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Review article

Peroxisomal disorders in neurology

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SUMMARY

Although peroxisomes were initially believed to play only a minor role in mammalian metabolism, it is now clear that they catalyse essential reactions in a number of different metabolic pathways and thus play an indispensable role in intermediary metabolism. The metabolic pathways in which peroxisomes are involved include the biosynthesis of ether phospholipids and bile acids, the oxidation of very long chain fatty acids, prostaglandins and unsaturated long chain fatty acids and the catabolism of phytanate and (in man) pipecolate and glyoxylate.

The importance of peroxisomes in cellular metabolism is stressed by the existence of a group of inherited diseases, the peroxisomal disorders, caused by an impairment in one or more peroxisomal functions. In the last decade our knowledge about peroxisomes and peroxisomal disorders has progressed enormously and has been the subject of several reviews. New developments include the identification of several additional peroxisomal disorders, the discovery of the primary defect in several of these peroxisomal disorders, the recognition of novel peroxisomal functions and the application of complementation analysis to obtain information on the genetic relationship between the different peroxisomal disorders.

The peroxisomal disorders recognized at present comprise 12 different diseases, with neurological involvement in 10 of them. These diseases include: (1) those in which

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peroxisomes are virtually absent leading to a generalized impairment of peroxisomal functions (the cerebro-hepato-renal syndrome of Zellweger, neonatal adrenoleukodystrophy, infantile Refsum disease and hyperpipecolic acidaemia); (2) those in which peroxisomes are present and several peroxisomal functions are impaired (the rhizomelic form of chondrodysplasia punctata, combined peroxisomal β-oxidation enzyme protein deficiency); and (3) those in which peroxisomes are present and only a single peroxisomal function is impaired (X-linked adrenoleukodystrophy, peroxisomal thiolase deficiency (pseudo-Zellweger syndrome), acyl-CoA oxidase deficiency (pseudo-neonatal adrenoleukodystrophy) and probably, the classic form of Refsum disease.

Key words: Zellweger syndrome; Peroxisome; Peroxisomal disorder; Adrenoleukodystrophy; Inborn error; Fatty acid

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INTRODUCTION

In 1964 Bowen, Lee, Zellweger and Lindenberg described a familial syndrome of multiple congenital defects in two pairs of sibs (3 girls and a boy). Prenatal history and delivery were unremarkable. At birth hypotonia and a number of congenital anomalies were noted including bilateral glaucoma with corneal opacities, bilateral epicanthal folds, abnormal ears, a high arched palate, wide fontanels, open metopic and lambdoid sutures, clitoris hypertrophy, camptodactyly and simian creases. Unaware of this publication Smith, Opitz and Inhorn (1965) described "a syndrome of multiple developmental defects including polycystic kidneys and intrahepatic biliary dysgenesis in two sibs", who presented with a large number of comparable defects including severe hypotonia, high forehead, shallow supraorbital ridges, camptodactyly, minor anomalies of the eyes, ears, palate and hands and failure to thrive. Two years later Passarge and McAdams (1967) described 5 sisters with similar clinical and pathological features and introduced the term cerebro-hepato-renal syndrome. In 1969 Opitz and colleagues introduced the name Zellweger syndrome (Opitz et al. 1969). In an editorial comment McKusick (1969) suggested that the two designations proposed by Passarge and McAdams (1967) and Opitz et al. (1969) be combined giving rise to the "cerebro-hepato-renal syndrome of Zellweger". Following these initial reports more than 150 patients have now been described in the literature.

A major hallmark in research on the Zellweger syndrome was the discovery by Goldfischer et al. (1973) that morphologically distinguishable peroxisomes are absent in hepatocytes and renal tubule cells from Zellweger patients. Since at that time peroxisomes were thought to play only a minor role in mammalian metabolism, the full significance of this finding was appreciated only a decade later. Initial attention was focused much more on the finding reported in the same paper of morphologically distorted mitochondria which appeared to be defective in electron transport. Now we know, however, that peroxisomes are indispensable organelles carrying out a diverse set

of reactions. The properties, distribution and characteristics of peroxisomes relevant for human pathophysiology will be described first.

PEROXISOMES: IDENTIFICATION, MORPHOLOGY AND DISTRIBUTION

Peroxisomes are now known to be constituents of every eukaryotic cell except the mature erythrocyte. They were first described by Rhodin in 1954 as "spheric or oval bodies" present in the cytoplasm of mouse proximal kidney tubules. Rouiller and Bernard (1956) identified similar organelles in rat hepatocytes and suggested that they were precursors (progenitors) of mitochondria rather than distinct cell organelles sui generis. The addition of microbodies to the group of biochemically defined organelles is closely related to the development of cell fractionation techniques. Indeed, the conclusion that catalase, urate oxidase and D-amino acid oxidase are located in distinct particles different from lysosomes, microsomes and mitochondria was reached on the basis of differential and isopycnic gradient centrifugation studies (for review, see de Duve and Baudhuin 1966). The concomitant occurrence of hydrogen peroxide-producing enzymes and catalase in a single particle prompted de Duve and coworkers to introduce the name peroxisome. Combined morphological and biochemical investigations by Baudhuin et al. (1965) provided unequivocal evidence that microbodies are identical to peroxisomes.

The identification of peroxisomes in virtually every mammalian cell type analyzed so far has greatly been stimulated by the development of cytochemical procedures. The most widely used method is the 3,3′-diaminobenzidine (DAB) procedure in which peroxisomes are visualized by virtue of the peroxidative activity of catalase at alkaline pH. Using this methodology it has become clear that the size, shape and number of peroxisomes can vary widely. Peroxisomes vary greatly in size from a diameter of 0.5–1.0 μm, as found in rat liver for instance, to 0.05–0.2 μm as found in skin fibroblasts and blood cells (neutrophils, granulocytes, monocytes, platelets). In liver and kidney peroxisomes appear as round or oval organelles whereas in sebaceous glands, for instance, peroxisomes do not exist as individual organelles but are interconnected and organized into an elaborate structure called "peroxisome reticulum" (Gorgas 1987). A similar picture has been found in regenerating liver (Yamamoto and Fahimi 1987). Furthermore, the abundance of peroxisomes in different cell types varies considerably. In liver and kidney peroxisomes are present in high numbers, whereas in skin fibroblasts, for instance, relatively few peroxisomes are present (Böck et al. 1980). Peroxisomes are also numerous in cells specialized in lipid metabolism such as present in sebaceous glands, brown fat and other tissues, and also in nervous tissue. It is especially interesting to note their abundance in oligodendrocytes, in which their number is about 40 times higher than in neurons or astrocytes (Arnold and Holzman 1978; Holzman 1982). In oligodendroglial cells, which produce myelin in the central nervous system, microperoxisomes are often located near the developing myelin sheaths. Furthermore, Adamo et al. (1987) have found that microperoxisomes are most abundant in oligodendroglial cells at the peak of myelin formation suggesting a major role of microperoxisomes in myelinogenesis.

Peroxisomes can be visualized not only by histochemical or cytochemical procedures but also by immunochemical techniques. In recent years immuno-electronmicroscopy has become one of the most powerful methods in analyzing the ultrastructural localization of enzymes within a cell by virtue of their reactivity with a specific antibody. This technique has also been applied to peroxisomes. For instance, Yokota et al. (1987) recently visualized peroxisomes by the protein A–gold technique using antibodies against eight different peroxisomal proteins.

FUNCTIONS OF PEROXISOMES IN MAMMALIAN CELLS

Each type of subcellular organelle (mitochondrion, peroxisome, lysosome) plays a distinct role in cellular metabolism. As a consequence each organelle houses a characteristic set of enzyme activities. In recent years peroxisomes have been discovered to play an important role in a number of metabolic pathways (Lazarow 1987). The major functions are:

1) H_2O_2 metabolism

Peroxisomes were originally defined by de Duve and coworkers as organelles containing at least one oxidase which forms H_2O_2, and catalase which decomposes it (de Duve and Baudhuin 1966). Since that time a variety of different oxidases has been identified in peroxisomes, including D-amino acid oxidase, L-α-hydroxyacid oxidase A, urate oxidase, acyl-CoA oxidase, glutaryl-CoA oxidase and polyamine oxidase. The most recent addition to this list is L-pipecolic acid oxidase, a peroxisomal enzyme in man (Mihalik and Rhead 1988; Wanders et al. 1988d).

2) Ether–phospholipid biosynthesis

Peroxisomes play an essential role in ether–phospholipid biosynthesis since the two enzyme activities responsible for the introduction of the ether bond in ether–phospholipids, i.e. acyl-CoA : dihydroxyacetone phosphate acyltransferase (DHAPAT) and alkyldihydroxyacetone phosphate synthase (alkyl DHAP synthase) are localized in peroxisomes at least in rodent liver and brain (Hajra et al. 1979; Hajra and Bishop 1982; for reviews, see Hajra 1984 and Lazarow 1987).

In mammals the main end products of ether–phospholipid biosynthesis are the plasmalogens (1-*O*-alk-1′-enyl-2-acylphosphoglycerides), which are characterized by the presence of an α, β-unsaturated ether bond at the *sn*-1 position of the glycerol backbone. Plasmalogens have long been known to be widely distributed in mammalian cell membranes making up 5–20% of total phospholipids. They are particulary abundant in electrically active tissues such as brain. Indeed, plasmalogens constitute as much as 80–90% of ethanolamine phospholipids in brain white matter (Wykle 1977). The physiological function of plasmalogens and, in fact, of ether phospholipids in

general has not been clear until recently, except for platelet activating factor (PAF). Indeed, PAF induces platelet and leukocyte aggregation and degranulation and is implicated in several pathological processes such as inflammation, asthma, anaphylaxis, hypotension and thrombocytopenia.

