Annu. Rev. Nutr. 1989. 9:229–51

NUTRIENT SUPPLY AND MITOCHONDRIAL FUNCTION

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INTRODUCTION

In recent years, considerable advances have occurred in understanding the genetics and cell biology of mitochondria. This knowledge provides the basis for a more complete analysis of nutrient effects on mitochondrial function than was possible from earlier knowledge of vitaminic cofactor requirements for mitochondrial enzymes and electron transport systems. In this chapter, we review the nutrient requirements of mitochondria, the plasticity of mitochondrial form and function, and the modulation of mitochondrial form and function by nutrients. These considerations suggest new directions for research to determine the contribution of nutrition to tissue-specific energy production and other mitochondrial functions.

0199-9885/89/0715-0229$02.00

MITOCHONDRIAL PLASTICITY

Organization and Function of Mitochondria

Mitochondria are the powerhouses of the cell. The enzymes and enzyme complexes vital for mitochondrial function are organized in a compact form in the different compartments of the organelle. The functions that are common to most, if not all, mitochondria include the synthesis of ATP, terminal oxidation of pyruvate derived from carbohydrate and amino acid catabolism, β-oxidation of fatty acids, and oxidation of acetate (acetyl CoA) derived from the diet and from ethanol, fatty acid, protein, and carbohydrate oxidations (119). Other specialized functions include oxidation of branched chain amino acids, maintenance of nitrogen homeostasis and urea formation, oxidation of sulfite, activation of vitamin D_3, and required steps in the synthesis of heme, steroid hormones, bile acids, and numerous other essential biochemical components (119). Thus, in addition to the essential function in biologic oxidations and energy metabolism, mitochondria contain many other biochemical systems that are vital to organismic survival.

Because mammalian cells are specialized in their functions, many of the specialized systems within mitochondria are expressed differentially. The complexity of this differential expression is only now being fully appreciated. Relatively little is known about the determinants of this differential expression, i.e. about what determines whether the mitochondria of hepatocytes contain the enzyme activities essential for bile acid production, whether the mitochondria from kidney cortex contain an enzyme essential for the final activation of vitamin D_3, or whether the mitochondria from adrenal cortex have enzymes essential for glucocorticoid production. Even less is known about the factors that determine the dynamic responses of mitochondrial functions to nutrient intake (e.g. changes from high-carbohydrate to high-protein or high-fat diets) or the factors that determine the differential content of various metabolic systems of mitochondria in cells with different localizations relative to blood supply (e.g. periportal versus pericentral hepatocytes, 117) or with different localizations within cells (e.g. subsarcolemmal versus intramyofibrillar mitochondria in muscle, 74). Recent advances provide methodology for uncovering such refined knowledge of mitochondrial function and indicate that nutritional factors are of primary importance in modulating the heterologous nature of mitochondrial functions in mammalian tissues.

To maintain normal function of the mitochondria, the matrix compartment is isolated from the cytoplasmic compartment by a special coupling membrane that is impermeable to most solutes (69). This membrane contains solute transport systems that function to allow the energy available from electron transport to be captured in the form of an electrochemical gradient and to allow utilization of this electrochemical gradient to drive ATP synthe-

sis from ADP and inorganic phosphate (Pi). A feature frequently overlooked, however, is that the electrochemical H^+ gradient is essential for virtually every aspect of mitochondrial function. The supply of oxidizable substrates and precursors (pyruvate, glutamate, fatty acyl carnitine, ADP, Pi) and the elimination of products (ATP, carnitine) are governed by either the electrical ($\Delta\psi$) or chemical (ΔpH) components of the electrochemical H^+ gradient (68, 69). In addition, the import of most matrix, inner membrane, and intermembrane space proteins, which are encoded by the nuclear genome and synthesized in the cytosol, requires the presence of the electrochemical potential (96, 120). Because new mitochondria are formed only by growth and division, only functional mitochondria can give rise to new mitochondria. Thus, continued function of mitochondria, especially maintenance of the electrochemical potential, appears to be essential for cell survival and for provision of functional mitochondria for new cells.

Earlier studies indicated that mitochondrial production of ATP may be under near-equilibrium control (126), i.e. that the amount of energy available from the oxidative reactions in the mitochondria, as captured in the electrochemical H^+ gradient, is totally available for ATP synthesis. More recent studies have shown that a variety of control features exist to regulate production of Δp from oxidative reactions and utilization of Δp for ATP synthesis (Figure 1). These mechanisms include rate limitation at the adenine nucleotide transporter (delivery of ADP and removal of ATP from the matrix) (66, 110), supply of NADH from dehydrogenases (31), transfer of electrons through cytochrome oxidase to O_2 (63), and utilization of the Δp by the ATP synthase (9). Through these mechanisms, the mitochondria can maintain a relatively stable Δp while at the same time can accommodate large variations in use of oxidizable substrates and in use of Δp for osmotic, electrical, and chemical work. Failure to maintain a relatively stable Δp would lead to loss of ATP synthesis and also to the potentially more serious losses of the ability to take up essential precursors for oxidative phosphorylation and to take up and process newly synthesized proteins.

Viewed in this context, there are two important aspects to nutrient supply to support mitochondrial function, namely, supply that affects the ability to maintain functional mitochondria and supply that optimizes the ability of functional mitochondria to support diverse, specialized functions in different sites within an organism. Both aspects require consideration of the factors that determine expression of enzyme activities in mitochondria and the heterogeneous distribution and functions of mitochondria.

Expression of Enzyme Activities in Mitochondria

Although mitochondria contain their own genome and transcription and translation components for protein synthesis, the number of proteins syn-

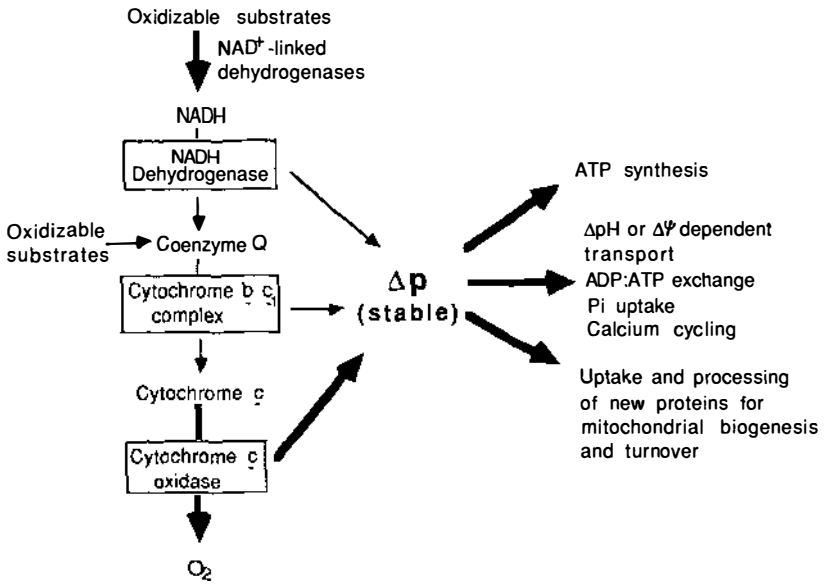

Figure 1 Sites of regulation in mitochondrial oxidative phosphorylation. Regulated steps are indicated *(bold arrows)*. Several NAD^+-linked dehydrogenases are regulated by Ca^{2+} (73), and cytochrome oxidase contains several subunits thought to function in regulation (63). Thus, generation of energy to drive Δp can be regulated by control of the oxidation of NAD^+-linked substrates. Most of the major pathways that consume Δp are also regulated. ATP synthase is regulated by an endogenous inhibitory protein (127). The adenine nucelotide carrier, phosphate carrier, and Ca^{2+} transport systems are also regulated (9). Similarly, the cytosolic synthesis of new mitochondrial proteins is regulated and coordinated with need (96, 120). Thus, regulatory mechanisms exist to stabilize Δp and eliminate a precise coupling (thermodynamic equilibrium) of the cytosolic [ATP]/[ADP] [Pi] and Δp.

thesized within the mitochondria is modest (~8–12). Most proteins are encoded by nuclear genes, synthesized as precursor forms on free cytosolic ribosomes, and imported into mitochondria (96, 120). Import of proteins occurs by vectorial processing, i.e. most imported proteins undergo covalent modification, interaction with coenzymes, and activation during this process. Inadequate availability of a coenzyme can thus arrest the import and processing of a protein. If this coenzyme is a critical enzyme or transporter for maintenance of the Δp, the resultant functional loss could lead to mitochondrial failure. Because both mitochondrial and nuclear gene products are required for functional mitochondria, the expression of gene products from the two genomes must be coordinated (119). The lack of a required vitaminic or mineral coenzyme can disrupt this coordination and result in mitochondria with diverse and aberrant properties. In this way, nutrient deficiency can have

a dramatic and generalized effect on mitochondrial formation and, consequently, on function.

