Disorders of Peroxisomal Fatty Acid \( \beta \)-Oxidation

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The functional significance of a second fatty acid \( \beta \)-oxidizing system localized in peroxisomes has remained obscure for some time. In recent years, however, much has been learned about the physiological importance of the peroxisomal \( \beta \)-oxidation system. The recognition of a group of inherited diseases in humans associated with a dysfunction in one or more steps involved in peroxisomal \( \beta \)-oxidation stresses the importance of this \( \beta \)-oxidation system.

As discussed in detail elsewhere (1), peroxisomes are now known to be involved in the \( \beta \)-oxidative chain shortening of a distinct set of compounds. In case of some of these compounds, notably very long chain fatty acids (VLCFA) and the bile acid intermediates di- and trihydroxycholestanoic acid (DHCA and THCA), oxidation takes place virtually exclusively in peroxisomes, whereas in other cases (long chain fatty acids, mono- and polyunsaturated fatty acids, prostaglandins, dicarboxylic acids), both mitochondria and peroxisomes contribute to their \( \beta \)-oxidation.

**ENZYMES INVOLVED IN THE PEROXISOMAL \( \beta \)-OXIDATION OF VERY LONG CHAIN FATTY ACIDS AND DI- AND TRIHYDROXYCHOLESTANOIC ACIDS**

\( \beta \)-Oxidation of Very Long Chain Fatty Acids

As discussed in more detail elsewhere (1,2) the peroxisomal enzymes involved in fatty acid \( \beta \)-oxidation are different from their mitochondrial counterparts, except for the long chain fatty acyl-CoA synthetase. This enzyme, present on the cytosolic site of the peroxisomal membrane (3), is identical to the enzyme present in the mito-
chondrial outer membrane and the membrane of the endoplasmic reticulum (4). Recently, it has become clear that there is a separate acyl-CoA synthetase involved in the activation of very long chain fatty acids. This enzyme activity is present in peroxisomes and endoplasmic reticulum but not in mitochondria (5,6). That the enzyme is, indeed, different from the long chain fatty acyl-CoA synthetase, is concluded from their different subcellular localization (5,6), kinetic properties (7), competition experiments (5), their behavior toward various detergents (8), immunological studies (9), and the findings in X-linked adrenoleukodystrophy (see below). Following their activation, the very long chain fatty acyl-CoA esters are subsequently β-oxidized via the concerted action of acyl-CoA oxidase, bifunctional protein with enoyl-CoA hydratase and 1,3-hydroxyacyl-CoA dehydrogenase activity, and peroxisomal thiolase.

**β-Oxidation of Di- and Trihydroxycholestanolic Acid**

Recent studies by the groups of Mannaerts and Pedersen, respectively, have shed new light on the pathway of di- and trihydroxycholestanolic acid oxidation in peroxisomes. First, activation of trihydroxycholestanolic acid does not occur in peroxisomes but instead is catalyzed by a specific activating enzyme present in the endoplasmic reticulum (10,11). Furthermore, it is now clear that the first step in the subsequent β-oxidation of the THCA-CoA ester is carried out by a distinct trihydroxycholestanoxyl-CoA oxidase (12,13). The findings in some of the disorders of peroxisomal β-oxidation (see below) suggest that, at least in humans, subsequent oxidation of the unsaturated Δ⁵₄-THCA-CoA is catalyzed by the same enzymes as those involved in the oxidation of α,β-unsaturated VLCFA-CoA esters (i.e., bifunctional protein and peroxisomal thiolase; see Fig. 1).

**INBORN ERRORS OF PEROXISOMAL β-OXIDATION**

The inborn errors of peroxisomal β-oxidation known today are listed in Table 1. These include diseases in which peroxisomal β-oxidation is impaired due to a deficiency of all peroxisomal β-oxidation enzyme proteins resulting from the (virtual) absence of peroxisomes (group A), diseases in which peroxisomal β-oxidation is impaired due to the loss of multiple peroxisomal β-oxidation enzyme activities (group B), and diseases in which a single peroxisomal β-oxidation enzyme activity is lost (group C). In the latter two groups, morphologically distinguishable peroxisomes are present in normal amounts. The characteristics of these disorders are described below.

