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Immunolocalization of a 43 kDa peroxisomal membrane protein in the liver of patients with generalized peroxisomal disorders

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The presence of peroxisomal membrane ghosts was examined in liver biopsies from eleven patients presenting the clinical and biochemical picture of a generalized peroxisomal disorder (Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease and variants of these syndromes). A polyclonal antibody raised against the membrane of human liver peroxisomes and recognizing a 43 kDa peroxisomal membrane protein (PMP) was used. In human control liver the antibodies react in a distinct and specific way with the peroxisomal membrane.

Two types of organelles with an immunoreactive membrane were identified in the liver parenchymal cells of the patients: organelles containing an electron-dense core and organelles with electron transparent contents. Both types may co-occur in the same patient; in two patients they were found in the same cell. The organelles are rare, and their number varies between patients. The first type possibly corresponds to the previous morphological description of aberrant peroxisomes in the liver of patients with Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease. The empty looking organelles have not been reported previously in the liver, some of the "empty" organelles seem to be enclosed by a double membrane.

Morphometrical analysis in three patients indicated that both types of organelles (corrected mean d-circle 0.271–0.306 μm for the "empty" and the dense core organelles, respectively) are smaller than the peroxisomes in postnatal control liver and in fetal liver.

In one patient (infantile Refsum disease) immunoreactive organelles were not detected. The organelles with the electron-dense core were not found in two patients. In one of them, described clinically and biochemically as a mild Zellweger variant, very few cells showed numerous small organelles enclosing a morphologically normal matrix which contained at least the peroxisomal matrix proteins catalase, acyl-CoA oxidase, 3-ketoacyl-CoA thiolase, and alanine/glyoxylate aminotransferase; their membrane showed 43 kDa PMP immunoreactivity.

The dense core organelles and the empty-looking organelles were not found in human control livers, in which solely the membrane of normal peroxisomes was labeled. It is therefore suggested that both types of organelles, identified by their immunoreactivity for the 43 kDa PMP, represent aberrant peroxisomes. The finding of morphologically empty vesicles is in accordance with the concept that the defect in these disorders is at the level of the assembly of import competent peroxisomes. On the other hand, the findings also indicate that at least in one patient the defect is not present in all parenchymal cells: peroxisomes containing at least four matrix proteins were present in a minority of the cells.

Introduction

Peroxisomes are single membrane-limited cell organelles, which are involved in various anabolic and catabolic processes (for review, see [35]). The importance of their role in human metabolism has become clear after the recognition of a distinct group of inherited diseases, the peroxisomal disorders [15]. These disorders are subdivided in three groups, reflecting the deficiency of all (group I), multiple (group II) or a single (group III) peroxisomal enzyme function [39]. In the Cerebro-Hepato-Renal syndrome (CHRIS; Zellweger syndrome; peroxisomal disorder group I), which is the prototype of the peroxisomal disorders and represents the most severe clinical condition, morphologically recognizable peroxisomes are absent in the liver and kidney [8].
In cultured skin fibroblasts from CHRS patients, the peroxisomal matrix and membrane proteins are normally synthesized [26, 32]. Some of the enzymes remain localized in the cytoplasm where they may preserve their enzyme activity, e.g. catalase and alanine/glyoxylate aminotransferase [37, 38]; others are readily degraded [33].

In these cells large and rare vesicles were found with a membrane that was immunoreactive for an antisera raised against peroxisomal membrane proteins. These organelles, which looked largely empty and were designated as "membrane ghosts", were considered to represent peroxisomes which are unable to import their matrix components, and an import defect was suggested to be at the base of these disorders [22, 23]. So far, two different peroxisomal targeting signals which are necessary to direct matrix proteins to the peroxisome in mammalian cells, were identified: a carboxy-terminal SKL-tripeptide (PTSII) and an amino-terminal leader sequence (PTSII); the involved translocation mechanisms remain as yet unknown (for review, see [30]).

Since the work by Santos et al. [22, 23], membrane ghosts have also been identified in cultured skin fibroblasts from patients with infantile Refsum disease, neonatal adrenoleukodystrophy and hyperpigecolic acidemia [4]. Experimental evidence for an import defect in these cells was provided by microinjection experiments of synthetic proteins carrying the carboxy-terminal SKL signal [36].