Tissue-Specific Mitochondrial Distribution

Microcompartmentation of cell structures and functions is a common occurrence in mammalian cells (59). In differentiated mammalian cells, the distribution of mitochondria occurs in distinct patterns that are characteristic for different cell types. In transport epithelia such as renal proximal tubule cells and gastric parietal cells, the mitochondria are positioned adjacent to the pump-containing plasma membrane (21, 39). In contractile and motile cells, such as muscle and sperm cells, the mitochondria are positioned adjacent to contractile elements (39). In different cell types, mitochondria have numerous other specific associations, such as with gap junctions in cardiac myocytes (41), synaptic terminals in neurons (60), and junctional complexes in ependymal cells (94). This spatial distribution of mitochondria is due to apparently specific molecular interactions with cytoskeletal elements (85, 90) and, in cases for which it has been studied in detail, is reflected in the heterologous and the functional characteristics of the mitochondria.

Two aspects of this heterogeneous distribution are noteworthy, namely that the position of mitochondria within a cell or along a capillary can affect the accessibility of substrates from capillaries and that the clustering of mitochondria can create a unique microenvironment that can affect accessibility of nutrients from other regions of the cell or from capillaries. Among the clearest examples of the former are studies on the characteristics of subsarcolemmal and intermyofibrillar mitochondria of skeletal muscle (74). In myocytes, mitochondria are located in three different regions of the cell: adjacent to the plasma membrane (subsarcolemmal), between the myofibrils (intermyofibrillar), and adjacent to the nuclei (perinuclear). The intermyofibrillar population has higher cytochrome content, greater respiration rate, and higher activities for Ca^{2+} transport (74). The subsarcolemmal population responds preferentially to endurance training and to disuse atrophy (54), which indicates that this population is responsive to energy demands or to exogenous signals. Further, a genetic defect affecting only the intermyofibrillar population was reported (53). Thus, the respiratory and nutrient requirements for different mitochondria can vary even within single cells. A similar heterogeneity in mitochondria occurs between hepatocytes found in the periportal region of the liver sinusoid and those found in the centrilobular region (70). Morphometric studies of Loud (70) showed that the fractional volume of mitochondria is greater in the periportal region and that the mitochondria are morphologically distinct. Other studies have shown that there are significant differences in enzymatic activities along the sinusoid (117) and that this pattern can be rapidly reestablished in vitro by placing isolated cells in a column, where

a nutrient gradient can be established by slow perfusion with medium (88). Thus, cells with different positions relative to blood supply are exposed to different nutrient supplies, and the nutrient supply is a factor in determining the enzyme content and metabolic characteristics of the cells.

Nutrient supply to mitochondria can also vary because of the clustering of mitochondria. Clusters of mitochondria are common in adult mammalian cells and are a major determinant of the O_2 dependence of cellular respiration (60, 64). Clustering results in the occurrence of radial O_2 gradients, which can limit mitochondrial oxygenation (58). This inhomogeneity in mitochondrial distribution further results in a heterogenous supply of ATP under conditions of decreased ATP production such that sites of utilization peripheral to mitochondria are particularly vulnerable to ATP limitation (11). In kidney proximal tubule cells, the clustering and asymmetric distribution of mitochondria create a heterogeneity of pH within the aqueous cytoplasm (13) and may be responsible for the microcompartmentation of glycine metabolism within these cells (45, 46). Microcompartmentation of carbohydrate metabolism (59, 71, 86) is a particularly important aspect to the balance of nutrient supply to mitochondria. Studies of Lynch & Paul (71) and Paul (86) show that in smooth muscle, glycolytic activities are partitioned so that the supply of nutrients to the mitochondria is essentially independent of the supply of nutrients to glycolysis. Bereiter-Hahn (15) found that such partitioning in cultured cells changes during the cell cycle, i.e. the nutrient requirements cycle between more glycolytic and less glycolytic phases.

These examples illustrate a subtlety in nutrient requirements for mitochondria that is critical for optimal cell growth and cell function. The tissue specificity of mitochondrial plasticity results in distinctly different nutritional requirements, both qualitatively and quantitatively, in different mitochondria in various cell types. Although considerable research will be required to effectively use this knowledge for nutritional therapy, the information provides the basis for a more detailed understanding of the differential requirements of nutrients in different tissues and for a better understanding of how marginally replete dietary regimens can contribute to pathologic processes.

MODULATION OF MITOCHONDRIAL FORM AND FUNCTION BY NUTRIENTS

Nutrient Supply and Maintenance of Functional Mitochondria

A noticeable response of mitochondria to metabolic and environmental alterations such as aging, endurance training, and pathologic processes (26, 115) is their propensity to enlarge. Deficiencies in a variety of nutrients, including riboflavin (112, 114), vitamin D (115), iron (30), and protein (107), as well

as starvation (91) also resulted in mitochondrial gigantism in animals. Giant mitochondria (at least 10 μm in diameter) induced by nutrient deficiency exhibit degenerative changes in cristae structure, development of an electron-lucent matrix (112, 114, 115), and decreased oxidation of numerous oxidizable substrates (115). Thus, insufficiency of nutrient supply can have a deleterious effect on the biogenesis of functional mitochondria.

VITAMIN EFFECTS AND MITOCHONDRIAL BIOGENESIS Formation of giant mitochondria consequent to a vitamin deficiency is best documented in riboflavin-deficient animals (112, 114, 115). Riboflavin is an essential component of the prosthetic group of many mitochondrial flavoproteins that function in the citric acid cycle, the respiratory chain, and β-oxidation of fatty acids. Mitochondrial function related to the requirement of riboflavin as cofactors for function of these enzymes has been well studied (43). The effect of ariboflavinosis on mitochondrial ultrastructural changes, though documented, is less well understood.

Studies in mice fed a riboflavin-free diet showed that changes in hepatic mitochondrial size were evident after 4 weeks of vitamin deficiency, but the most striking increase in size and volume occurred beyond 5 weeks on the deficient diet (112). Some mitochondria attained diameters larger than the nucleus and volumes 125 times that of normal mitochondria. A progressive alteration of cristae structure also occurred; this alteration included an increase in number and size of the cristae, many of which were tightly packed to resemble myelin figures (112). In other studies with mice, supplementation with galactoflavin, an antagonist of riboflavin, accelerated the symptom of ariboflavinosis, and megamitochondria were observed as early as 2 weeks after administration (113, 115). The ultrastructural changes in megamitochondria are rapidly reversed upon refeeding with riboflavin diets (112, 114).

Changes in form and structure of mitochondria have also been observed in riboflavin-deficient rats (114) and in rats (107) and primates (81) receiving protein-poor diets. The profound effect of these nutrient deficiencies on mitochondrial structure suggests that studies to elucidate the mechanism of action of nutrient deficiency and repletion using current molecular and cellular approaches may provide a basis to understand currently idiopathic mitochondrial defects that are associated with morphologic abnormalities.

MITOCHONDRIAL DISORDERS AND NUTRIENT THERAPY Abnormalities of muscle mitochondria are common manifestations of many mitochondrial respiratory disorders (33, 83, 105, 111). Examples are the Kearns-Sayre syndrome, myoclonus epilepsy with ragged-red fibers, and mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS). Mitochondrial myopathies are typically considered defects of

substrate utilization, oxidative phosphorylation coupling, or the respiratory chain. These defects can occur in either the mitochondrial or nuclear genome, the former transmitted by maternal inheritance and the latter often transmitted by Mendelian inheritance (33, 111).

