>Infantile Refsum</td>
<td>3-Oxoacyl thiolase deficiency</td>
<td></td>
</tr>
<tr>
<td>Hyperpipecolic acidemia</td>
<td>Thiolase deficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bifunctional enzyme deficiency</td>
<td></td>
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<tr>
<td></td>
<td>Acyl-CoA oxidase deficiency</td>
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ALD, adrenoleukodystrophy.

levels serve as diagnostic markers for 8 of the 12 disorders that are now assigned to the peroxisome disease category (Table 1).

DISORDERS OF PEROXISOME BIOGENESIS

The disorders of peroxisome biogenesis comprise a group of genetic diseases in which peroxisomes are deficient in number or lacking altogether. The peroxisome is a subcellular organelle which normally is present in all cells other than the mature erythrocyte (13,14). There is increasing evidence that all of the dysfunction in this group of disorders is attributable to the peroxisome defect. Study of these disorders thus can contribute to the understanding of the normal role of this subcellular organelle. That the peroxisome has a significant role in humans is evidenced by the fact that patients with the Zellweger syndrome, the most severe phenotype, show abnormalities in nearly all organs and tissues and rarely survive beyond the 4th month. An analogous peroxisome defect has recently been described in a Chinese hamster ovary cell mutant (15).

Structural Organelle Defect

In tissue sections peroxisomes appear as round or oval structures that are bounded by a single membrane and have a diameter that varies from 0.1 to 0.5 μm. At least 40 enzymes have been localized to the peroxisome (16), and additional functions continue to be assigned. Catalase has long been known to be located in the peroxisome, and cytochemical evidence (17) that an organelle contains this enzyme has long been used as a criterion for its identification as a peroxisome. It was with the use of this technique that the deficiency of peroxisomes was first demonstrated in the liver and kidney of patients with the Zellweger syndrome (11). More recently it
was shown that cultured skin fibroblasts of Zellweger disease patients do have membranous structures that contain the proteins characteristic of peroxisomal membranes, but that they lack proteins that are normally found in the matrix. These empty or partially empty membranous structures are referred to as peroxisome ghosts (18).

The Biogenesis Defect

Peroxisome proteins are encoded by nuclear genes and synthesized on free polyribosomes in the cytosol (19). The newly synthesized proteins diffuse through the cytosol and are imported post-translationally into pre-existing peroxisomes. Peroxisomal integral membrane proteins are not deficient in the liver (20) or cultured skin fibroblasts of Zellweger disease patients (18). Furthermore chase studies in cultured skin fibroblasts of Zellweger disease patients have shown that matrix enzymes are synthesized, but that they are degraded with abnormal rapidity (21). These observations have led to the hypothesis that the basic defect in the disorders of peroxisome biogenesis involves the mechanisms that target to the peroxisome the proteins that are normally destined to enter that organelle.

Except for 3-oxoacyl-CoA thiolase, peroxisomal enzymes are synthesized in the mature form (19). A carboxy terminal serine-lysine-leucine sequence (22) appears to be involved in the targeting or 40% of more of peroxisomal proteins (23). Other targeting sequences have been identified (24). As will be discussed, complementation analysis indicates that the disorders of peroxisome biogenesis can be subdivided into at least six groups. It is hypothesized that some or all of these mutants involve abnormalities of these targeting or import mechanisms.

Biochemical Consequences of Peroxisome Deficiency

The disorders of peroxisome biogenesis have in common a panel of morphological and biochemical abnormalities that are listed in Table 2. All can be traced to the defect in peroxisome structure. Ultrastructural and immunocytochemical studies of liver biopsy or cultured skin fibroblasts have revealed a deficiency or absence of catalase-containing particles (11). Unlike many other peroxisomal enzymes, catalase