**The Peroxisome Deficiency Disorders**

The clinical and biochemical characteristics of this group of disorders have recently been described in detail (14,15) and will only be summarized briefly here, especially
since these disorders cannot be taken to be true disorders of peroxisomal $\beta$-oxidation. The prototype of the group of peroxisome deficiency disorders, alternatively named disorders of peroxisome biogenesis (15), is the cerebro-hepato-renal syndrome of Zellweger. In patients suffering from the classical form of Zellweger syndrome there are multiple abnormalities, including craniofacial, neurological, ocular, hepatological, renal, skeletal, and other aberrations [see (14,15) for details].

Morphologically distinguishable peroxisomes are also (virtually) absent in neonatal adrenoleukodystrophy, infantile Reffsum disease, and the four cases of hyperpilocolic acidemia described in the literature. Without going into detail, it can be concluded that disorders of group A are overlapping syndromes, the clinical course being mildest in patients suffering from infantile Reffsum disease (16).

The (virtual) absence of peroxisomes in these patients is associated with a generalized loss of peroxisomal functions, including peroxisomal fatty acid $\beta$-oxidation. Indeed, immunoblotting experiments have shown that the three peroxisomal $\beta$-ox-
<table>
<thead>
<tr>
<th>Peroxisomal disorder</th>
<th>Peroxisomal β-oxidation defect</th>
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<tbody>
<tr>
<td><strong>A. Peroxisomes absent, generalized dysfunction</strong></td>
<td></td>
</tr>
<tr>
<td>Cerebro-hepato-renal (Zellweger) syndrome</td>
<td>Generalized</td>
</tr>
<tr>
<td>Neonatal adrenoleukodystrophy</td>
<td>Generalized</td>
</tr>
<tr>
<td>Infantile Refsum disease</td>
<td>Generalized</td>
</tr>
<tr>
<td>Hyperpipecolic acidemia</td>
<td>Generalized</td>
</tr>
<tr>
<td><strong>B. Peroxisomes present, multiple defects</strong></td>
<td></td>
</tr>
<tr>
<td>&quot;Zellweger-like&quot; syndrome</td>
<td>Multiple defects (acyl-CoA oxidase, bifunctional protein, and peroxisomal thiolase)</td>
</tr>
<tr>
<td><strong>C. Peroxisomes present, single defect</strong></td>
<td></td>
</tr>
<tr>
<td>X-linked adrenoleukodystrophy</td>
<td>Peroxisomal very long chain acyl-CoA synthetase</td>
</tr>
<tr>
<td>Acyl-CoA oxidase deficiency (pseudo-NALD)</td>
<td>Acyl-CoA oxidase</td>
</tr>
<tr>
<td>Peroxisomal thiolase deficiency (pseudo-ZS)</td>
<td>Peroxisomal thiolase</td>
</tr>
<tr>
<td>Bifunctional protein deficiency</td>
<td>Bifunctional protein</td>
</tr>
</tbody>
</table>