Recent clinical studies have shown that nutritional therapies can be beneficial in restoring mitochondrial power and muscle function in patients with mitochondria that have defects in the respiratory chain. A combination of biochemical and molecular approaches (33, 83, 111) has revealed abnormalities in three segments of the mitochondrial electron transport chain, namely, Complex I, III, and IV. Complex I defect appeared to involve the nonheme iron sulfur proteins of the NADH dehydrogenase complex (80, 83, 111), and in several instances, Complex I deficiency was found to be associated with Complex IV deficiency, a defect in cytochrome oxidase (33, 83, 111). Recent studies showed that the genes for the two proteins are under coordinate regulation (96, 120). Complex III deficiency, a defect of the ubiquinone cytochrome c_1 complex, appeared to be independently regulated (83).

Knowledge of the molecular bases for these defective segments of the electron transport chain in patients with mitochondrial myopathies allows for precise diagnosis by biochemical and immunochemical means and for exploration of methods to treat the defects. For instance, clinical administration of coenzyme Q_{10} (48, 128) or succinate (83) to patients with MELAS, a defect localized to Complex I, resulted in disappearance of the strokelike episodes. Electrons are transferred from succinate via $FADH_2$ to the respiratory chain at Complex II, a site distal to Complex I, thereby enabling uninterrupted electron flow to O_2 and ATP synthesis (see Figure 1). Similarly, oral administration of vitamin K_3 (menadione) (35, 67, 83, 111) or ubiquinone (83) is effective in alleviating symptoms associated with defects in Complex III or IV. In the latter cases, the electrons from Complex I and II are transferred to O_2 via protein-bound ubiquinol and free ubiquinone. Though less efficient, this bypass allows continued electron transfer to drive ATP production (see Figure 1) and partial restoration of mitochondrial function.

Nutrient treatment has also been successful in treating disorders of mitochondrial dehydrogenases such as multiple acyl CoA dehydrogenases (MADD, 47, 121), biotin-responsive multiple carboxylase deficiency (108), and syndromes of lipid myopathies associated with carnitine deficiency (36, 37). Patients presented with MADD or glutaric aciduria type II exhibit atrophic type I fibers with excess numbers of lipid droplets within muscle fibers and excessive amounts of ethylmalonate, glutarate, and 2-hydroxy-glutarate in the 24-hr urine sample (121). Administration of riboflavin (30–100 mg/day) stimulated acyl CoA dehydrogenase activities, reduced urinary excretion of oxidation products, and led to substantial clinical improvement (121).

The clinical and biochemical response to biotin administration was similarly dramatic in patients with biotin-responsive multiple carboxylase deficiency, a condition characterized by an abnormally high K_m value for holocarboxylase synthetase (108) and deficiencies in the mitochondrial pyruvate, propionyl CoA, and 3-methylcrotonyl-CoA carboxylases (40, 49, 84, 89, 93). At a dose of 10 mg biotin per day (200 times normal intake), plasma biotin concentration was elevated to that above the K_m value for the synthetase, which allows effective conversion of the mitochondrial apocarboxylases to the holoenzymes (40, 84, 108). Concomitantly, the clinical symptoms disappeared. In contrast, isolated deficiencies of pyruvate, propionyl CoA, and 3-methylcrotonyl-CoA carboxylases were not responsive to biotin therapy (108), but the biochemical basis for this lack of response is not clear.

Syndromes of lipid myopathies associated with carnitine deficiency (36) and carnitine palmityl transferase deficiencies (23) have been described. The primary carnitine deficiencies, which include myopathic and systemic syndromes, are characterized by decreased free and total carnitine in muscle (myopathic) or in muscle, heart, liver, and plasma [systemic, see (102)] and are essentially reversed by carnitine administration. The secondary deficiency of carnitine is characterized by an abnormally high mitochondrial acyl CoA : CoA ratio, probably a consequence of an excessive matrix production of organic acid intermediates that depletes coenzyme A (37). The efficacy of carnitine treatment in patients with a variety of disorders of organic acid metabolism such as isovaleric acidemia, methylmalonic aciduria, and medium-chain acyl CoA dehydrogenase deficiencies (32, 65) can therefore be ascribed to the removal of accumulated acyl CoA, formation of the corresponding acylcarnitine, and subsequent excretion in the urine. Additionally, the regeneration of free CoA from the CoA: carnitine transferase-catalyzed reactions has been shown to be essential for optimal α-ketoglutarate dehydrogenase activity (55) for oxidation of pyruvate, fatty acid, and branched-chain α-keto acid. Moreover, earlier in vitro studies with deenergized kidney mitochondria revealed that catalytic amounts of acetylcarnitine initiate the mitochondrial oxidation of fatty acids (103) by providing the initial energy to activate the substrates. Thus, in analogy to the phosphate reserve of muscle phosphocreatine, acylcarnitine may be regarded a readily metabolizable acyl reservoir.

Despite the complexity and lack of a complete understanding of the various disease states and the efficacy of nutrient treatment, nutrient administration to restore near-normal state of function in mitochondrial disorders has been remarkably effective. Future endeavors, especially those that apply current technologies of molecular and cell biology to define the molecular mechanisms and tissue specificity of mitochondrial defects, should provide a basis for continued improvement in nutrient therapy.

Nutrient Supply and Optimal Mitochondrial Function

As discussed above, mitochondria are dynamic organelles that are responsive to numerous factors. In the following, we present examples of nutritional, developmental, physiologic, and pathologic factors important in understanding the nutrient supply for optimal mitochondrial function. Note that these are interacting factors and that we are far from a detailed knowledge of the molecular bases for these effects.

NUTRITIONAL EFFECTS Variation in protein content of the diet has a major effect on mitochondrial volume and number (17) and also specifically affects activities of numerous mitochondrial enzymes (125). In early studies of the adaptation of urea cycle enzymes in the rat to variation in dietary protein consumption, Schimke (98) found that the liver content of all urea cycle enzymes is directly proportional to the daily consumption of protein after 4–8 days. He showed that these changes were increases in enzyme protein rather than simply activation processes. Acute decrease in protein intake resulted in a net decrease in enzymes that was not maximal until after 8 days. Starvation also resulted in a net increase in the urea cycle enzymes, which showed that the urea cycle enzyme content was a function of the rate of urea synthesis and not simply a function of liver protein content or protein intake (97). Although these conditions resulted in coordinate changes in all of the enzymes, other studies showed that expression of two of the enzymes could be regulated separately (99) and that the rate of degradation of enzymes is also important in determining the enzyme content (100). The separate regulation of the enzymes probably occurs because some of the enzymes function in the supply of arginine as well as in urea formation; however, the molecular basis for this difference remains undefined (104) and can be examined with currently available molecular techniques.

Other dietary manipulations also affect the mitochondrial composition, activities of mitochondrial enzymes, and content of mitochondrial proteins. Variation in carbohydrate diet has a large effect on the enzymes of energy metabolism, especially those related to triglyceride synthesis, and can affect function, at least indirectly, through changes in mitochondrial phospholipid composition (122). Iron supplementation or chemical destruction of heme results in stimulation of δ-aminolevulinic acid synthetase (50). Rats receiving a diet containing 20% (w/w) fat in the form of soybean oil had only 70% of the monoamine oxidase activity of those receiving the same amount of lard (28). Dietary protein intake alters the reversible phosphorylation-dephosphorylation of the branched-chain keto acid dehydrogenase complex in adult rat liver and skeletal muscle mitochondria and thereby regulates activity of the complex (76).

Nutrients may play a more subtle yet fundamentally important role in defining the characteristics of mitochondria in different regions of a cell or in cells with different localizations relative to blood supply. This concept is illustrated by the metabolic zonation of the liver, where the zonation of mitochondria relative to blood supply (e.g. periportal versus pericentral cells) is associated with different metabolic functions (117). The periportal zone predominantly catalyzes reactions of glucose production, oxidative energy metabolism, amino acid utilization, urea formation, and bile acid and heme metabolism, while the pericentral zone has a predominant function in glucose uptake and glutamine formation (62). This zonal heterogeneity establishes, during a single passage of blood through the liver acinus, either decreasing or increasing periportal-to-pericentral concentration gradients of glucose, amino acids, fatty acids, glycerol, ketone bodies, and other components (62). The magnitude and direction of nutrient gradients varies with the diurnal rhythm, i.e. during absorptive and postabsorptive phases (62). The significance of differential functions has been studied for the role of O_2 and hormones (insulin and glucagon) in induction of parenchymal zonation (62). Less is known about the regulatory importance of nutrient concentration gradients in control of periportal and pericentral gene expression, and this influence could be important in modulation of the heterogeneity of mitochondrial function. Furthermore, the nature of the diet (i.e. high carbohydrate, protein, or fat) could contribute to dynamic changes in these gradients and consequently in the mitochondrial composition and function (62).

