Inborn Errors of Cellular Organelles: Peroxisomes and Mitochondria

edited by R. J. Pollitt,
A. H. van Gennip, C. J. de Groot,
G. M. Addison and R. A. Harkness

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Preface and Retrospect

The 24th Annual Symposium of the SSIEM was held at Amersfoort in the Netherlands. It was the first time that we had the honour of organising this meeting, which is becoming increasingly important. It is encouraging to count a growing number of active participants (291) coming from 25 countries. They submitted as many as 184 free communications. This memorably great participation reflects the need for communication in the field of inborn errors of metabolism and, at the same time, proves the viability of the SSIEM!

As the theme of the symposium the scientific committee chose "Inborn Errors of Cellular Organelles". This comprehensive subject emphasizes the relationship between structural defects of cell compartments and metabolic abnormalities. However, a comprehensive treatment of the subject was not possible in the time available. It was therefore decided to focus on peroxisomes and mitochondria; inevitably lysosomes had to be left out of the programme despite considerable innovations in this area.

Much progress has been made in the elucidation of peroxisomal disorders during the last decade and in particular during the last few years. Typical peroxisomal functions are catabolism of very long chain fatty acids, β-oxidation of dicarboxylic acids and catabolism of phytanic acid, breakdown of pipecolic acid, the oxidation of polyamines, the synthesis of bile acids and of plasmalogens. Diseases due to one impaired function and those resulting from a more generalized impairment have now been recognized. Peroxisomes may be absent or significantly reduced in number. Also abnormal morphology occurs. However, in most of the diseases the basic biochemical lesion remains to be established.

Invited speakers from New York, Baltimore, Amsterdam and Nijmegen shared their expertise, whilst in various free communications valuable additional results were provided.

Mitochondrial defects are a most interesting subject because of their far reaching clinical and metabolic consequences. Most of us are confronted with the differential diagnosis of persistent lactic acidosis. There are many patients with a defect of the respiratory chain, which has to be localized by what could be called "intracellular screening". But this kind of biochemical diagnosis is still the work of specialists. Not only the biochemical aspects but also the morphology is crucial for the characterization of the disorders.

The Symposium Lecture was dedicated to the mitochondrial myopathies and we were delighted that an expert in this field, Professor S. DiMauro from New York, could give this lecture. Invited speakers from Groningen, Nijmegen, Newcastle-upon-Tyne and Rotterdam discussed the biogenesis of mitochondria and the genetics of defects, morphological observations, the expression of defects, secondary pathology, defects of fatty acid oxidation in muscle diseases and therapy in mitochondrial

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disorders. A great number of free communications provided complementary information.

It was thought that magnetic resonance spectroscopy (MR) analysis should have a place in our programme. ^{31}P MR, especially, is becoming a promising tool for both non-invasive *in vivo* and *in vitro* metabolic investigations. It gives access to organs such as the central nervous system, liver and muscle, which were all practically inaccessible for metabolic studies until recently. We hope that this introduction will stimulate our members to incorporate the possibilities offered by MR in their research.

A wealth of new information was presented in the form of numerous free communications not related to the main theme of the symposium. This was a good demonstration of the growing activities of the SSIEM members! Poster presentations are extremely useful because they bring the latest news and offer the opportunity for starting cooperation and the exchange of experience on new subjects.

K. M. Gibson and co-workers from the University of California San Diego, La Jolla received the D. N. Raine award 1986 for their excellent work on mevalonic aciduria.

We are grateful for financial support from many sources – as explicitly acknowledged in the Symposium programme. This support has considerably contributed to the success of the Symposium.

We now look forward to the 25th Annual Symposium in Sheffield, 1987. This meeting will be a Silver Jubilee which, we trust, will bring along an exceptionally attractive programme.

S. K. Wadman
M. Duran

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Inborn Errors of Cellular Organelles: an Overview

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Metabolic processes in the cell are catalysed by enzymes and enzyme systems present in discrete intracellular compartments consisting of the cytosol and various intracellular organelles. Three well defined groups of genetic diseases in man can now be recognized in which the functions of an intracellular organelle are impaired: lysosomal storage diseases, mitochondrial disorders and peroxisomal diseases. Extensive studies carried out during the last decade on the biogenesis of intracellular organelles have contributed to an understanding of the molecular basis of the lesions leading to these three groups of genetic disorders. The results of the studies have stressed that such lesions can arise not only through mutations in the structural genes for the proteins in an organelle but also through mutations in the genes coding for components required for the specific transport and incorporation of proteins into organelles.