Oxidation enzyme proteins (acyl-CoA oxidase, bifunctional protein, and peroxisomal thiolase) are strongly deficient in Zellweger patients as well as in other patients in whom there is a major deficiency of peroxisomes (see ref. 1 for references). This explains the strong impairment in the oxidation of very long chain fatty acids such as tetracosanoic acid (24:0) and hexacosanoic acid (26:0), as first described by Singh and co-workers (17). Oxidation of palmitate and stearate was found to proceed normally in these cells in accordance with the view that oxidation of these fatty acids occurs primarily in mitochondria. The deficiency of bifunctional protein and peroxisomal thiolase also explains the impairment in THCA-CoA β-oxidation (18,19) in Zellweger patients, leading to their accumulation in plasma and other body fluids. Recent studies have shown that the oxidation of other fatty acids is also impaired in patients lacking peroxisomes. Indeed, Christensen and co-workers (20,21) recently reported that the oxidation of erucic acid [C22:1 (n-9)] and adrenic acid [C22:4 (n-6)] is deficient in fibroblasts from Zellweger (20) and neonatal adrenoleukodystrophy patients, whereas the oxidation of linoleic acid [18:3 (n-3)], arachidonic acid [20:4 (n-6)], and eicosapentaenoic acid [C20:5 (n-3)] was found to proceed normally (21). In a recent study by Street et al. (22) oxidation of tetracosatetraenoic acid [C24:4 (n-6)] was also found to be deficient in Zellweger patients (see ref. 1).

Abnormalities in plasma and other body fluids of Zellweger patients are not restricted to very long chain fatty acids and bile acid intermediates, but also include various medium and long chain dicarboxylic acids. Although not related to peroxisomal β-oxidation directly, it is interesting to note that Martínez (23) recently reported remarkable abnormalities in the content of some polyunsaturated fatty acids, notably C22:6 (n-3) and C22:5 (n-6), in tissues from a Zellweger infant.
Disorders of Peroxisomal β-Oxidation Characterized by the Multiple Loss of Peroxisomal β-Oxidation Enzyme Activities

The only peroxisomal disorder known to belong to this category is “Zellweger-like syndrome,” so far described in two patients (24,25). In these patients, who had a clinical presentation indistinguishable from Zellweger syndrome, the three peroxisomal β-oxidation enzyme proteins were found to be deficient upon immunoblotting. Furthermore, the activity of the peroxisomal enzyme acyl-CoA:dihydroxyacetonephosphate acyltransferase was found to be deficient in accordance with a deficiency of plasmalogens. Morphological studies, however, revealed the normal presence of peroxisomes.

Disorders of Peroxisomal β-Oxidation Associated with a Deficiency of Only One Peroxisomal β-Oxidation Enzyme Activity

X-Linked Adrenoleukodystrophy (Adreno-testiculo-leuko-myelo-neuropathic Complex)

The presentation of adrenoleukodystrophy (ALD) in its classical form is that of a boy who develops normally for the first years of life and then presents with signs of central nervous system involvement as manifested in behavioral abnormalities, visual and auditory disturbances, abnormal gait, loss of school performances, and other derangements (see refs. 26 and 27 for reviews). Studies in the 1970s have established that there is an accumulation of very long chain fatty acids in tissues as well as in fibroblasts and plasma of ALD patients. The discovery of a biochemical marker for ALD has enabled Moser and co-workers to study the phenotype of ALD, which they have shown to vary enormously, not only between families but also within the same pedigree (26).

Recent studies have identified the enzyme defect in ALD. A key finding in this respect was the observation by Hashmi et al. (28), who reported that the oxidation of lignoceroyl-CoA was unimpaired in ALD fibroblasts in contrast to the oxidation of lignoceric acid itself. Studies by Singh and co-workers (29) and ourselves (30) have shown that the defect in ALD is indeed at the level of a deficient activity of the peroxisomal enzyme which activates very long chain fatty acids to their CoA esters. In microsomes, however, a normal VLCFA-CoA synthetase activity was found (29,30). This suggests that the two VLCFA-CoA synthetases have different functions in the cell and that the VLCFA-CoA ester synthesized at the site of the endoplasmic reticulum is not available for β-oxidation in the peroxisome. This situation closely resembles the situation in the yeast *Candida lipolytica* (31). Interestingly, Lazo et al. (31) recently suggested that the inability of the microsomal VLCFA-CoA synthetase to generate VLCFA-CoA esters for β-oxidation in the peroxisome is due to the hydrolysis of VLCFA-CoA esters by acyl-CoA hydrolases present in the cytosol.
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Acyl-CoA Oxidase Deficiency (Pseudo-neonatal Adrenoleukodystrophy)