The complexities of coordinating mitochondrial and nuclear gene expression, importation and processing of protein precursors, and covalent and noncovalent regulation require that detailed molecular studies be performed to understand the various effects of diet on individual proteins. Although earlier studies were limited mostly to activity measurements, numerous immunochemical and molecular approaches are now available to define these interactions. Thus, substantial advances in obtaining systematic knowledge of dietary effects on mitochondrial composition and function could occur in the near future.

DEVELOPMENTAL EFFECTS Increases in mitochondrial density and oxidative activities of mitochondrial enzymes and the achievement of specific distribution patterns of mitochondria are associated with normal growth and differentiation (5). In fetal liver, cytochrome oxidase and cytochrome *c* reductase activities parallel the increase in mitochondrial number (57, 87), and increases in mitochondrial enzyme activities are preceded by high rates of protein synthesis in the cytoplasm and the mitochondria (57, 75). Induction of mitochondrial cytochrome oxidase and succinate dehydrogenase activities in

neonatal brain occurs simultaneously with the enzymes of glycolysis (22). Other enzymes such as pyruvate dehydrogenase (29), however, are not simultaneously increased. Pyruvate dehydrogenase controls entry of pyruvate into the citric acid cycle, and its activity is determined in the adult predominantly by the phosphorylation state of the enzyme (124). Phosphorylation is an important control mechanism in the fetus and neonate (25); however, the limited fetal and neonatal activity is further limited by a low content of the pyruvate dehydrogenase complex (16).

Recent studies showed that the basal respiratory rate in newborn rat hepatocytes is low and is stimulated up to twofold by glucose, lactate, pyruvate, ketone bodies, and fatty acids (Figure 2) (12). The NADH-linked substrates (malate, glutamate) gave little stimulation (Figure 2), consistent with a low perinatal NADH dehydrogenase activity (57). In contrast, succinate stimulated O_2 consumption 15-fold above the basal rate (Figure 2). Inhibition by antimycin A, oxaloacetate, and malonate indicates that the succinate-induced O_2 consumption involves mitochondrial succinate dehydrogenase (12). This remarkable ability of neonatal liver cells to utilize succinate coincides with a high content of succinate dehydrogenase (2, 62) and a relatively poor ability to oxidize NADH. The physiologic significance of the succinate effect is not clear but may reflect a sparing effect of succinate on NADH utilization by the electron transport chain. This sparing effect would allow increased transhydrogenation (92) to supply NADPH for biosynthetic reactions. The presence of active transport systems in the placenta (44) and intestine (77) for succinate and the high concentration of succinate in fetal serum (750 μM, F. C. Kauffman, personal communication) are consistent with the idea that circulating plasma succinate is a critical respiratory substrate and/or a specific

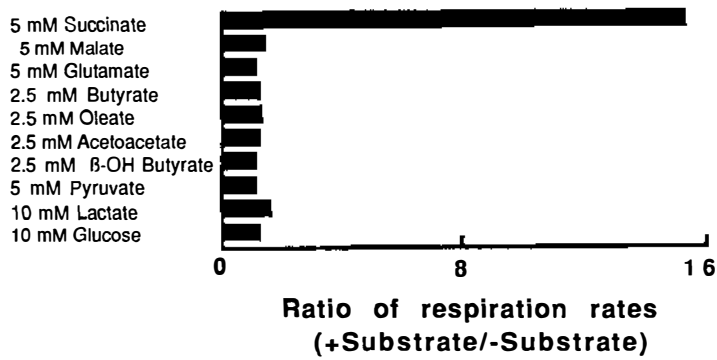

Figure 2 Effect of substrates on respiration rates in hepatocytes from newborn rats. Cellular O_2 consumption rates were determined polarographically in control cells and in cells incubated in the presence of various substrates (2.5 mM– 10 mM). Values are expressed as ratios of rates + substrate/− substrate. Data from Aw & Jones (12).

stimulator of perinatal cellular O_2 consumption. Therefore succinate may be a unique and preferred substrate for neonatal respiration and ATP production.

Studies over the past several years have revealed that cytosolic-to-matrix distribution of adenine nucleotides can also have a primary role in determining mitochondrial activity in the neonate (6). The dramatic increase in mitochondrial respiration in newborn rat liver mitochondria parallels a marked increase in cellular adenine nucleotides and a shift in the cytosolic-to-mitochondrial distribution of the nucleotides within 2 to 3 hr of birth (4, 6, 7). The shift results in a net matrix accumulation of the adenine nucleotides that is accomplished by an ATP-Mg/P_i carrier (6) distinct from the classic ATP/ADP translocator (66). This carrier is stimulated by submicromolar concentrations of calcium (51) and is responsive to insulin:glucagon ratios (73). The sensitivity to hormonal and Ca^{2+} changes in the physiologic range that occurs postnatally (6, 51, 73) suggests a role for the carrier as a regulator for matrix adenine nucleotide uptake and enhanced mitochondrial activity.

Studies of substrate utilization for glycolysis and mitochondrial oxidation in cell cultures have revealed that following plating, cells undergo a stimulation of aerobic glycolysis that is associated with actin polymerization and stress-fiber formation (15). Stress-fiber formation stimulates Na^+, K^+-ATPase activity prior to initiation of DNA synthesis. The increased demand for energy of these latter processes requires a stimulation of mitochondrial oxidative activity (15). Thus, cells apparently undergo a cycling between relatively high glycolytic and mitochondrial oxidative activities during cell proliferation. This cycling suggests that nutrient availability may be generally important for cell division and turnover. Such effects of diet on mitochondria could have important long-term health implications that would not be readily apparent from short-term functional studies.

PHYSIOLOGIC EFFECTS The physiologic state of an organism determines the nutrient requirements and energetic efficiency of mitochondrial function. Numerous factors, such as hypoxia, endurance training, and obesity can significantly affect the substrate utilization. During acclimatization to chronic hypoxia, mitochondrial volume and number are increased (19, 82) and redistribution occurs (27). An increase in activities of cytochrome oxidase and other mitochondrial enzymes also generally occurs (20). Such changes may reflect the increased utilization of nonglucose energy substrates by mitochondria (18) and are consistent with the mobilization of protein from muscle to other tissues (106) and higher plasma levels of free fatty acids (123).

Biochemical and morphologic characteristics of skeletal muscle mitochondria also change in response to submaximal endurance exercise training. The principal change is an increase in number and size of the mitochondria (52). This increase is limited to muscles that participate in training, which indicates

that it is not a consequence of circulating hormones or metabolites. Increases occur in pyruvate oxidation, citric acid cycle enzymes, electron transport activity, cytochrome contents, and fatty acid oxidation (116, 125). With increased mitochondrial respiratory function and oxidative capacity following training, substrate utilization is shifted from carbohydrate to fat (1). This shift spares glucose and allows a greater efficiency of energy-utilizing processes with less glucose depletion. Thus, both the type of nutrients oxidized by mitochondria and the ability of mitochondria to maintain energy supply during prolonged submaximal exercise is enhanced by exercise training.

This adaptation effectively provides for improved efficiency of energy expenditure. Several lines of evidence indicate that the efficiency of coupling of the mitochondrial protonmotive force available from biological oxidation with ADP phosphorylation is variable and under physiologic control. Regulation of the ATP synthase at constant magnitude of the protonmotive force (see Figure 1) and constant rate of ATP utilization determines the steady state phosphorylation potential ([ADP] [Pi]/[ATP]). At a constant flux through ATP-utilizing reactions, the energy lost from ATP hydrolysis increases as [ADP] [Pi]/[ATP] decreases. In addition, ion flux that is determined by membrane potential or ATP concentration dissipates a larger amount of potential energy under this condition. Thus, metabolism becomes inherently less efficient as [ATP] increases relative to [ADP] and [Pi]. This means that modulation of ATP synthase can function to provide a metabolic set point for energetic efficiency of metabolism.