However, Raetz and coworkers recently reported that plasmalogens protect animal cell membranes against damage by reactive oxygen species such as singlet oxygen (Zoeller et al. 1988; Morand et al. 1988). This was concluded from experiments with Chinese hamster ovary cells deficient in plasmalogen biosynthesis. Exposure of the mutant cells to the fluorescent fatty acid analogue, 12-(1-pyrene)-dodecanoic acid and ultraviolet light resulted in 100% killing of the mutants whereas the wild-type cells remained viable. Restoration of plasmalogen levels by growing the cells on I-*O*-hexadecyl-*sn*-glycerol rendered them resistant to the damaging effect of the treatment. According to Raetz and coworkers the vinyl-ether linkage in plasmalogens participates directly as a scavenger of reactive oxygen species resulting in the selective decomposition of the plasmalogen phospholipid (Morand et al. 1988).

3) β-Oxidation of fatty acids and other compounds

Enzymic organization

Cooper and Beevers (1969) discovered that a fatty acid β-oxidation system is present in glyoxysomes of germinating castor bean endosperm. Glyoxysomes are catalase-containing organelles closely related to peroxisomes. Subsequently, Lazarow and de Duve (1976) showed that rat liver peroxisomes are also capable of fatty acid β-oxidation. Indeed, peroxisomes contain the full machinery required for β-oxidation. First, peroxisomes are capable of activating fatty acids with chain length of 12 carbons and more to the corresponding acyl-CoA esters via a membrane-bound acyl-CoA synthetase. Available evidence suggests that peroxisomes contain a distinct synthetase catalyzing the activation of very long chain fatty acids (Singh et al. 1987; Wanders et al. 1987a,c). Following activation fatty acyl-CoA esters are oxidized via successive steps of dehydrogenation, hydration, dehydrogenation and thiolytic cleavage. These reactions are catalyzed by the specific peroxisomal β-oxidation enzyme proteins acyl-CoA oxidase, the bifunctional protein with enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities and 3-oxoacyl-CoA thiolase. Hashimoto and coworkers have purified and characterized these enzyme proteins from rat liver (Hashimoto 1987).

Apart from the enzyme proteins involved in the actual β-oxidation process, peroxisomes contain the auxiliary enzymes 2,4-dienoyl-CoA reductase (Dommes et al. 1981), Δ^3,Δ^2-enoyl-CoA isomerase (Kärki et al. 1986) and 3-hydroxyacyl-CoA epimerase (Chu and Schulz 1985) necessary for the degradation of unsaturated fatty acids (Schulz and Kunau 1987). Finally, peroxisomes contain carnitine acetyltransferase and carnitine octanoyl transferase activity. Since fatty acids are not oxidized to completion in peroxisomes, these two enzymes probably play a role in the transfer of the end-products of peroxisomal β-oxidation (acetyl-CoA and medium chain acyl-CoA esters) out of the peroxisomes so that oxidation can be completed in the mitochondria.

Initially it was thought that the peroxisomal β-oxidation system was just an

auxiliary system assisting in fatty acid oxidation under conditions of high fatty acid supply. However, peroxisomes contribute only little to total palmitate oxidation. Now, however, it is clear that the peroxisomal and mitochondrial β-oxidation systems are involved in the β-oxidation of different compounds. The major substrates undergoing β-oxidation in peroxisomes are discussed below.

Very long chain fatty acids, dicarboxylic acids and mono- and polyunsaturated fatty acids

Mainly through work from Moser and coworkers (Singh et al. 1984) it has become clear that saturated very long chain fatty acids (VLCFAs) such as tetracosanoic acid (C24 : 0) and hexacosanoic acid (C26 : 0) are primarily catabolized in peroxisomes. Since acyl-CoA oxidase, the first enzyme of the peroxisomal β-oxidation sequence, is only reactive towards fatty acyl-CoA esters containing 6 carbon atoms or more, very long chain fatty acids are not oxidized to completion but only chain-shortened to yield shorter chain acyl-CoA esters and acetyl-CoA.

Recent evidence suggests that peroxisomes also play an important role in the β-oxidation of poly-unsaturated fatty acids (Hiltunen et al. 1986; Hovik and Osmundsen 1987). Furthermore, it has been suggested that chain-shortening of long-chain dicarboxylic acids to shorter-chain dicarboxylic acids is primarily carried out in peroxisomes (Kolvraa and Gregersen 1986; Vamecq and Draye 1987).

Prostaglandins

It has recently been shown that peroxisomes are capable of β-oxidative chain-shortening of the prostaglandins $F_{2\alpha}$ and E_2 to the respective tetranor compounds (Diczfalusy et al. 1987; Schepers et al. 1988). It is not yet clear, however, whether this is an exclusive property of peroxisomes or not.

Di- and trihydroxycholestanoic acid

Mainly through work from the group of Pedersen, Björkhem and coworkers it has become clear that β-oxidation of trihydroxycholestanoic acid to cholic acid and of dihydroxycholestanoic acid to chenodeoxycholic acid is primarily carried out in peroxisomes (see Pedersen et al. (1987) for review).

Xenobiotics

It is becoming increasingly clear that the peroxisomal β-oxidation system is capable of chain-shortening different xenobiotics including many drugs. Yamada et al. (1986) have shown for instance that xenobiotic compounds with an acyl side chain are subject to very efficient chain-shortening in peroxisomes.

4) Glyoxylate metabolism

Studies by Noguchi and coworkers (for review, see Noguchi (1987)) have shown that peroxisomes are the major site of glyoxylate catabolism in man since alanine glyoxylate aminotransferase, a pyridoxal phosphate dependent enzyme, is a peroxisomal enzyme in man. This view is strengthened by the finding that this enzyme activity is

deficient in hyperoxaluria type 1, a disease in which there is an accumulation of glyoxylate and oxalate in tissues and body fluids (Danpure and Jennings 1986).

5) Polyamine catabolism

Höltta (1978) and Beard et al. (1985) have shown that peroxisomes contain an oxidase capable of oxidizing different polyamines.

6) Cholesterol and dolichol synthesis

Although 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase was long thought to reside exclusively in the endoplasmic reticulum of mammalian cells, it is now clear that the enzyme is also present in peroxisomes (Keller et al. 1985; Krisans et al. 1987). Available evidence suggests that HMG-CoA reductase is a soluble matrix protein in peroxisomes in contrast to the situation in microsomes where it is a 97 kDa transmembrane protein. Recent studies by Krisans and coworkers (Thompson et al. 1987) have shown that peroxisomes are capable of de novo cholesterol synthesis thus providing a role for peroxisomal HMG-CoA reductase. It remains to be established what the importance of a second cholesterol biosynthesizing organelle is.

Finally, recent studies by Appelkvist and Dallner (1987) have shown that peroxisomes are also capable of synthesizing dolichol.

7) Phytanic acid and pipecolic acid degradation

Phytanic acid is known to accumulate in body fluids from patients lacking peroxisomes. Accordingly, it has been suggested (Poulos et al. 1984a) that peroxisomes are the true site of phytanic acid degradation and that the earlier conclusion that phytanic acid oxidation occurs in mitochondria, is invalid since crude mitochondrial fractions contaminated with peroxisomes were used. Skjeldahl and Stokke (1987) recently reinvestigated the subcellular localization of phytanic acid oxidase in rat liver and obtained unequivocal evidence that α-oxidation of phytanic acid occurs solely in mitochondria at least in rat liver. The question of whether phytanic acid oxidation is mitochondrial in man, too, can only be answered if the subcellular site of phytanic acid oxidation is studied in human liver since the subcellular localization of an enzyme can be very different in various species as shown for alanine glyoxylate aminotransferase by Noguchi and coworkers (see Noguchi 1987).

Pipecolic acid is elevated in body fluids from Zellweger patients. It has thus been inferred that at least one enzymic step in pipecolic acid degradation takes place in peroxisomes. We have recently discovered that in man the first step in L-pipecolic acid degradation is catalyzed by the enzyme L-pipecolic acid oxidase, present in peroxisomes (Wanders et al. 1988d). In line with these results Mihalik and Rhead have reported that in man the conversion of L-[^{3}H]pipecolic acid to α-[^{3}H]aminoadipic acid takes place in peroxisomes (Mihalik and Rhead 1988).

BIOGENESIS OF PEROXISOMES

Peroxisomes were long thought to form by budding from the endoplasmic reticulum. This was based upon morphological observations by Novikoff and Shin (1964) on the one hand showing extensive continuities between peroxisomes and endoplasmic reticulum, and biochemical evidence by Higashi and Peters (1963) on the other hand showing that catalase first enters the endoplasmic reticulum before its transfer into peroxisomes.

Now, some 20 years later, there is a general consensus of opinion that this hypothesis is not true. Instead abundant evidence suggests that peroxisomes multiply by fission of pre-existing peroxisomes (like mitochondria and chloroplasts), peroxisomal proteins being synthesized on free polysomes and transported into the peroxisome posttranslationally (see Lazarow and Fujiki 1985 for review). That peroxisomes do, indeed, multiply by enlargement of pre-existing peroxisomes followed by fission was observed morphologically by Veenhuis et al. (1978) in the yeast *Hansenula polymorpha.*

Studies by Fujiki et al. (1982) have clearly shown that the peroxisomal membrane differs from the membrane of the endoplasmic reticulum not only with regard to its phospholipid composition, but also with regard to the pattern of proteins. Most prominent among the peroxisomal membrane polypeptides are two proteins with a molecular mass of 22 kDa and 70 kDa, respectively (Fujiki et al. 1982).

Furthermore, it is now clear that in contrast to the early conclusions by Higashi and Peters (1963), peroxisomal proteins are made on free polyribosomes. This includes not only all matrix enzyme proteins studied so far but also the core protein urate oxidase and an integral membrane protein. Peroxisome biogenesis thus resembles the biogenesis of mitochondria and chloroplasts in key respects. An important distinction, however, is that peroxisomal proteins are generally synthesized in their final size whereas mitochondrial and chloroplast proteins are generally synthesized as larger precursors with cleavable, amino-terminal targeting sequences. It appears that the only exception to this rule is the peroxisomal thiolase which is synthesized as a larger precursor. Available evidence suggests that proteolytic processing is not linked with import of thiolase into the peroxisome (see Hashimoto 1987).