This condition has only been described in two patients within the same family (32). Both patients presented with early seizures, muscle hypotonia, progressive hearing loss, and visual impairment. There was psychomotor retardation but no facial dysmorphism. These and other clinical findings led to the diagnosis of neonatal ALD. Peroxisomes were found to be normally present, however. Subsequent immunoblotting studies revealed a deficiency of acyl-CoA oxidase (32). This was associated with elevated plasma VLCFA levels, whereas the bile acid intermediates were normal, which is in line with the recent findings of a separate THCA-CoA oxidase (12, 13).

Bifunctional Protein Deficiency

Recently, Watkins et al. (33) identified the first case of bifunctional protein deficiency in a patient who showed a variety of clinical abnormalities resembling those found in Zellweger syndrome and neonatal ALD. There was no dysmorphism, however. The patient remained hypotonic and showed no developmental progress. Interestingly, a brain biopsy at 6 weeks of age revealed polymicrogyria. Morphologically distinguishable peroxisomes were found to be normally present in this patient. In plasma accumulation of VLCFAs and bile acid intermediates was found. Bifunctional protein was subsequently found to be deficient upon immunoblotting. We have recently identified a second patient with this disorder (see below).

Peroxisomal 3-Oxoacyl-CoA Thiolase Deficiency (Pseudo-Zellweger Syndrome)

In 1986, Goldfischer et al. (34) described a patient with all the clinical and pathological features of Zellweger syndrome, including facial dysmorphism, hypotonia at birth, and neuronal heterotopia. Abnormal VLCFA and bile acid intermediates levels were found in plasma. Subsequent studies revealed the presence of hepatic peroxisomes in normal amounts. Furthermore, plasmalogen biosynthesis was found to proceed normally. The defect in this patient turned out to be at the level of peroxisomal 3-oxoacyl CoA thiolase (35).

Unidentified Disorders of Peroxisomal β-Oxidation

In recent years several patients with a defect in peroxisomal β-oxidation of unknown etiology have been described (see ref. 1 for a review). In one of these patients we have recently found that the defective VLCFA β-oxidation is due to a deficient activity of bifunctional protein. Interestingly, the protein itself was found to be nor-
normally present upon immunoblotting, which suggests that the mutation affects the active site of the enzyme without resulting in reduced enzyme protein levels.

DIAGNOSIS OF INBORN ERRORS OF PEROXISOMAL β-OXIDATION

It is clear from the data described above (see also Table 2) that there is accumulation of very long chain fatty acids in all disorders of peroxisomal β-oxidation known so far, which allows identification by means of gas chromatographic analysis of plasma VLCFAs. If, indeed, VLCFAs are found to accumulate in plasma from a particular patient, additional analyses in plasma (bile acid intermediates, pipecolic acid, phytanic acid), platelets (activity measurements of DHAPAT), and fibroblasts (plasmalogon biosynthesis, VLCFA β-oxidation, phytanic acid oxidation, and particle-bound catalase) will have to be done to find out whether the impairment in peroxisomal VLCFA β-oxidation is the result of a deficiency of peroxisomes (as in group A) or due to the loss of multiple (group B) or single (group C) β-oxidation enzyme activities (see ref. 1 for details).