Several aspects of this regulation are of great importance for physiologic adaptation and response to pathologic conditions. An efferent signal that directs an increase in this set point increases the ATP production prior to an increase in demand for ATP. This mechanism thus provides a cellular "fight or flight" response, i.e. it anticipates the increased energy need. Similarly, an increase in the set point linked to signal transduction processes that activate cellular functions, i.e. secretion, phagocytosis, or cell division, activates the energy-producing machinery in concert with activation of other cell systems. An example of this mechanism is increased cytosolic Ca^{2+}, which activates many cytoplasmic processes, releases the inhibition of ATP synthase by the endogenous inhibitor (127), and stimulates the activities of critical NAD^{+}-linked dehydrogenases (31, 73).

Exercise conditioning or inactivity could similarly effect changes in energetic efficiency. An individual with a higher [ATP] would have greater immediately available utilizable energy than would one with a low concentration. The expense of this greater capacity is a reduced energy efficiency, i.e. more oxidizable substrate is needed to do the same productive work. Such variations in tissue phosphorylation state may be obscured by cell heterogeneities in tissues and analytic difficulties; however, even a subtle

change in cellular energy efficiency can have a remarkable effect on long-term energy balance.

The converse of activation by increasing the set point is suppression of metabolism such as occurs during neahypoxia (8, 10, 61). Decreased $\Delta\psi$ following exposure of cells to short-term anoxia results in loss of mitochondrial Ca^{2+} (3, 9). Suppression of metabolism is mediated partly by the binding of the endogenous inhibitor to the ATP synthase (127). The decrease in mitochondrial Ca^{2+} results in increased binding of the inhibitor and decreased ATP synthase activity (9). An analogous process could result from inactivity: lowering the phosphorylation state makes energy utilization more efficient but reduces functional capacity. This process may contribute to differences in basal metabolic rate between individuals and also to changes that occur during periods of inactivity.

Variations in nutrient intake can modulate the coupling of the ATP synthase with Δp, thereby affecting the steady-state phosphorylation state and energetic efficiency of metabolism. Studies with rats, mice, and hamsters showed that an enhancement of thermogenic response of brown fat mitochondria (101, 109, 118) occurred with overconsumption of a rich "cafeteria diet" high in carbohydrates but low in protein. The molecular basis for this energetic inefficiency is that of increased synthesis of the uncoupling protein (38) of the mitochondrial inner membrane that functions to dissipate the electrochemical gradient (79). The mechanism by which low-protein diets elicit an elevated mitochondrial thermogenic function, though not clear, appeared to be under adrenergic control; inhibition of norepinephrine production accordingly decreased the response (95). An understanding of the influence of protein feeding on mitochondrial energetics allows for implementation of dietary regimens and macronutrient feeding patterns that may be useful in the treatment of certain types of human obesities.

Other physiologic factors can also alter the function of mitochondria. In isolated kidney proximal tubule cells, inhibition or stimulation of function of the membrane-bound Na^+, K^+-ATPase by ouabain or nystatin, respectively, decreased or increased the O_2 required for half-maximal oxidation of mitochondrial cytochromes (14). Mitochondrial function is also affected by thyroid hormones. Administration of T_4 to rats resulted in cytoplasmic production of tissue-specific modulators of mitochondrial protein synthesis (56). In liver, enhanced synthesis of mRNA of many proteins, including that of malic enzyme, appeared to be controlled by T_3 (78). In the human breast cancer cell line MCF-7, mitochondrial α-glycerolphosphate dehydrogenase activity was stimulated by T_3 (34).

PATHOLOGIC AND TOXICOLOGIC EFFECTS Cell and tissue injury affect mitochondrial function either directly, by altering the function of mitochon-

drial components, or indirectly, by increasing the cellular requirements for energy for repair processes. Because of the central role of mitochondria in metabolism, analyses of the effects on mitochondrial function are frequently among the first aspects of a pathologic process to be investigated. However, cell functions altered because of either injury or increased energy requirement for repair can be expected to affect mitochondrial function under nearly all pathologic and toxicologic conditions. Distinguishing between these general responses and specific effects on mitochondria is not simple, for the production and maintenance of normal mitochondria require coordinated function of mitochondrial and nuclear gene expression, as described above. In addition, maintenance of proper lipid composition of the inner membrane, supply of carbohydrate and amino acid precursors, and a suitable ionic environment (pH and $[Ca^{2+}]$) is necessary for function. To provide a framework to distinguish between the diverse indirect effects and the direct effects of injury on mitochondrial function, one can consider the functions of mitochondria and the developmental and physiologic responses of mitochondria, as discussed above.

Adjustment of the activity of the mitochondria by regulation of the ATP synthase provides a mechanism to increase ATP concentration and enhance repair. Because of a general need for repair during and following injury, activation of this reaction is common and associated with a hypermetabolic state (24). The signaling processes involved in activation are not completely resolved but probably include an increase in cytosolic Ca^{2+} that is known to stimulate this reaction. This reaction may be mediated by central mechanisms and may occur in some cells or tissues while others undergo metabolic suppression (10, 61).

The ability of individual cells to respond to signals with an increase or decrease in metabolism cannot be overemphasized. Whole-body or whole-organ measurements of metabolism that indicate a hypermetabolic or a hypometabolic state are only an average of the cellular responses. Such measurements may obscure a great heterogeneity in function, in which some cells are activated and others inhibited. Recent studies show that in addition to the activation processes elicited by a transient rise in cytosolic Ca^{2+}, a sustained rise in cytosolic Ca^{2+} concentration can elicit programmed cell death (apoptosis, 72). Thus, what is tantamount to excessive activation of a cell may be a signal that the cellular homeostasis is severely perturbed and that elimination of this cell is advantageous to reduce the burden of injury to the whole organism. Similarly, a suppression of functions in noninjured tissues may make more nutrients available for mitochondria in damaged tissues. Thus, both hypermetabolism and hypometabolism are expected

tissue-specific responses to injury, and additional research is necessary to understand the nutrient requirements of these diverse conditions.

Direct damage to mitochondria from various chemicals, metabolic inhibitors, ischemia, and oxidative stress is well known. One of the most general effects is damage to the mitochondrial inner membrane that increases ionic conductance and interferes with the electrochemical coupling mechanism. Vitamin E, selenium, and ubiquinone are critical dietary components to prevent mitochondrial free-radical injury; these components function with the mitochondrial GSH pool to inhibit processes such as lipid peroxidation. Mitochondrial GSH is maintained in the reduced form by reduction of GSSG by NADPH. Under conditions in which lipid peroxidation increases, the demand for NADPH is increased. Supply of NADPH in the mitochondria depends upon oxidation of NADH or substrates normally used for NADPH generation. Therefore, increased NADPH demand competes with the need for NADH for oxidative phosphorylation. The increased demand for reducing equivalents is partially compensated by enhanced oxidation of succinate or fatty acids (42) that supply electrons to the respiratory chain at the level of ubiquinone. A similar shift in substrate utilization occurs because of increased demand for NADPH for biosynthetic and repair reactions. Thus, pathologic and toxicologic processes can result in important changes in nutrient requirements. However, because of the diversity of these processes and the lack of detailed knowledge on the nutrient requirements to enhance recovery in individual cells, further research is needed to provide optimal nutrition for mitochondrial function during and after cell injury.

CONCLUSIONS AND PERSPECTIVE

Newer data on the molecular and cell biology of mitochondria reinforce the concept that mitochondria are complex, dynamic organelles that are highly responsive to nutriture. Numerous vitamin and mineral deficiency states result in aberrant expression of protein precursors and abnormal mitochondrial structure and function. These abnormalities frequently can be rapidly reversed by nutrient repletion. Similarly, abnormalities occur as a result of genetic defects in both the mitochondrial and nuclear genomes. Nutritional supplementation provides a remarkably effective therapy for some of these defects, which suggests that nutritional supplementation may be useful for other mitochondrial myopathies.

Advances in the understanding of the heterologous character of mitochondrial distribution and the dynamic responses of mitochondria to nutritional variations further indicate that the integral composition and function of

mitochondria are closely related to the nutritional state. Variations in nutrition can affect the sensitivity of cells to pathologic and toxicologic processes specifically through the modulation of mitochondrial characteristics. Understanding such effects may allow improved definition of regional and temporal specificity of injury and lead to nutritional manipulations that minimize cell death and enhance tissue repair processes.

