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# MINI-REVIEW

# The Biochemical Basis of Mitochondrial Diseases

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## Abstract

Disfunctioning of human mitochondria is found in a rapidly increasing number of patients. The mitochondrial system for energy transduction is very vulnerable to damage by genetic and environmental factors. A primary mitochondrial disease is caused by a genetic defect in a mitochondrial enzyme or translocator. More than 60 mitochondrial enzyme deficiencies have been reported. Secondary mitochondrial defects are caused by lack of compounds to enable a proper mitochondrial function or by inhibition of that function. This may result from malnutrition, circulatory or hormonal disturbances, viral infection, poisoning, or an extramitochondrial error of metabolism. Once mitochondrial ATP synthesis decreases, secondary mitochondrial lesions may be generated further, due to changes in synthesis and degradation of mitochondrial phospholipids and proteins, to mitochondrial antibody formation following massive degradation, to accumulation of toxic products as excess acyl-CoA, to the depletion of Krebs cycle intermediates, and to the increase of free radical formation and lipid peroxidation.

**Key Words:** Beta-oxidation; carnitine deficiency; coenzyme Q; mitochondrial diseases; mitochondrial myopathies; NADH-CoQ reductase deficiency; oxidative phosphorylation; respiratory chain defects; therapy of mitochondrial diseases.

## The Functions of Mitochondria

The most important function of mitochondria in all kinds of tissues and cells is oxidative phosphorylation: the oxidation of fuel molecules by oxygen and the concomitant energy transduction into ATP. Table I summarizes properties of the complexes involved in oxidative phosphorylation. Figure 1

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**Table I.** Properties of Complexes Involved in Oxidative Phosphorylation[a]

| Complex | Site | Name | Molecular weight (protein part) | Function: Electron transport or reaction | Function: Proton flow | Subunits: N-DNA | Subunits: Mt-DNA | Prosthetic groups |
|---|---|---|---|---|---|---|---|---|
| I | 1 | NADH-CoQ reductase, flavoprotein$_1$ | 800,000 | NADH to CoQ | Outwards | 25 | 7 | FMN, Fe–S clusters |
| II | | Succinate-CoQ reductase, flavoprotein$_2$ | 140,000 | Succinate to CoQ | None | 4–5 | 0 | FAD, Fe–S clusters $b_{560}$ heme |
| III | 2 | Ubiquinol-cytochrome *c* reductase, cytochrome $bc_1$ | 250,000 | CoQ to cytochrome *c* | Outwards | 9–10 | 1 | $b_{562}$, $b_{566}$, $c_1$ hemes, Fe–S cluster |
| IV | 3 | Cytochrome *c* oxidase, cytochrome $aa_3$ | 162,000 | Cytochrome *c* to $O_2$ | Outwards | 8 | 3 | $aa_3$ hemes, $Cu_a$, $Cu_{a_3}$ |
| V | | ATP synthetase, $Mg^{2+}$-ATPase | 500,000 | ADP + $P_i$ → ATP | Inwards | 12–14 | 2 | |

[a]N, nuclear; Mt, mitochondrial. After the table from Hatefi (1985), with number of Mt-DNA encoded subunits of complex I from Chomyn *et al.* (1985, 1986). The molecular ratio of the complexes in mitochondria is I : II : III : IV : V = 1 : 2 : 3 : 6–7 : 3–4. The purified complexes contain lipid as well, which is needed for appropriate functioning. The phospholipid composition (mol. %) is the same as in mitochondrial inner membranes: phosphatidylcholine, 40; phosphatidylethanolamine, 35; cardiolipin, 15; others, 10 (Hatefi, 1985).

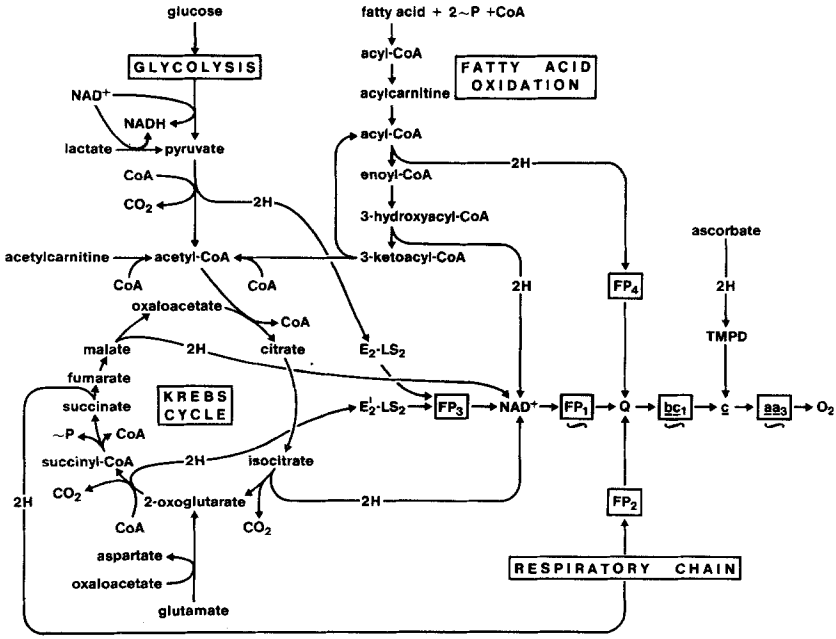


**Fig. 1.** The function of the respiratory chain in the oxidation of pyruvate, acetylcarnitine, fatty acids, glutamate, and Krebs cycle intermediates in skeletal muscle mitochondria. The reducing equivalents produced are indicated by 2H, which are oxidized by the respiratory chain to $H_2O$. $E_2$-$LS_2$ = dihydrolipoyltransacetylase, $E'_2$-$LS_2$ = dihydrolipoyltranssuccinylase, $FP_3$ = lipoamide dehydrogenase, $FP_1$ = NADH-CoQ reductase, $FP_2$ = succinate dehydrogenase, $FP_4$ = acyl-CoA dehydrogenases-electron transfer flavoprotein-electron transfer flavoprotein dehydrogenase, Q = CoQ, and $bc_1$, $c$, and $aa_3$ are the corresponding cytochromes. Taken with permission from Scholte *et al.* (1981).

shows the flow of reducing equivalents during the oxidation of pyruvate, acetylcarnitine, fatty acids, Krebs cycle intermediates, glutamate, and ascorbate to oxygen. The energy-transducing complexes in the respiratory chain (I, III, and IV) and in the phosphorylation (V) are transmembrane oriented in the inner mitochondrial membrane. The former complexes eject protons from the matrix space to the cytosol during the transport of reducing equivalents. Cytochrome $a_3$ ($Fe^{2+}$) also contributes to the generation of a proton gradient in the reaction with oxygen, which requires matrix protons. Mitochondrial ATP synthesis occurs during the reverse flow of electrons through complex V, a proton channel (in proteins $F_0$ and $F_1$), by the action of ATP synthetase located in $F_1$. The synthesized ATP is used for energy-requiring reactions in the matrix or exported to the cytosol by the adenine nucleotide translocator in exchange for cytosolic ADP. *In vivo*, the mitochondrial respiratory rate is determined by the rate of ADP production.