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Peroxisome deficiency disorders</th>
<th>Zellweger-like syndrome</th>
<th>X-linked ALD</th>
<th>Pseudo-Zellweger syndrome</th>
<th>Pseudo-neonatal ALD</th>
<th>Bifunctional protein deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolites in body fluids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Very long chain fatty acids</td>
<td>Elevated</td>
<td>Elevated</td>
<td>Elevated</td>
<td>Elevated</td>
<td>Elevated</td>
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</tr>
<tr>
<td>Bile acid intermediates</td>
<td>Elevated</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
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</tr>
<tr>
<td>Pipecolic acid</td>
<td>Elevated*</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Phytanic acid</td>
<td>Elevated*</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
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<tr>
<td>Plasmalogon synthesis</td>
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<td>Deficient</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>DHAPAT</td>
<td>Deficient</td>
<td>Impaired</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Alkyl DHAP synthase</td>
<td>(Virtually)</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>De novo synthesis</td>
<td>&lt;5</td>
<td>n.d.</td>
<td>&gt;65</td>
<td>&gt;65</td>
<td>&gt;65</td>
<td>&gt;65</td>
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<tr>
<td>Hepatic peroxisomes</td>
<td></td>
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<td></td>
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<tr>
<td>Particle-bound catalase (%) of total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Peroxisomal β-oxidation</td>
<td>Deficient</td>
<td>Deficient</td>
<td>Deficient</td>
<td>Deficient</td>
<td>Deficient</td>
<td>Deficient</td>
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<tr>
<td>Activity with C26:0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Enzyme proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acyl-CoA oxidase</td>
<td>Deficient</td>
<td>Normal</td>
<td>Normal</td>
<td>Deficient</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Bifunctional protein</td>
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<td>Normal</td>
<td>Normal</td>
<td>Deficient</td>
<td>Normal</td>
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</tr>
<tr>
<td>Peroxisomal thiocase</td>
<td>Deficient</td>
<td>Normal</td>
<td>Deficient</td>
<td>Deficient</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*Age dependent.  
n.d., not done.
PRENATAL DIAGNOSIS

Prenatal diagnosis is possible in any of the inborn errors of peroxisomal β-oxidation known today (see ref. 1 for a review). This can be done in the first trimester of pregnancy via analyses in chorionic biopsy material or cultured chorionic villous fibroblasts.

PATHOGENIC ASPECTS

Although our knowledge regarding disorders of peroxisomal β-oxidation is of rather recent date, it is clear that they are all devastating diseases with severe neurological involvement. If we restrict the discussion to the true disorders of peroxisomal β-oxidation (i.e., group C), the available information suggests that the severity of the disease is related to the exact site of the enzymic defect. Indeed, the deficiency of peroxisomal thiolase is associated with a clinical phenotype indistinguishable from the Zellweger syndrome, whereas a deficiency of acyl-CoA oxidase is associated with a much milder phenotype (32). This is even more so if X-linked adrenoleukodystrophy is concerned. A possible explanation for this relationship is that a deficiency of peroxisomal VLCFA-CoA synthetase or peroxisomal thiolase affects VLCFA β-oxidation to the same extent but affects the peroxisomal β-oxidation of other compounds differently. Indeed, peroxisomal VLCFA-CoA synthetase is probably involved in activation of very long chain fatty acids only, whereas peroxisomal thiolase is not only involved in VLCFA β-oxidation, but also in the β-oxidation of THCA-CoA and (most likely) mono- and polyunsaturated fatty acyl-CoA esters, prostaglandin CoA-esters, and other compounds yet to be identified. The same probably applies to acyl-CoA oxidase, which is not only involved in VLCFA β-oxidation but also in the β-oxidation of other compounds, such as prostaglandin CoA-esters (but not THCA-CoA).

With regard to the relationship between biochemical abnormalities on the one hand and clinical and pathological aberrations on the other, little is known even in the case of X-linked ALD, in which the accumulation of VLCFAs is the only known abnormality. Recent studies by Whitcomb et al. (36) suggest that very long chain fatty acids are toxic to the cell, changing the microviscosity of the membrane and subsequently its biological properties. This was concluded from experiments with adrenocortical cells which showed a decreased rate of ACTH-induced cortisol production upon addition of hexacosanoic acid. Interestingly, Meyer et al. (37) reported that leukocytes from ALD patients lack detectable ACTH-binding sites.