**TABLE 2. Diagnostically significant abnormalities in disorders of peroxisome biogenesis**

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Description</th>
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<tbody>
<tr>
<td>Peroxisomes absent or reduced in number</td>
<td></td>
</tr>
<tr>
<td>Catalase in cytosol</td>
<td></td>
</tr>
<tr>
<td>Defective oxidation and abnormal accumulation of very long chain fatty acids</td>
<td></td>
</tr>
<tr>
<td>Deficient synthesis and reduced tissue levels of plasmalogens</td>
<td></td>
</tr>
<tr>
<td>Deficient oxidation and age-dependent accumulation of phytic acid</td>
<td></td>
</tr>
<tr>
<td>Defects in certain steps of bile acid formation and accumulation of bile acids</td>
<td></td>
</tr>
<tr>
<td>Defects in oxidation and accumulation of L-pipecolic acid</td>
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</table>
is not degraded rapidly in the cytosol. Total catalase activity is not diminished, and may even be increased, but it is located in the cytosol rather than the particulate fraction (25).

Immunoblot studies have shown that livers and cultured skin fibroblasts of Zellweger or neonatal ALD patients lack or are deficient in one or more of the peroxisomal β-oxidation enzymes (26). Since these enzymes are synthesized in Zellweger fibroblasts (21) but are mislocated, their absence or low concentration is attributable to their rapid degradation in the cytosol. The deficiency of these enzymes can account for the abnormally high levels of VLCFA that are characteristic for this group of disorders. The pattern of VLCFA accumulation is complex. Unlike X-linked ALD, where only saturated VLCFA accumulate, unsaturated VLCFA also accumulate in the Zellweger syndrome (10,27), including polyenic fatty acids with a chain length up to C38 (28).

The other biochemical abnormalities can also be traced to peroxisomal dysfunction. The low tissue levels of plasmalogens (29) are due to defective function of the first three steps of plasmalogen synthesis which are known to take place in the peroxisome (30). The accumulation of dihydroxy- and trihydroxycholestanolic acid (31) results from a deficiency in the peroxisomal steps of bile acid formation (32). The moderate accumulation and impaired oxidation of phytanic acid (33) appears to be due to impaired oxidation of pristanic acid (34,35), which is the product of α-oxidation of phytanic acid, and thus differs from the defect in classical Refsum disease, where the defect involves the α-oxidation of phytanic acid itself (36). The increased levels of pipecolic acid (37) are attributable to the deficiency of peroxisomal L-pipecolic acid oxidase (38).

Phenotype-Genotype Correlations

The names assigned to the disorders of peroxisome biogenesis are historically based. The Zellweger syndrome was first described in 1963 (39), long before the peroxisomal defect was recognized. Neonatal ALD (40), infantile Refsum disease (41), and hyperpipecolic acidemia (42) were so named because initial metabolic studies pointed to a single abnormality. It was only years later that all of these disorders were found to display the panel of abnormalities listed in Table 2. The disorders differ in respect to severity, the Zellweger syndrome being the most severe, followed by neonatal ALD, with infantile Refsum relatively milder, but still severely disabling. Hyperpipecolic acidemia is not considered to be a distinct entity (14). The clinical features of these disorders have been reviewed (14). The main characteristics of the Zellweger syndrome are profound hypotonia, virtual absence of psychomotor development, neonatal seizures, multiple malformations, eye abnormalities, renal cysts, and cirrhosis of the liver. The children rarely survive beyond the 4th month. The neurological abnormalities appear to be due to a striking and characteristic defect of neuronal migration (43). Patients with neonatal ALD or infantile Refsum disease may survive to the third decade and possibly even longer. Nevertheless they are
severely disabled. All are mentally retarded, usually in the severe or profound range; they have impaired hearing, retinal degeneration, liver disease, and often have seizures. Dysmorphic features are usually present.