Although most mitochondrial enzymes and translocators are related to their main function, ATP production, they contain many more enzymes and translocators. Their enzymatic equipment is related to the function of the cell, and each cell type possesses unique mitochondria with a unique enzymatic outfit. For organ- and cell-specific mitochondrial activities, see Scholte and Veerkamp (1981).

## Failing Mitochondria

Failing mitochondria may be a cause or a consequence of disease. A mitochondrial disease is caused by a biochemically defined defect in mitochondrial functioning and results from a variety of genetic disorders and environmental factors. The disease may affect one tissue only, usually skeletal muscle (mitochondrial myopathy or myopathy with abnormal mitochondria), but also one or more other tissues as liver, brain, retina, nerves, heart, kidneys, small intestinal epithelium, thrombocytes, and chorionic villi. The defect may also be expressed in cultured muscle cells, skin fibroblasts, and amniotic fluid cells. This organ and cell specificity of the disease often cannot be explained by the existence of tissue specific (iso)enzymes. The clinical involvement of one organ does not imply that the defect is not expressed in other tissues. Investigation of skeletal muscle mitochondria proved to be effective in the elucidation of defects affecting the central nervous system or the heart, also in patients with minor or no signs of myopathy.

Most conditions are detected by pediatricians or neurologists on the basis of clinical signs and symptoms. Myopathic patients have chronic problems in skeletal muscle function such as easy fatiguability, muscular weakness, or hypotonia and stiffness and/or cramps after exercise, which are often caused by mitochondrial defects, but muscle AMP deaminase deficiency and disorders in carbohydrate metabolism are other possibilities. Patients with mitochondrial defects often show one or more of the following abnormalities:

a. Increased blood lactate in rest and after exercise
b. Increased respiratory quotient
c. Increase of abnormal organic acids in urine
d. Increase of abnormal carnitine esters in urine and decrease in blood free carnitine
e. Abnormal muscle morphology by abnormal accumulation of mitochondria, which gives rise to the appearance of so-called ragged red fibers in Gomori's trichrome staining of muscle tissue sections; by increase in intramuscular fat droplets which may appear when the

mitochondrial beta oxidation is inhibited; by an increased staining of $Mg^{2+}$-ATPase, which reflects the activity of the mitochondrial ATP synthetase and indicates the presence of loosely coupled mitochondria; by ultrastructural changes of the mitochondria.

The age of onset is variable. When the mitochondrial disease is also expressed in liver, the symptoms usually appear earlier and the disease is more severe, with episodes of vomiting and stupor, failure to thrive, and sudden death. Due to the rich detoxification metabolic repertoire of the liver, these mitochondrial diseases often give rise to the formation of abnormal organic acids in the urine. The specific pattern of these acids detected by coupled gas chromatography and mass spectrometry enabled the diagnosis of many mitochondrial and extramitochondrial enzyme deficiencies. This group of disorders is known as the "organic acidurias," and research was stimulated by the finding that several of the mitochondrial diseases were expressed in leucocytes and cultured skin fibroblasts. Most mitochondrial defects at the level of acyl-CoA metabolizing enzymes are expressed in these cells. An important metabolic consequence of such a deficiency is the development of carnitine deficiency, which stressed the importance of this compound for health and its research.

Mitochondrial diseases have been reviewed in several recent papers and books (DiMauro, 1979; Land and Clark, 1979; Stumpf, 1979; Carafoli and Roman, 1980; Bethlem, 1981; Busch *et al.*, 1981a; Scholte *et al.*, 1981; Morgan-Hughes, 1982; Scarlato and Cerri, 1983; Walter, 1983; Clark *et al.*, 1984; Morgan-Hughes *et al.*, 1984a, b, 1985; Sengers *et al.*, 1984a; Byrne and Trounce, 1985; Cornelio and DiDonato, 1985; Fischer, 1985; Scholte *et al.*, 1985b, 1986a, b, 1987a, b; DiMauro *et al.*, 1985a, b; Bianchi *et al.*, 1986; Engel and Banker, 1986; Morgan-Hughes, 1986a, b; Petty *et al.*, 1986; Bethlem and Knobbout, 1987). In these articles the historical, morphological, biochemical, and clinical aspects of these diseases are discussed. This paper presents an overview of the biochemical mechanisms that lead to mitochondrial diseases and to classify all known mitochondrial enzyme defects.

## On the Nature of Mitochondrial Diseases

### *Primary and Secondary Mitochondrial Defects*

For the explanation of the cause of mitochondrial dysfunction, it is important to distinguish primary and secondary diseases of mitochondria (see also Sengers, 1986). A primary mitochondrial disease is caused by a genetic defect in a mitochondrial enzyme or translocator. The mutation can

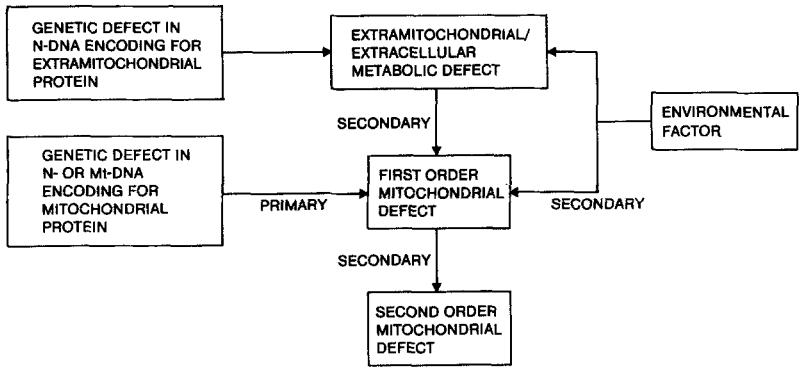


**Fig. 2.** The genesis of primary and secondary mitochondrial defects. N = nuclear and Mt = mitochondrial.

occur in the primary transcript, in one of the enzymes which catalyze post-translational modification, or in receptors functioning in the import pathway (see, e.g., Hartl *et al.*, 1986). It may affect the catalytic activity (e.g., by increased $K_m$ for a substrate, decreased $V$, or increased $K_{0.5}$ for an activator), the import of the protein into the mitochondria, or its degradation (Hurt and van Loon, 1986).

The causes of all other diseases involving mitochondrial dysfunction are regarded to be secondary (Fig. 2). They are caused by genetic defects or environmental factors leading to the obstruction of normal mitochondrial metabolism by lack of components needed for mitochondrial functioning or by the action of inhibitors. Secondary mitochondrial lesions are likely to occur, or have been detected, in the following conditions, which are not completely mutually exclusive:

1. Defects in the circulation, including shock (e.g., Shimahara *et al.*, 1981; Corbucci *et al.*, 1985, 1986) and other causes of ischemia and anoxia, which arrest the oxidative phosphorylation. Tissue ischemia is likely to be the most important cause of mitochondrial dysfunction. Early lesions were reported in the oxidation of $NAD^+$-linked substrates (Rouslin and Millard, 1980), probably caused by reversible inhibition of the adenine nucleotide translocator (Piper *et al.*, 1985) by long-chain acyl-CoA (see below). Longer periods of ischemia caused disappearance of the cytochromes and the adenine nucleotide translocator (Piper *et al.*, 1985).

