

Review

The development of mitochondrial medicine

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ABSTRACT Primary defects in mitochondrial function are implicated in over 100 diseases, and the list continues to grow. Yet the first mitochondrial defect—a myopathy—was demonstrated only 35 years ago. The field's dramatic expansion reflects growth of knowledge in three areas: (i) characterization of mitochondrial structure and function, (ii) elucidation of the steps involved in mitochondrial biosynthesis, and (iii) discovery of specific mitochondrial DNA. Many mitochondrial diseases are accompanied by mutations in this DNA. Inheritance is by maternal transmission. The metabolic defects encompass the electron transport complexes, intermediates of the tricarboxylic acid cycle, and substrate transport. The clinical manifestations are protean, most often involving skeletal muscle and the central nervous system. In addition to being a primary cause of disease, mitochondrial DNA mutations and impaired oxidation have now been found to occur as secondary phenomena in aging as well as in age-related degenerative diseases such as Parkinson, Alzheimer, and Huntington diseases, amyotrophic lateral sclerosis and cardiomyopathies, atherosclerosis, and diabetes mellitus. Manifestations of both the primary and secondary mitochondrial diseases are thought to result from the production of oxygen free radicals. With increased understanding of the mechanisms underlying the mitochondrial dysfunctions has come the beginnings of therapeutic strategies, based mostly on the administration of antioxidants, replacement of cofactors, and provision of nutrients. At the present accelerating pace of development of what may be called mitochondrial medicine, much more is likely to be achieved within the next few years.

In 1959, the first biochemical studies of a cell organelle in humans were undertaken, following observations made at the bedside of a patient with striking symptoms, never before encountered. These clinical observations, first, led to an idea about the origin of the symptoms and, second, to studies of the particular organelle: the mitochondrion (1). The pathophysiology of the mitochondria developed gradually over the years as relevant discoveries were made in biochemistry, cell biology, and molecular biology. During the past few years the field *mitochondrial medicine* has expanded dramatically, in several directions. I here

provide a short review concentrating on those aspects most relevant to clinical medicine. In the accompanying review (174), Wallace describes the molecular, biological, and evolutionary implications of mitochondrial diseases.

The Birth of Mitochondrial Medicine (1959–1962): Luft Disease

The first patient found to have a mitochondrial disease was a 30-year-old woman who developed clinical symptoms at the age of 7. Her dominant symptoms were *enormous perspiration* combined with markedly increased fluid intake but without polyuria; *extremely high caloric intake* (above 3000 kcal per day) at a stable body weight of 38 kg and a body height of 159 cm; and *general weakness*, particularly prominent in her musculature. The dominating laboratory finding was a basal metabolic rate (BMR, a measure of oxygen consumption) of +180%. Thyroid function was normal. Subtotal thyroidectomy with administration of thyroid-depressing drugs was followed by classical myxedema but with a BMR of +100%.

Following the idea that the patient's enormously elevated BMR must involve mechanisms regulating oxygen consumption at the cellular level, studies were undertaken that focused on the mitochondria of skeletal muscle. By 1960, studies on rat liver mitochondria had already shown that this organelle is site of cell respiration and respiration-regulated phosphorylation. Uptake of oxygen by mitochondria was known to be controlled by the components of ATP production (inorganic phosphate, P_i , and the phosphate acceptor, ADP). This respiratory control allows the body to adapt oxygen consumption to actual energy need. The patient's condition, *a priori*, could then be ascribed to a derangement of respiratory control.

Biochemical studies of isolated skeletal muscle mitochondria from the patient (Figs. 1 and 2) demonstrated a nearly maximal rate of respiration in the presence of substrate alone without addition of ADP + P_i , but an almost normal phosphorylating efficiency (expressed as the P/O ratio) in the presence of ADP and P_i . The mitochondria also exhibited high ATPase activity, which was only slightly stimulated by 2,4-dinitrophenol, a known uncoupler of respiration from phosphor-

ylation. These features of "loosely coupled" respiration—deficient respiratory control with a partially maintained ability to synthesize ATP—accounted for the symptoms of the patient: abnormal production of heat, which the body tried to relieve by increased perspiration, and enormous caloric intake to compensate for the increased combustion.

The mitochondria in this patient were also insensitive to oligomycin, a drug which interferes with the tight coupling of electron transport to phosphorylation without inhibiting ATP synthesis. A tentative explanation for this observation was an "energy leak" above the level of the phosphorylating system. Such a proposal was supported by the observation in the second patient with this disease, Luft disease (2–4), of an energy-dissipating futile cycle of Ca^{2+} uptake and release—i.e., a waste of energy without a change in calcium concentration. Other remarkable findings in the first patient's mitochondria were a high level of cytochrome oxidase, a relatively low level of coenzyme Q, and a high content of RNA in a muscle homogenate, one piece of evidence for increased mitochondrial synthesis.

Electron microscopy (Fig. 3) of the mitochondria revealed striking structural abnormalities: large accumulations of mitochondria of highly variable size in the perinuclear zone of the muscle cells and vast paracrystalline inclusions, possibly composed of lipofuscin granules.