One of the most striking neuropathological abnormalities in Zellweger patients is the greatly disturbed neuronal migration. This disordered migration leads to unique cytoarchitectonic abnormalities which involve the cerebral hemispheres, the cerebellum, and the inferior olivary complex (26). In the cerebral hemispheres, neurons that are destined to migrate to outer cortical layers remain scattered within the inner cortical layers. The finding that a deficiency of peroxisomal thiolase leads to the
same abnormalities (34) suggests a direct causal relationship between the disturbance in neuronal migration and the deficiency of peroxisomal thiolase, although there is no information on the exact mechanism. According to this rationale, the deficiency of plasmalogens is not of major pathogenic significance in this respect.

THERAPY IN DISORDERS OF PEROXISOMAL β-OXIDATION

In 1986, Rizzo et al. (38) discovered that addition of oleic acid to the culture medium of ALD fibroblasts leads to a drastic reduction in C26:0 levels. Since very long chain fatty acids originate primarily from endogenous fatty acids via chain elongation, it was suggested that oleic acid exerted its effect via competition at the level of chain elongation. These findings inspired Rizzo et al. (39) and Moser et al. (40) to try to reduce very long chain fatty acids in patients via a combined approach consisting of the use of a diet low in VLCFAs together with the administration of a glycerol trioleate oil. This combined approach would reduce exogenous as well as endogenous sources of the VLCPA burden in ALD. The results obtained so far indicate that plasma C26:0 levels in ALD patients can be reduced significantly via this regimen (39,40). However, clinical and neurological evaluation revealed little or no improvement. Along the same lines both Moser and Rizzo have instituted a new therapy involving the use of erucate rather than oleate. Preliminary results show that plasma C26:0 levels normalize almost completely. Whether or not this will be beneficial to ALD patients is studied intensively at this moment.

ACKNOWLEDGMENTS

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DISCUSSION

*Dr. Van Hooft:* Detection of a specific defect of the peroxisomal β-oxidation of fatty acids can be difficult. Demonstration of the presence of an enzyme using specific antibodies does not imply that this enzyme is active *in vivo*. If biochemical techniques are used, one has to cope with the existence of isoenzymes, most of them present in mitochondria. Distinction between acyl-CoA dehydrogenase and acyl-CoA oxidase is easy because only the latter forms H₂O₂. But what with the peroxisomal bifunctional protein and thiolase?

*Dr. Wanders:* In general, that is true. It is very difficult but not impossible. We have developed a technique with which you can fractionate fibroblasts in mitochondria and in peroxisomes. You can then assay bifunctional protein and peroxisomal thiolase in the peroxisomes and in fact that was the way in which we discovered the patients with an isolated enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase deficiency. You can also use antibodies to selectively immunoprecipitate enzyme proteins, for example bifunctional protein or the peroxisomal thiolase, and you can assay the enzymic activity of these proteins on the Sepharose beads to which the proteins are now attached. So it is difficult but it is not impossible (1).

*Dr. Duran:* It looks so easy if you just say that very long chain fatty acids are increased. Could you be a bit more specific? What do we need? Do we need the ratio C26 to C22? Do we need absolute concentrations? Do we need the unsaturated C26? What about the bifunctional protein deficiency? Do we see increased unsaturated or hydroxy acids?