Definition of the genotype has been aided by complementation analysis. To perform such analyses cultured skin fibroblast cell lines from two patients are fused with polyethylene glycol and a Ficoll gradient is used to separate multinucleate from mononuclear cells. Complementation is present when fused cell lines acquire capacities that the individual cell lines lack. The following properties have been used to assess complementation: acquisition of the capacity to synthesize plasmalogens (44,45), to oxidize phytanic acid (46), to oxidize VLCFA (47), or to assemble peroxisomes based upon the appearance of organelles that contain catalase (48). Results with these assays have been congruous for the most part. In this way cell lines from patients with disorders of peroxisome biogenesis have been subdivided into at least six complementation groups (45), one large and five smaller groups that so far include only one or two patients. It is presumed that each of these groups represents a distinct genotype, each of which possibly involves a different defect in the previously mentioned targeting-import mechanisms. Current research is aimed to define these defects by cell and molecular biology techniques. So far it has not been possible to establish correlations between genotype and phenotypes as they are currently defined. The large complementation group includes cell lines from patients with Zellweger syndrome, neonatal ALD, infantile Refsum disease, and hyperpippecolic acidemia, while the most common phenotype (Zellweger syndrome) was represented in four of the five small groups (45).

**GROUP 2: DEFECTS THAT INVOLVE A SINGLE Peroxisomal Enzyme**

These disorders differ fundamentally from the disorders of peroxisome biogenesis. Peroxisome structure is normal, and there is a mutation that involves a single enzyme. Table 1 lists the disorders that have been identified so far.

**X-Linked Adrenoleukodystrophy**

X-linked ALD is characterized by the abnormal accumulation of saturated VLCFA, particularly hexacosanoic acid (C26:0), but also tetracosanoic (C24:0), pentacosanoic (C25:0), and those with still longer chain lengths (7,49). The accumulation is due to impaired capacity to oxidize VLCFA, a reaction that normally takes place in the peroxisome (12). While ALD patients have an impaired capacity to oxidize free VLCFA, they metabolize their coenzyme derivative at a normal rate. This led to the hypothesis that the defect involves the coenzyme ligase for VLCFA, and experimental support for a defective function of this enzyme has been provided by two research groups (50,51). The ligase for VLCFA appears to be distinct from that for palmitic acid (52). Palmitoyl-CoA ligase is located in mitochondria, while
that for VLCFA (lignoceroyl-CoA ligase) is present in microsomes and peroxisomes. The activity of peroxisomal lignoceroyl-CoA ligase is reduced in X-linked ALD cells, but the microsomal lignoceroyl-CoA activity is normal (50,51). The difference between the microsomal and peroxisomal enzyme activity is unexplained; possibly there are two distinct VLCFA CoA ligases, or the defect involves the import of the enzyme or the substrate to the organelle. The VLCFA CoA ligases have not yet been purified. The X-linked ALD gene has been mapped to Xq28, the terminal segment of the long arm of the X-chromosome (53).

X-linked ALD must be differentiated sharply from neonatal ALD. Neonatal ALD has an autosomal recessive mode of inheritance and is one of the disorders of peroxisome biogenesis. The two disorders have never occurred in the same family. X-linked ALD has several phenotypes, which do often occur in the same family. Approximately 50% of patients have the childhood cerebral form, a serious disease which presents most commonly between 4 and 8 years of age as a learning disability or dementing illness. It often progresses rapidly so that the child is left in an apparently vegetative state within 1 to 4 years, and dies within a few years thereafter. The neurological disturbance is due to a cerebral demyelination which begins in the parieto-occipital region and resembles multiple sclerosis because of intense perivascular lymphocyte infiltration in the actively demyelinating regions (49). In approximately 25% of patients there is slowly progressive involvement of the long tracts in the spinal cord, referred to as adrenomyeloneuropathy. These patients present with progressive paraparesis and sphincter and sexual disturbances beginning in the third or fourth decade which are slowly progressive over decades. The remainder of the patients may have adrenocortical insufficiency with little or no nervous system involvement and thus are diagnosed as having Addison disease. Still others have brain or cerebellar involvement in adulthood, and may be misdiagnosed as Alzheimer disease or brain tumor. A small proportion of men with the biochemical defect of X-linked ALD remain asymptomatic even in middle age or later. Approximately 15% of female heterozygotes develop neurological disability that resembles adrenomyeloneuropathy, but usually of later onset and milder.