At present much work is being done to unravel the mechanism whereby newly synthesized peroxisomal proteins are targeted to peroxisomes. In a recent report Small and Lazarow (1987) presented presumptive evidence suggesting that in the case of acyl-CoA oxidase this topogenic information resides in the carboxy-terminal portion of the enzyme protein. Gould et al. (1987) recently reported that a 12 amino acids containing carboxy-terminal fragment of firefly luciferase, a peroxisomal enzyme, was able to target chloramphenicol acetyltransferase to peroxisomes. Furthermore, Swinkels et al. (1988) recently found that the topogenic signal in the glycosomal form of phosphoglycerate kinase of *Crithidia fasciculata* also resides in a carboxy-terminal extension.

An important question with regard to peroxisome biogenesis is whether energy is required for the translocation of peroxisomal enzyme proteins synthesized in the cytosol into and across the peroxisomal membrane, as is the case for proteins destined for mitochondria and chloroplasts. Recent results from Lazarow and coworkers indi-

cate that import of acyl-CoA oxidase into peroxisomes requires ATP, but no membrane potential (Imanaka et al. 1987). Finally, there is evidence that import requires the presence of certain membrane proteins possibly acting as receptors (Imanaka et al. 1987).

"CLASSICAL" ZELLWEGER SYNDROME

Since the original description by Bowen and coworkers (1964) more than 150 Zellweger patients have now been described in the literature. In order to obtain information on the frequency of the different clinical, pathological and biochemical abnormalities reported to occur in the Zellweger syndrome we reviewed 114 cases described in the literature (Heymans 1984). The data are presented in Table 1 taken from Heymans (1984). It is clear that the clinical presentation of Zellweger patients is dominated by the typical craniofacial dysmorphia and the neurological abnormalities exemplified by the presence of a high forehead, large fontanels, shallow orbital ridges, low/broad nasal bridge, epicanthus, high arched palate, external ear deformities, micrognathia, redundant folding of the neck, severe hypotonia, abnormal Moro response, hypo-/areflexia, epileptic seizures, and psychomotor retardation in virtually every patient (Table 1). Other clinical abnormalities frequently encountered include ocular abnormalities. This is especially clear if the results of electrophysiological techniques are considered. An extinguished electroretinogram (ERG) was found in virtually all patients studied (Stanescu and Dralands 1972; de Léon et al. 1977; Hittner et al. 1981; Kretzer et al. 1981; Arneson et al. 1982; Garner et al. 1982; Govaerts et al. 1985). Upon roentgenological examination the stippled scimitar shaped calcifications of the patellae are the most impressive and most frequently described abnormalities (incidence: 30/40 (75%)) although calcific stippling is not confined to the patellae only.

Pathological investigations in Zellweger patients have revealed a great number of abnormalities in various organs most notably in the brain (Table 2). Macroscopic abnormalities include deviant sulci and gyri such as deep, almost vertical parietal clefts and pachymicrogyria. Microscopic abnormalities fundamentally represent two types of abnormality: dysontogenetic and degenerative. The former type characteristically affects neuromigration at different levels and in different areas. The migration defect in the neocortex is compound and consists of medium and large sized neurons immediately beneath the molecular layer, marginal glial heterotopia, simple microgyri with apparently fused first layers, microgyria of the cloverleaf type and subcortical and deep nodular neuronal heterotopia. Germinolytic cysts in the subependymal areas of the cerebral hemispheres relate to early loss of cells inhabiting the germinal matrix. Purkinje cell heterotopia and dysplastic inferior olivary nuclei similarly represent a neuromigrational abnormality. The degenerative disorder compounds storage as well as cell degeneration and cell death. The storage consists of neutral fat in astrocytes, glycogen in cortical neurons, lamellar-lipid and lamellar membrane inclusions in lateral cuneate and Clarke's column neurons, while cortical neuronal loss, fibrous gliosis, globoid cells, lipid phagocytes and glial nodules are widespread (Volpe and Adams 1972; Vuia et al. 1973;

Garzuly et al. 1974; Agamanolis et al. 1976; Mei Liu et al. 1976; de Léon et al. 1977; Brun et al. 1978; Evrard et al. 1978; Agamanolis and Patre 1979; Aubourg et al. 1985; Powers et al. 1985, 1987).

TABLE 1

CLINICAL MANIFESTATIONS IN THE CEREBRO-HEPATO-RENAL SYNDROME OF ZELLWEGER

Table 1 is based on the information presented in the following papers as compiled by Heymans (1984): Bowen et al. 1964; Smith et al. 1965; Passarge and McAdams 1967; Punnett and Kirkpatrick 1968; Opitz et al. 1969; Taylor et al. 1969; Vitale et al. 1969; Jan et al. 1970; Poznanski et al. 1970; Patton et al. 1972; Stanescu and Dralands 1972; Volpe and McAdams 1972; Williams et al. 1972; Goldfischer et al. 1973; Vincens et al. 1973; Vuia et al. 1973; Bernstein et al. 1974; Garzuly et al. 1974; Gomez et al. 1974; Sommer et al. 1974; Danks et al. 1975; Agamanolis et al. 1976; Gilchrist et al. 1976; Haddad et al. 1976; Mei Liu et al. 1976; Variend et al. 1976; Camera et al. 1977; de Léon et al. 1977; Bartoletti et al. 1978; Brun et al. 1978; Carlson and Weinberg 1978; Evrard et al. 1978; Agamanolis and Patre 1979; Hanson et al. 1979; Pfeiffer and Sandhage 1979; Mathis et al. 1980; Della Giustina et al. 1981; Hittner et al. 1981; Hong et al. 1981; Kretzer et al. 1981; Müller-Höcker et al. 1981; Toussaint et al. 1981; Arneson and Ward 1982; Garner et al. 1982; Govaerts et al. 1982; Govaerts et al. 1983; Gustaffson et al. 1983; Sarnat et al. 1983. In the 49 papers analyzed 117 patients were described; however, 3 cases were excluded because of uncertainty about their diagnosis (case 3 from Sommer et al. 1974; case 4 from Bartoletti et al. 1978; case 3 from Bernstein et al. 1974).

Number of cases analysed	114
Males/females	55/65 (3 not recorded)
Mean age at death (weeks – no. patients)	19.7/102

	Incidence*	(%)**		Incidence	(%)
Craniofacial features			*Ocular features*		
High forehead	58/60	(97)	Optic disc pallor	17/23	(74)
Large fontanels, wide sutures	55/57	(96)	Cataract/cloudy cornea	30/35	(86)
Shallow orbital ridges	33/33	(100)	Brushfield spots	5/6	(83)
Low/broad nasal bridge	23/23	(100)	Glaucoma	7/12	(58)
Epicanthus	33/36	(92)	Abnormal retinal pigmentation	6/15	(40)
High arched palate	35/37	(95)			
External ear deformity	39/40	(97)	*Limbs*		
Micrognathia	18/18	(100)	Foot deformity	39/43	(93)
Redundant neck foldings	13/13	(100)	Simian crease(s)	29/37	(78)
			Camptodactyly	12/14	(86)
Neurological features					
Severe hypotonia	94/95	(99)	*Genitals*		
Abnormal Moro response	26/26	(100)	Cryptorchidism	20/29	(69)
Hypo-, areflexia	56/57	(98)	Clitoris hypertrophy	6/10	(60)
Poor sucking	74/77	(97)			
Gavage feeding	26/26	(100)	*Other clinical features*		
Epileptic seizures	56/61	(92)	Failure to thrive	50/53	(94)
Psychomotor retardation	45/45	(100)	Hepatomegaly at birth	14/47	(30)
Nystagmus	30/37	(81)			

* The two numbers reflect the number of cases in which the abnormality studied was found to be present and the number of cases in which information about the abnormality was available, respectively.

** Percentage of cases in which the feature studied was found to be abnormal.

TABLE 2

PATHOLOGICAL FINDINGS IN THE CEREBRO-HEPATO-RENAL SYNDROME OF ZELLWEGER

	Incidence	(%)		Incidence	(%)
Central nervous system			*Liver*		
Deep parietal cleft	5/5	(100)	Hepatomegaly	21/27	(78)
Germinolytic cysts	29/29	(83)	Fibrosis	47/62	(76)
Hypomyelination	19/20	(95)	Micronodular cirrhosis	11/30	(37)
Demyelination	7/8	(88)	Cholestasis	20/34	(59)
Widened ventricles	31/36	(86)	Intrahepatic bile duct hypoplasia	12/26	(46)
Gyral abnormalities	53/57	(93)	Hepatic siderosis	40/55	(73)
Cortical layering abnormalities	22/26	(85)			
Neuronal heterotopias	27/31	(87)	*Other pathological findings*		
Purkinje cell heterotopias	18/19	(95)	Renal cysts	78/80	(97)
Abnormal configuration of dentate nucleus and/or inferior olives	20/26	(77)	Patent ductus Botalli	14/28	(50)
Polydendritric Purkinje cells	7/7	(100)	Pneumonia	30/32	(94)
Non specific neuronal degeneration	7/7	(100)	Atelectasis	10/11	(91)
(Astra) glial neutral fat storage	23/23	(100)	Lung hypoplasia	9/10	(90)
			Thymic aplasia	2/8	(25)
			Thymic hypoplasia	8/16	(50)
			Pancreatic islet hyperplasia	11/19	(58)

Information was obtained from the papers referred to in Table 1.

Ocular histopathological studies have also revealed various abnormalities including: corneal edema, iridocorneal adhesion, lens changes, vitreous cells, ganglion cell atrophy, photoreceptor cell atrophy, retinal pigment epithelium changes, optic atrophy with reactive gliosis and demyelination (Jan et al. 1970; Volpe and Adams 1972; Haddad et al. 1976; Hittner et al. 1981; Kretzer et al. 1981; Toussaint et al. 1981; Garner et al. 1982; Cohen et al. 1983).