Chronic progressive external ophthalmoplegia (CPEO) was assumed to be a mitochondrial disease (Bastiaensen, 1978) (for discussions, see Morgan-Hughes, 1986a; Scholte *et al.*, 1987a). Recent work has shown that it is more likely that the disease is caused by a circulatory defect. Accumulation of (abnormal) mitochondria was observed only in a distinct zone of the length

of the muscle fiber, with an increased amount of capillaries, which had a thicker wall than in normal muscle (Sengers *et al.*, 1986a). Driessen-Kletter *et al.* (1987) investigated seven CPEO patients and found a different degree of mitochondrial dysfunction in the patients, which did not correlate with increased blood lactate levels during exercise or the clinical condition, but perfectly with postexercise creatine kinase levels in the blood. Biochemical investigation of isolated mitochondria showed an oxidation rate of $NAD^+$-linked substrates ranging from 24–100% of controls. The patient with the lowest rate had a combined deficiency of rotenone-sensitive NADH oxidase and cytochrome *c* oxidase in the muscle homogenate, while in the others no abnormal mitochondrial enzyme activities were detected. In two other patients there was also an inability to oxidize $NAD^+$-linked substrates, while the oxidation of succinate + rotenone and ascorbate + TMPD was less impaired. Two patients showed increased blood lactate with normal oxidative phosphorylation, suggesting that increased lactate efflux occurs before mitochondrial dysfunction can be assessed. These experiments suggest that CPEO is not a primary mitochondrial disease, and that secondary mitochondrial disfunction develops in the course of the disease and causes postexercise muscle damage.

2. Malnutrition causes the depletion of components essential for mitochondrial functioning.

3. Defects at the level of hormones and neurotransmitters may cause abnormalities in mitochondrial metabolism or its regulation. Untreated diabetes mellitus causes a change in mitochondrial oxidative substrate, circulatory defects, and carnitine deficiency (Genuth and Hoppel, 1979). In hypothyroid myopathy by autoimmune disease, mitochondrial beta-oxidation is decreased in activity and normalizes after supplementation with thyroid hormone (Scholte, H. R., Busch, H. F. M., and Luyt-Houwen, I. E. M., unpublished observation). In animal experiments thyroidectomy induced loose coupling (Meijer, 1972). This was also encountered in patients with myasthena gravis, with a defect in the nerve–muscle transmission (Lousa *et al.*, 1983). In human muscle denervated for 2 w, a decrease in cytochrome *c* oxidase was observed (Nicolai *et al.*, 1983). Carnitine and carnitine palmitoyltransferase II were decreased in neurogenic atrophy (Scholte *et al.*, 1981), infantile spinal muscular atrophy, and in denervated rats (Bresolin *et al.*, 1984).

4. Viral infection is an important etiological factor in acute childhood encephalopathies (Kennedy *et al.*, 1986) and in juvenile cardiomyopathies (Schultheiss *et al.*, 1985, 1986), where massive disruption of heart mitochondria was shown. Reye's syndrome is considered to be a virus-associated disease, although preexisting mitochondrial defects and compounds toxic to mitochondria like salicylate and dicarboxylates may also play a role in the

pathophysiology (Crocker and Bagnell, 1981; Brown and Forman, 1982; Trauner, 1982; La Montagne, 1983; Tonsgard, 1986).

5. Poisoning by exogenous components as alcohol (Rubin *et al.*, 1970; Baraona and Lieber, 1979; Gordon, 1984), its catabolite acetaldehyde (Cederbaum *et al.*, 1975), antitumor drugs as adriamycin (see, e.g., Muhammed *et al.*, 1982; Goormaghtigh and Ruysschaert, 1984; Porumb and Petrescu, 1986), antibacterial drugs as chloramphenicol and tetracyclines inhibiting both bacterial and mitochondrial protein synthesis (De Vries and Kroon, 1970; Biancaniello *et al.*, 1981; Nau *et al.*, 1981; Van den Bogert and Kroon, 1981; Fripp *et al.*, 1983), bacterial toxins as diphteria toxin (Marques *et al.*, 1984), anticonvulsants as barbiturates and valproate (Sherratt and Veitch, 1984), fruit toxins like hypoglycin (Sherratt, 1986), and the long list of natural occurring or laboratory-made compounds, which are familiar to most biochemists since they played an important role in the elucidation of mitochondrial metabolism (antimycin, azide, bongkrekic acid, carbon monoxide, (carboxy)atractylate, cyanide, fluoroacetate, fluorocitrate, lewisite, malonate, oligomycin, rotenone, etc.).

6. Extramitochondrial or extracellular inborn or acquired errors of metabolism cause depletion of essential components for mitochondrial functioning such as phosphate (e.g., in fructose intolerance; Gitzelmann *et al.*, 1983), adenine nucleotides (e.g., in Lesch-Nyhan syndrome; Kelley and Wijngaarden, 1983), oxygen (e.g., hemoglobin disorders; Winslow and Anderson, 1983; Kan, 1983; myoglobin disorders; Sengers *et al.*, 1986b), copper (Menke's disease; Danks, 1983), etc.

There are also errors which cause the formation of mitochondrial inhibitors. Some examples are the formation of phenylpyruvate in phenylketonuria which inhibits the mitochondrial monocarboxylate translocator for pyruvate and ketone bodies (Clark and Land, 1974; Halestrap *et al.*, 1974; Land *et al.*, 1976; Patel *et al.*, 1977). Another toxic metabolite that may affect brain function in this disease is phenylacetyl-CoA, a potent inhibitor of choline acetyltransferase (Potempska *et al.*, 1984). Accumulated protoporphyrin in heme synthesis disorders cause loose coupling of oxidative phosphorylation (Stumpf *et al.*, 1979). In the given examples the metabolic relationship between the extramitochondrial and mitochondrial defect is clear. Yet unexplained are defects at the level of CoQ (binding protein) in Zellweger's disease (which is caused by deficiency of peroxisomes (Goldfisher *et al.*, 1973; Trijbels *et al.*, 1983a). In a patient with the adult form of Pompe's disease, a glycogen storage disease caused by a deficiency of lysosomal alpha glucosidase, which gives rise to lysosomal storage of glycogen, our group found a similar respiratory chain defect at the level of CoQ (Scholte *et al.*, 1987a). In Duchenne dystrophy, which is probably caused by deficiency of an extramitochondrial protein, loose coupling of oxidative

phosphorylation was detected (Ionasescu *et al.*, 1967) and, in most patients, decreased respiratory rate with most of the measured substrates and a low carnitine level in muscle in all patients (Scholte *et al.*, 1981). In fibroblast mitochondria from patients with cystic fibrosis a decreased affinity was found of cytochrome *c* oxidase for cytochrome *c* (Battino *et al.*, 1986).

### *Mechanisms of Mitochondrial Doom*

In both primary and secondary mitochondrial diseases further mitochondrial deterioration has to be expected in the course of the disease (Fig. 2). These secondary (or tertiary) lesions may also be called "second order" defects. They are the consequence of (a combination of) the following processes:

*1. Changes in Synthesis and Degradation of Mitochondrial Proteins and Phospholipids*

Very low cellular ATP is not compatible with life, and is a biochemical definition of death. Without ATP, synthesis of proteins and phospholipids and maintenance of a proper cytosolic ionic composition (low $Ca^{2+}$, high $K^+/Na^+$) is impossible. $Ca^{2+}$ overload will stimulate proteases and phospholipases (see, e.g., Jackson *et al.*, 1984). Lysosomes, often increased in myopathies, also contribute to breakdown of proteins and phospholipids. Our group received much frozen muscle biopsies from patients suspected from having a mitochondrial disease. In several of these specimen, we found very low activities of cytosolic and mitochondrial enzymes. Although such material is not suited for biochemical diagnosis, it may reflect the poor metabolic state of the biopsied muscle. But alternative explanations are replacements of the muscle by connective or fatty tissue (Scholte and Busch, 1980), or an (unwanted/unknown) extra cycle of thawing and freezing of the muscle specimen.