*Dr. Wanders:* Basically, for most disorders of peroxisomal β-oxidation it is very simple. It is simple because both the absolute amount of C26:0 and the ratio are greatly increased in the patient’s plasma: they are four times higher than the normal values. The biggest problem is, of course, X-linked adrenoleukodystrophy, which is sometimes very hard to detect. In this case we do two things. We look at the C26:0 level itself, and also the C26:0/C22:0 ratio. Sometimes the ratio is in the high normal range, but the C26:0 level exceeds the normal range and in those cases in which only one of the two results is abnormal, we proceed to investigate fibroblasts
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in which we can measure C26:0 β-oxidation as well as the C26:0 levels and the C26/C22 ratio. We have only missed one case of X-linked adrenoleukodystrophy by looking at very low chain fatty acids in plasma. So saying that it is simple is an overstatement, but at least the results look much more unequivocal than in case of the mitochondrial β-oxidation disorders. We have adopted the procedure of Moser and co-workers (2) which is a very good procedure, but I know that other people use other methods and then it might be more problematic. For example, with different assay methods there may be great difficulty in discriminating between controls and X-linked adrenoleukodystrophy patients.

Bile acid intermediates are elevated in those peroxisomal disorders of β-oxidation in which either bifunctional protein or the peroxisomal thiolase is deficient, simply because the pathways of very long chain fatty acid oxidation and bile acid intermediate oxidation converge at the level of bifunctional protein. Only in case of acyl-CoA oxidase deficiency and X-linked adrenoleukodystrophy are the bile acid intermediates normal, as in Dr. Saudubray’s patients. To summarize, we always perform these measurements because they contribute to the diagnosis and we can immediately say the defect is at the level of acyl-CoA oxidase, or at the level of bifunctional protein or peroxisomal thiolase.

Dr. Leroy: I have two questions. In infantile or juvenile adrenoleukodystrophy, as well as in adult adrenomyeloneuropathy, the functional deficiency of peroxisomal fatty-acyl-CoA synthetase has been demonstrated. There is no apparent enzymatic difference between these two types of patients. It is my experience and that of others that in children the adrenal symptoms may precede the neurological symptoms, and vice versa. Is there any way of explaining this kind of variability? My second question is the following. Did I hear correctly from Dr. Mannaerts that the peroxisomal very long chain fatty acid CoA synthetase is a peroxisomal membrane enzyme? Do we already know anything of the distribution of that enzyme within the peroxisomal membrane? Can differences in distribution be the explanation of the fascinating but disturbing fact of clinical differences between males in the same pedigree? One type has a devastating neurological disorder from early childhood and the other type of male patient can, at least for some time, lead a reasonably normal life and have children.

Dr. Wanders: It is most difficult to understand the relationship between a biochemical abnormality and the clinical and pathological presentation of a patient. The second part of the question is less difficult than the first, in that we know that this enzyme (i.e., very long chain acyl-CoA synthetase, VLCFA-CoA synthetase) is most probably located at the peroxisomal membrane. Recent experiments that we have done suggest that the enzyme sticks to the cytosolic site of the peroxisome, like palmitoyl-CoA synthetase, as shown by Professor Mannaerts, but I doubt whether this actually gives a clue to the variation between X-linked ALD as you see it in young boys and AMN patients. With regard to the first part of the question concerning the ACTH receptors, I have no information about studies that have been done in ALD as compared to AMN patients.

Dr. Hobbs: How about detecting the carriers? Have you any experience on hair follicles, for example? Have you any test sensitive enough to work on single hair follicles?

Dr. Wanders: I must rely on the data from Moser again because he has the greatest experience in this field. According to his information, you can do carrier detection by looking at very long chain fatty acids in plasma and in fibroblasts. You should combine the two because carrier detection in plasma is very difficult, and is successful in only 85% of cases. You can increase this percentage by doing additional assays in fibroblasts and of course there is now the DNA analysis where you can detect carriers using the ST14 probe.

Dr. Saudubray: The two most intriguing categories of patients with peroxisomal disorders are undoubtedly chondrodysplasia punctata and the so-called Zellweger-like syndrome. You did
not speak about chondrodysplasia punctata because it was outside the scope of your topic. Could you give some additional information on phytic acid oxidation in both these disorders, and also comment on the two enzymes necessary for the synthesis of plasalogens? You spoke about the first enzyme, dihydroxyacetonephosphate acyltransferase, but what about the other enzyme?