In 83 out of 114 patients studied from literature, information about the pathology of the liver was available (see Table 2). The liver was found to be enlarged in most patients studied. Histology of the liver varied from near normal to diffuse fibrotic or micronodular cirrhotic, a phenomenon probably related to the age of the patients. In the first two months of life microscopic abnormalities were reported to be absent or only mild (Passarge and McAdams 1967; Kohn and Mundel 1969; Poznanski et al. 1970; Sommer et al. 1974; Danks et al. 1976) including a.o. mid portal and/or lobular fibrosis, cholestasis, intrahepatic bileduct hypoplasia and altered lobular architecture. In patients between 8 and 20 weeks of age fibrosis and distortion of liver architecture was found to be more severe. In almost all patients who were 20 weeks of age or older, there was severe fibrosis and micronodular cirrhosis (Passarge and McAdams 1967; Punnett and Kirkpatrick 1968; Gomez et al. 1974; Gilchrist et al. 1976; de León et al. 1977; Carlson and Weinberg 1978; Pfeiffer and Sandhage 1979; Hong et al. 1981; Arneson et al. 1982; Sarnat et al. 1983).

Increased tissue iron levels have been found in many patients although not in all. Gilchrist et al. (1976) found that the iron content of the liver was increased manyfold in patients between 5 and 18 weeks of age but declined gradually in infants 20 weeks of age or older. In addition to the abnormalities described above microscopical studies have invariably revealed distorted mitochondria (see below) and the total absence or strong reduction of peroxisomes in liver from Zellweger patients as first shown by Goldfischer et al. (1973).

Among the various pathological abnormalities observed in other organs of Zellweger patients the most conspicuous is the occurrence of renal cysts in 97% of the Zellweger patients studied (Table 2).

Upon routine laboratory investigation elevated transaminases, (conjugated) hyperbilirubinaemia, hypoprothrombinemia and elevated serum iron levels are found in the majority of patients. Apart from these non-specific abnormalities, however, there is an accumulation of pipecolic acid (Danks et al. 1975) and the bile acid intermediates di- and trihydroxycholestanoic acid (Hanson et al. 1978) in body fluids (serum, urine and cerebrospinal fluid) of Zellweger patients. Since it was generally thought at that time that at least the conversion of di- and trihydroxycholestanoic acid to chenodeoxycholate and cholate, respectively, took place in mitochondria (Masui and Staple 1966), it was assumed that these abnormalities were due to failing mitochondria. Several authors have reported that mitochondria were, indeed, not only ultrastructurally distorted in the Zellweger syndrome, but also functionally defective since oxidation of the respiratory substrates glutamate plus malate and succinate was found to be strongly impaired Goldfischer et al. 1973; Pfeiffer and Sandhage 1979; Mathis et al. 1980; Kelly and Corkey 1983; Sarnat et al. 1983; Trijbels et al. 1983; Müller-Höcker et al. 1984). Now we know, however, that virtually all biochemical abnormalities found in Zellweger patients, are directly attributable to the absence of peroxisomes as first reported by Goldfischer et al. (1973).

BIOCHEMICAL ABNORMALITIES IN THE ZELLWEGER SYNDROME

The absence of peroxisomes in patients suffering from Zellweger syndrome is associated with a number of biochemical abnormalities as specified below.

Impaired synthesis of plasmalogens

Following the finding (Hajra and Bishop 1982) that key steps involved in ether-phospholipid biosynthesis take place in peroxisomes, Borst (1983) hypothesized that the synthesis of plasmalogens might be impaired in Zellweger patients. Heymans et al. (1983, 1984) found that the levels of phosphatidylethanolamine and phosphatidylcholine plasmalogens are, indeed, strongly deficient in tissues from Zellweger patients. Subsequent studies revealed that the activity of DHAPAT was strongly deficient in tissues, cultured skin fibroblasts, platelets and leukocytes from Zellweger patients (Datta et al. 1984; Schutgens et al. 1984; Wanders et al. 1985). Furthermore, Schrakamp et al.

(1985a) observed that alkyl DHAP synthase is also deficient in Zellweger patients. In accordance with these findings there is an impairment in de novo synthesis of plasmalogens as shown by the reduced incorporation of [^{14}C]hexadecanol into plasmalogens in the patients (Roscher et al. 1985; Schrakamp et al. 1985b). Synthesis of plasmalogens from 1-*O*-alkyl-*sn*-glycerol which only requires the participation of the microsomal steps involved in plasmalogen biosynthesis, was subsequently found to proceed normally in Zellweger fibroblasts (Roscher et al. 1985; Schrakamp et al. 1985b).

The combined use of [^{14}C]hexadecanol plus 1-*O*-[^{3}H]alkyl-*sn*-glycerol has proved to be a valuable tool in evaluating the extent of peroxisomal dysfunction in the different peroxisomal disorders in which there is an impairment in plasmalogen biosynthesis (Roscher et al. 1985; Schrakamp et al. 1988).

Defective peroxisomal β-oxidation system

Singh et al. (1984) were the first to demonstrate that oxidation of the very long chain fatty acid, lignoceric acid (C24 : 0), is strongly deficient in Zellweger fibroblasts (see also Poulos et al. 1986; Wanders et al. 1987a,b). In 1985, Tager et al. showed that the absence of peroxisomes in Zellweger patients was associated with a deficiency of the three peroxisomal β-oxidation enzyme proteins acyl-CoA oxidase, bifunctional protein and peroxisomal thiolase (see also Lazarow et al. 1985; Suzuki et al. 1986; Chen et al. 1987). These findings immediately explain why very long chain fatty acids accumulate in Zellweger patients and, since the peroxisomal thiolase is required for the side chain cleavage of di- and trihydroxycholestanoic acid (Schram et al. 1987), why there is an accumulation of these bile acid intermediates in Zellweger patients.

Accumulation of pipecolic acid

Danks et al. (1975) first reported that pipecolic acid accumulates in body fluids from Zellweger patients. Dancis and Hutzler (1986) have shown that the pipecolic acid which accumulates is of the L-configuration and that L-pipecolic acid levels in Zellweger patients increase with age, being normal in Zellweger infants analyzed at 4 and 10 days of age (Lam et al. 1986). Oral loading tests by Trijbels et al. (1979) suggested that the accumulation was due to an impairment in pipecolic acid degradation. Recent studies by Mihalik and Rhead (1988) and Wanders et al. (1988d) indicate that this is, indeed, true.

Accumulation of phytanic acid

Several authors have reported on the accumulation of phytanic acid in body fluids from Zellweger patients (Poulos et al. 1984a; Wanders et al. 1987e). The accumulation of phytanic acid in Zellweger patients was found to be age-related (Wanders et al. 1987e). Since phytanic acid is almost exclusively derived from dietary sources, this is most probably the resultant of dietary intake combined with a defective catabolism of this compound. Studies in cultured skin fibroblasts have, indeed, established that

[^{14}C]CO_2 release from [1-^{14}C]phytanic acid is strongly reduced in Zellweger fibroblasts (Poulos et al. 1984b; Skjeldahl et al. 1986; Stokke et al. 1986).

Normal activity and aberrant subcellular distribution of catalase and other peroxisomal matrix enzymes

Several peroxisomal enzymes have been found to show normal activity in liver and fibroblasts from Zellweger patients. These include catalase, D-amino acid oxidase, L-α-hydroxy acid oxidase A and alanine: glyoxylate aminotransferase (Wanders et al. 1984, 1987d; Lazarow et al. 1985; Santos et al. 1985). Subcellular fractionation studies in liver and cultured skin fibroblasts have shown that the enzymes are present in the soluble cytoplasm. Apparently, these enzymes are stable in the cytosol and are not rapidly degraded as in the case of the peroxisomal β-oxidation enzyme proteins (Schram et al. 1986).

Mitochondrial abnormalities

In the classic paper by Goldfischer et al. (1973) in which the absence of peroxisomes in Zellweger patients was first reported, mitochondria were shown to be defective in electron transport. Studies by a number of other workers (Mathis et al. 1980; Kelley and Corkey 1983; Sarnat et al. 1983; Trijbels et al. 1983; Müller-Höcker et al. 1984) have confirmed the existence of mitochondrial abnormalities in Zellweger syndrome. It remains to be established what the significance of these findings for the pathophysiology of Zellweger syndrome is. The finding of normal lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios (Trijbels et al. 1983) would suggest that the consequences of the mitochondrial abnormalities in vivo are mild.

ZELLWEGER SYNDROME: ONLY ONE OF A GROUP OF PEROXISOMAL DISORDERS

In recent years it has become clear that the Zellweger syndrome is only one of a group of genetic diseases caused by an impairment in one or more peroxisomal functions. Identification of the different peroxisomal disorders was in some cases based upon certain similarities in clinical abnormalities (e.g., rhizomelic chondrodysplasia punctata) whereas in other cases a certain disease could be classified as a disorder of the peroxisome on the basis of a biochemical abnormality known to occur in Zellweger patients (e.g., the accumulation of VLCFAs in X-linked adrenoleukodystrophy (Brown et al. 1982)). The peroxisomal disorders identified so far can be classified into 3 groups depending upon whether there is a generalized, multiple or single loss of peroxisomal functions (Table 3).

A) Peroxisomal disorders characterized by a generalized loss of peroxisomal functions

The disorders belonging to this category are Zellweger syndrome, neonatal adrenoleukodystrophy (ALD), infantile Refsum disease and hyperpipecolic acidemia.