Literature Cited

1. Ahlborg, G., Felig, P., Hagenfeldt, L., Hendler, R., Wahren, J. 1974. Substrate turnover during prolonged exercise in man: splachnic and leg metabolism of glucose, free fatty acids, and amino acids. *J. Clin. Invest.* 53:1080–90
2. Andersen, B., Zierz, S., Jungermann, K. 1983. Perinatal development of the distributions of phosphoenolpyruvate carboxykinase and succinate dehydrogenase in rat liver parenchyma. *Eur. J. Cell Biol.* 30:126–31
3. Andersson, B. S., Aw, T. Y., Jones, D. P. 1987. Mitochondrial transmembrane potential and pH gradient during anoxia. *Am. J. Physiol.* 252:C349–55
4. Aprille, J. R. 1981. Net uptake of adenine nucleotides by newborn rat liver mitochondria. *Arch. Biochem. Biophys.* 207:157–64
5. Aprille, J. R. 1986. Perinatal development of mitochondria in rat liver. In *Mitochondrial Physiology and Pathology*, ed. G. Fiskum, pp. 66–99. New York: Van Nostrand Reinhold
6. Aprille, J. R. 1988. Regulation of the mitochondrial adenine nucleotide pool size in liver: mechanisms and metabolic role. *FASEB J.* 2:2547–56
7. Aprille, J. R., Asimakis, G. K. 1980. Postnatal-development of rat-liver mitochondria: state-3 respiration, adenine nucleotide translocase activity and the net accumulation of adenine nucleotides. *Arch. Biochem. Biophys.* 201:564–75
8. Aw, T. Y., Andersson, B. S., Jones, D. P. 1987. Mitochondrial transmembrane ion distribution during anoxia. *Am. J. Physiol.* 252:C356–61
9. Aw, T. Y., Andersson, B. S., Jones, D. P. 1987. Suppression of mitochondrial respiratory function after short-term anoxia. *Am. J. Physiol.* 252:C362–68
10. Aw, T. Y., Andersson, B. S., Kennedy, F. G., Jones, D. P. 1986. Intracellular O_2 supply to support mitochondrial function. In *Biochemical Aspects of Physical Exercise*, ed. G. Benzi, L. Packer, N. Siliprandi, pp. 101–12. Amsterdam/New York: Elsevier
11. Aw, T. Y., Jones, D. P. 1985. ATP concentration gradients in cytosol of liver cells during hypoxia. *Am J. Physiol.* 249:C385–92
12. Aw, T. Y., Jones, D. P. 1987. Respiratory characteristics of neonatal rat hepatocytes. *Pediatr. Res.* 21:492–96
13. Aw, T. Y., Jones, D. P. 1989. Heterogenerity of pH in the aqueous cytoplasm of renal proximal tubule cells. *FASEB J.* 3:52–58
14. Aw, T. Y., Wilson, E., Hagen, T. M., Jones, D. P. 1987. Determinants of mitochondrial O_2 dependence in kidney. *Am. J. Physiol.* 253:F440–47
15. Bereiter-Hahn, J. 1988. Involvement of microcompartmentation in the regulation of cell proliferation. See Ref. 59, pp. 55–69
16. Berger, R., Hommes, F. A. 1975. Regulation of pyruvate metabolism in fetal rat liver. In *Normal and Pathological Development of Energy Metabolism*, ed. F. A. Hommes, C. J. Van den Berg, pp. 97–107. London: Academic
17. Berlin, J., Castro, C. E., Bailey, F., Sevall, J. S. 1982. Adaptation of rat parenchymal hepatocyte to nutritional variation: quantitation by stereology. *Nutr. Res.* 2:51–63
18. Blume, F. D. 1983. Metabolism and nutrition at altitude. In *Hypoxia, Exercise, and Altitude*, ed. J. R. Sutton, C. S. Houston, N. L. Jones, pp. 311–16. New York: Liss
19. Boutellier, U., Howald, H., di Prampero, P. E., Giezendanner, D., Cerretelli, P. 1983. Human muscle adaptations to chronic hypoxia. See Ref. 18, pp. 273–81
20. Bouverot, P. 1985. Adaptation to altitude-hypoxia in vertebrates. In *Zoophysiology*, ed. B. Heinrich, K. Johansen, H. Langer, G. Neuweiler, D. J. Randall, 16:120–41. Berlin/Heidelberg: Springer-Verlag

21. Bulger, R. E., Dobyan, D. C. 1982. Recent advances in renal morphology. *Annu. Rev. Physiol.* 44:147–79
22. Carey, E. M. 1982. The biochemistry of fetal brain development and myelination. See Ref. 57, pp. 287–336
23. Carroll, J. E., Brooke, M. H., DeVivo, D. C., Kaiser, K. K., Hagberg, J. M. 1978. Biochemical and physiologic consequences of carnitine palmityltransferase deficiency. *Muscle Nerve* 1:103–10
24. Cerra, F. B. 1987. The hypermetabolism organ failure complex. *World J. Surg.* 11:173–81
25. Chitra, C. I., Cuezva, J. M., Patel, M. S. 1985. Changes in the activity of active pyruvate dehydrogenase complex in the newborn of normal and diabetic rats. *Diabetologia* 28:148–52
26. Coleman, R., Silbermann, M., Gershon, D., Reznick, A. Z. 1987. Giant mitochondria in the myocardium of aging and endurance-trained mice. *Gerontology*. 33:34–39
27. Costa, L. E., Boveris, A., Koch, O. R., Taquini, A. C. 1988. Liver and heart mitochondria in rats submitted to chronic hypobaric hypoxia. *Am. J. Physiol.* 255:C123–29
28. Crane, S. B., Greenwood, C. E. 1987. Dietary fat source influences neuronal mitochondrial monoamine oxidase activity and macronutrient selection in rats. *Pharmacol. Biochem. Behav.* 27:1–6
29. Cremer, J. E., Teal, H. M. 1974. The activity of pyruvate dehydrogenase in rat brain during postnatal development. *FEBS Lett.* 39:17–20
30. Dallman, P. R., Goodman, J. R. 1971. The effects of iron deficiency on the hepatocyte: a biochemical and ultrastructural study. *J. Cell Biol.* 48:79–90
31. Denton, R. M., McCormack, J. G. 1980. On the role of the calcium transport cycle in heart and other mammalian mitochondria. *FEBS Lett.* 119:1–8
32. Di Donato, S., Rimoldi, M., Garavaglia, B., Uziel, G. 1984. Propionyl carnitine excretion in propionic and methylmalonic acidurias: a cause of carnitine deficiency. *Clin. Chim. Acta* 139:13–21
33. DiMauro, S., Bonilla, E., Zeviani, M., Nakagawa, M., DeVivo, D. C. 1985. Mitochondrial myopathies. *Ann. Neurol.* 17:521–38
34. Draves, D. P., Ruzicka, F. J., Rose, D. P. 1983. Thyroid hormone-inducible mitochondrial α-glycerolphosphate dehydrogenase in the MCF-7 human-breast cancer cell-line. *Eur. J. Cancer Clin. Oncol.* 19:1485–86
35. Eleff, S., Kennaway, N. G., Buist, N. R. M., Darley-Usmar, V. M., Calpaldi, R. A., et al. 1984. ^{31}P NMR study of improvement in oxidative-phosphorylation by vitamins-K_3 and C in a patient with a defect in electron transport at complex-III in skeletal-muscle. *Proc. Natl. Acad. Sci. USA* 81:3529–33
36. Engel, A., Angelini, C. 1973. Carnitine deficiency of human skeletal muscle associated with lipid storage myopathy: a new syndrome. *Science* 79:899–902
37. Engel, A. G., Rebouche, C. J. 1984. Carnitine metabolism and inborn errors. *J. Inherit. Metab. Dis.* 7 (Suppl. 1):38–43
38. Falcou, R., Bouillaud, F., Mory, G., Apfelbaum, M., Ricquier, D. 1985. Increase of uncoupling protein and its mRNA in brown adipose tissue of rats fed on cafeteria diet. *Biochem. J.* 231:241–44
39. Fawcett, D. W. 1981. *The Cell.* Philadelphia, PA: Saunders. 862 pp.
40. Feldman, G. L., Wolf, B. 1981. Deficient acetyl CoA carboxylase activity in multiple carboxylase deficiency. *Clin. Chim. Acta* 111:147–51
41. Forbes, M. S., Sperelakis, N. 1982. Association between mitochondria and gap junctions in mammalian myocardial cells. *Tissue Cell* 14:25–37
42. Frayn, K. N. 1985. Substrate turnover after injury. *Br. Med. Bull.* 41:232–39
43. Frisell, W. R., Cronin, J. R., Mackenzie, C. G. 1966. Electron-transfer flavoprotein and its relation to the respiratory chain. *Flavins and Flavoproteins*, ed. E. C. Slater, pp. 367–90. Amsterdam: Elsevier
44. Ganapathy, V., Ganapathy, M. E., Tiruppathi, C., Miyamoto, Y., Mahesh, V. B., et al. 1988. Sodium-gradient-driven, high-affinity, uphill transport of succinate in human placental brush-border membrane vesicles. *Biochem. J.* 249:179–84
45. Garfinkel, D. 1963. A metabolic inhomogeneity of glycine in vivo. II. Computer simulation. *J. Biol. Chem.* 238:2435–39
46. Garfinkel, D., Lajtha, A. 1963. A metabolic inhomogeneity of glycine in vivo. I. Experimental determination. *J. Biol. Chem.* 238:2429–34
47. Gilkeson, G. S., Caldwell, D. S. 1988. Riboflavin-responsive multiple acyl coenzyme-A dehydrogenase deficiency presenting as a proximal myopathy in a