Complementation analysis with fibroblasts revealed that the generalized peroxisomal disorders segregate in at least nine complementation groups; there is no obvious correlation between the genotype and the phenotype [27]. It has recently been shown that in cultured fibroblasts from complementation group IV the import via the amino-terminal presequence of 3-ketoacyl-CoA thiolase is unaffected [17].

Immunoblotting studies in the liver of patients with a generalized peroxisomal disorder have demonstrated that the peroxisomal membrane proteins (PMPs) were normally present [14, 28] or that they were present in variable, diminished amounts and associated with a membrane fraction [7, 40]. Other studies reported the absence of peroxisomal membrane proteins in several patients [1, 31]. These studies do not exclude the possibility that the PMPs, even when they are found in association with a membranous structure, have inserted into a wrong subcellular compartment [28]. To address this question, we have examined by immunocytochemistry the subcellular localization of a 43 kDa PMP which was recently isolated from human liver peroxisomes [24] – in the liver parenchymal cells of eleven patients with a generalized peroxisomal disorder.

### Materials and methods

#### Antibodies

Polyclonal antisera were raised in rabbits against the membrane of human liver peroxisomes isolated from fresh and frozen human liver according to Alvarez et al. [2]. On immunoblots one serum cross-reacted with PMPs with a molecular mass of 112, 69 and 43 kDa. From this serum monospecific antibodies against the 43 kDa PMP were obtained by affinity purification. The affinity-purified antibodies reacted mainly with the 43 kDa PMP and to a much lesser extent with the 69 kDa PMP [24].

The following polyclonal antibodies, raised in rabbits, against peroxisomal matrix proteins were used: catalase (IgG fraction from serum raised against bovine liver catalase; Rockland Laboratories, Gilbertsville, PA/USA, Code No. 200-4151); acyl-CoA oxidase (IgG fraction raised against the rat liver enzyme; supplied by Dr. A. Völk, Heidelberg/Germany), 3-ketoacyl-CoA thiolase (IgG fraction raised against the rat liver enzyme; supplied by Dr. T. Hashimoto, Nagano/Japan) and alanine/glyoxylate aminotransferase (full serum raised against the human liver enzyme; supplied by Dr. R. J. A. Wanders and Dr. R. B. H. Schuengens, Amsterdam/The Netherlands).

#### Immunocytochemical procedures

In a first experiment, preembedding immunolabeling in human control liver was tested because this method (i) maximally preserves antigenicity by avoiding embedding, and (ii) it allows a sensitive control to check for a possible cross-reaction with the membrane of other cell organelles like the endoplasmic reticulum and the mitochondria. For this purpose two biopsies from human control liver were fixed with 4% commercial formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) during 24 h at room temperature. Cryostat sections (24 μm) were immunolabeled following the procedure described in [4]. Protein A colloidal gold (10 nm), prepared according to Slot and Geuze [29], was used as a label. The following polyclonal antibodies, raised in rabbits, against human liver PMPs were used:

**Table 1.** Examined patients and types of 43 kDa PMP immunoreactive organelles.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at biopsy</th>
<th>Diagnosis</th>
<th>Type of 43 kDa PMP immunoreactive organelles</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE</td>
<td>M</td>
<td>9 years</td>
<td>neonatal adrenoleukodystrophy; patient 2 described in [34]</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>OG</td>
<td>M</td>
<td>11 days</td>
<td>classical Zellweger syndrome, died at the age of 5 months</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>MM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M</td>
<td>1st: 6 months</td>
<td>mild Zellweger variant</td>
<td>Empty vesicles</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2nd: 27 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEGC</td>
<td>F</td>
<td>10 years</td>
<td>mild Zellweger variant</td>
<td>Empty vesicles and small, numerous peroxisomes</td>
<td>B</td>
</tr>
<tr>
<td>MD</td>
<td>M</td>
<td>1.5 months</td>
<td>generalized peroxisomal disorder</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>AC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F</td>
<td>11 months</td>
<td>Infantile Refsum disease</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>CC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M</td>
<td>2 years</td>
<td>Infantile Refsum disease</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>DL</td>
<td>M</td>
<td>6 months</td>
<td>generalized peroxisomal disorder</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>IB</td>
<td>F</td>
<td>7.5 months</td>
<td>generalized peroxisomal disorder</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>MN&lt;sup&gt;a&lt;/sup&gt;</td>
<td>M</td>
<td>20 years</td>
<td>Infantile Refsum disease</td>
<td>Dense core organelles and empty vesicles</td>
<td>A</td>
</tr>
<tr>
<td>ON&lt;sup&gt;a&lt;/sup&gt;</td>
<td>F</td>
<td>15.5 years</td>
<td>Infantile Refsum disease</td>
<td>Immunoreactive organelles not detected</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>See Discussion. <sup>b</sup>Patient MM showed normal levels of phytanic acid. Phytanic acid accumulation in patients with a generalized peroxisomal disorder is age and diet dependent [39]. Patients AC and CC and patients MN and CN are siblings. The latter were clinically and biochemically described [21].
was used for immunodetection. Subsequently the sections were post-fixed with 1 % osmium tetroxide and embedded in LX-112 resin (Ladd Research Industries, Burlington, VT/USA). Ultrathin sections, collected on 300 mesh copper grids, were conventionally poststained with uranyl acetate and lead citrate and examined in a JEOL 100B electron microscope.

A postembedding on-grid immunolabeling procedure was applied to ultrathin sections of control and patient livers. Samples, fixed as mentioned above, were postfixed with 0.5 % glutaraldehyde during 1 h at 4°C and further processed for embedding in Lowicryl K4M (Balzers Union/Liechtenstein) or in Unicryl (BioCell International, Cardiff/UK), a recently introduced acrylate resin [25]. The labeling procedure, making use of 15-nm colloidal conjugated to protein A, has been described elsewhere [5]. Negative controls were incubated with normal rabbit serum or the IgG fraction of this serum (Dakopatts, Copenhagen/Denmark).

Liver specimens
The control specimens for preembedding immunolabeling were two biopsies from adult females, suffering from chronic hepatitis B and an unknown etiology (history of cervix carcinoma, followed by radiotherapy; recently, impaired liver transferase functions were found). The control specimens for postembedding were, besides the above mentioned samples, two biopsies from adult males with alcoholic steatosis.

The examined patients are listed in Table I. They all showed the clinical picture of a generalized peroxisomal disorder which was confirmed in all cases by biochemical analysis (i.e. reduced plasmalogon levels, increased levels of very long chain fatty acids, phytanic and pipercolic acid, and an accumulation of bile acid intermediates).

Diaminobenzidine cytochemistry
Sections from all the patients' samples were incubated for the cytochemical demonstration of catalase activity according to Roels et al. [18]. Catalase containing peroxisomes could not be detected at the light and electron microscopy level in none of the samples (not shown).

Morphometry
In four patients morphometric analysis was done on calibrated electron micrographs of immunolabeled ultrathin Unicryl sections, according to previously described procedures [3].

![Fig. 1. Preembedding immunolabeling for the 43 kDa peroxisomal membrane protein in a control liver, revealing the high specificity of the antibodies for the peroxisomal membrane. The gold particles (10 nm) only decorate the membrane of the peroxisomes (arrows). Labeling at the membrane of the mitochondria (M) or of the endoplasmic reticulum is absent. Glycogen rosettes (arrowheads) and a lipid droplet (L) are seen. - Bar 0.5 μm. - 37 400×.](image1)

![Fig. 2. Postembedding labeling (15-nm gold particles) for the 43 kDa peroxisomal membrane protein in a control liver (ultrathin Unicryl section). The membrane of the peroxisomes (arrows) is labeled; the labeling density is lower in comparison with the preembedding labeling. The organelle indicated by the asterisk possibly represents an unlabeled peroxisome. - Bar 0.5 μm. - 50 000×.](image2)
Immunochemistry. Two types of immunoreactive membrane containing an electron-dense core that is separated from specific immunoreactivity of the peroxisomal membrane was found in both samples. The membrane of other cell organelles remained unlabeled (Fig. 2). Control incubations with normal rabbit serum were negative in all samples and experiments (not shown).