TABLE 3

CLASSIFICATION OF PEROXISOMAL DISORDERS

	Enzyme defect
A) *Generalized loss of peroxisomal functions*	
Cerebro-hepato-renal syndrome of Zellweger	Generalized
Neonatal adrenoleukodystrophy	
Infantile Refsum disease	
Hyperpipecolic acidaemia	
B) *Loss of multiple peroxisomal functions*	
Rhizomelic chondrodysplasia punctata	DHAPAT, alkyl DHAP synthase, phytanic acid oxidase
Combined peroxisomal β-oxidation enzyme deficiency (Zellweger-like syndrome)	peroxisomal β-oxidation enzyme proteins
C) *Loss of a single peroxisomal function*	
Adrenoleukodystrophy	Peroxisomal very-long-chain fatty acyl-CoA synthetase
Thiolase deficiency (pseudo-Zellweger syndrome)	Peroxisomal thiolase
Acyl-CoA oxidase deficiency (pseudo-neonatal ALD)	Acyl-CoA oxidase
Classical Refsum disease	Phytanic acid oxidase
Hyperoxaluria type I	Alanine:glyoxylate aminotransferase
Acatalasaemia	Catalase

Neonatal adrenoleukodystrophy

Neonatal ALD was first described by Ulrich et al. (1978) as connatal ALD in a boy who presented at birth with hypotonia, convulsions, absent grasp reflex, slight Moro response, and little spontaneous movements. The patient showed all signs diagnostic of adrenoleukodystrophy including the characteristic demyelination of the central nervous system (CNS) white matter, atrophy of the adrenal cortex, ballooned adrenocortical cells and splinter-like lamellar elements composed of electron-dense leaflets separated by a clear space. However, there were also a number of striking CNS abnormalities not described in the X-linked form of ALD such as cerebral heterotopia, micropachygyria and other gray matter changes reflecting general cerebral maldevelopment. In subsequent years more than 30 additional cases of neonatal ALD have been described (for a recent review, see Kelley et al. 1986). Despite the fact that the central nervous system and adrenal gland abnormalities in neonatal and X-linked ALD show similarities, it is clear that the two forms of ALD differ sharply and are in fact completely different disorders (Kelley et al. 1986). First, the mode of inheritance is different in the two disorders. Second, the onset of symptoms in neonatal ALD is before 1 year of age, whereas X-linked ALD patients are asymptomatic at least up to 3 years of age. Third, there are important differences in clinical presentation. Benke et al. (1981) were the first to point out the clinical similarity between neonatal ALD and Zellweger

syndrome and it is now clear that Zellweger syndrome and neonatal ALD resemble one another in their principal clinical and biochemical features although differences have been reported. Facial dysmorphia, for instance, a characteristic finding in Zellweger syndrome (Table 1) is less frequent in neonatal ALD. Furthermore, in contrast to most Zellweger patients neonatal ALD patients show some psychomotor development and develop certain milestones before regression sets in. Computerized tomography may show contrast enhancement around demyelinating areas. As in the Zellweger syndrome, neuropathological abnormalities in neonatal ALD are diverse, being dysontogenetic and degenerative in nature. This is reflected in the finding of a.o. demyelination in the cerebral hemispheres, cerebellum and brainstem, neuronal migration disturbances, neocortical microgyria and perivascular lymphocytic infiltrates.

Kelley and coworkers (1986) have suggested criteria to discriminate between Zellweger syndrome and neonatal ALD. According to these criteria patients with neonatal ALD would demonstrate adrenal atrophy, cerebral demyelination, systemic infiltration by abnormal lipid-laden macrophages and elevated levels of saturated VLCFAs (notably C26 : 0) whereas Zellweger patients would have chondrodysplasia, glomerulocystic disease of the kidney, CNS dysmyelination rather than demyelination and elevated levels of both saturated and unsaturated VLCFAs (C26 : 0 and C26 : 1) (see also Molzer et al. 1986). It should be noted, however, that adrenal atrophy, for instance, is also found in Zellweger patients (Gilchrist et al. 1976; Goldfischer et al. 1983).

Partin and McAdams (1983) were the first to report that peroxisomes were absent in liver from a neonatal ALD patient. In another patient studied by Goldfischer et al. (1985) peroxisomes were found to be scarce and much smaller as in control liver. As a result of the deficiency of peroxisomes the same set of biochemical abnormalities is seen in neonatal ALD as in Zellweger syndrome including the deficiency of DHAPAT and alkyl DHAP synthase in fibroblasts, the impaired oxidation of very long chain fatty acids due to the deficiency of all peroxisomal β-oxidation enzyme proteins and the accumulation of very long chain fatty acids, bile acid intermediates, phytanic acid and pipecolic acid in body fluids (Brown et al. 1982; Kelley and Moser 1984; Aubourgh et al. 1986; Kelley et al. 1986; Chen et al. 1987).

Infantile Refsum disease

Infantile Refsum disease was first described in 1982 (Boltshauser et al. 1982; Scotto et al. 1982). The patients showed mental retardation, minor facial dysmorphia, retinitis pigmentosa, failure to thrive and hypocholesterolaemia. Phytanic acid levels were found to be elevated in serum from the patients thus suggesting that the patients were suffering from an infantile form of phytanic acid storage disease (Refsum disease). Later it was found that biochemical abnormalities included the accumulation not only of phytanic acid, but also of very long chain fatty acids, abnormal bile acids and pipecolic acid (Poll-Thé et al. 1986). Similar findings were obtained in other patients (Poulos and Sharp 1984; Poulos and Whiting 1985; Budden et al. 1986). It is now clear that these abnormalities result from a deficiency of peroxisomes as has been shown in liver (Ogier et al. 1985; Budden et al. 1986; Roels et al. 1986) and fibroblasts (Wanders et al. 1986b).

Poll-Thé et al. (1987) recently discussed the clinical presentation of infantile Refsum disease in relation to neonatal ALD and the Zellweger syndrome. The absence of distinct abnormalities in the neonatal period, together with the fact that dysmorphia was only minor and patients were able to explore objects bimanually and to walk independently, indicates that the clinical course of infantile Refsum disease is milder than that of Zellweger syndrome or neonatal ALD. The patients described by Poll-Thé et al. (1987) are still alive at 9, 10 and 12 years of age, respectively. The patients described by Budden et al. (1986) were very similar to the patients described by Poll-Thé et al. (1987) although regressive changes such as retinopathy and sensorineural hearing loss started earlier (see also Weleber et al. 1983).

Recently, Torvik et al. (1988) described the first autopsy findings in a patient affected by infantile Refsum disease who died at 12 years of age. Evaluation of 11 years of age showed severe growth retardation with normocephaly. His face was typical with sunken eyes, high prominent nasal bridge, numerous ectatic veins, redundant skin folds in the neck and a small mouth. The patient did not speak, gave no reaction to visual or sound stimuli and could not walk. Other abnormalities included tapetoretinal degeneration, bilateral simian creases and spasticity with myoclonic fits. Whereas in the Zellweger syndrome cerebral malformations with micropolygyria, cerebral and cerebellar heterotopias and olivary dysplasia are almost invariable findings, the case of Torvik et al. (1988) showed no malformations of the cerebral cortex and no heterotopias of the cerebral or cerebellar white matter. Furthermore, there were no cortical renal cysts, skeletal changes or liver damage. The case of Torvik et al. (1988) was also found to differ from neonatal ALD in a number of respects. Indeed, whereas cases with neonatal ALD show mild cerebral malformations, active demyelination, degenerative changes of the adrenals, liver changes and bilamellar inclusions in macrophages, the case by Torvik et al. (1988) lacked active demyelination, cerebral cortical malformations and adrenal degeneration.

Recently, Schrakamp et al. (1988) studied de novo plasmalogen biosynthesis in fibroblasts from Zellweger, neonatal ALD and infantile Refsum patients using the sensitive double-label test described above. They found that the impairment in plasmalogen biosynthesis was on the average most severe in Zellweger syndrome, followed by neonatal ALD and finally, infantile Refsum disease.

Hyperpipecolic acidemia

Hyperpipecolic acidemia was first described by Gatfield et al. (1968) in a male infant with hepatomegaly, retinopathy and a progressive, neurologic disorder. The patient's serum was subsequently found to contain an unusual compound, pipecolic acid. Three additional patients have been reported in literature (Thomas et al. 1975; Burton et al. 1981). Several authors have pointed out that there is a strong resemblance in clinical presentation between the hyperpipecolic acidemia patients described and Zellweger patients. We have recently studied fibroblasts from the patient described by Thomas et al. (1975) and found a generalized loss of peroxisomal functions due to a deficiency of peroxisomes (Wanders et al. 1988c). Recent studies by Moser and coworkers (H. W. Moser, personal communication) in fibroblasts from the patients

described by Gatfield et al. (1968) and Burton et al. (1981) have shown that the same is true for these patients. Burton et al. (1981), however, reported the presence of peroxisomes in liver material from one of the patients. The reason for this discrepancy is not clear.

B) Peroxisomal disorders characterized by an impairment in several peroxisomal functions

Chondrodysplasia punctata represents a genetically heterogeneous group of bone dysplasias whose common feature is stippling of the epiphyses in infancy. According to Spranger et al. (1971) two major types can be recognized: the rhizomelic type with an autosomal-recessive mode of inheritance (RCDP) and the Conradi-Hunermann type with autosomal dominant inheritance. The rhizomelic type is clinically characterized by a disproportionally short stature primarily affecting the proximal parts of the extremities, typical facial appearance, congenital contractures, characteristic ocular involvement and severe mental and growth retardation (Spranger et al. 1971). Radiological studies reveal shortening, metaphyseal cupping and disturbed ossification of humeri and/or femora, together with epiphyseal and extra-epiphyseal calcifications (Spranger et al. 1971). Kretzner et al. (1981) suggested the involvement of a related biochemical defect in RCDP and Zellweger syndrome based upon the striking resemblance in ocular abnormalities. Indeed, Heymans et al. (1985, 1986) found that in RCDP, as in the Zellweger syndrome, plasmalogen levels in erythrocytes are reduced, and that the activity of DHAPAT in blood platelets of RCDP patients is deficient. Furthermore, phytanic acid levels were found to be strongly elevated in RCDP patients. Studies in cultured skin fibroblasts have shown that DHAPAT and alkyl DHAP synthase and, in addition, phytanic acid oxidase (Hoefler et al. 1988; Schutgens et al. 1988) are deficient in the patients' fibroblasts. Furthermore, studies by Hoefler et al. (1988) and ourselves (Heikoop et al. 1988) have shown that in RCDP peroxisomal 3-oxoacyl-CoA thiolase is present in the 44 kDa precursor form rather than in its mature 41 kDa form. We have carried out ultrastructural studies in liver biopsy material from 2 RCPD patients (Dingemans and Heymans, unpublished observations). In most hepatocytes peroxisomes were undetectable whilst in others an increased number of irregularly shaped peroxisomes were found. Additional studies will have to be carried out in order to ascertain the reason for the heterogeneous picture in the liver.