Dr. Wanders: First I did not speak about rhizomelic chondrodysplasia punctata because it is not a β-oxidation disorder, but it is interesting, of course. In this disease there is a deficiency of the two enzymes involved in plasalogen biosynthesis, DHAPAT (dihydroxyacetonephosphate acyltransferase) and alkyl dihydroxyacetonephosphate synthase. Furthermore, this disorder is characterized by a deficient activity of phytic acid oxidation, and next to that there is an abnormality in the peroxisomal thiolase. As to the other question with regard to phytic acid oxidation in all the β-oxidation disorders: we know that in acyl-CoA oxidase deficiency, bifunctional protein deficiency, and thiolase deficiency, phytic acid oxidation is normal. We have found, however, that pristanic acid is increased in all these cases. We have just started these studies, but it looks as if phytic acid breakdown to pristanic acid is normal, but that subsequent oxidation of pristanic acid is deficient. This might contribute, of course, to the pathogenesis of these disorders.

Dr. Hobbs: Xenobiotic toxicity shows an idiosyncratic susceptibility. Has anyone studied peroxisomal carriers or defects in victims of the condition?

Dr. Van Hoof: We know that more than 100 cytochromes P450 and P448 exist. Genetic differences in these multiple isoenzymes might contribute to the heterogeneity in the individual response to xenobiotics. Some of these cytochromes are needed for the formation of dicarboxylic acids, which will later be β-oxidized, mainly in peroxisomes. May I remind you of the observation made by Van den Branden et al. (3). Rats receiving high amounts of phytol in their diet accumulate very long chain fatty acids in their tissues. Phytol is the precursor of phytate. Part of it is oxidized in peroxisomes and the action of β-oxidation enzymes is required for this. It is likely that very long chain fatty acid accumulation can result from a competition between substrates of the peroxisomal β-oxidation. The same competition is expected to occur in conditions in which high amounts of dicarboxylic acids are formed [e.g., in subjects receiving medium chain triglycerides (MCT)]. One of the conclusions that should be drawn from this is that phytol-, phytic acid- or MCT-containing foods should be avoided hours or days before collecting blood for the search of heterozygotes for X-linked adrenoleukodystrophy.

Dr. Krywawyych: I come back to the point you have just raised. We have looked at the tissues from a patient with a dicarboxylic aciduria which we suspect was due to a lesion in the oxidation of long chain fatty acids and found an accumulation of very long chain fatty acids present only in cardiac tissue.

Dr. Hobbs: I am intrigued by the difference between “brown fat” persons and others. I would like to ask both Dr. Wanders and Dr. Mannaerts whether anybody has studied either peroxisomal function or malonyl-CoA functions to explore such differences.

Dr. Mannaerts: There have been some studies on mitochondria and peroxisomes in brown adipose tissue, mainly from the laboratory of Barbara Cannon in Sweden. Brown adipose tissue contains peroxisomes, which are inducible in rodents. Most of the thermogenesis is mitochondrial, however, and peroxisomes contribute to an only minor degree.

Dr. Van Hoof: I agree. We failed to demonstrate a significant activity of acyl-CoA oxidase in brown fat, and there is good evidence that mitochondria are responsible for the thermogenesis, which is important in newborns.

Dr. Mannaerts: At the beginning at least, the idea of peroxisomes being involved in thermogenesis was attractive, since the energy that is released during the first step of peroxisomal
β-oxidation is completely lost as heat. That was the main reason why people thought about the role of peroxisomes in terms of thermogenesis. The regulatory role of malonyl-CoA I think has been proven only for liver and not for extrahepatic tissues. Malonyl-CoA is present in extrahepatic tissues, and it inhibits extrahepatic carnitine palmitoyltransferase I in vitro, but it is not known whether its concentration fluctuates, or fluctuates in the right direction. There is some indication that it might have a regulatory role in the heart.

REFERENCES