Patients

Immunocytochemistry. Two types of immunoreactive membranous structures were identified. The first type is an organelle containing an electron-dense core that is separated from an immunoreactive membrane. These organelles were numerous. - Fig. 7. Patient IB. A vesicle with an immunoreactive membrane, which seems to enclose a very dispersed matrix. The size of this organelle is similar to that of a normal peroxisome. - Fig. 8. Patient CC. “Empty” vesicle with an immunoreactive membrane. - Bars 0.2 µm (Figs. 3-6, 8), 0.5 µm (Fig. 7). - 79 000 × (Figs. 3-5), 63 200 × (Figs. 6, 8), 56 800 × (Fig. 7).

Results

Control liver

In the preembedding immunolabeling experiments using the affinity-purified antibodies against the 43 kDa PMP, a highly specific immunoreactivity of the peroxisomal membrane was found in both samples. The membrane of other cell organelles remained unlabeled (Fig. 1). In the postembedding labeling the peroxisomal membrane was labeled at a lower density as compared with preembedding labeling; it is possible that some organelles remained unlabeled (Fig. 2). Control incubations with normal rabbit serum were negative in all samples and experiments (not shown).

Figs. 9 to 14. Patient MM. - Fig. 9. “Empty” vesicle with a (double?) 43 kDa PMP immunoreactive membrane. - Fig. 10. Numerous, small organelles with a (single) 43 kDa PMP immunoreactive membrane and enclosing a normal appearing matrix, were present in a few cells. - Figs. 11 to 14. The organelles shown in (Fig. 10) contain catalase (Fig. 11), acyl-CoA oxidase (Fig. 12), 3-ketoacyl-CoA thiolase (Fig. 13) and alanine/glyoxylate aminotransferase (Fig. 14). The labeling pattern for catalase (Fig. 11) reveals numerous gold particles over the cytoplasm. The mitochondrial profiles (M) and the cisternae of the endoplasmic reticulum (arrowheads) are devoid of gold particles. - Bars 0.2 µm (Figs. 9, 10), 0.20 µm (Figs. 11-14). - 47 400 × (Figs. 9, 10), 37 900 × (Figs. 11-14).
the membrane by an electron-lucent halo. It was found in patients DE, NEGC, OG, MD, AC, CC, DL, and MN. The shape and appearance of these organelles is remarkably similar in all patients (Figs. 3, 4).

The second type are organelles which most often look empty, i.e., their contents are electron translucent (Figs. 5-9). They were found in all the patients, except for CN. Occasionally some vesicles seem to contain a very dispersed matrix (Fig. 7). In contrast to the organelles with the electron-dense core, which are delineated by a single membrane, some organelles of the second type seem to be enclosed by a double membrane (Figs. 5, 9).

Both types of organelles may co-occur in the same patient (see Tab. I and Figs. 4 and 5); in patients NEGC, DL and OG they were found in the same cell. In patients MM and IB only organelles of the second type were found. The results are summarized in Table I.

As evaluated from the ultrathin sections, the number of labeled organelles varies between the patients. In most samples the organelles are very rare, and many cells are seen without immunoreactive organelles.

None of these organelles were found in the control livers. Control incubations with normal rabbit serum were negative in all samples (not shown).

Besides the empty vesicles in patient MM (Fig. 9), the antibody reacted in a very few cells with the membrane of numerous small organelles enclosing a homogeneous matrix and appearing as small peroxisomes (Fig. 10). The peroxisome nature of these organelles was further confirmed by immunocytochemistry: catalase, alanine/glyoxylate aminotransferase, acyl-CoA oxidase and 3-ketoacyl-CoA thiolase were localized in these organelles (Figs. 11-15). Besides the peroxisomal localization of catalase in these cells, there was also evidence for a cytoplasmic localization of catalase: numerous gold particles were present in the cytoplasm while the mitochondrial profiles and the cisternae of the endoplasmic reticulum were devoid of label (Fig. 11).

Morphometry. The mean diameter (d-circle) of the 43 kDa PMP immunoreactive organelles was measured in patients OG, MD and DL and of the "empty" organelles and the peroxisomes in patient MM; the data are summarized in Table II. The d-circle of the 43 kDa PMP immunoreactive organelles varies between 0.196 μm (dense core organelle in patient DL) and 0.311 μm ("empty" organelle in patient MM). Student's t-test indicated that in patients MD and OG the mean d-circle of the organelles with the electron-dense core is significantly smaller (P ≤ 0.015) than that of the empty-looking organelles. ANOVA analysis showed that the d-circle of both types of organelles does not significantly differ between the three patients. Both types are smaller than the peroxisomes in human embryonic and postnatal liver.