It is likely that ATP- and ubiquitin-dependent protein degradation plays an important role in normal mitochondrial turnover (Rapoport and Schewe, 1986). This, together with an increased mitochondrial metabolic demand, provides an explanation for the fact that in many patients with mitochondrial defects the concentration of mitochondria is increased as shown by histopathological and biochemical studies. In the past the increase of mitochondria, as shown by the occurrence of ragged-red fibers, was generally accepted as proof of the existence of a mitochondrial disease. Now we know that several patients with primary mitochondrial defects can also have a normal amount of mitochondria, and that ragged-red fibers also occur in extramitochondrial diseases. In several multibiopsied patients with mitochondrial respiratory chain defects, the amount of ragged-red fibers paralleled the severity of the disease (H. F. M. Busch, unpublished observation).

The degradation of NADH oxidase and succinate oxidase activities by added proteases or phospholipase was found to increase when they were not functioning by inhibition of the respiratory chain (Luzikov and Romashina, 1972; Luzikov, 1973). It is likely that the same increase in susceptibility occurs when the adenine nucleotide translocator is inhibited by a mitochondrial antibody (see Paragraph 2) or long-chain acyl-CoA (see Paragraph 3), since this will also give rise to inactivity of the respiratory chain.

*2. The Formation of Mitochondrial Antibodies*

After massive mitochondrial degradation by viral attack, tissue-specific autoantibodies are formed against the mitochondrial adenine nucleotide translocator (Schultheiss *et al.*, 1983; Schultheiss and Bolte, 1985). In patients with dilated or congestive cardiomyopathy, antibody binding on myocardial cell surface and mitochondrial inner membrane was demonstrated by immunofluorescent and immunoelectron-microscopic techniques, suggesting an inhibitory action of circulating antibodies on the remaining intact mitochondria. This was confirmed in animal studies (Schultheiss *et al.*, 1986). It is feasible that this autosuicidal mechanism can also be triggered by nonviral mitochondrial lesions. In patients with this condition, prevention of mitochondrial degradation by the action of $Ca^{2+}$-entry blockers, membrane stabilizers, and vitamin E could have therapeutic value. Vitamin E was found to be an inhibitor of platelet phospholipase A (Douglas *et al.*, 1986).

The pathophysiology of other mitochondrial antibodies is summarized by Berg and Klein (1986).

*3. Formation of Toxic Levels of Metabolites*

*a. Increased Matrix Acyl-CoA and Resulting Carnitine Deficiency.* Increase in acyl-CoA in the mitochondrial matrix is likely to occur in many mitochondrial defects when the metabolism of acyl-CoA is blocked as in deficiencies in one of the acyl-CoA dehydrogenases and in respiratory chain defects. The increased acyl-CoA may inhibit a variety of mitochondrial enzymes and translocators, depending upon the nature of the acyl group. Striking examples are the inhibition of the adenine nucleotide carrier by long-chain acyl-CoA (Lauquin *et al.*, 1977; Shug *et al.*, 1978), and the inhibition of glutamate dehydrogenase by palmitoyl-CoA (Fahien and Kmiotek, 1981). An inhibition in the mitochondrial metabolism of an acyl-CoA ester causes carnitine deficiency by an increase in acylcarnitine release and excretion. When this condition continues, free carnitine depletion can cause the accumulation of all CoA-esters (Scholte, 1983; Engel and Rebouche, 1984; Engel, 1986; Scholte and De Jonge, 1987). This may be prevented by carnitine supplementation.

*b. The Formation of Other Inhibitors.* Accumulating 2-oxocaproate in maple syrup urine disease caused by branched-chain keto acid dehydrogenase deficiency inhibits the monocarboxylate carrier as phenylpyruvate

(see above for references). Increased glutarate and related metabolites were found to inhibit brain glutamate decarboxylase, which may be important in the glutaric acidurias caused by glutaryl-CoA dehydrogenase and multiple acyl-CoA dehydrogenase deficiency (Stokke *et al.*, 1976).

*4. Decrease in Krebs Cycle Intermediates*

This occurs when the Krebs cycle intermediates are more rapidly removed than formed. Acetyl-CoA can no longer be oxidized via the Krebs cycle, and hepatic encephalopathy may occur by decreased hepatic gluconeogenesis and urea synthesis, respectively, causing low blood glucose and high blood ammonia. The latter compound is toxic to the brain, probably because it removes Krebs cycle intermediate 2-oxoglutarate to glutamate (and glutamine). This mechanism was demonstrated in pyruvate carboxylase deficiency (Robinson *et al.*, 1984; Greter *et al.*, 1985), but may be also operative in other mitochondrial defects such as propionyl-CoA carboxylase deficiency or respiratory chain disorders. Isocitrate, citrate, propionate, and glycogenic amino acids are of potential value for increasing the Krebs cycle intermediates in hepatocytes.

*5. Lipid Peroxidation and Formation of Free Radicals*

Oxygen radicals produced by lipoxygenase destroy the respiratory chain at the level of CoQ-binding proteins (QP-N and QP-S; King, 1982) and cytochrome *c* oxidase in submitochondrial particles (Schewe *et al.*, 1981). The damage at the level of CoQ-binding proteins is irreversible. The cytochrome *c* oxidase inhibition is caused by lipid peroxidation in phospholipids that are needed for activation of the enzyme. This inhibition is reversible. Normal phospholipids restore the enzyme activity (Wiesner *et al.*, 1981). Lipoxygenase-induced mitochondrial degradation only occurs in reticulocytes during their maturation process (Rapoport and Schewe, 1986). In other tissues the free radicals may be generated by the mitochondrial respiratory chain at NADH-CoQ reductase and CoQ-cytochrome $bc_1$ reductase, especially when the mitochondrial respiratory chain has been blocked (Turrens and Boveris, 1980; Nohl *et al.*, 1982). To prevent this slow but resistant damage in patients, the therapeutic possibilities of free radical scavengers like vitamin E (Jackson *et al.*, 1985) and lipid peroxidation inhibitors as $Zn^{2+}$ (Girotti *et al.*, 1985) could be considered. Recently Toifl *et al.* (1986) described the beneficial effects of dietary measures, carnitine, and vitamin E in a patient with multiple respiratory chain defects.

Some examples of multiple mitochondrial defects which could be explained by "second-order" mitochondrial changes are the following:

1. In granulocytes of patients with 5-aminolevulinic synthetase deficiency, decreased activities were detected of cytochrome *c* oxidase and ATP synthetase, while citrate synthase activity was normal. These defects in oxidative phosphorylation were not present in lymphocytes (Aoki, 1980).