Recently, Holmes et al. (1987b) reported on a partial deficiency of DHAPAT in fibroblasts from a patient with the Conradi-Hunermann form of chondrodysplasia punctata. Studies in the authors' laboratories, however, have failed to reveal peroxisomal abnormalities in fibroblasts from patients with the Conradi-Hunermann or X-linked dominant type of the disease (Schutgens et al. 1988).

Combined oxidase-bifunctional protein-thiolase deficiency (Zellweger-like syndrome)

Recently Paturneau-Jouas et al. (1987) and Suzuki et al. (1988) reported on two patients with a clinical presentation indistinguishable from classical Zellweger syndrome but showing abundant peroxisomes in liver. In both patients the three peroxisomal β-oxidation enzyme proteins were found to be absent as shown by immunoblotting. Other peroxisomal enzyme activities, however, were found to be normal.

C) Peroxisomal disorders characterized by an impairment in only one peroxisomal function

Adrenoleukodystrophy (adreno-testiculo-leuko-myelo-neuropathic complex)

The phenotypic presentation of X-linked adrenoleukodystrophy varies considerably. Childhood ALD is the most stereotypic form of ALD occurring in 41% of the 409 cases studied by Moser et al. (1987). The typical presentation is that of a boy who progresses normally for the first 5 years of life and then presents with CNS signs and symptoms such as behaviour abnormalities, visual or auditory disturbances and abnormal gait. The mean age of onset is 7.2 ± 1.7 years (Moser et al. 1987). The course of the disease is progressive, culminating within a few years in dementia, blindness, quadriplegia and death. Bronzing of the skin secondary to adrenal dysfunction may occur. Seizures are usually a late manifestation in ALD.

In the childhood form of ALD, the most striking pathological changes occur in the adrenal glands and in the central white matter (for review, see Powers 1985). Schaumburg et al. (1975) described three areas of CNS abnormalities: areas of lipid-laden macrophages and myelin destruction, areas of perivascular mononuclear cell response (frontal edge of the lesion), and the largest area consisting of dense glial fibrils and scattered astrocytes without evidence of an active process. In adrenal glands, the adrenocortical cells are ballooned and striated due to accumulations of lamellae, lamellar-lipid profiles and fine lipid clefts. The histologic and ultrastructural lesion can exist without evidence of disturbed cortisol production even following adrenocorticotropin (ACTH) stimulation. Similar inclusions have been found in the cytoplasm of CNS macrophages, peripheral Schwann cells and Leydig cells (Powers 1985).

Visual disturbances are an early feature in ALD. Upon histopathological studies Cohen et al. (1983) observed that the characteristic bileaflet inclusions described above are present in optic nerve macrophages and retinal neurons. Furthermore, there was loss of ganglion cell and never fiber layers.

Adrenomyeloneuropathy (AMN) is the second most commonly observed ALD phenotype occurring in 21% of the 409 cases analyzed by Moser et al. (1987) with primary involvement of the spinal cord and peripheral nerves. Patients have signs of myelopathy with polyneuropathy and bladder dysfunction. The onset of neurological symptoms may be preceded by signs of Addison disease.

Other forms of the disease include adolescent ALD (10%), adult cerebral ALD (3%), Addison disease without neurologic involvement (7%) and those individuals without overt clinical symptoms but who demonstrate the biochemical abnormality (Moser et al. 1987). Furthermore, females heterozygous for ALD may also be symptomatic presenting with a chronic non-progressive spinal cord disorder. It is remarkable that these different ALD phenotypes may occur within the same pedigree (see Moser et al. 1987).

It is now well established that there is an accumulation of very long chain fatty acids in tissues, blood cells and body fluids from ALD patients. Studies by Singh et al. (1984) have clearly shown that the VLCFA accumulation results from an impairment in their degradation which normally occurs in peroxisomes. Based upon the finding that the CoA-esters of VLCFAs are oxidized at normal rates in X-ALD fibroblasts, Hashmi

et al. (1986) suggested that the activation of very long chain fatty acids to their CoA-esters is deficient in X-ALD. We have recently demonstrated directly that the deficient oxidation of VLCFAs in X-ALD fibroblasts is, indeed, due to an impaired ability of peroxisomes to activate very long chain fatty acids (Wanders et al. 1988b).

Peroxisomal 3-oxoacyl-CoA thiolase deficiency (pseudo-Zellweger syndrome)

Goldfischer et al. (1986) described a girl from consanguinous parents, showing marked facial dysmorphia, muscle weakness and hypotonia at birth. Intractable seizures developed soon after birth and the patient showed no psychomotor development during her life of 11 months. At autopsy the patient had renal cysts, atrophic adrenals with striated cells, minimal liver fibrosis, hypomyelination in the cerebral white matter, foci of neuronal heterotopia, and a sudanophilic leukodystrophy. Based upon these findings the patient was diagnosed as suffering from Zellweger syndrome. However, upon morphological analysis the patient's liver was found to contain abundant peroxisomes. Peroxisomal involvement was indicated, however, by the finding of elevated very long chain fatty acids in the patient's plasma and bile acid intermediates in a duodenal aspirate. Further studies revealed that the defect in this patient was at the level of a deficiency of the peroxisomal 3-oxoacyl-CoA thiolase enzyme protein as shown by immunoblotting (Schram et al. 1987). Other peroxisomal parameters (plasmalogen biosynthesis, phytanic acid oxidation, particle-bound catalase) were normal.

Refsum disease (phytanic acid storage disease)

Phytanic acid storage disease (Refsum disease) in its classical presentation is an autosomal recessive disorder of lipid metabolism characterized by the accumulation of phytanic acid in blood and tissues due to a deficient oxidation of this 20-carbon branched chain fatty acid (for review, see Steinberg 1983). This disease is characterized by retinitis pigmentosa, peripheral polyneuropathy, cerebellar ataxia, and elevated CSF protein levels, although this classical tetrad of abnormalities is not seen in every patient. In a recent paper by Skjeldahl et al. (1987), for instance, cerebellar ataxia was found to be present in only 5 of the 17 patients studied. Furthermore, phenotypic variants of Refsum disease have been described lacking retinitis pigmentosa and cerebellar ataxia (Dotti et al. 1985).

In patients with Refsum disease accumulation of phytanic acid is the only known abnormality (Wanders et al. 1988a). It remains to be established whether or not Refsum disease belongs to the group of peroxisomal disorders since at present there is only indirect evidence suggesting that phytanic acid oxidase is localized in peroxisomes in man.

Acyl-CoA oxidase deficiency (pseudo-neonatal adrenoleukodystrophy)

Recently Poll-Thé et al. (1988a) described two siblings born from consanguinous parents with clinical manifestations similar to those observed in patients suffering from neonatal ALD. Extreme muscular hypotonia and absence of reflexes were present at birth. There was no dysmorphia in either patient. During the first year of life the older sibling was hospitalized several times for stridor, apneic spells, and short tonic seizures.

Although psychomotor development was severely delayed, both patients showed some progress in the first 2 years of life, after which there was a progressive neurological regression. A sensorineural hearing deficit was apparent in both patients. The electroretinograms were abnormal and flash-evoked visual responses virtually absent. These findings led to the diagnosis neonatal ALD. However, peroxisomes were found to be present in the liver. In fact, hepatic peroxisomes were increased in number and of enlarged size. Subsequent studies revealed that there was a deficiency of acyl-CoA oxidase, the first enzyme involved in peroxisomal β-oxidation. This finding explains the accumulation of VLCFAs in these patients. Other peroxisomal functions were found to be normal (Poll-Thé et al. 1988a).

There was no accumulation of bile acid intermediates in these patients indicating that acyl-CoA oxidase, in contrast to peroxisomal thiolase, is not involved in the side-chain cleavage of di- and trihydroxycholestanoic acid.

Hyperoxaluria type I and acatalasia

Since there is no neurological involvement in these two peroxisomal disorders, we will not discuss them in depth. Suffice it to say that hyperoxaluria type I is associated with a deficiency of the peroxisomal enzyme alanine glyoxylate aminotransferase (Danpure and Jennings 1986), whereas in acatalasia catalase is deficient.

Apart from the peroxisomal disorders discussed above, there are several disorders such as cerebrotendinous xanthomatosis (CTX) and Canavan disease suggested to belong to the group of peroxisomal disorders (Goldfischer and Reddy 1984). However, available evidence suggests that CTX is due to a deficient mitochondrial 26-hydroxylase activity (Oftebro et al. 1980), whereas in Canavan disease the defect is at the level of a deficient activity of *N*-acetylaspartate hydrolase (Matalon et al. 1988).

Clinical guidelines for the identification of peroxisomal disorders

In Table 4 a summary is given of the clinical manifestations in the various peroxisomal disorders.

Although the history of the peroxisomal disorders is relatively young, it is clear that the clinical presentation of the peroxisomal disorders can vary greatly. This is not only true for X-linked ALD and classical Refsum disease (see above), but especially so for the peroxisomal disorders characterized by the (virtual) absence of morphologically distinct peroxisomes. Indeed, the (virtual) absence of peroxisomes can be associated with the full spectrum of clinical abnormalities as in classical Zellweger syndrome (Table 1) but can also result in a much milder clinical presentation as in some patients with infantile Refsum disease (Poll-Thé et al. 1986; Budden et al. 1986) or mild variants of Zellweger syndrome (Barth et al. 1985, 1987; Bleeker-Wagemaker et al. 1986; Ek et al. 1986).

An important aspect is that the clinical symptomatology is very much dependent upon the age of the patient at presentation (Poll-Thé et al. 1987). Indeed, at birth the clinical picture is dominated by severe hypotonia and craniofacial dysmorphia whereas in patients 6 months of age or older major symptoms are psychomotor retardation, failure to thrive, epileptic seizures and hepatomegaly (Heymans 1984).