OTHER DEFECTS OF PEROXISOMAL FATTY ACID OXIDATION

Figure 1 shows the pathways of peroxisomal β-oxidation and their interrelation with certain steps of bile acid synthesis. The genes for acyl-Co oxidase (54), bifunctional enzyme (54), and 3-oxo-acyl CoA thiolase (55) have been cloned. The bifunctional enzyme has recently been shown to be trifunctional. In addition to enoyl-CoA hydratase and 3-hydroxy-acyl CoA dehydrogenase it also has δ-3, δ-2 enoyl-CoA isomerase activity (56).

Disease states have now been identified for each of these enzymatic steps. They are acyl-CoA deficiency, also referred to as pseudo-neonatal ALD (57), bifunctional enzyme deficiency (58), and 3-oxo-coenzyme A thiolase deficiency (59), also referred to as pseudo-Zellweger syndrome (60). An unexplained and interesting feature is that
FIG. 1. Pathway of peroxisomal fatty acid and bile acid degradation. Note that for the CoA ligase and oxidase reactions the fatty acids and bile acids are processed by separate enzymes, while for the last two steps the two substrates are processed by the same enzymes.

the phenotype of patients with these single enzyme defects resembles that of the disorders of peroxisome biogenesis (as evidenced even by the names assigned). The patients have profound neurological defects at birth, including defects of neuronal migration. In the oxidase deficiency patients, LCFA accumulation is the only substrate abnormality. In the two other disorders there is also accumulation of bile acid intermediates. It appears that the accumulation of VLCFA and of bile acid intermediates may have serious deleterious effects on nervous system development.

OTHER PEROXISOMAL DISORDERS

The other peroxisomal disorders do not affect fatty acid metabolism, and will only be discussed briefly. In hyperoxaluria type 1 there is a deficiency of hepatic
peroxisomal alanine: glyoxalate aminotransferase. The main complications are oxalate renal stones and renal failure. Neurological function is not impaired. Most patients with acatalasemia are asymptomatic. Rhizomelic chondrodysplasia punctata is associated with three biochemical defects: a severe impairment of peroxisomal plasmalogen synthesis, impaired oxidation of phytanic acid, and failure to process peroxisomal 3-oxo-acyl thiolase (61). VLCFA, pipecolic, and bile acid metabolism are normal, and peroxisomes are present although their structure may not be normal. Clinical manifestations are shortening of proximal limbs, cataracts, mental retardation, ichthyosis, and chondrodysplasia punctata. Its nosology is uncertain. More than one peroxisomal function is impaired but the organelle is present; possibly it will be shown to involve a unique set of targeting or import mechanisms.

**DIAGNOSIS OF PEROXISOMAL DISORDERS**

Prompt and accurate diagnosis of the peroxisomal disorders is important since they are genetically determined. X-linked ALD is an X-linked disorder; all others are autosomal recessive, so that recurrence risk is 25%. All of the disorders can be identified prenatally and genetic counseling is possible and essential.

The diagnosis of peroxisomal disorders may be suspected in the following clinical settings:

1. Neonates with hypotonia, seizures, and failure to thrive or with liver disease
2. Infants with chondrodysplasia punctata
3. A boy with a dementing illness
4. Males with Addison disease
5. Men with progressive paraparesis, with or without adrenal insufficiency
6. Women with progressive paraparesis
7. Oxalic acid urinary stones and renal failure

Measurement of the levels of VLCFA in plasma (62) is the most widely used diagnostic assay for X-linked ALD, the disorders of peroxisome biogenesis, and the defects of peroxisomal β-oxidation. We have performed this assay in 19,000 persons and have identified more than 2,200 patients with peroxisomal disorders (Table 3).