Several explanations for the loose coupling were tested, using techniques then available. These studies suggested that a short circuit of the flow of protons in the inner membranes had occurred, partly inhibiting ATP production—but preserving electron transport. No uncoupling agent—e.g., thermogenin (5)—was found in muscle homogenates.

Another assumption was that the lack of respiratory control might be due to en-

Abbreviations: KSS, Kearns–Sayre syndrome; MERRF, myoclonus epilepsy and ragged red fibers (syndrome); LHON, Leber hereditary optic neuropathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; CPEO, chronic progressive external ophthalmoplegia; CNS, central nervous system; LDL, low density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; MHC, major histocompatibility complex.

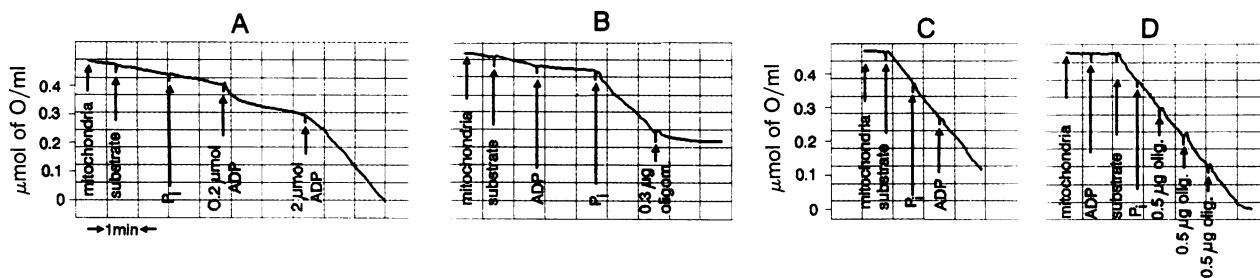


FIG. 1. Effect of P_i , ADP, and oligomycin on respiration of skeletal muscle mitochondria from a normal subject (A and B) and from the hypermetabolic patient (C and D).

hanced proliferation of mitochondria, with the formation of a component necessary for maintaining tight coupling between respiration and phosphorylation having failed to keep pace with the proliferation (6). Coenzyme Q might be a candidate for this—in the first patient its level was decreased relative to cytochrome oxidase.

The biochemical and morphological findings in Luft disease would have an impact on the further development of mitochondrial pathophysiology with the growth of the field in the 1970s.

Growth of the Field of Mitochondrial Disease

At the beginning of the 1970s it was realized that aberrations of the respiratory chain with or without structurally abnormal mitochondria of the type observed in Luft disease also occurred in certain other myopathies not associated with elevated oxygen consumption (7). In 1970–1972, respiratory chain deficiencies in disorders mainly involving central nervous system (CNS) and skeletal muscles were reported (8–10). In the following year, the first examples were reported of myopathies due to isolated deficiencies of muscle carnitine (11) and carnitine palmitoyltransferase (12).

These additional clinical discoveries were the starting point for rapid expansion in the field of mitochondrial pathophysiology. By 1988, Scholte's comprehensive

review of the biochemical basis of mitochondrial diseases classified more than 120 entities (13). All were based on alterations in mitochondrial biochemistry.

From Scholte's and subsequent reviews several basic principles in mitochondrial pathology emerged. First, some mitochondrial diseases affect only one tissue, most often skeletal muscle and brain but also liver, heart, kidneys, or endocrine glands. Other organs may be involved secondarily. The disease may originate as a specific defect in mitochondrial function, but a variety of genetic and environmental factors may contribute to the phenotype.

Despite the diversity of clinical phenomena and mitochondrial pathology, seven syndromes have been particularly important in advancing our understanding of mitochondrial medicine: (i) *Kearns-Sayre syndrome* (KSS), with ophthalmoplegia, retinal pigmentary degeneration, sometimes heart block, ataxia, hyperparathyroidism, and short stature; (ii) *myoclonus epilepsy and ragged red fibers syndrome* (MERRF), with intense myoclonus, epilepsy, progressive ataxia, muscle weakness and wasting, deafness, and dementia; (iii) *Leber hereditary optic neuropathy* (LHON), with blindness in men, at times movement disorders and encephalomyopathy, electrocardiogram abnormalities, and retinal microangiopathy; (iv) *mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes*

(MELAS), with episodic vomiting, lactic acidosis, and myopathy with ragged red fibers, sometimes dementia, generalized seizures, deafness, and short stature; (v) *Leigh disease* or subacute necrotizing encephalomyopathy, with respiratory abnormalities, weak cry, impaired feeding, impaired vision and hearing, ataxia, weakness, and hypotension; (vi) *chronic progressive external ophthalmoplegia* (CPEO) and mitochondrial myopathy, with symptoms similar to those in KSS but also ocular myopathy, retinitis pigmentosa, and central nervous system (CNS) dysfunction; and (vii) *Alper syndrome* or progressive infantile poliodystrophy, with seizures, dementia, spasticity, blindness, and liver dysfunction accompanied by specific cerebral degeneration.