Tab. II. Size parameters of the 43 kDa immunoreactive organelles in four patients and of peroxisomes in control liver.

<table>
<thead>
<tr>
<th>Patients</th>
<th>d-circle (μm)</th>
<th>Corrected mean</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± S.E.M.)a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OG</td>
<td>&quot;Empty&quot; organelles (n=24)b</td>
<td>0.267 (±0.013)</td>
<td>0.306</td>
</tr>
<tr>
<td></td>
<td>Organelles with core (n=10)b</td>
<td>0.201 (±0.011)</td>
<td>0.235</td>
</tr>
<tr>
<td></td>
<td>&quot;Empty&quot; organelles (n=22)b</td>
<td>0.231 (±0.010)</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>Organelles with core (n=16)b</td>
<td>0.212 (±0.008)</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td>&quot;Empty&quot; organelles (n=48)b</td>
<td>0.237 (±0.009)</td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td>Organelles with core (n=22)b</td>
<td>0.196 (±0.010)</td>
<td>0.231</td>
</tr>
<tr>
<td></td>
<td>&quot;Empty&quot; organelles (n=14)b</td>
<td>0.311 (±0.016)</td>
<td>0.367</td>
</tr>
<tr>
<td></td>
<td>Peroxisomes (n=108)</td>
<td>0.324 (±0.007)</td>
<td>0.387</td>
</tr>
<tr>
<td>Controls</td>
<td>Postnatal adult (n=951)c</td>
<td>0.525 (±0.017)</td>
<td>0.643</td>
</tr>
<tr>
<td></td>
<td>Postnatal infantd</td>
<td>0.445 (±0.013)</td>
<td>0.555</td>
</tr>
<tr>
<td></td>
<td>4 months (n=135)</td>
<td>0.518 (±0.012)</td>
<td>0.640</td>
</tr>
<tr>
<td></td>
<td>Embryonicd</td>
<td>0.334 (±0.008)</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td>7 weeks (n=87)</td>
<td>0.348 (±0.007)</td>
<td>0.415</td>
</tr>
</tbody>
</table>

* Standard error of the mean. – a Frequency of labeled organelles explains the low numbers measured. – b Seven samples (age 46±7 years), from De Craemer et al. [3]. – c Postmenstrual age, from Espeel et al. [6].
In patient MM, the empty organelles and the genuine peroxisomes have a similar mean d-circle which is smaller than in postnatal control liver, but of a similar magnitude as in embryonic liver. The empty organelles in MM are significantly larger (P < 0.015) than in the three other patients.

Discussion

With polyclonal antibodies against a 43 kDa peroxisomal membrane protein isolated from human liver peroxisomes, two types of organelles with an immunoreactive membrane in the liver of patients with a generalized peroxisomal disorder were identified. By immunolabeling in human control livers, it was shown that the antibodies react in a distinct and highly specific way with the peroxisomal membrane only.

The first type of immunoreactive organelles, i.e. the organelles with the electron-dense core, corresponds to the description “sparse, scattered and small organelles, with a dense matrix that was separated from the membrane” in the liver of patients with Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease [9, 11, 13, 16, 34]. In a recent immunocytochemical study [13] such organelles were found in two of four Zellweger patients, in one NALD and two IRD patients; the diameter (uncorrected d-circle) varies between 0.20 and 0.26 μm. Only in the IRD patients the membrane of the organelles was immunoreactive for an antibody against the 68 kDa PMP from mouse liver peroxisomes [13]. In agreement with these observations, we did not find the dense core organelles in all the patients.