Demonstration of diminished plasmalogen levels in red blood cells (63) or of impaired peroxisomal plasmalogen synthesis in cultured skin fibroblasts (64) is a valuable diagnostic technique for rhizomelic chondrodysplasia punctata and the disorders of peroxisome biogenesis. Other important diagnostic techniques are the measurement of the levels of pipecolic acid (65) and phytanic acid (66) and of bile acid intermediates (67). Table 4 lists the clinical indications for these various assays. Techniques for prenatal diagnosis are discussed in a recent review (14).

**TREATMENT OF PEROXISOMAL DISORDERS**

Therapy for the disorders of peroxisome biogenesis is limited because of the severe damage already present at time of birth. This limitation does not apply to X-linked
TABLE 3. Experience with VLCFA assay at the Kennedy Institute, October 1990

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients tested since 1980</td>
<td>19,000</td>
</tr>
<tr>
<td>X-linked ALD hemizygotes</td>
<td>874</td>
</tr>
<tr>
<td>X-linked ALD heterozygotes</td>
<td>1031</td>
</tr>
<tr>
<td>Other peroxisomal disorders</td>
<td></td>
</tr>
<tr>
<td>Zellweger syndrome</td>
<td>180</td>
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<tr>
<td>Neonatal ALD</td>
<td>85</td>
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<tr>
<td>Infantile Refsum</td>
<td>19</td>
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<tr>
<td>Hyperpipecolic acidemia</td>
<td>5</td>
</tr>
<tr>
<td>RCDP</td>
<td>29</td>
</tr>
<tr>
<td>Single β-oxidation defects</td>
<td>18</td>
</tr>
<tr>
<td>Unusual phenotypes</td>
<td>9</td>
</tr>
<tr>
<td>Phenotype uncertain</td>
<td>37</td>
</tr>
<tr>
<td>Total peroxisome disease patients identified</td>
<td>2287</td>
</tr>
</tbody>
</table>

VLCFA, very long chain fatty acids; ALD, adrenoleukodystrophy; RCDP, rhizomelic chondrodysplasia punctata.

TABLE 4. Peroxisomal disorders: biochemical diagnostic assays

<table>
<thead>
<tr>
<th>Disease</th>
<th>Assay</th>
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<tbody>
<tr>
<td>Disorders of peroxisome biogenesis:</td>
<td></td>
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<tr>
<td>Zellweger syndrome, neonatal ALD, infantile</td>
<td>Plasma: VLCFAs</td>
</tr>
<tr>
<td>Refsum disease, hyperpipecolic acidemia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma/RBCs: VLCFAs</td>
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<tr>
<td></td>
<td>Fibroblasts: VLCFAs</td>
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<tr>
<td></td>
<td>DNA probe</td>
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<tr>
<td>X-linked ALD hemizygote</td>
<td></td>
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<tr>
<td></td>
<td>RBCs: Plasmalogens</td>
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<tr>
<td></td>
<td>Fibroblasts: Plasmalogens synthesis</td>
</tr>
<tr>
<td></td>
<td>Catalase subcellular localization</td>
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<tr>
<td>Rhizomelic chondrodysplasia punctata</td>
<td>Plasma: Phytanic acid</td>
</tr>
<tr>
<td></td>
<td>RBCs: Plasmalogens</td>
</tr>
<tr>
<td></td>
<td>Fibroblasts: Plasmalogens synthesis</td>
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<tr>
<td></td>
<td>Phytanic acid oxidation</td>
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<tr>
<td>Classic Refsum disease</td>
<td>Plasma: Phytanic acid</td>
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<tr>
<td></td>
<td>Fibroblasts: Phytanic acid oxidation</td>
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<tr>
<td>Isolated defects of VLCFA degradation</td>
<td>Plasma: VLCFAs</td>
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<tr>
<td></td>
<td>Fibroblasts: VLCFAs</td>
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<tr>
<td></td>
<td>VLCFA oxidation</td>
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<tr>
<td></td>
<td>Immunoblot of peroxisomal fatty acid</td>
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<td>oxidation enzymes</td>
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<td>Hyperoxaluria, type I</td>
<td>Urine: Organic acids</td>
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<tr>
<td></td>
<td>Liver: Alanine: Glyoxalate amino</td>
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<tr>
<td></td>
<td>transferase in percutaneous liver biopsy</td>
</tr>
<tr>
<td>Acatalasemia</td>
<td>RBCs: Catalase</td>
</tr>
</tbody>
</table>