TABLE 4
CLINICAL CHARACTERISTICS OF THE PEROXISOMAL DISORDERS

General	ZS	NALD	IRD	HPA	RCDP	ALD	Pseudo-ZS	Pseudo-NALD	Refsum
Mode of inheritance	Aut.rec.	Aut.rec.	Aut.rec.	Aut.rec.	Aut.rec.	X-linked	Aut.rec.	Aut.rec.	Aut.rec.
Age of onset	At birth	At birth 1 y	1–6 m	At birth	At birth	Childhood	At birth	At birth	Childhood
Age of death	Usually <1 y	$1\frac{1}{2}$–10 y	>$2\frac{1}{2}$–>13 y	$1\frac{1}{2}$–>6 y	>1 y–>16 y	within 2 y after onset	<1 y	>$2\frac{1}{2}$–5 y	Adult life
Craniofacial dysmorphia									
Wide open fontanels	+ + +	+ +	+	+ +		−	+ + +	−	−
High forehead	+ + +	+ +	+	+	+ + +	−	+ + +	−	−
Epicanthus	+ + +	+ +	+ +	+	+ + +	−	+ + +	−	−
External ear deformities	+ + +	+ +	+	+ +		−	+ + +	−	−
High arched palate	+ + +	+ +	+	+ +		−	+ + +	−	−
Neurological abnormalities									
Severe hypotonia	+ + +	+ +	+	+ +	+ +	−	+ + +	+ + +	−
Hypo-, areflexia	+ + +	+ +	+	+		−	+ + +	−	+ +
Seizures	+ + +	+ +	+	+ +		+ +	+ + +	+ + +	−
Nystagmus	+ + +	+ +	+	+ +		+	+ +	+ + +	+
Impaired hearing	+ + +	+ + +	+ + +	+ +	+ + +	+	+ + +	+ + +	+ +
Neurological deterioration	−	+ +	+	+ +		+ + +	+ + +	+ +	+ +
Ocular abnormalities									
Cataract	+ + +	+	−		+ + +	−	+ + +	−	+ +
Abnormal retinal pigmentation	+ + +	+ + +	+ + +	+ + +		−	+ + +	+ + +	+ + +
Optic nerve dysplasia/atrophy	+ + +	+ + +	+	+ + +		+ +	+ + +	+ + +	+
Liver abnormalities									
Hepatomegaly	+ + +	+ +	+	+ +		−	+ + +	+	−
Fibrosis/micronodular cirrhosis	+ + +	+ +	+	+ +		−	+	−	−
Lamellar inclusions	+ +	+ + +	+ + +			−	+	−	−
Renal abnormalities									
Renal cysts	+ + +	−	−	−		−	+	−	−
Adrenal abnormalities									
Adrenal atrophy	+	+ + +		−		+ + +	+		−
Lamellar inclusions	+	+ + +		+		+ + +	+	−	−
Diminished response upon ACTH	+ +	+ +	+	−		+ + +		+	−
Other abnormalities									
Cryptorchidism/cliteromegaly	+ + +	+ +	+	+		−	+	+	−
Chondrodysplasia calcificans	+ +	−	−	−	+ + +	−		−	−

Symbols: + + + = feature almost invariably present; + + = frequently present; + = present; − = absent.
Abbreviations: ZS = Zellweger syndrome; NALD = neonatal ALD; IRD = infantile Refsum disease; HPA = hyperpipecolic acidaemia.

Monnens and Heymans (1987) have suggested that biochemical investigations of peroxisomal functions should be done if a patient shows two or more of the following abnormalities:

cranio-facial abnormalities;

neurological abnormalities (hypotonia, seizures, nystagmus, hearing deficiencies, white matter degeneration);

ocular abnormalities (cataract, chorioretinopathy, abnormal ERG, optic nerve dysplasia/atrophy);

hepatological abnormalities (hepatomegaly, liver function disturbances, fibrosis/cirrhosis);

skeletal abnormalities (calcific stippling, rhizomelic shortening of the limbs).

POSTNATAL DIAGNOSIS OF PEROXISOMAL DISORDERS

The biochemical abnormalities associated with the peroxisomal disorders are summarized in Table 5. In 8 of the 10 peroxisomal disorders in which there is neurological involvement, there is an accumulation of VLCFAs which suggests that VLCFA analysis should be used as a primary test rather than analysis of pipecolic acid or phytanic acid, or bile acid intermediates (Clayton et al. 1987; Gustafsson et al. 1987; Van Elderen et al. 1987).

When VLCFA analysis is used as a screening method, rhizomelic chondrodysplasia punctata and adult Refsum disease will be missed. However, the clinical presentation of these entities is usually (see, however, Skjeldal et al. 1987) clear enough to avoid difficulties. Hence, very long chain fatty acid analysis via gaschromatography remains the method of choice. Most laboratories utilize plasma or serum for VLCFA analysis although Nishio et al. (1986) recently described that blood spotted onto filter paper and dried, can also be used.

In order to elucidate whether the accumulation of VLCFAs in a certain patient's plasma results from the absence of peroxisomes or is caused by a defect in one of the peroxisomal β-oxidation enzyme activities, additional investigations in plasma or other body fluids (bile acid intermediates, pipecolic acid and phytanic acid), blood cells (DHAPAT in platelets, plasmalogen levels in erythrocytes), cultured skin fibroblasts (DHAPAT, de novo plasmalogen synthesis, C26:0 β-oxidation, phytanic acid oxidation, measurement of the amount of particle-bound catalase) or liver (immunoblotting, L-pipecolate oxidase activity) will have to be carried out. If these studies indicate that the defect is restricted to the peroxisomal β-oxidation system as in pseudo-Zellweger syndrome, pseudo-neonatal ALD, X-linked ALD and Zellweger-like syndrome, subsequent immunological and enzymic studies will have to be done to elucidate the specific enzyme defect. A word of caution is necessary with regard to immunological studies. A mutation can effect the active site of an enzyme in such a way that activity is lost without resulting in reduced enzyme protein levels as detected by immunoblotting. For this reason one should always measure the activity of the individual peroxisomal β-oxidation enzymes. At present this can be done only in liver material. Specific antisera

TABLE 5

BIOCHEMICAL CHARACTERISTICS OF THE PEROXISOMAL DISORDERS

Parameter measured	Disease					
	PDDs	RCDP	ALD	Pseudo-ZS	Pseudo-NALD	Refsum
Metabolites in body fluids						
Very long chain fatty acids	Elevated	Normal	Elevated	Elevated	Elevated	Normal
Bile acid intermediates	Elevated	Normal	Normal	Elevated	Normal	Normal
Pipecolic acid	Elevated*	Normal	Normal	Normal	Normal	Normal
Phytanic acid	Elevated*	Elevated	Normal	Normal	Normal	Elevated
Plasmalogen synthesis						
DHAPAT	Deficient	Deficient	Normal	Normal	Normal	Normal
Alkyl DHAP synthase	Deficient	Deficient	Normal	Normal	Normal	Normal
De novo synthesis	Impaired	Impaired	Normal	Normal	Normal	Normal
Peroxisomes						
Hepatic peroxisomes	(Virtually) absent		Normal	Normal	Normal	Normal
Particle-bound catalase in fibroblasts (% of total)	<5	>65	>65	>65	>65	>65
Peroxisomal β-oxidation						
VLCFA β-oxidation in fibroblasts	Deficient	Normal	Deficient	Deficient	Deficient	Normal
Enzyme proteins						
Acyl-CoA oxidase	Deficient	Normal	Normal	Normal	Absent	Normal
Bifunctional protein	Deficient	Normal	Normal	Normal	Normal	Normal
Peroxisomal thiolase	Deficient	Normal**	Normal	Absent	Normal	Normal

* Age-dependent.

** Present as 44 kDa precursor. Abbreviations used: PDDs = peroxisome deficiency disorders (Zellweger syndrome, neonatal ALD, infantile Refsum disease, hyperpipecolic acidaemia). Other abbreviations: see text.

are used to eliminate the mitochondrial β-oxidation enzymes by immunoprecipitation and this is followed by activity measurements of the peroxisomal β-oxidation enzymes using specific enzyme assays (Suzuki et al. 1986).

Heterozygote detection

At present heterozygote detection is only possible in X-linked ALD using VLCFA analysis or restriction fragment lengths polymorphism (RFLP) using the DNA probe St14 (Aubourg et al. 1987). In Zellweger syndrome and the other peroxisome deficiency disorders, no heterozygote detection is possible at present since the abnormalities observed in the patients are secondary effects resulting from a primary lesion the nature of which is not yet known. In the peroxisomal β-oxidation enzyme deficiencies it should, in principle, be possible to detect heterozygotes by measuring the activity of the enzyme involved.

Prenatal diagnosis

Various methods are available for prenatal diagnosis of any of the peroxisomal disorders known today. These are listed in Table 6. Especially in the case of the disorders with a generalized loss of peroxisomal functions, there are several methods available. Since some of these tests can only be done in chorionic villous fibroblasts and not in chorionic villous biopsy material, we recommend that chorionic villous fibroblasts be used whether or not preceded by more limited investigations in biopsy material (Hajra et al. (1985): DHAPAT activity measurements; Rocchiccioli et al. (1987): VLCFA analyses; Roels et al. (1987): visualization of peroxisomes and/or plasmalogens).

TABLE 6

PRENATAL DIAGNOSIS IN PEROXISOMAL DISORDERS

Peroxisomal disorder	Material	Type of analysis
Peroxisome deficiency disorders	CB	VLCFA, DHAPAT, peroxisomes, plasmalogens
	CVF, AC	VLCFA, DHAPAT, plasmalogen biosynthesis, particle-bound catalase, phytanic acid oxidase
Rhizomelic chondrodysplasia	CB	DHAPAT, plasmalogens
punctata	CVF, AC	DHAPAT, de novo plasmalogen biosynthesis, thiolase (immunoblotting)
Adrenoleukodystrophy	CB	VLCFA, RFLP-studies
	CVF, AC	VLCFA, C26:0 β-oxidation, RFLP-studies (St14 probe)
Thiolase-deficiency	CB	VLCFA
	CVF, AC	VLCFA, C26:0 β-oxidation, thiolase (immunoblotting)
Acyl-CoA oxidase deficiency	CB	VLCFA
	CVF, AC	VLCFA, C26:0 β-oxidation, acyl-CoA oxidase (immunoblotting)
Hyperoxaluria type I	Fetal liver biopsy	Alanine glyoxylate aminotransferase

Abbreviations: CB = chorion biopsy material; CVF = chorionic villous fibroblasts; AC = cultured amniocytes; VLCFA = very long chain fatty acids.