2. In liver specimen from patients with propionyl-CoA carboxylase and methylmalonyl-CoA mutase deficiency obtained after autopsy, the activity of cytochrome *c* oxidase was considerably reduced (Hayasaka *et al.*, 1982). It is likely that this enzyme in patients with mitochondrial diseases is more rapidly degraded after death than in other patients (Rodriges Pereira, R., De Klerk, J. B. C., Barth, P. G., and Scholte, H. R., unpublished observations in several deceased patients). In liver from a patient with propionyl-CoA carboxylase deficiency obtained after autopsy, our group also found a decrease in antimycin-sensitive succinate-cytochrome *c* reductase, a normal pyruvate carboxylase and pyruvate dehydrogenase activity, and a highly increased activity of 2-ketoglutarate dehydrogenase (unpublished observation).

3. In young patients with glutaryl-CoA dehydrogenase (unpublished result) and multiple acyl-CoA dehydrogenase deficiency (Mooy *et al.*, 1984), the function of skeletal muscle mitochondria was severely impaired. The oxidation with all substrates was reduced. Fortunately the diagnosis could be made on the basis of an investigation of urinary organic acids, which was then confirmed in cultured fibroblasts.

4. In postmortem liver of a patient with lipoamide dehydrogenase deficiency a severe reduction was found in the activity of the glycine cleavage enzyme complex, which did not normalize after addition of purified lipoamide dehydrogenase from human liver. This experiment suggests that the lipoamide dehydrogenase activity of the glycine cleavage enzyme complex is different from the lipoamide functioning in the keto acid dehydrogenase complexes (Yoshino *et al.*, 1986).

5. In several patients with defects in the oxidative phosphorylation, we found a deficiency of muscle medium-chain acyl-CoA dehydrogenase activity. The absence of suggestive urinary metabolites like dicarboxyl glycine conjugates suggested a normal hepatic beta oxidation (Scholte *et al.*, 1986a). In a patient with carnitine palmitoyltransferase II deficiency in muscle, fibroblasts, thrombocytes, and leucocytes, deficiencies were found in muscle mitochondria of long- and medium-chain acyl-CoA dehydrogenase, while the activity of butyryl-CoA dehydrogenase was normal (Scholte *et al.*, 1986a). Also in this patient no abnormal urinary fatty acids were found.

Table II summarizes secondary changes in enzyme activities that are frequently encountered in patients with mitochondrial diseases.

It may be stated that mitochondrial diseases are secondary, unless their primary nature has been assessed. Detection of a deficient mitochondrial protein does not necessarily imply that the nature of the defect is primary, but it is important evidence that can be used for the development of a rational treatment. Additional evidence for a primary nature is obtained by assessment of the inheritance pattern of the defect, knowledge of which is important for the patient and his family and may enable genetic counselling

**Table II.** Secondary Enzyme Changes in Mitochondrial Defects (Summary of Frequently Encountered Secondary Changes of Enzyme Activities in Muscle and Liver of Patients with Mitochondrial Defects)

| Decrease | Increase or no change |
|---|---|
| NADH-CoQ reductase | Uncoupler-stimulated $Mg^{2+}$-ATPase |
| CoQ-Binding proteins | Palmitoyl-CoA dehydrogenase |
| Cytochrome *c* oxidase | Butyryl-CoA dehydrogenase |
| Adenine nucleotide carrier | 2-Ketoglutarate dehydrogenase |
| Coupling becomes loose | Succinate dehydrogenase |
| Medium-chain acyl-CoA dehydrogenase | Carnitine palmitoyltransferase I |
| Tissue carnitine | Carnitine palmitoyltransferase II |
| | Carnitine acetyltransferase |
| | Propionyl-CoA carboxylase |
| | Malonyl-CoA decarboxylase |
| | Palmitoyl-CoA synthetase |

and prevention of the disease by abortion or treatment of the condition of the affected child before and after birth. When the defect is expressed in cultured cells, abnormalities in synthesis, processing, import, and degradation of the enzyme can be studied. Final proof for the primary nature of the disease is obtained by the demonstration of a mutation in the base sequence of isolated nuclear or mitochondrial DNA. This contributes to basic scientific knowledge of the molecular nature of the defect and enables detection and prevention of that mutation in one family.

The line of scientific research to the nature of mitochondrial diseases moves from clinical investigation, detection of abnormal metabolites in blood and urine, morphological investigations, functional studies in isolated mitochondria, and enzymology in homogenates of tissues and cultured cells, attempts to treat and follow improvement of the condition, immunoblotting studies in tissues and cultured cells to investigate the identity of a missing protein (subunit), complementation studies in cultured cells, to genome cloning and DNA sequence analysis.

These studies require the cooperation of several expert groups of investigators, initiated and coordinated by the clinician.

## A Biochemical Classification of Mitochondrial Diseases

A systematic approach would be to take the list of the Enzyme Committee of the International Union of Biochemistry, to select the mitochondrial enzymes and indicate which enzyme deficiencies have been reported. Unfortunately the enzyme list is not complete. Several mitochondrial enzymes do not have a separate entry (e.g., acyl-CoA dehydrogenases). So this enterprise is work for the future.

**Table III.** Biochemical Classification of Mitochondrial Defects

| | | | |
|---|---|---|---|
| 1. | Defects in oxidative phosphorylation | | |
| 1.1. | Respiratory chain defects | | |
| 1.1.1. | NADH-CoQ reductase deficiency | 25539[a] | Senior and Jungas, 1974; Morgan-Hughes *et al.*, 1979; Kerr *et al.*, 1980; Busch *et al.*, 1981b; Land *et al.*, 1981; Prick *et al.*, 1981; Scholte *et al.*, 1983; Sherratt *et al.*, 1984; Morgan-Hughes *et al.*, 1985; Fischer *et al.*, 1986a; Kobayashi *et al.*, 1986; Robinson *et al.*, 1986; Scholte *et al.*, 1986a; Tanaka *et al.*, 1986b; Van Erven *et al.*, 1986; Nishizawa *et al.*, 1987; Scholte *et al.*, 1987a |
| | Deficiency of all Fe–S clusters | | Moreadith *et al.*, 1984 |
| | Riboflavin-responsive patients | | Arts *et al.*, 1983; Busch *et al.*, 1986; Scholte *et al.*, 1987a |
| | Form with high $K_m$ for NADH | | Scholte *et al.*, 1987a |
| 1.1.2. | Defect at the level of CoQ (binding proteins)[b] | | Morgan-Hughes, 1983; Morgan-Hughes *et al.*, 1984; Scholte *et al.*, 1987a In Zellweger's disease: Goldfischer *et al.*, 1973; Trijbels *et al.*, 1973 |
| | CoQ-responsive patients | | Folkers *et al.*, 1985; Ogasahara *et al.*, 1986 |
| 1.1.3. | Ubiquinol-cytochrome *c* reductase deficiency | | Sengers *et al.*, 1983; Behbehani *et al.*, 1984; Riggs *et al.*, 1984; Morgan-Hughes, 1985 |
| | Cytochrome *b* deficiency | | Spiro *et al.*, 1970; Morgan-Hughes *et al.*, 1977, 1982 |
| | Cytochrome $c_1$ deficiency | | Przyrembel *et al.*, 1983, 1984; Scholte *et al.*, 1983; Barth *et al.*, 1983 (Table 6); Scholte *et al.*, 1987a |
| | Fe–S cluster deficiency[b] | | Morgan-Hughes, 1983; Reichmann *et al.*, 1986 |
| | Cytochromes *b* + $c_1$ deficiency | | Morgan-Hughes *et al.*, 1984 |
| | Cytochrome *b* + Fe–S cluster deficiency | | Darley-Usmar *et al.*, 1983; Kennaway *et al.*, 1984; Argov *et al.*, 1986 |
| 1.1.4. | Cytochrome *c* oxidase deficiency | 22011 | Willems *et al.*, 1977; Rimoldi *et al.*, 1982; Miyabayashi *et al.*, 1983; Müller-Höcker *et al.*, 1983; Prick *et al.*, 1983; Trijbels *et al.*, 1983b; Bresolin *et al.*, 1985; Byrne *et al.*, 1985; DiMauro *et al.*, 1985b; Zeviani *et al.*, 1985; Angelini *et al.*, 1986; Robinson *et al.*, 1986; Arts *et al.*, 1987; Servidei *et al.*, 1987. In Menkes syndrome: French *et al.*, 1972; Maehara *et al.*, 1983 |
| | Benign reversible form | | DiMauro *et al.*, 1983; Zeviani *et al.*, 1987 |
| 1.1.5. | Multiple respiratory chain defects | | |
| | At sites 1 + 2: NADH-CoQ reductase + cytochrome $bc_1$ | | Papadimitrou *et al.*, 1984 |
| | At sites 1 + 3: NADH-CoQ reductase + cytochrome *c* oxidase | | Roodhooft *et al.*, 1986 |
| | At sites 2 + 3: Cytochrome *b* + cytochrome *c* oxidase | | Van Biervliet *et al.*, 1977; DiMauro *et al.*, 1980; Stansbie *et al.*, 1982; Boustany *et al.*, 1983; Minchom *et al.*, 1983; Sengers *et al.*, 1984b |