ALD, adrenoleukodystrophy; VLCFA, very long chain fatty acids; RBC, red blood cells.
ALD since boys with this disorder are entirely normal until age 4 years or later. Diagnosis can be achieved prenatally or at birth. There thus is a 4-year or longer "window of opportunity" for preventive therapy.

Two approaches are under active investigation. There now exists a dietary regimen that can normalize the levels of saturated VLCFA in plasma within 4 weeks (68). The regimen combines dietary restriction of VLCFA with the administration of large amounts of oils that contain the monounsaturated fatty acids esterified with glycerol. The fatty acids are oleic acid (C18:1) and erucic acid (C22:1). The monounsaturated fatty acids have been shown to inhibit the synthesis of saturated VLCFA in ALD cultured skin fibroblasts (69). Figure 2 shows the effects of this regimen on the plasma level of C26:0 in 75 patients with X-linked ALD. The regimen has resulted in a statistically significant improvement of peripheral nerve function in patients with adrenomyeloneuropathy, and an international trial is now in progress to determine whether it can prevent the onset of neurological disability in persons with the biochemical defect of ALD who are neurologically intact. Dietary therapy does not alter

![Graph showing the effects of GTO and GTE-GTO diets on plasma C26:0 levels.](image-url)

**FIG. 2.** Effect of GTO and GTE-GTO diets on plasma C26:0 levels. The abscissa shows duration of dietary therapy in months. Plasma C26:0 levels were measured as described previously (62). The figure compares the mean C26:0 levels in 15 male AMN patients (open circles) who continued their customary diet, 16 AMN patients who received GTO oil and a VLCFA-restricted diet (squares), and 75 male AMN or childhood ALD patients who received the GTE/GTO oils and the VLCFA-restricted diet (solid circles). The horizontal dotted lines indicate the zone of normal plasma C26:0 levels (0.33 ± 0.18 μg/ml). Note the rapid normalization of the C26:0 level in the GTE/GTO treated group. The mean baseline level in the control and GTO group happened to be identical. The baseline value in the GTE/GTO group was lower than in the other two groups because the last group included some individuals who had previously been on the GTO diet. GTE/GTO oil normalized plasma C26:0 levels irrespective of prior dietary history.
the course of the rapid neurological progression of boys with the severe cerebral childhood form of ALD.

Much interest has been evoked by a recent report that bone marrow transplantation brought about reversal of early neurological abnormalities in a 7½ year old boy with X-linked ALD (70). Bone-marrow-derived cells contain the enzyme that is deficient in ALD and do cross the blood-brain barrier at least to some extent. It was of particular interest that following the transplant plasma VLCFA levels were normalized without the need for a special diet. Bone marrow transplant was ineffective in more advanced cases and under those circumstances appeared to aggravate the pre-existing neurological deficit. Because of the need for matched donors and the risk of the procedure, bone marrow transplant is considered only under carefully selected circumstances. The highly favorable outcome of this approach in the one case, combined with the boys' normal status before symptoms begin and the serious prognosis of the disease, make X-linked ALD a strong potential candidate for gene therapy.

FUTURE DIRECTIONS

The combined incidence of the genetically determined peroxisomal disorders is estimated at 1:25,000. It is anticipated that general awareness of these disorders will increase, that their incidence will be reduced through genetic counseling, and that effective therapy will be developed for those forms in which pathological changes commence postnatally.