The clinical expressions of these and other mitochondrial syndromes may vary considerably. Overlapping between the syndromes is common and may make diagnosis difficult. Clearly, tissues with a high demand for ATP and oxidative turnover are preferentially affected in different combinations. In some syndromes, endocrine glands are involved with signs of diabetes, hypoparathyroidism, stunted growth, etc. Inherited factors are present in some, if not all, of these syndromes. Maternal inheritance is well established in MERRF and LHON.

A common feature in this group of inborn metabolic errors is the involvement of specific enzymes in the pathway of aerobic energy production in the mitochondria. Thus, there is a defect in coenzyme Q metabolism in KSS; reduced activities of respiratory complexes I and IV in MERRF; reduced activity in complex I and cytochrome *c* oxidase in MELAS; and reduced activity in cytochrome *c* oxidase in Leigh syndrome.

The Discovery of Specific Mitochondrial DNA (1963–1964) and of Mutations in It (1988)

That DNA is present in mitochondria (mtDNA) was first clearly shown in 1963 by Nass and Nass (14) in chick embryos and by Schatz *et al.* (15), who isolated DNA from purified yeast mitochondria. By 1981 the complete sequence of human

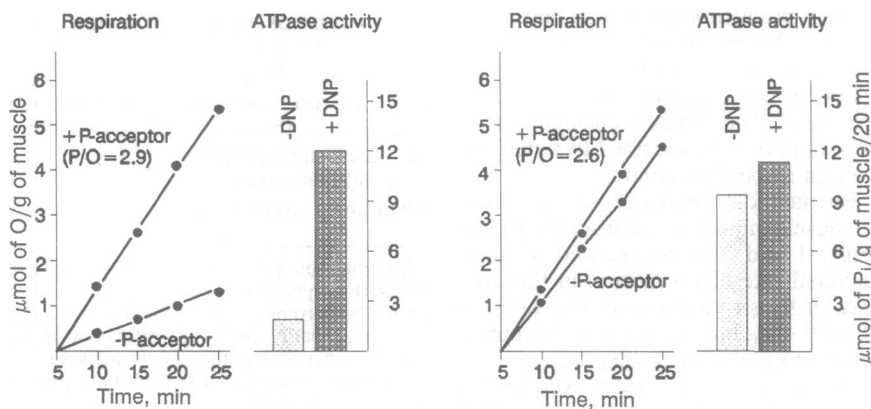


FIG. 2. Respiratory control, phosphorylation efficiency, and ATPase activity of isolated skeletal muscle mitochondria from a normal subject (Left) and from the hypermetabolic patient (Right). DNP, 2,4-dinitrophenol.

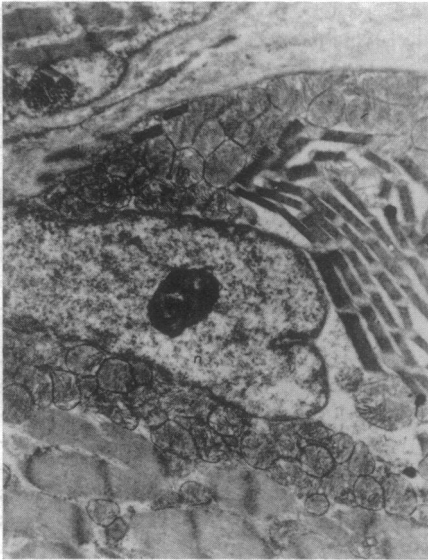


FIG. 3. Electron micrograph from a muscle fiber of the hypermetabolic patient. Cell nucleus (n) and a multitude of mitochondria (m) surrounding it. On the right is a bundle of dense cell inclusions. ($\times 4700$.)

mtDNA was elucidated (16). Unlike nuclear DNA, there are thousands of copies of mtDNA in every nucleated cell, each mitochondrion containing 2–10 copies. In normal individuals, these copies are identical, each containing the genes encoding 13 proteins, all of which are subunits of the respiratory chain enzyme complexes, 22 tRNAs, and 2 rRNAs (for review, see refs. 17 and 18). The absence of introns makes mtDNA compact. The only non-coding part of mtDNA is the D loop (displacement loop) of about 1000 bp, containing the origin of replication of the H strand (heavy strand) of the mtDNA and the promoters for L- (light strand) and H-strand transcription (19). One of the fascinating features of mtDNA is that it undergoes mutations 5–10 times faster than nuclear DNA (20). This increased rate of mutation is due to at least two factors. (i) There is a lack of histone proteins to protect mtDNA (21). (ii) The mitochondria are not efficient in repairing DNA damage (22). About 90% of oxygen in the cell is consumed by mitochondria and, as a result, there can be extensive oxidative damage to mtDNA (23).

Mitochondria are the only known source of extranuclear DNA in humans. Since, during egg fertilization, the sperm contributes only its nuclear DNA to the zygote (19), the entire mitochondrial genotype in both males and females is maternally inherited. Thus, only the mothers transmit mtDNA to the children, and only the daughters can transmit mtDNA to the next generation. This inheritance does not follow Mendelian laws.