INTRODUCTION

Intracellular compartmentation forms the basis of metabolic regulation in living organisms. The number of different intracellular compartments recognized to be present in eukaryotic cells has increased dramatically in the last 20 years due to advances made in various fields of cell biology. In mammalian cells these compartments include the plasma membrane, the nucleus, the endoplasmic reticulum, the Golgi apparatus, the lysosomal apparatus, secretory and storage vesicles, the mitochondria, the peroxisomes, the cytoskeleton and the cytosol. Most of these compartments can be subdivided into several subcompartments.

This introduction to the topic Inborn Errors of Cellular Organelles will be restricted to a consideration of three organelles: mitochondria, peroxisomes and lysosomes. Particular attention will be paid to certain aspects of the biogenesis of the organelles since knowledge of these aspects is essential for understanding the molecular basis of inborn errors of metabolism involving cellular organelles.

MITOCHONDRIAL DISEASES

Mitochondrial diseases can be divided into two categories: those in which the genetic defect leads to an impairment of oxidative phosphorylation and those in

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which the genetic defect involves other mitochondrial functions (deficiency of carbamoylphosphate synthase, ornithine transcarbamoylase, *N*-acetylglutamate synthase, etc.). An impairment of mitochondrial oxidative phosphorylation can arise from a defect in mitochondrial oxidative phosphorylation, a defect in the respiratory chain or a defect in energy transduction (DiMauro *et al.*, 1985b; Morgan-Hughes, 1986).

The essential features of the biogenesis of mitochondria are summarized in Table 1.

Table 1 Biogenesis of intracellular organelles

Organelle	*Genetic information*	*Protein synthesized*	*Organelle arises*	*Expression of genes*
Mitochondrion	In nuclear and mitochondrial DNA	On free ribosomes in cytosol and mitochondrial ribosomes	By division	Tissue-specific
Peroxisome	In nuclear DNA	On free ribosomes in cytosol	By division	Tissue-specific
Lysosome	In nuclear DNA	On ribosomes bound to endoplasmic reticulum	By formation of vesicles in Golgi region	In all tissues and cells

1. The interaction between the nuclear and mitochondrial genomes in providing the genetic information for the biogenesis of mitochondria is the subject of the paper by Kroon and Van den Bogert (1987). Several instances of maternal inheritance of mitochondrial diseases have been reported (see, e.g., Egger and Wilson, 1983).

Mammalian mitochondria display considerable heterogeneity in enzymic composition (see Scholte and Veerkamp, 1981, for a review). An obvious example of such tissue-specific heterogeneity is the presence in liver mitochondria of enzymes of the ornithine cycles and gluconeogenesis. However, the heterogeneity extends even further.

Kadenbach and coworkers (reviewed in Kadenbach *et al.*, 1986) were the first to show that tissue-specific forms of cytochrome *c* oxidase occur in several mammalian species. Cytochrome *c* oxidase comprises 13 subunits, three of which (subunits I, II and III) are encoded for by mitochondrial DNA and the others (subunits IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc and VIII) by nuclear DNA. Tissue-specific differences occur in the subunits encoded for by nuclear DNA, whereas subunits I, II and III are identical in isoenzymes from all tissues in one particular species. This is also true of human cytochrome *c* oxidase. DiMauro and colleagues (1985a) have shown that a monoclonal antibody raised against subunit IV of human heart cytochrome *c* oxidase does not cross-react with the corresponding subunit in the skeletal muscle enzyme and Sinjorgo and colleagues (1987) have found that the forms of subunit VI present in the heart and skeletal muscle enzymes behave differently in sodium dodecyl sulphate–polyacrylamide gels.

These findings should provide an explanation for the fact that a deficiency of cytochrome *c* oxidase (and of other respiratory chain complexes) can be restricted

to certain tissues. For instance Rimoldi and colleagues (1982) showed that cytochrome *c* oxidase in a floppy infant was deficient in skeletal muscle but not in heart, brain or fibroblasts. Obviously the mutation must have affected the expression of a skeletal muscle-specific form of a subunit of cytochrome *c* oxidase and not that of the form(s) present in heart, brain and fibroblasts. This topic is pursued further in the papers by DiMauro (1987) and Scholte *et al.* (1987).