| | | | |
|---|---|---|---|
| | Cytochrome $bc_1$ + cytochrome $c$ oxidase | | Hayes *et al.*, 1984 |
| | At sites 1 + 2 + 3: NADH-CoQ reductase, cytochromes $c_1$ + $aa_3$ | 30982 | Barth *et al.*, 1983 |
| | NADH-CoQ reductase, cytochromes $bc_1$, $c$, $aa_3$ | | Tanaka *et al.*, 1986a |
| 1.2. | Defects in energy transduction | | |
| 1.2.1. | ATP synthetase ($Mg^{2+}$-ATPase) deficiency | | Schotland *et al.*, 1976; Clark *et al.*, 1984 |
| 1.2.2. | Disfunction of the adenine nucleotide translocator | | Tomasi *et al.*, 1980 |
| | Inhibition by long-chain acyl-CoA[b] | | Lauquin *et al.*, 1977; Scholte *et al.*, 1987a |
| | Inhibition by mitochondrial antibody | | Schultheiss *et al.*, 1986 |
| 1.2.3. | Loose coupling (severe) in Luft's disease | 23880 | Luft *et al.*, 1962; Ernster and Luft, 1963; Afifi *et al.*, 1972; DiMauro *et al.*, 1976 |
| | Loose coupling without hypermetabolism | | Van Wijngaarden *et al.*, 1967; Hülsmann *et al.*, 1967; Schellens and Ossentjuk, 1969; Worsfold *et al.*, 1973; Meijer and Vloedman, 1980; Meÿer and Van Wÿngaarden, 1983; Meijer *et al.*, 1985; Müller-Höcker *et al.*, 1986 |
| 1.2.4. | Defective proton pumping by complexes I and II *in vitro* | | Trockel *et al.*, 1986 |
| 1.3. | Defective mitochondriogenesis[b] | | Demonstrated in human fibroblast line by Constantopoulos *et al.*, 1986; Suspected in several patients with relative normal muscle creatine kinase and low activities of all tested mitochondrial enzymes. |
| 2. | Deficiencies of mitochondrial dehydrogenases | | |
| 2.1. | Defects in ETF-linked Acyl-CoA dehydrogenases | | |
| 2.1.1. | Long-chain fatty acyl-CoA dehydrogenase deficiency | 20146 | Hale *et al.*, 1985; Moon and Rhead, 1987 |
| 2.1.2. | Medium-chain fatty acyl-CoA dehydrogenase deficiency | 20145 | Kølvraa *et al.*, 1982; Divry *et al.*, 1983; Duran *et al.*, 1983; Stanley *et al.*, 1983; Gregersen, 1984; Huijmans *et al.*, 1984; Amendt and Rhead, 1985; Coates *et al.*, 1985; Duran *et al.*, 1985; Roe *et al.*, 1986; Moon and Rhead, 1987 |
| 2.1.3. | Butyryl-CoA dehydrogenase deficiency | 20147 | Turnbull *et al.*, 1984 |
| 2.1.4. | Glutaryl-CoA dehydrogenase deficiency | 23167 | Goodman and Kohlhoff, 1975; Christiansen and Brandt, 1978 |
| 2.1.5. | Isovaleryl-CoA dehydrogenase deficiency | 24350 | Rhead and Tanaka, 1980; Tanaka and Rosenberg, 1983 |
| 2.1.6. | Sarcosine dehydrogenase deficiency | 26890 | Gerritsen and Waisman, 1978 |

**Table III.** Continued

| | | | |
|---|---|---|---|
| 2.1.7. | Multiple acyl-CoA dehydrogenase deficiency by deficiency of electron-transferring flavoprotein (ETF) or ETF dehydrogenase | 23168 | Przyrembel *et al.*, 1976; Goodman *et al.*, 1980; Tanaka and Rosenberg, 1983; Christensen, 1984; Christensen *et al.*, 1984; Goodman and Frerman, 1984; Mooy *et al.*, 1984; Frerman and Goodman, 1985; Gregersen, 1985a, b; Amendt and Rhead, 1986; De Visser *et al.*, 1986; DiDonato *et al.*, 1986; Moon and Rhead, 1987 |
| 2.2. | Deficiencies of keto acid and dehydrogenase complexes | | |
| 2.2.1. | Pyruvate dehydrogenase and deficiency<br>Dephosphorylation defect in the α-subunit | 20880 | Blass, 1983; McKay *et al.*, 1986; Stansbie *et al.*, 1986<br>Wicking *et al.*, 1986 |
| 2.2.2. | Pyruvate dehydrogenase phosphatase deficiency | | Robinson and Sherwood, 1975; DeVivo *et al.*, 1979; Koster *et al.*, 1978 |
| 2.2.3. | Dihydrolipoyl transacetylase deficiency[b] | | Blass *et al.*, 1972; Cederbaum *et al.*, 1976 |
| 2.2.4. | 2-Ketoglutarate dehydrogenase deficiency | | Schutgens *et al.*, 1980; Kohlschrütter *et al.*, 1982; Abboud *et al.*, 1985 |
| 2.2.5. | Branched-chain keto acid dehydrogenase deficiency | 24860 | Tanaka and Robenberg, 1983; Duran and Wadman, 1985 |
| 2.2.6. | Branched-chain keto acyltransferase deficiency | | Danner *et al.*, 1985 |
| 2.2.7. | Lipoamide dehydrogenase deficiency | 24690 | Haworth *et al.*, 1976; Robinson *et al.*, 1977; Matalon *et al.*, 1984 |
| 2.2.8. | Multiple keto acid dehydrogenase deficiency in beriberi or defective thiamine metabolism | | Suspected in one patient (Bakker, H. *et al.*, unpublished observations) |
| 2.3. | Deficiencies of other dehydrogenases | | |
| 2.3.1. | Glutamate dehydrogenase deficiency | 16460 | Plaitakis *et al.*, 1982; Duvoisin and Chockroverty, 1984; Finocchiaro *et al.*, 1986 |
| 2.3.2. | Glycine cleavage enzyme complex deficiency<br>by threonine hydratase deficiency<br>by T-enzyme deficiency | | Nyhan, 1983<br>Krieger and Booth, 1984<br>Schutgens *et al.*, 1986 |
| 2.3.3. | Proline oxidase deficiency | 23950 | Scriver *et al.*, 1983 |
| 2.3.4. | Succinic semialdehyde dehydrogenase deficiency | 27198 | Gibson *et al.*, 1983 |
| 2.3.5. | 4-Aminobutyrate dehydrogenase deficiency | | Gibson *et al.*, 1985 |
| 3. | Defects in mitochondrial transport processes | | |
| 3.1. | Defects in the carnitine system for acyl transport | | |