young adult. *Arthritis Rheum.* 31:695–96
48. Goda, S., Hamada, T., Ishimoto, S., Kobayashi, T., Goto, I., et al. 1987. Clinical improvement after administration of coenzyme Q_{10} in a patient with mitochondrial encephalomyopathy. *J. Neurol.* 234:62–63
49. Gompertz, D., Draffan, G. H., Watts, J. L., Hull, D. 1971. Biotin-responsive β-methylcrotonylglycinuria. *FEBS Lett.* 32:13–14
50. Granick, S., Urata, G. 1963. Increase in activity of δ-aminolevulinic acid synthetase in liver mitochondria induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine. *J. Biol. Chem.* 238:821–27
51. Haynes, R. C. Jr., Picking, R. A., Zaks, W. J. 1986. Control of mitochondrial content of adenine nucleotides by submicromolar calcium concentrations and its relationship to hormonal effects. *J. Biol. Chem.* 261:16121–25
52. Holloszy, J. O., Coyle, E. F. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56:831–38
53. Hoppel, C. L., Tandler, B., Parland, W., Turkaly, J. S., Albers, L. D. 1982. Hamster cardiomyopathy: a defect in oxidative phosphorylation in the cardiac interfibrillar mitochondria. *J. Biol. Chem.* 257:1540–48
54. Hoppeler, H., Luthi, P., Classen, H., Weibel, E. R., Howald, H. 1973. The ultrastructure of normal human skeletal muscle. *Pfleugers Arch.* 344:217–32
55. Hulsmann, W. C., Siliprandi, D., Ciman, M., Siliprandi, N. 1964. Effect of carnitine on the oxidation of α-oxoglutarate to succinate in the presence of acetoacetate or pyruvate. *Biochim. Biophys. Acta* 93:166–68
56. Ichikawa, K., Hashizume, K., Kobayashi, M., Yamada, T. 1985. Evidence for induction by thyroid hormone of cytosolic proteins which control mitochondrial protein synthesis. *Endocrinology* 117:1749–58
57. Jones, C. T. 1982. The development of the metabolism in the fetal liver. In *Biochemical Development of the Fetus and Neonate,* ed. C. T. Jones, pp. 287–336. Amsterdam/New York: Elsevier Biomedical
58. Jones, D. P. 1984. Effect of mitochondrial clustering on O_2 supply in hepatocytes. *Am. J. Physiol.* 267:C83–89
59. Jones, D. P., ed. 1988. *Microcompartmentation.* Boca Raton, Fla: CRC Press. 261 pp.
60. Jones, D. P., Aw, T. Y. 1988. Mitochondrial distribution and O_2 gradients in mammalian cells. See Ref. 59, pp. 37–53
61. Jones, D. P., Kennedy, F. G., Andersson, B. S., Aw, T. Y., Wilson, E. 1985. When is a mammalian cell hypoxic? Insights from studies of cells versus mitochondria. *Mol. Physiol.* 8:473–82
62. Jungermann, K. 1986. Zonal signal heterogeneity and induction of hepatocyte heterogeneity. In *Regulation of Hepatic Metabolism: Intra- and Intercellular Compartmentation,* ed. R. G. Thurman, F. C. Kauffman, K. Jungermann, pp. 445–69. New York/London: Plenum
63. Kadenbach, B. 1986. Regulation of respiration and ATP synthesis in higher organisms: Hypothesis. *J. Bioenerg. Biomemb.* 18:39–54
64. Kennedy, F. G., Jones, D. P. 1986. Oxygen dependence of mitochondrial function in isolated rat cardiac myocytes. *Am. J. Physiol.* 250:C374–83
65. Kim, C. S., Dorgan, D. R., Roe, C. R. 1984. L-carnitine: therapeutic strategy for metabolic encephalopathy. *Brain Res.* 310:149–53
66. Klingenberg, M., Heldt, H. W. 1982. The ADP/ATP translocation in mitochondria and its role in intracellular compartmentation. In *Metabolic Compartmentation,* ed. H. Sies, pp. 101–22. London: Academic
67. 1988. Lactic acidosis and mitochondrial myopathy in a young woman. *Nutr. Rev.* 46:157–63
68. LaNoue, K. F. 1984. Metabolite transport in mammalian mitochondria. In *Bioenergetics,* ed. L. Ernster, pp. 221–68. Amsterdam: Elsevier
69. LaNoue, K. F., Schoolwerth, A. C. 1979. Metabolite transport in mitochondria. *Annu. Rev. Biochem.* 48:871–922
70. Loud, A. V. 1968. A quantitative stereological description of the ultrastructure of normal rat liver parenchymal cells. *J. Cell Biol.* 37:27–46
71. Lynch, R. M., Paul, R. J. 1983. Compartmentation of glycolytic and glycogenolytic metabolism in vascular smooth muscle. *Science* 222:1344–46
72. McConkey, D. J., Hartzell, P., Nicotera, P., Wyllie, A. H., Orrenius, S. 1988. Stimulation of endogenous endonuclease activity in hepatocytes exposed to oxidative stress. *Toxicol. Lett.* 42:123–30
73. McCormack, J. G., Denton, R. M. 1986. Ca^{2+} as a second messenger with-