In 1988, a breakthrough in mitochondrial pathophysiology occurred with the report of an association of different spo-

radic human encephalomyopathies with large deletions of mtDNA (24) and a G-to-A transition mutation at nucleotide pair 11778 in the mtDNA of patients with LHON (25). A constant feature in LHON has been the coexistence of mutant and wild-type mtDNA (heteroplasmy). Following these reports, other clinical syndromes were soon linked to specific mutations, deletions, and duplications of mtDNA, impairing protein synthesis of the mitochondrial subunits of the respiratory chain complexes—e.g., point mutations of the tRNA^{Lys} gene in the MERRF syndrome (26) and a point mutation of the tRNA^{Phe} gene in MELAS (27). Most of the “classical” mitochondrial disorders have since been submitted to detailed studies (for reviews, see refs. 28–31). A special feature of these tRNA mutations is that they have indistinguishable consequences at the biochemical level, producing partial defects in the mtDNA-dependent respiratory complexes. In sporadic adult-onset CPEO with ragged red fibers, large-scale deletions ranging from 1.3 to 7.6 kb (32–35) or duplications (36) were observed in about 50% of the patients, and in nearly 100% of patients with KSS. No other mitochondrial encephalomyopathies had deletions (37). In some instances, evolution from a tissue-specific to a multisystem disorder (KSS) could be observed, and probably could be explained by an increase in the mutated mtDNA fraction with age (38).

In earlier studies, nuclear rather than mitochondrial mutations were thought responsible for the above defects. Rather, variation in the ratio of wild-type to mutant mtDNA in different tissues probably explains the tissue specificity of the mitochondrial myopathies (39). Similarly, alterations in the tissue distribution of the proportion of mutant mtDNA with time may explain some of the age dependency. However, disturbances in interactions between nucleus and mitochondria were recently reported in families with mitochondrial disease (CPEO-like syndrome) with autosomal dominant inheritance (for review, see ref. 31). The activities of respiratory complexes I and IV were markedly reduced, and there were multiple deletions of mtDNA spanning several kilobases. This autosomal dominant disease implied mutation in a nucleus-encoded gene (31). The abnormal product of this nuclear gene was supposed to interact with mtDNA to cause accumulation of multiple lesions in the molecule. This particular area was recently enriched by similar reports on CPEO syndromes and multiple deletions of mtDNA with autosomal recessive and autosomal dominant transmission (39–42). The next step towards better understanding the pathogenesis of these diseases must include studies of nuclear gene products interfering with mtDNA or

its gene products. AIDS patients undergoing long-term treatment with azidothymidine (AZT) developed destructive mitochondrial myopathy with ragged red fibers and markedly reduced amounts of mtDNA in skeletal muscle, and this depletion was reverted in one patient after withdrawal of AZT (43).

Free Radicals, Oxidative Damage, and Antioxidants

Essential for the discussion of mitochondrial pathophysiology is a brief summary of oxidative processes in mitochondria and the consequences of abnormalities in those processes. Molecular oxygen has the ability to take up electrons (e^-) from the surroundings, and these electrons are easily exchangeable. During normal aerobic respiration, mitochondria consume O_2 , reducing it stepwise to form H_2O (Fig. 4).

During this process, four electrons are added, and energy released is conserved as ATP. The chemical oxidants, $\cdot O_2$ and $\cdot OH$, are normal products of the oxidative process. Entities with such unpaired electrons and with reactive properties are called *radicals*. They may be harmful when produced in increased amounts and not neutralized by the normally occurring antioxidants. Then leakage may lead to damage of membrane lipids, DNA, proteins, and other macromolecules.

Other sources of radicals are destruction of cells during chronic infections (44) with bursts of NO , $\cdot O_2$, H_2O_2 , and OCl^- ; degradation of fatty acid and other molecules by peroxisomes (45); and by-products of processes acting as defense mechanisms against toxic substances (44). Exogenous additions to such endogenous contributors to the load of oxidants are, e.g., oxides of nitrogen (NO) in cigarette smoke, generation of radicals from peroxides promoted by iron and copper compounds (Fenton reaction), and products from normal food intake (46, 47). As a matter of fact, the effects of some anticancer drugs are based on this principle. Thus, Adriamycin targets cancer by producing reactive oxygen species.

Defense mechanisms try to minimize the levels of harmful oxidants and the damage they inflict. Several enzymes

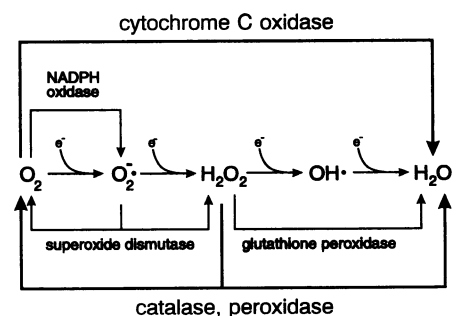


FIG. 4. Cellular formation of free radicals.

such as superoxide dismutase, catalase, and glutathione peroxidase are part of these mechanisms. The body also has developed natural lipophilic and hydrophobic antioxidants: vitamin E, the quinones (coenzyme Q), and carotenoids, typically located in membranes and in lipoproteins. Water-soluble antioxidants include vitamin C and thiols such as glutathione. Many of these antioxidants also are dietary products. The significance of vitamins Q and E as antioxidants has gained enormous attention during the last few years, especially coenzyme Q, located as it is in the electron transport system by linking complexes I and III of the respiratory chain. In its reduced form it serves as an antioxidant, preventing lipid peroxidation in biological membranes and low density lipoproteins (LDL) and, thereby, playing an active role in cellular defense against oxidative damage. Accordingly, coenzyme Q has been given to patients suffering from mitochondrial diseases.