The second type of organelles has not been described in the liver previously. As most of these organelles do not contain a morphologically recognizable matrix, their appearance is in agreement with the original description of “empty” membrane ghosts in cultured skin fibroblasts from a CHRS patient [23]. Remarkably, some of these organelles seem to be enclosed by a double membrane. The resolution of the colloidal gold labeling combined with the relatively poor contrast of membranes in acrylate resin sections does not allow to determine unequivocally whether both membranes are labeled. The following points with respect to the presence of a double membrane should be mentioned: 1) at least some of the peroxisomal ghosts in cultured fibroblasts are enclosed by a double or multiple membrane (personal communication by Dr. A. Motley, Amsterdam; cited with permission); 2) the image of a vesicle with a double membrane might be the result of a section through a collapsed, single membrane-limited organelle; 3) referring to the observations in CHRS fibroblasts that multiple membrane (personal communication by Dr. A. Motley, Amsterdam; cited with permission); 4) the density of the membrane ghosts in cultured fibroblasts might be increased even more, so that the membrane ghosts have the appearance of a double membrane.

In an attempt to classify the types and occurrence of immunoreactive organelles in the examined samples, three groups can be considered: group A (patients DE, NEG, OG, MD, AC, CC, DL and MN), in which dense core organelles and empty vesicles co-occur, group B (patient MM) with the presence of empty vesicles in the major part of the parenchymal cells and numerous small peroxisomes in few cells, and group C (patient IB) with the empty vesicles only. It might be interesting to compare the classification with the complementation groups to which the patients belong; this awaits further experiments.

With a polyclonal antibody against the 68 kDa mouse liver PMP, Hughes et al. [13] found no immunoreactive empty-looking organelles in the liver of their patients with a generalized peroxisomal disorder. In a previous study with a polyclonal antibody against a 70 kDa peroxisomal membrane protein from rat liver peroxisomes, we could not demonstrate immunoreactive organelles in the liver from six patients with a generalized peroxisomal disorder, including the present cases MM, OG and DE [21]. Similar negative results from immunocytochemistry with the same antibody probe were obtained in three Zellweger patients by another group (Dr. S. Yokota, personal communication; cited with written permission). It should also be remembered that in liver immunoblots from several patients, in which the same antibodies were used, absence of the 70 kDa peroxisomal membrane protein was reported [1, 31]. In addition to patient CN, in two patients with multiple defects of peroxisomal metabolism and showing a mosaic distribution of peroxisome occurrence in the liver parenchyma, we could not detect 43 kDa immunoreactive organelles in the cells without peroxisomes (Espeel et al., in press, Hepatology).

The results from morphometry in three patients show that both types of organelles are smaller than the peroxisomes in infant and adult control liver. In early embryonic human liver, peroxisomes also are of reduced size. At this stage, catalase activity shows a pronounced heterogeneity between organelles, many being negative and the peroxisomal β-oxidation enzymes cannot yet be detected [6]. This suggests that the aberrant organelles in the patients did not undergo a normal growth during ontogeny. The data differ from the membrane ghosts in fibroblasts, which were described as enlarged. In peroxisomal disorder patients from group II and III (see Introduction), the hepatic peroxisomes are, except for primary hyperoxaluria type I and X-linked adrenoleukodystrophy, enlarged (for review, see [19]); extreme enlargement was reported in rhizomelic chondrodysplasia punctata and variants of this syndrome [5, 12, 20].

No immunolabeling was detected in the dense core organelles and over the profiles corresponding to empty-looking organelles after incubation for catalase and alanine/glyoxylate aminotransferase in three patients studied in this respect (OG, DL, MD). Also after incubation for catalase activity with diaminobenzidine no reaction product was found in the dense core organelles. This is in agreement with Hughes et al. [13], who found no labeling for catalase in the dense core organelles of the CHRS and NALD patients; in the IRD patients these authors observed a reduced labeling density for catalase.

The presence of import competent peroxisomes in a few cells in the liver of patient MM, illustrates that the defect is not fully expressed in all cells. Moreover, the fact that catalase, acyl-CoA oxidase, 3-ketoacyl-CoA thiolase, and alanine/glyoxylate aminotransferase are imported (acyl-CoA oxidase and 3-ketoacyl-CoA thiolase make use of PTS1 and PTSII, respectively), indicates that the routing and translocation processes for these proteins are functioning. The question remains by which mechanisms these cells have accomplished the restoration of the defect. This restoration appears to be incomplete as catalase was also visualized in the cytoplasm of these cells.

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