PEROXISOMAL DISEASES

Recently a new group of genetic diseases in man has been defined in which peroxisomal functions are impaired (for reviews see Goldfischer and Reddy, 1984; Kelley *et al.*, 1986; Moser, 1986; Schutgens *et al.*, 1986). The prototype of the peroxisomal disorders is the cerebro-hepato-renal (Zellweger) syndrome in which the entire organelle is deficient (Goldfischer *et al.*, 1973) and in which there is a generalized impairment of peroxisomal functions. Other diseases with a generalized impairment of peroxisomal functions are the infantile form of Refsum disease and the neonatal form of adrenoleukodystrophy (Table 2). In the rhizomelic form of chondrodysplasia punctata some, but not all, peroxisomal functions are impaired and peroxisomes are present (Table 2).

Table 2 Characteristics of some peroxisomal diseases[a]

Parameter	*Zellweger syndrome*	*Infantile Refsum disease*	*Neonatal ALD*	*Hyperpipecolic acidaemia*	*Chondro-dysplasia punctatum, rhizomelic*
Metabolites in body fluids					
C_{26}/C_{22} fatty acids	Elevated	Elevated	Elevated	Elevated	Normal
Pipecolic acid	Elevated	Elevated	Elevated	Elevated	Normal
Bile acid intermediates	Elevated	Elevated	Elevated	Elevated	Normal
Phytanic acid	Elevated	Elevated	Elevated		Elevated
Plasmalogen biosynthesis					
DHAP acyltransferase	Deficient	Deficient	Deficient	Deficient	Deficient
Alkyl DHAP synthase	Deficient	Deficient	Deficient		Deficient
De novo synthesis	Decreased	Decreased	Decreased		Decreased
Peroxisomes					
Number in liver	Absent	Absent	Decreased		
Percent particle-bound catalase	<5	<5	<5		>65
β-Oxidation proteins					
Acyl-CoA oxidase	Deficient	Deficient			Normal
Bifunctional protein	Deficient	Deficient			Normal
Thiolase	Deficient	Deficient			Normal

[a]For details see Schutgens *et al.*, (1986).
ALD = adrenoleukodystrophy; DHAP = dihydroxyacetone-phosphate

We have investigated the genetic relationship between these diseases by complementation analysis following somatic cell fusion of cultured skin fibroblasts from patients with peroxisomal disorders. Restoration of the activity of acyl-CoA:

dihydroxyacetone-phosphate acyltransferase, one of the two peroxisomal enzymes involved in the synthesis of ether phospholipids was used as an index of complementation. The results are summarized in Table 3 (see Tager *et al.*, 1987). These results indicated that the cell lines studied can be divided into three complementation groups (Table 4).

Table 3 Complementation analysis of peroxisomal diseases

Cell genotypes fused	*Complementation*
RCDP × ZS	+
RCDP × IRD	+
RCDP × NALD	+
RCDP × HPA	+
ZS × IRD	−
ZS × HPA	−
ZS × NALD	+
IRD × NALD	+
HPA × NALD	+

RCDP = chondrodysplasia punctata (rhizomelic); ZS = Zellweger syndrome; IRD = infantile Refsum disease; NALD = neonatal adrenoleukodystrophy; HPA = hyperpipecolic acidaemia

Table 4 Complementation groups in peroxisomal diseases

Complementation group	*Phenotypes*
1	Rhizomelic chondrodysplasia punctata
2	Zellweger syndrome; infantile Refsum disease; hyperpipecolic acidaemia
3	Neonatal adrenoleukodystrophy

Thus at least three genes must be involved in the biogenesis of peroxisomes. One gene codes for a protein required for the expression of phytanic acid oxidase, acyl-CoA: dihydroxyacetone-phosphate acyltransferase and alkyldihydroxyacetone-phosphate synthase. The other two genes coded for proteins, possibly membrane proteins, required for the assembly of functional peroxisomes. Roscher *et al.* (1987) have obtained analogous results; they have identified four different complementation groups among the cell lines they studied, two from patients with the Zellweger syndrome and two from patients diagnosed as having the neonatal form of adrenoleukodystrophy.