The Aging Process and the Mitochondria

Many diseases related to aging may involve oxygen radicals at some stage in their development. In these diseases, it has been proposed that mutations of mtDNA and changes in cellular bioenergetics contribute in some way to the aging process and to the development of degenerative diseases. Thus, the capacity for oxidative phosphorylation declines with age (29) due to accumulation of defective mtDNA, nuclear DNA, or both. ATP production can decline below a level critical for the function of the cell. There is evidence that the "normal" aging process is accompanied by damage of molecules, including mtDNA, and that such damage accumulates with age (48–51). A 5-kb common deletion of mtDNA was found to accumulate with age in human brain and skeletal muscle (52). Furthermore, diseases associated with alterations in mtDNA progress with age, and this progression is associated with an increasing proportion of deleted molecules. In heart muscle, mtDNA deletions—especially a 3.6-kb deletion—have been shown to accumulate after 35 years of age (53–55), as has a 3-kb deletion in skeletal muscle (56). In addition, the concentration of mitochondrial mRNA and rRNA declined with age in rat brain and heart (57) and was associated with a 50% decrease in transcription rate (58). In ischemic hearts, hypoxemic inhibition of oxidative phosphorylation was accompanied by an increase in mtDNA damage (59).

As a sign of the aging process, the number of cytochrome *c* oxidase-negative skeletal and heart muscle fibers increased with age (60, 61), and enzyme activities of complexes I and IV declined

progressively with age in human skeletal muscle and liver (62, 63). In addition, evidence has been presented for age-related changes in coenzyme Q levels in several tissue: by Beyer *et al.* (64) in rats, and by Kalén *et al.* (65) in humans.

While more substantial studies are needed, these data—concerning mtDNA, oxidation, and coenzyme Q—seem to favor the idea that aging may be associated with a disturbed balance between oxidative and antioxidative forces, leading to a decline in oxidative phosphorylation below some "organ-specific threshold" and to mtDNA damage. A morphologic consequence could be the age-related increase in lipofuscin granules, also termed "age pigments" (66).

Age-Related Degenerative Diseases and Defects in Oxidative Phosphorylation

In a search for diseases possibly connected with defects in oxidative phosphorylation and with alterations in mtDNA, it seems appropriate to look at tissues that are critically dependent on a large supply of ATP for their specific functions—e.g., CNS, heart and skeletal muscle, kidney, liver, retina, and pancreatic islets.

In this connection, some degenerative disorders—such as Parkinson disease and cardiomyopathies—are associated with deletions of the mitochondrial genome, in contrast to the classical encephalomyopathies (e.g., MERRF, MELAS, and LHON), characterized by distinct mutations. Ozawa *et al.* (67) recently expanded this knowledge by demonstrating that patients with degenerative disorders (Parkinson disease and dilated or hypertrophic cardiomyopathy) and classical encephalomyopathies (MERRF and MELAS), which carry some distinct but partly overlapping symptoms and pathologies, also carry similar clustering of point mutations of mtDNA. They emphasize, first, that these patients, while having phenotypically different disorders, belong to the same mtDNA gene family and, second, that not one particular mutation but the type and total number of mutations of a patient is an important factor for the expression of the disease. Brown *et al.* (68) supported this suggestion by reporting on synergistically interacting mutations of mtDNA in LHON, indicating that the clinical manifestations of the disease are the product of an overall decrease in mitochondrial energy production rather than a defect in a specific mitochondrial enzyme.

The Cardiovascular System. mtDNA deletions and depressed activities of the enzymes in oxidative phosphorylation in aging heart muscle could pave the way for a disturbed balance of oxidation/antioxidation. The imbalance could lead to free radical-mediated lipid peroxidation, including that of LDL. The aldehyde prod-

ucts of lipid hydroperoxide breakdown are responsible for the modification of LDL apoprotein. Aldehyde-modified apolipoprotein B alters receptor affinity and, therefore, is subjected to endocytosis via the scavenger receptor pathway of macrophages (69) and accumulates (70, 71). This accumulation can initiate foam cell formation and the appearance of atherosclerotic plaques (72–74). The oxidation of LDL may be prevented by endogenous antioxidant compounds, mainly α -tocopherol and coenzyme Q (75).

The approach to reduce such an assumed imbalance has been based on the general theme that defective energy supply—due to lack of substrate and cofactor and decreased utilization of oxygen—may lead to the progression of various myocardial diseases. This approach is supported by the findings that low levels of vitamins C and E, two of the natural plasma antioxidants, may contribute to the high incidence of ischemic heart disease (76, 77) and that cardiovascular diseases are accompanied by low plasma concentrations of vitamin C, α -tocopherol and β -carotene (78, 79), and coenzyme Q (80). The latter appears to protect human LDL more efficiently against lipid peroxidation than does α -tocopherol (81), demonstrating that LDL-associated coenzyme Q may be an important anti-risk factor. Again, these studies must be considered preliminary.