Genetic relationship between Zellweger syndrome and the other peroxisome deficiency disorders

In order to obtain information on the question of whether Zellweger syndrome, neonatal ALD, infantile Refsum disease and hyperpipecolic acidaemia are phenotypic variations of the same allelic mutation, complementation studies have been carried out (Roscher et al. 1986; Wanders et al. 1986a; Tager et al. 1987; Brul et al. 1988). In these studies fibroblasts from one patient (A) are fused with fibroblasts from another patient (B) using Sendai virus or polyethylene glycol giving rise to multinucleate cells. If the mutations in A and B affect different genes, then one would expect restoration of the deficient activity in the heterokaryons. If, however, the mutation in A affects the same

gene as in B, then no restoration of enzyme activity is expected. In the case of Zellweger syndrome and the other peroxisome deficiency disorders the occurrence of complementation can be tested by measuring de novo plasmalogen biosynthesis (Roscher et al. 1986), DHAPAT activity (Brul et al. 1988), particle-bound catalase (Brul et al. 1988) or phytanic acid oxidase activity (Poll-Thé 1988b) and in principle any of the enzyme activities deficient in Zellweger syndrome.

We have identified 4 different complementation groups within the group of peroxisomal disorders characterized by the (virtual) absence of peroxisomes (see Table 7). It should be added that Roscher et al. (personal communication) identified two complementation groups within neonatal ALD. These data demonstrate unequivocally that there is not only genetic heterogeneity within the group of peroxisome deficiency disorders but also within the Zellweger syndrome. It is intriguing that all the Zellweger cell lines from complementation group 1 are from patients ranging from 2 to 6 years of age. In contrast, the other Zellweger cell lines (ZS2 and ZS3) from complementation group 2 and 4 were from patients who died at the age of 3 and 5 months, respectively, showing all characteristics of classical Zellweger syndrome. These results indicate that mutations in two different genes can lead to the same clinical and biochemical phenotype.

TABLE 7

COMPLEMENTATION GROUPS WITHIN THE PEROXISOME DEFICIENCY DISORDERS

Complementation group	Phenotype
1	ZS 1 (4), IRD, HPA
2	ZS 2
3	Neonatal ALD
4	ZS 3

Data from Brul et al. (1988). The number within parenthesis indicates the number of different fibroblast cell lines.

ON THE PRIMARY DEFECT IN ZELLWEGER SYNDROME AND RELATED PEROXISOME DEFICIENCY DISORDERS

As discussed in previous sections several enzyme activities are deficient in disorders characterized by a deficiency of peroxisomes. It has been shown that at least in the case of the peroxisomal β-oxidation enzymes synthesis of the proteins occurs normally but that they are rapidly degraded in the cytosol of these peroxisome deficient cells (Schram et al. 1986). This could simply be due to the absence of a peroxisomal membrane. Lazarow et al. (1986), however, have found that the 22 kDa integral membrane protein found only in peroxisomes, is normally present in liver material from

Zellweger patients. Furthermore, this membrane protein was not found free in the cytosol as is the case with, e.g., catalase (see before), but is present within a membrane. Recently, using immunofluorescence microscopy Santos et al. (1988) have provided convincing evidence that the 22 kDa membrane protein (as well as other membrane proteins) is located in unusual, empty membrane structures, called "peroxisomal ghosts" with a size larger than that of normal peroxisomes. We have obtained similar results in Zellweger fibroblasts using an antiserum directed against the 69 kDa integral membrane protein from rat liver (Wiemer et al. 1988).

These results suggest that the primary defect in the Zellweger syndrome and the other peroxisome deficiency disorders is an inability to import proteins into peroxisomes. Most likely the defect is not at the level of the peroxisomal proteins themselves but at the level of the machinery required for translocation of peroxisomal proteins across the membrane. As discussed above, information about the uptake machinery of peroxisomes is at present sparse. There is probably the involvement of an ATP-utilizing protein during import and presumably receptor proteins are present (Imanaka et al. 1987).

PATHOGENESIS OF THE PEROXISOMAL DISORDERS

Much has been learned recently about the biochemical defects in the various peroxisomal disorders. However, the relationship between the biochemical abnormality and the pathogenesis of the disease has largely remained enigmatic even in the case of disorders such as X-linked ALD in which the only abnormality is the deficient oxidation of VLCFAs, leading to their accumulation in tissues and bodyfluids. Recent studies by Whitcomb et al. (1988) suggest that the adrenal insufficiency and atrophy in ALD is due to an increase in membrane microviscosity in adrenocortical cells brought about by the increase in VLCFAs. This effect of VLCFAs has, indeed, been shown experimentally (Knazek et al. 1983; Whitcomb et al. 1988). Since it is well known that an increase in membrane microviscosity results in a decreased number of hormone receptors Whitcomb et al. (1988) postulated that the adrenal insufficiency was due to a decreased ability to respond to ACTH. Supportive evidence for this hypothesis has recently been brought forward by Meyer et al. (1987) who reported that leukocytes from ALD patients lacked detectable ACTH binding sites in contrast to control leukocytes.

It has been suggested that the myelinolysis in ALD is due to the abnormalities in myelin lipids resulting from the high content of VLCFAs (Powers 1985). Gangliosides are substantial components of myelin and neuronal membranes. Hence, gangliosides containing high amounts of VLCFAs may play a major role in the induction of myelin instability. It is also argued (Powers 1985) that VLCFA-containing gangliosides are highly immunogenic and elicit an immune reaction. Macrophages and lymphocytes are then summoned to the myelinolytic region, where they attack the myelinated fibers remaining and the oligodendrocytes, thus leading to a fulminating and widespread lymphocyte-mediated destruction of cerebral myelin (Powers 1985).

In Zellweger syndrome one of the major abnormalities relates to the

neuromigrational defect which is already present in Zellweger fetuses (Powers et al. 1985). Since plasmalogens are major constituents of brain (see Wyckle 1977), we suspected that the neuromigrational defect might be due to the impairment in plasmalogen biosynthesis in Zellweger patients. However, the same abnormality was found in the pseudo-Zellweger patient described by Goldfischer et al. (1986). In this patient plasmalogen biosynthesis was normal. Due to a deficiency of peroxisomal 3-oxoacyl-CoA thiolase there was an accumulation of VLCFAs and bile acid intermediates in this patient, however. This suggests that there is no causal relationship between the impairment in plasmalogen biosynthesis and the neuromigrational defect. The data suggest that the neuromigrational defect is related to the accumulation of VLCFAs or bile acid intermediates. Since neuronal migration is normal in X-linked ALD, these data might be taken to indicate that the bile acid intermediates are responsible for the defect in neuronal migration in Zellweger syndrome. However, there are also differences in the extent of the accumulation of VLCFAs as well as in the pattern of VLCFAs that accumulate in X-linked ALD on the one hand and Zellweger syndrome on the other hand.

The same reasoning applies to other abnormalities such as fibrosis and cirrhosis of the liver observed in Zellweger patients (Table 1). In pseudo-Zellweger syndrome but not in X-linked ALD and acyl-CoA oxidase deficiency, fibrosis has been found. This might indicate that bile acid intermediates are involved in the liver damage. That bile acid intermediates, notably trihydroxycholestanoic acid are, indeed, very deleterious was shown by Lee et al. (1965). In rat liver mitochondria trihydroxycholestanoic acid was found to be a very effective inhibitor of oxidative phosphorylation.

It is clear that much remains to be learned about the relation between biochemical abnormalities and pathogenesis of the clinical signs and symptoms observed in the peroxisomal disorders.

THERAPY IN PEROXISOMAL DISORDERS

It is well known that dietary restriction of phytanic acid is clinically beneficial to patients with Refsum's disease. Indeed, peripheral nerve function improves and there is stabilisation of the retinal lesion and deficits resulting from central nervous involvement (Steinberg 1983).

In initial studies in X-linked ALD patients in which dietary intake of VLCFAs was restricted, there was no reduction in plasma C26 : 0 levels or clinical improvement. It is likely that the difference between Refsum disease and ALD relates to the fact that phytanic acid is solely derived from exogenous sources whereas VLCFAs originate from both endogenous as well as exogenous sources. Endogenous VLCFAs originate from fatty acids with shorter chain lengths via chain elongation.

Recently, Moser et al. (1987) and Rizzo et al. (1987) reported that C26 : 0 plasma levels in ALD patients could be reduced significantly by administration of glycerol trioleate (C18 : 1) to patients. Oleic acid probably exerts its effect by inhibiting the chain elongation of fatty acids to VLCFAs (Rizzo et al. 1986). It is not yet clear whether the

reduction in C26:0 levels in ALD patients on trioleate therapy, leads to clinical improvement or not. A large scale double-blind study is now being carried out by Moser and coworkers.

Recently, therapeutic measures have been taken in patients suffering from one of the peroxisome deficiency disorders. Holmes et al. (1987) succeeded in obtaining partial normalization of erythrocyte plasmalogen levels in one patient affected with a mild form of Zellweger syndrome. A therapeutic regime, consisting of a reduced intake of VLCFAs and phytanic acid plus administration of alkylglycerol to try and normalize plasmalogen levels, is at present being tried by Greenberg et al. (1987) in mildly affected Zellweger patients. Robertson et al. (1988) recently reported on 2 patients with infantile Refsum disease who were treated with a diet low in phytanic acid for 2 years. Some beneficial effect was noted. However, these efforts must be viewed with some caution since abnormalities already arise in utero (e.g., neuromigration defect).

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