| | | | |
|---|---|---|---|
| 3.1.1. | Carnitine deficiency | | |
| | by renal leak | 21214 | Waber *et al.*, 1982 |
| | by defective transport into skeletal muscle | | Rebouche and Engel, 1984 |
| | secondary to defects in mitochondrial acyl-CoA metabolizing enzymes | | Scholte, 1983; Scholte *et al.*, 1983; Engel and Rebouche, 1984; Stumpf *et al.*, 1985; Engel, 1986; Scholte and De Jonge, 1987 |
| | developmental or acquired deficiency | | Engel and Rebouche, 1984; Scholte and De Jonge, 1987 |
| 3.1.2. | Carnitine palmitoyltransferase I deficiency | 25512 | Hoppel *et al.*, 1980; Bonnefont *et al.*, 1985 |
| 3.1.3. | Defective mitochondrial carnitine carrier[b] | | Scholte *et al.*, 1986a |
| 3.1.4. | Carnitine palmitoyltransferase II deficiency | 25511 | Patten *et al.*, 1979; Scholte *et al.*, 1979a, 1985; Angelini *et al.*, 1981; Pula *et al.*, 1981; Trevisan *et al.*, 1984; Bonnefont *et al.*, 1985; Zierz and Engel, 1985; DiMauro and Papadimitriou, 1986 |
| 3.1.5. | Combined carnitine palmitoyltransferase I and II deficiency[b] | | DiMauro and Melis DiMauro, 1973 |
| 3.1.6. | Carnitine acyltransferase deficiency | | DiDonato, *et al.*, 1979 |
| 3.1.7. | Multiple defects in the carnitine system | | Ionasescu *et al.*, 1980 |
| | Carnitine plus carnitine palmitoyltransferase deficiency | | |
| 3.2. | Other mitochondrial transport defects | | |
| 3.2.1. | Monocarboxylate translocator defect[b] | | DiMauro *et al.*, 1973; Kark *et al.*, 1978 |
| 3.2.2. | Ornithine translocator defect[b] | 23897 | Hommes *et al.*, 1982; Shih *et al.*, 1982; Fell *et al.*, 1974 |
| 3.2.3. | Lysine translocator defect[b] | | Oyanagi *et al.*, 1986 |
| 3.2.4. | 2-Oxoglutarate malate carrier in aspartate malate shuttle[b] | | Hayes *et al.*, 1986 |
| 4. | Deficiencies of enzyme catalyzing the synthesis of oxidative substrates (non-redox reactions) | | |
| 4.1. | Deficiencies of mitochondrial carboxylases | | |
| 4.1.1. | Propionyl-CoA carboxylase deficiency | 23200, 5 | Rosenberg, 1983; Watkins and Rosenblatt, 1986 |
| 4.1.2. | Pyruvate carboxylase deficiency | 26615 | Blass, 1983; Bartlett *et al.*, 1984; Robinson *et al.*, 1984 |
| 4.1.3. | 3-Methylcrotonoyl-CoA carboxylase deficiency | 21020 | Tanaka and Rosenberg, 1983; Beemer *et al.*, 1982 |
| 4.1.4. | Multiple carboxylase deficiency | | Suormala *et al.*, 1985 |
| | by mitochondrial holocarboxylase synthetase | 25327 | Sherwood *et al.*, 1982; Bartlett *et al.*, 1985; Burri *et al.*, 1985 |
| | by biotinidase deficiency | 25326 | Baumgartner *et al.*, 1985; Wolf *et al.*, 1985; Sweetman and Nyhan, 1986 |
| | by unknown defect in Rett syndrome | 31275 | Bachmann *et al.*, 1986 |
| 4.2. | Defects in other mitochondrial CoA-linked enzymes | | |

**Table III.** Continued

| | | | |
|---|---|---|---|
| 4.2.1. | Methylmalonyl-CoA mutase deficiency | | Rosenberg, 1983 |
| | by mutation in the enzyme | 25100 | Rosenberg, 1983 |
| | by defective adenosylcobalamin synthesis | 25110, 1 | Gravel *et al.*, 1975 |
| | by defective adenosyl- and methylcobalamin synthesis (presenting with homocysteinuria) | | Willard *et al.*, 1978 |
| | by defective $B_{12}$ release from lysosomes | | Rosenblatt *et al.*, 1985 |
| 4.2.2. | Acetoacetyl-CoA thiolase deficiency ($K^+$-stimulated enzyme, also active with 3-methylacetoacetyl-CoA) | 20375 | Robinson and Sherwood, 1975; Schutgens *et al.*, 1982; Tanaka and Rosenberg, 1983; Middleton *et al.*, 1986 |
| 4.2.3. | 3-Methylglutaconyl-CoA hydratase deficiency | 25095 | Duran *et al.*, 1982; Narisawa *et al.*, 1986 |
| 4.2.4. | 3-Hydroxyisobutyryl-CoA deacylase deficiency | | Brown *et al.*, 1982 |
| 4.2.5. | 3-Hydroxy-3-methyl-glutaryl-CoA lyase deficiency | 24645 | Tanaka and Rosenberg, 1983; Wysocki and Hähnel, 1986 |
| 4.2.6. | 3-Ketoacyl-CoA transferase deficiency | 24505 | Tildon and Cornblath, 1972 |
| 4.2.7. | Malonyl-CoA decarboxylase deficiency | | Brown *et al.*, 1984; Haan *et al.*, 1986 |
| 4.3. | Other deficiencies | | |
| 4.3.1. | Fumarase deficiency | | Zinn *et al.*, 1986 |
| 4.3.2. | Hexokinase I deficiency | 23570 | Enzyme is bound to outer membrane porin: Feik *et al.*, 1982; Magnani *et al.*, 1986 |
| 4.3.3. | Glycerol kinase deficiency | 30703 | Enzyme is bound to outer membrane porin: Feik *et al.*, 1982; McGabe, 1983 |
| 4.3.4. | Glutamate decarboxylase deficiency | 26610 | Yoshida *et al.*, 1971 |
| 5. | Mitochondrial defects in hormone metabolism | | |
| 5.1. | Steroidogenetic enzyme defects | | |
| 5.1.1. | Cholesterol side-chain cleavage enzyme deficiency[c] | 20171 | New *et al.*, 1983; Degenhart, 1984 |
| 5.1.2. | Steroid 11-beta-hydroxylase deficiency | 20201 | New *et al.*, 1983 |
| 5.1.3. | Steroid 18-hydroxylase deficiency | 20340 | New *et al.*, 1983 |
| 5.1.4. | Steroid 18-hydroxy dehydrogenase deficiency | 20341 | New *et al.*, 1983 |
| 5.1.5. | Steroid 26-hydroxylase deficiency | 21370 | Miki *et al.*, 1986; Skrede *et al.*, 1986 |
| 5.1.6. | 25-Hydroxycholecalciferol-1-hydrolase | 26470 | Morris and Sebastian, 1983 |
| 5.2. | Defects in other hormone-metabolizing enzymes | | |