Dilated Cardiomyopathy. Dilated cardiomyopathy is the most common cause of severe cardiomyopathies in young and middle-aged people. Deletions in mtDNA in heart muscle from such patients have been demonstrated (82, 83). Most of these cardiac myopathies are familial (84), and in some families an X-chromosomal inheritance has been suggested (85). Interestingly, in a patient with familial dilated cardiomyopathy (86) mtDNA with deletions of different sizes made up about 50% of total mtDNA in heart muscle. This suggests the presence of some nuclear gene defect leading to multiple mtDNA deletions (see above).

Recently, a rapidly escalating number of reports have appeared on attempts to treat "cardiomyopathy" with compounds with antioxidant action, in particular coenzyme Q (87–92). While some of the reports may be promising, such treatment is yet to be established.

The CNS. The CNS derives its energy almost exclusively from oxidative phosphorylation and thus consumes a large amount of oxygen. Hydrogen peroxide (H_2O_2) is a normal by-product of the function of several enzymes of importance for the CNS—e.g., monoamine oxidase and tyrosine hydroxylase—and of the autooxidation of several endogenous substances (ascorbic acid and catecholamines). Any disturbances in the equilibrium between oxidation and antioxi-

tion in the CNS tissues could disrupt the efficiency of electron transport. This could decrease ATP availability to cellular functions in the CNS such as ATP-regulated K channels, Ca^{2+} pumps, Na^+/K^+ pumps, exocytosis, and various phosphorylation processes.

Another possible process leading to oxidative stress in the CNS could involve the major excitatory neurotransmitter glutamate (for review, see ref. 93) (Fig. 5). There is increasing evidence that glutamate may be a major mediator of oxidative stress in the CNS, primarily through its activation of ionotropic receptors, distinguished by specific agonists. Activation of glutamate receptors by these agonists in tissue culture leads to neuronal degeneration (94, 95). The processes include receptor-mediated influx of Na^+ and Ca^{2+} , which brings about a series of events including initiation of the arachidonic acid cascade, activation of proteases, and stimulation of NO synthase. The depolarization increases ATP consumption induced by Na^+/K^+ ATPase, increasing oxidative phosphorylation with superoxide radicals as a by-product. These radicals, as well as arachidonic acid, enhance the release of glutamate and inhibit its inactivation, thereby promoting the harmful events (96, 97). Furthermore, NO released in the above process interferes with many events, including oxidative phosphorylation, with a reduction in ribonucleotide reductase activity (98) and with forma-

tion of $\cdot\text{OH}$ from O_2 (99), ultimately leading to degeneration of neurons (100).

Thus, stimulation of receptors for neurotransmitters—as in this case glutamate—may activate processes in the CNS leading to an imbalance in oxidation/antioxidation. This in turn could be accompanied by cumulative damage to DNA, proteins, and lipids, and eventually to degeneration of neurons. The results would be especially harmful if, for some reason, antioxidant defenses are compromised, as during aging.

However, systemic treatment with a variety of free radical scavengers did not protect against striatal lesions produced by intracerebral injection of glutamate receptor agonists (101, 102).

Neurodegenerative Diseases. Oxidative stress, perhaps partly glutamate mediated, has also been implicated in some neurodegenerative diseases. In *Parkinson disease* there is degeneration of dopaminergic neurons projecting into the caudate-putamen. The dopaminergic system may be at risk for oxidative stress (103), since the oxidation of catecholamines by monoamine oxidase, which increases with age, is a source of oxygen radicals (104). Enzyme assays in brains from Parkinson patients did reveal a reduction of complex I activity, especially in the substantia nigra (105–107), also observed in blood platelets (108) and skeletal muscle mitochondria (109, 110). Furthermore, some of the mtDNA-encoded subunits of complex I were de-

creased in the nigrostriatal region of brains from Parkinson patients (95). The amount of deletion-bearing relative to normal mtDNA in such patients was about 10 times larger than in controls (111). Parkinson disease has been suggested to appear when the genomes that have undergone deletions surpass a certain threshold, or the deletions are concentrated to a specific neuronal subtype of the striatum (31).

In addition, Parkinsonism is induced by a specific toxin of the substantia nigra, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a strong inhibitor of NADH-coenzyme Q reductase (complex I) and generator of oxygen radicals (112–114). Treatment with glutamate receptor agonists protected against the dopaminergic degeneration induced by an MPTP metabolite (115, 116). These data suggest a possible link between oxidative stress and glutamate neurotransmission in this system (93). For a while, emphasis was put on a 5-kb deletion of mtDNA in the striatum (111), but that could not be confirmed (115–117).

Amyotrophic lateral sclerosis (ALS). ALS is accompanied by progressive degeneration of motor neurons in the brain stem and spinal cord. In about 10% of the patients, ALS is inherited as an autosomal dominant trait with high penetrance after the sixth decade (118, 119). There are some data suggesting that oxidative stress and activation of glutamate-gated cation channels may be involved in ALS (93). Eleven different missense mutations in the gene encoding one form of cytosolic superoxide dismutase (SOD1)—responsible for the degradation of the toxic superoxide anion O_2^- to O_2 and H_2O —were observed in families suffering from the autosomal dominant form of ALS (120). In addition, the content of protein carbonyl, a measure of protein oxidation, was elevated in patients with sporadic ALS as compared with controls—at least suggesting oxidative stress as a feature of ALS (93). These data, while limited, may carry some important implications for future therapy in ALS (121).