The similarities and differences in the biogenesis of peroxisomes and mitochondria are shown in Table 1. Like mitochondria, peroxisomes arise by budding on fission of pre-existing peroxisomes (Lazarow and Fujiki, 1985; Borst, 1986). Thus the absence of complementation between cell lines in complementation group 2 (see Table 3) could be due to the absence of pre-existing peroxisomes; experiments involving cytoplasts as a source of peroxisomes are at present being carried out in order to test this possibility.

LYSOSOMAL DISEASES

Since the identification of glucogenosis type II as a lysosomal storage disease in 1963, about 40 hereditary lysosomal storage diseases have been described in man (Hasilik, 1980; Callahan and Lowden, 1981; Tager *et al.*, 1984; Tager, 1985).

The biogenesis of lysosomes differs from that of mitochondria and peroxisomes (Table 1). Lysosomal enzymes are glycoproteins, and the precursors of lysosomal enzymes are synthesized on ribosomes attached to the rough endoplasmic reticulum. The specific routing of soluble lysosomal enzymes to the lysosomes in cultured human skin fibroblasts involves formation of mannose-6-phosphate groups in the oligosaccharide chains and binding of the precursors containing mannose-6-phosphate to specific receptors in the Golgi apparatus; this leads to sequestration of the enzymes within primary lysosomes (see Von Figura and Hasilik, 1986, for a review).

Two enzymes are required for the formation of mannose-6-phosphate groups in precursors of lysosomal enzymes: *N*-acetylglucosaminyl phosphotransferase and a phosphodiester glycosidase. The phosphotransferase is deficient in mucolipidosis II (I-cell disease) and mucolipidosis III (for a review see Von Figura and Hasilik, 1986). Recently, a deficiency of the phosphodiester glycosidase has been discovered in one particular family (Alexander *et al.*, 1986). The absence of mannose-6-phosphate groups in a lysosomal enzyme may also be due to a mutation in the structural gene for the lysosomal enzyme itself, as has recently been shown for α-glucosidase by Reuser *et al.* (1985).

Glucocerebrosidase, the enzyme deficient in Gaucher disease, is a membrane-associated protein. Glucocerebrosidase is not deficient in I-cell disease, which implies that routing of the enzyme to the lysosomes does not depend on the mannose-6-phosphate pathway. Indeed, Aerts *et al.* (1986) have recently shown that the transport of glucocerebrosidase to the lysosomes requires the conversion of high-mannose type to complex type oligosaccharides

The following mechanisms may lead to deficiencies of lysosomal enzymes (Tager *et al.*, 1984):

(1) The precursor of the enzyme may not be synthesized or it may be synthesized at a diminished rate.

(2) Normal amounts of the precursor may be synthesized but rapid degradation of the enzyme may occur.

(3) The precursor may lack the mannose-6-phosphate recognition marker.

(4) The precursor of the mature form may have altered physicochemical and/or enzymological properties.

(5) The enzyme may be degraded because of the absence of a protective protein required for its stabilization.

(6) The deficiency may be due to the absence of a factor required for enzymic activity.

(7) Products that accumulate as a result of a deficiency of one enzyme may inhibit the activity of unrelated enzymes.

PERSPECTIVE FOR THE FUTURE

With regard to the mitochondrial diseases, an important area of research in the near future will obviously be the regulation of the tissue-specific expression of genes coding for subunits of mitochondrial respiratory chain complexes. Will the information obtained provide therapeutic possibilities? Could one, for instance, switch on a gene for a liver-specific subunit of cytochrome oxidase in muscle?

Studies on the biogenesis of peroxisomes are of importance for understanding the molecular basis of those diseases in which multiple peroxisomal functions are impaired. On the other hand the availability of mutant cell lines should facilitate identification and characterization of genes and gene products involved in the biogenesis and assembly of a fully functional peroxisome.

Much of the research on lysosomal diseases being carried out at present is focussed on cloning the genes for lysosomal enzymes and on characterizing the lesions at the molecular genetic level. Furthermore, transfection studies are being carried out with the aim of testing the feasibility of gene therapy in this group of diseases.

Finally, one area in which more information is urgently required concerns the pathophysiology of many of the genetic diseases involving subcellular organelles. Why, for instance, does accumulation of a particular compound in a lysosomal storage disease lead to the characteristic clinical symptoms associated with that disease? This should prove a fruitful and rewarding field of research with important clinical implications.

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