This chapter has focused on those reactions that take place mainly or exclusively in the peroxisome. For other reactions, such as the oxidation of long chain fatty acids, the peroxisomal pathway appears to represent a relatively minor duplication of the mitochondrial pathway. A recent and surprising finding is that the peroxisome also appears to be the site of a duplicate pathway for cholesterol biosynthesis (71). The physiological role and the mechanisms that regulate these duplicate pathways are not known. The observation that patients with disorders of peroxisome biogenesis have abnormally low plasma cholesterol and lipoprotein levels (41) suggests that the peroxisome is involved in cholesterol homeostasis. While the genetically determined peroxisome disorders discussed in this chapter are dramatic and at this time command all our personal research efforts, we believe that future research should focus to an increasing extent on the interaction between the peroxisome and other subcellular organelles in the control of normal metabolism.

ACKNOWLEDGMENT

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REFERENCES


**DISCUSSION**

*Dr. Galli:* Can these enzymes be induced by compounds such as clofibrate? And do n-3 fatty acids have an effect similar to erucic acid in your patients?

*Dr. H. W. Moser:* We have treated patients with X-linked ALD with clofibrate and saw no lowering of VLCFA. Lazarow’s group treated patients with Zellweger syndrome with clofibrate but without effect.

Oleic acid and erucic acid were chosen because in studies of cultured skin fibroblasts these
were the most effective in lowering the rate of synthesis of the saturated VLCFA. We have not tested the effect of other unsaturated acids.

**Dr. Bazan:** Is there any information on the reason why neuronal migration is altered? We know that migration is highly dependent on the glial surface for the navigation events and for signal recognition and signal information. Although it might not help these patients, this might be a unique opportunity to address this fundamental question. Do we know if the glial cells are affected?

**Dr. H. W. Moser:** Glial cells are affected. Dr. J. M. Powers showed that the radial glia in the fetus has inclusions characteristic of VLCFA. We have also compared neuronal migration in the various types of peroxisomal diseases. In classical rhizomelic chondrodysplasia, where there is a profound defect in plasmalogen synthesis but where VLCFA are metabolized normally, Margaret Norman in Vancouver found that there was no migrational defect. Conversely, in a patient with bifunctional enzyme defect, where VLCFA are increased but plasmalogens are normal, there was a profound neuronal migration abnormality. At this time, the VLCFA and the bile acid intermediates are under the greatest suspicion.

**Dr. Gualde:** Do these patients have any immunologic dysfunction?

**Dr. H. W. Moser:** Patients with X-linked ALD have profound perivascular accumulation of lymphocytes in the nervous system. The pattern is compatible with an immunologic reaction to an antigen within the nervous system. We also found that the ganglioside fatty acid composition is highly abnormal, as is the fatty acid composition of phosphatidylcholine. It is our working hypothesis that the abnormal brain fatty acid composition leads to an autoimmune response with a "final common pathway" resembling that in multiple sclerosis.

**Dr. Small:** In your statement about the viscosity of the red cell membranes you imply that the VLCFA are esterified into the phospholipids of the red cell membranes and that this increases the viscosity. If this is true these phospholipids must be in many others cells, in other organelles. Perhaps that is part of the problem.

**Dr. H. W. Moser:** When adrenal cells are cultured in a medium containing docosahexanoic acid in a concentration equivalent to that in ALD plasma their capacity to release cortisone is diminished. The hypothesis is that the abnormality in the plasma membrane interferes with ACTH receptor function. The cholesterol ester abnormality in the brain appears to be a secondary phenomenon. We have examined tissues from ALD patients in whom there were parts of the brain that were not yet affected pathologically. In that part of the brain the cholesterol esters were normal and the main excess of hexacosanoic acid was found in the phosphatidylcholine fraction. The abnormal phosphatidylcholine fatty acid composition may play a role in the pathogenesis.

**Dr. Heim:** Long chain dicarboxylic acid urea is a typical phenomenon in peroxisomal disorders. Does it occur in every kind of disorder or is it more characteristic of ALD?

**Dr. H. W. Moser:** It is a relatively small abnormality compared to that seen in the mitochondrial disorders and it is not always present. The abnormality is present in neonatal ALD but not in X-linked ALD.

**REFERENCES**