Huntington disease (HD). HD is an autosomal inherited disorder, characterized by disturbances in movement and progressive dementia and with onset at a mature age. Intrastriatal injection of a glutamate receptor agonist reproduced several aspects of the neuropathology of HD, indicating some dysfunction in the disposition of excitatory amino acids (122). The levels of glutamate in cerebrospinal fluid were reported to be elevated in HD (123). Pharmacological inhibition of complex I or complex II caused the same selective pattern of degeneration as seen with glutamate receptor agonists (124, 125). Neuronal susceptibility to complex II inhibition increases with age in animals, which may be germane to the

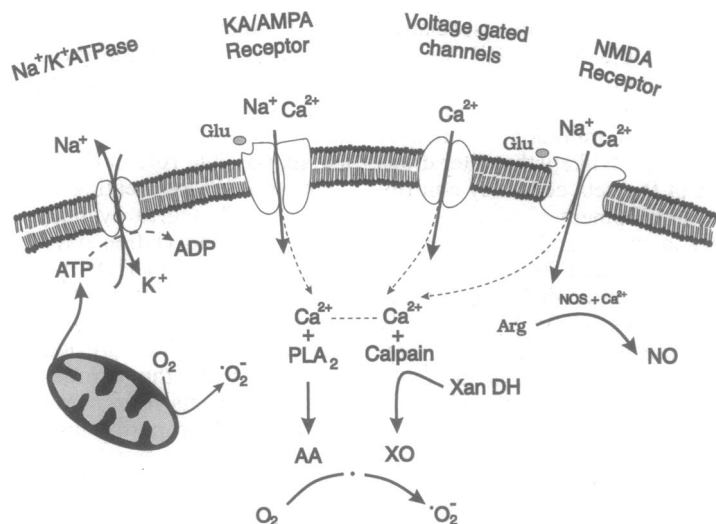


FIG. 5. Schematic representation of the glutamate receptor-mediated processes that may be involved in the generation of oxidative stress. This is a modification of the model presented by Coyle and Puttfarcken (93). Glutamate activates the kainic acid/ α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (KA/AMPA) receptor, which results in opening of channels through which Na^+ and Ca^{2+} flow. Depolarization activates voltage-gated Ca^{2+} channels, enabling Ca^{2+} influx and thereby increasing cytoplasmic free Ca^{2+} , $[\text{Ca}^{2+}]_i$. Under partially depolarizing conditions, Na^+ and Ca^{2+} flow through channels of the *N*-methyl-D-aspartate (NMDA) receptor. An increase in $[\text{Ca}^{2+}]_i$ may activate various enzymes such as phospholipase A_2 (PLA_2), proteases, and NO synthase (NOS), promoting the formation of $\cdot\text{OH}$ and NO. $\cdot\text{OH}$ is also produced as a by-product in the increased oxidative phosphorylation, which follows an increased ATP consumption by the Na^+/K^+ ATPase. AA, arachidonic acid; Xan DH, xanthine dehydrogenase; XO, xanthine oxidase.

delayed onset of neurodegeneration in HD (126). The ensuing disruption of the respiratory chain could lead to impaired oxidative phosphorylation. In addition, abnormal mitochondrial structures and accumulation of lipofuscin have been demonstrated in HD (127). A complex IV defect in the caudate, but not in the cortex, was found in brains from patients with HD (128), and also a complex I defect in blood platelet mitochondria (129). These and other data favor the possible involvement in HD of oxidative stress, perhaps in combination with dysfunction of glutamate metabolism.

Alzheimer disease. Alzheimer disease is an age-related dementia, characterized pathologically by neurofibrillary tangles, senile plaques, and amyloid deposits in the CNS. There are indications for defects in mitochondrial function in this disease: oxidative phosphorylation was not effectively coupled in homogenates of neocortex from patients (130); there were marked reductions in pyruvate dehydrogenase in frontal and occipital cortex (131) and in complex I activity in blood platelet mitochondria from patients (132); and distinct point mutations of mtDNA were reported in brain sections (133). Therefore, the development of Alzheimer disease to some extent involves components of mitochondrial energy production, including degeneration of synaptosomes because of impaired energy production of synaptosomal mitochondria (29).

On the whole, oxidative stress seems to represent one possible pathway—perhaps in part initiated by glutamate—leading to neuronal degeneration in a manner consistent with the course and pathology of some degenerative diseases of the CNS. However, antioxidants at best provided only partial protection, and oxidants can be generated by a number of mediators independent of glutamate (134). Furthermore, other pathologic processes may be the primary events enhancing the vulnerability to glutamate, such as the amyloid A4 peptide in Alzheimer disease (135). These and other observations and views demonstrate the gaps in our knowledge of the specific metabolic processes that may promote oxidative stress at the neuronal level, including glutamate receptor activation (93). Filling these gaps may lead to strategies for blocking pathways involved in neuronal degeneration.