| | | | |
|---|---|---|---|
| 5.2.1. | Monoamine oxidase[b] | | Changed properties repeatedly observed in a variety of diseases, recently in schizophrena: Moskvitina *et al.*, 1986 |
| 6. | Mitochondrial deficiencies not directly linked to ATP synthesis or hormone metabolism | | |
| 6.1. | Deficiencies in the mitochondrial part of the urea cycle | | |
| 6.1.1. | Carbamoyl phosphate synthetase I deficiency | 23730 | Walzer, 1983 |
| 6.1.2. | *N*-Acetylglutamate synthetase deficiency | 23731 | Walzer, 1983 |
| 6.1.3. | Ornithine transcarbamoylase deficiency | 31125 | Walzer, 1983 |
| | For ornithine translocator deficiency, see 3.2.2. | | |
| 6.2. | Defects in mitochondrial part of heme synthesis | | |
| 6.2.1. | 5-Aminolevulinic acid synthetase | 30130 | Aoki, 1980 |
| 6.2.2. | Coproporphyrinogen III oxidase deficiency | 12130 | Kappas *et al.*, 1983 |
| 6.2.3. | Protoporphyrinogen oxidase deficiency | 17620 | Kappas *et al.*, 1983 |
| 6.2.4. | Ferrochelatase or heme synthetase deficiency | 17700 | Kappas *et al.*, 1983 |
| 6.3. | Other enzyme deficiencies | | |
| 6.3.1. | Sulfite oxidase deficiency | 27230 | Irreverre *et al.*, 1967; Shih *et al.*, 1977 |
| | Combined molybdenum enzyme deficiency by Mb-cofactor defect | 25215 | Wadman *et al.*, 1983 |
| 6.3.2. | PEP carboxykinase deficiency | | Clayton *et al.*, 1986 |

[a] For more literature concerning these defects, see McKusick, 1986. The numbers refer to his classification.
[b] Indirect evidence of enzyme defect; awaits confirmation by direct assay of enzyme or translocator.
[c] Differs from peroxisomal enzyme that functions in bile acid synthesis. The mitochondrial cholesterol side chain cleavage enzyme complex cleaves $C_{22}$–$C_{27}$ from the cholesterol molecule, while the peroxisomal enzyme cleaves $C_{25}$–$C_{27}$.

A metabolic approach, then, is the preferred one, and must be based upon our knowledge of mitochondrial functions. Morgan-Hughes (1982, 1986a) classified mitochondrial deficiencies in four groups of defects: in mitochondrial substrate transport, substrate utilization, respiratory chain, and in energy conservation and transduction. Since, first, oxidation and phosphorylation are coupled, second, the defects at the level of substrate utilization can be separated in redox and nonredox reactions, and, finally, other known mitochondrial defects not directly related to mitochondrial energy delivery reactions must be considered, I prefer the classification shown in Table III.

## Defects in Oxidative Phosphorylation

The most frequently encountered defects in mitochondrial functioning are at the level of NADH-CoQ reductase, CoQ-cytochrome *c* oxidase, medium-chain acyl-CoA dehydrogenase, and in the carnitine system by carnitine deficiency.

The biochemical diagnosis of the respiratory chain defects is not easy. Several groups only study defects in homogenates from frozen muscle. Several defects, e.g., at the level of CoQ and in phosphorylation efficiency, may be overlooked (for a discussion, see Scholte *et al.*, 1987a). In contrast to measurement of rotenone-sensitive NADH oxidase (Fischer *et al.*, 1986b), assay of rotenone-sensitive NADH-cytochrome *c* reductase is not appropriate to measure defects in NADH-CoQ reductase, because the rotenone-insensitive activity of the latter assay is too high. When studying the capacity of isolated mitochondria to oxidize $NAD^+$-linked substrates, it is important to test enough substrates that are well oxidized by muscle mitochondria (see Table IV). Adequate testing is done with pyruvate + malate, glutamate + malate, and palmitoylcarnitine + malate. Testing of the first two substrate couples alone is not enough, since defects at the level of thiamine or lipoamide dehydrogenase will lower the activity with these substrates. In muscle, glutamate is oxidized preferentially via aspartate aminotransferase because the glutamate dehydrogenase activity is too low in this tissue. Further criteria for the assessment of NADH-CoQ reductase deficiency are presented by Scholte *et al.* (1986b).

$^{31}P$ nuclear magnetic resonance studies in muscle from myopathic patients with increased blood lactate and muscle mitochondria, some with proven respiratory chain defects, showed that the weakness of the patients does not result from depletion of ATP or from deleterious effects of intracellular acidosis. The calculated levels of cytosolic ADP were increased in most patients, also at rest. Weakness could be explained on the basis of inhibition of myosin-ATPase by ADP. Creatine phosphate was lowered in

**Table IV.** Oxidative Substrates for Liver and Skeletal Muscle Mitochondria in Man

| Class | Oxidative substrates | Mitochondria from | |
|---|---|---|---|
| | | Liver | Skeletal muscle |
| $NAD^+$-linked | Pyruvate + malate | Yes | Yes |
| | Acetylcarnitine + malate | Yes | Yes |
| | Pyruvate + carnitine | Yes | Yes |
| | Glutamate + malate | Yes[a] | Yes[b] |
| | 2-Ketoglutarate | Yes | Yes |
| | 3-Hydroxybutyrate | Yes | Poor |
| | Citrate | Yes | No |
| | Isocitrate | Yes | No |
| $NAD^+$-linked + CoQ-linked | Butyrylcarnitine + malate | Yes | Yes |
| | Hexanoylcarnitine + malate | Yes | Yes |
| | Palmitoylcarnitine + malate | Yes | Yes |
| CoQ-linked | Succinate + rotenone | Yes | Yes |
| | Some reduced CoQ analogs (e.g., duroquinol) | Yes | Yes |
| | Glycerol-3-phosphate | Yes | Poor[c] |
| Cytochrome *c*-linked | Ascorbate[d] | Yes | Yes |

[a]Via glutamate dehydrogenase plus aspartate aminotransferase.
[b]Via aspartate aminotransferase.
[c]Higher in mitochondria from type II muscle.
[d]Increased oxidation by *N*, *N*, *N'*, *N'*-tetramethyl-*p*-phenylenediamine.

the patients, especially after exercise, and its recovery took much more time in the patients. The drop in pH by exercise was lower in the patients than in controls, by an increased lactic acid efflux from the muscles in the patients (Arnold *et al.*, 1985).

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