Diabetes Mellitus

Non-insulin-dependent diabetes mellitus (NIDDM) is an age-related disease, which also exhibits features of a degenerative disorder. Can an increase in the incidence of insulin-dependent diabetes mellitus (IDDM) be on the basis of environmental exposure or other factors involving the mitochondrial genome?

There are several lines of evidence suggesting alterations in mtDNA in two of the major tissues involved in diabetes, pancreatic islets and skeletal muscle, both highly reliant on oxygen. Formation of free radicals such as NO and ·OH and alkylation of DNA and proteins also occur in the beta cells of the pancreatic islets, and the inadequately protected mitochondrial genome is open to attack from such chemicals. Various diabetogenic agents could operate by this route—e.g., interleukin 1 β , interferon γ , tumor necrosis factor α , alloxan, and streptozotocin (ref. 136; for review, see ref. 137). The action of some of these agents could be inhibited by antioxidants in animal models (138–141) as well as in humans (142). Such observations led Okamoto (143) to suggest that diabetogenic agents induce breaks of mtDNA in islets, ultimately followed by death of beta cells. Universal applicability of this hypothesis has been questioned (144).

Gerbitz (137) discusses whether mitochondrially encoded peptides can serve as MHC-restricted antigens. Some findings point in this direction (145, 146). If future research verifies this possibility, autoreactivity would enter the scheme leading to IDDM.

There is already some clinical evidence for the involvement of mtDNA in the development of diabetes. Patients with KSS and CPEO (see above) have an incidence of diabetes several times higher than in the general population (147–149). The earlier the onset of mitochondrial myopathy in these conditions, the more frequent was its association with IDDM (150). Also, MELAS and other mitochondrial cytopathies are sometimes associated with diabetes (151) and with a point mutation of mtDNA (152). This makes it likely that alterations of mtDNA of the beta cells may contribute to the development of diabetes.

Recently, a systemic 10.4-kb mtDNA deletion was reported in a family with maternally transmitted diabetes and sensor-neural deafness (153). Subsequently an A-to-G transition at nucleotide pair 3243, a conserved position in the mitochondrial gene for tRNA^{Leu}, also has been reported in families with diabetes (154–156). This mutation leads to impairment of mitochondrial transcription termination, which causes defects in mitochondrial protein synthesis (157). A similar mutation was found in insulin antibody-positive subjects, initially diagnosed as NIDDM, who progressed to IDDM (158). The mutation may be connected with a variable and progressive decrease in insulin secretory capacity.

It is unlikely that “common” NIDDM, constituting about 90% of the diabetic population, has its origin in specific mutations of mtDNA. However, an age-related decline in the capacity for oxida-

tive phosphorylation and its consequences could play a significant role in its pathophysiology.

Therapeutic Aspects

Understanding the mechanisms behind the development of mitochondrial diseases offers strategies for attempts at their treatment. Possibilities are supplementation of cofactors in the respiratory chain, addition of oxidizable substrates, and prevention of oxygen radical damage to the mitochondria.

Favorable results with antioxidants, “redox therapy,” were reported in a patient with a severe defect in complex III of the respiratory chain (159). Favorable results have also been reported in other circumstances (for reviews, see refs. 160 and 161): e.g., with coenzyme Q and succinate in a patient with KSS and a complex I defect (162) and with a prominent complex IV lesion (163); with coenzyme Q in ocular myopathy (164); and with coenzyme Q in five patients with KSS and low levels of coenzyme Q in serum and the mitochondrial fraction of skeletal muscle (164). Coenzyme Q also occupies a special place in the attempts to normalize oxidation/antioxidation abnormalities in age-related disease. Again, however, many of the reports on the use of coenzyme Q are anecdotal and require substantiation.

The rationale for treatment of cardiomyopathy with coenzyme Q rests, in part, on the finding of myocardial dysfunction and defective energy supply in biopsy samples from subjects with such pathology (165). While there are favorable reports on administration of coenzyme Q in that circumstance (166–169), additional studies are required to establish its possible role in that therapy.

Other ways of treating diseases that can be attributed to dysfunction of oxidative phosphorylation have been tried (170–173). One in LHON includes functional relocation of normal mitochondrial genes to the patient's nucleus so that their protein products are delivered to the organelle from the cytoplasm. In another, myoblasts from patients with mitochondrial myopathy have been explanted, and their mutant DNA has been replaced with normal DNA. The genotypically normal muscle cells have then been expanded and injected back into the patient's muscle, where they could fuse to existing myotubes, contributing more normal mtDNA and supplementing mitochondrial energy production.

Conclusions

We can anticipate expansion of the field of mitochondrial medicine in several directions: first, into some age-related diseases so far not approached; second, into

areas connected with new concepts in mitochondrial biochemistry and physiology—e.g., protein transport via the mitochondrial membrane. Of great interest are in-depth studies aiming at a better understanding of the processes underlying the mitochondrial defects and well-controlled studies on the place of nutritional supplements and replacement therapy with suitable redox compounds in the amelioration of disorders due to deficiencies in mitochondrial bioenergetics.

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