in mitochondria. *Trends Biochem. Sci.* 11:258–62
74. McMillin-Wood, J., Wolkowicz, P. E., Chu, A., Tate, C. A., Goldstein, M. A., et al. 1980. Calcium uptake by two preparations of mitochondria from heart. *Biochem. Biophys. Acta* 591:251–56
75. Meisami, E., Timiras, P. S. 1982. Normal and abnormal biochemical development of the brain after birth. See Ref. 57, pp. 758–821
76. Miller, R. H., Eisenstein, R. S., Harper, A. E. 1988. Effects of dietary protein intake on branched-chain keto acid dehydrogenase activity of the rat. Immunochemical analysis of the enzyme complex. *J. Biol. Chem.* 263:3454–61
77. Moe, A. J., Mallet, R. T., Jackson, M. J., Hollywood, J. A., Kelleher, J. K. 1988. Effect of Na^+ on intestinal succinate transport and metabolism in vitro. *Am. J. Physiol.* 255:C95–101
78. de Nayer, P. 1987. Thyroid hormone action at the cellular level. *Hormone Res.* 26:48–57
79. Nicholls, D. G., Locke, R. 1984. Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64:1–64
80. Nishizawa, M., Tanaka, K., Shinozawa, K., Kuwabara, T., Atsumi, T., et al. 1987. A mitochondrial encephalomyopathy with cardiomyopathy. A case revealing a defect of complex-I in the respiratory chain. *J. Neurol. Sci.* 78:189–201
81. Ordy, J. M., Samorajski, T., Zimmerman, R. R., Rady, P. M. 1966. Effects of postnatal protein deficiency on weight gain, serum proteins, enzymes, cholesterol, and liver ultrastructure in a subhuman primate *(Macaca mulatta). Am. J. Pathol.* 48:769–91
82. Ou, L. C., Tenney, S. M. 1970. Properties of mitochondria from hearts of cattle acclimated to high altitude. *Respir. Physiol.* 8:151–59
83. Ozawa, T., Tanaka, M., Suzuki, H., Nishikimi, M. 1987. Structure and function of mitochondria: Their organization and disorders, *Brain Dev.* 9:76–81
84. Packman, S., Caswell, N. M., Gonzalez-Rios, M. D. C., Kadlecek, T., Cann, H., et al. 1984. Acetyl CoA carboxylase in cultured fibroblasts: Differential biotin dependence in the two types of biotin-responsive multiple carboxylase deficiency. *Am. J. Hum. Genet.* 36:80–92
85. Pannese, E., Procacci, P., Ledda, M., Arcidiacono, G., Frattola, D., et al. 1986. Association between microtubules and mitochondria in myelinated axons of *Lacerta muralis*. A quantitative analysis. *Cell Tissue Res.* 245:1–8
86. Paul, R. J. 1983. Functional compartmentalization of oxidative and glycolytic metabolism in vascular smooth muscle. *Am. J. Physiol.* 244: C399–C409
87. Pollak, J. K., Duck-Chong, C. G. 1973. Changes in rat liver mitochondria and endoplasmic reticulum during development and differentiation. *Enzyme* 15:139–60
88. Quistorff, B. 1983. The use of a hepatocyte column in the study of metabolic zonation in the liver. In *Isolation, Characterization, and Use of Hepatocytes,* ed. R. A. Harris, N. W. Cornell, pp. 131–37. New York/Amsterdam: Elsevier Biomedical
89. Rasmussen, K., Ando, T., Nyhan, W. L., Hull, D., Cottom, D., et al. 1972. Excretion of tiglylglycine in propionic acidemia. *J. Pediatr.* 81:970–72
90. Rendon, A., Filliol, D., Jancsik, V. 1987. Microtubule-associated proteins bind to 30-kDa and 60-kDa proteins of rat-brain mitochondria: visualization by ligand blotting. *Biochem. Biophys. Res. Commun.* 149:776–83
91. Rohr, H. P., Riede, U. N. 1973. Experimental metabolic disorders and the subcellular reaction pattern. *Curr. Top. Pathol.* 58:1–48
92. Rydstrom, J. 1981. Mitochondrial nicotinamide nucleotide transhydrogenase. In *Mitochondria and Microsomes,* ed. C. P. Lee, G. Schatz, G. Dallner, pp. 317–35. London/Amsterdam: Addison-Wesley
93. Saunders, M., Sweetman, L., Robinson, B., Roth, K., Cohn, R., Gravel, R. A. 1979. Biotin-responsive organic-aciduria. Multiple carboxylase defects and complementation studies with propionicacidemia in cultured fibroblasts. *J. Clin. Invest.* 64:1695–1702
94. Santander, R. G., Cuadrado, G. M., Saez, M. R. 1987. High-energy adhering junctional complexes or with mitochondrial coupling. *Histol. Histopathol.* 2:153–61
95. Sawaya, A. L., Lunn, P. G. 1985. Evidence suggesting that the elevated plasma triiodothyronine concentration of rats fed on protein-deficient diets is physiologically active. *Br. J. Nutr.* 53:175–81
96. Schatz, G. 1981. The biogenesis of mitochondria. See Ref. 92, pp. 45–66
97. Schimke, R. T. 1962. Adaptive characteristics of urea cycle enzymes in the rat. *J. Biol. Chem.* 237:459–68

98. Schimke, R. T. 1962. Differential effects of fasting and protein-free diets on levels of urea cycle enzymes in rat liver. *J. Biol. Chem.* 237:1921–24
99. Schimke, R. T. 1963. Studies on factors affecting the levels of urea cycle enzymes in rat liver. *J. Biol. Chem.* 238:1012–17
100. Schimke, R. T. 1964. The importance of both synthesis and degradation in the control of arginase levels in rat liver. *J. Biol. Chem.* 239:3808–17
101. Schimmel, R. J., McCarthy, L. 1985. Brown adipose tissue in cafeteria-fed hamsters. *Am. J. Physiol.* 248:E230–35
102. Siliprandi, N. 1986. Transport and function of carnitine: Relevance to carnitine-deficient diseases. *Ann. NY Acad. Sci.* 488:118–26
103. Siliprandi, N., Siliprandi, D., Ciman, M. 1965. Stimulation of oxidation of mitochondrial fatty acids and of acetate by acetylcarnitine. *Biochem. J.* 96:777–80
104. Snodgrass, P. J., Lin, R. C. 1987. Differing effects of arginine deficiency on the urea cycle enzymes of rat liver, cultured hepatocytes and hepatoma cells. *J. Nutr.* 117:1827–37
105. Stadhouders, A. M., Sengers, R. C. A. 1987. Morphological observations in skeletal-muscle from patients with a mitochondrial myopathy. *J. Inherit. Metab. Dis.* 10(Suppl. 1):62–80
106. Surks, M. I., Chinn, K. S. K., Matoush, L. R. 1966. Alterations in body composition in man after acute exposure to high altitude. *J. Appl. Physiol.* 21:1741–46
107. Svoboda, D., Higginson, J. 1964. Ultrastructural changes produced by protein and related deficiencies in the rat liver. *Am. J. Pathol.* 45:353–79
108. Sweetman, L., Nyhan, W. L. 1986. Inheritable biotin-treatable disorders and associated phenomena. *Annu. Rev. Nutr.* 6:317–43
109. Swick, A. G., Swick, R. W. 1986. Enhanced thermogenic response to diet low but adequate in protein persists in older rats. *Am. J. Physiol.* 251:E438–41
110. Tager, J. M., Wanders, J. A., Groen, A. K., Kunz, W., Bohnensack, R., et al. 1983. Control of mitochondrial respiration. *FEBS Lett.* 151:1–9
111. Takamiya, S., Yanamura, W., Capaldi, R. A., Kennaway, N. G., Bart, R., et al. 1986. Mitochondrial myopathies involving the respiratory chain: A biochemical analysis. *Ann. NY Acad. Sci.* 488:33–43
112. Tandler, B., Erlandson, R. A., Wynder, E. L. 1968. Riboflavin and mouse hepatic cell structure and function. I. Ultrastructural alterations in simple deficiency. *Am. J. Pathol.* 52:69–95
113. Tandler, B., Hoppel, C. L. 1974. Ultrastructural effects of dietary galactoflavin on mouse hepatocytes. *Exp. Mol. Pathol.* 21:88–101
114. Tandler, B., Hoppel, C. L. 1980. Ultrastructural effects of riboflavin deficiency on rat hepatic mitochondria. *Anat. Rec.* 196:183–90
115. Tandler, B., Hoppel, C. L. 1986. Studies on giant mitochondria. *Ann. NY Acad. Sci.* 488:65–81
116. Terjung, R. L., Kaciuba-Uscilko, H. 1986. Lipid metabolism during exercise: influence of training. *Diabetes Metab. Rev.* 2:35–51
117. Thurman, R. G., Kauffman, F. C., Jungermann, K. 1986. *Regulation of Hepatic Metabolism: Intra- and Intercellular Compartmentation*. New York/London: Plenum. 489 pp.
118. Tyzbir, R. S. 1984. Altered brown adipose tissue mitochondrial function in neonates born to rats overfed foods of various protein content. *J. Nutr.* 114:234–37
119. Tzagoloff, A. 1982. *Mitochondria*. New York/London: Plenum. 342 pp.
120. Tzagoloff, A., Myers, A. M. 1986. Genetics of mitochondrial biogenesis. *Annu. Rev. Biochem.* 55:249–85
121. de Visser, M., Scholte, H. R., Schutgens, R. B. H., Bolhuis, P. A., Luyt-Houwen, I. E. M., et al. 1986. Riboflavin-responsive lipid-storage myopathy and glutaric aciduria type-II of early adult onset. *Neurology* 36:367–72
122. Wander, R. C., Berdanier, C. D. 1985. Effects of dietary carbohydrate on mitochondrial composition and function in two strains of rats. *J. Nutr.* 115:190–99
123. Whitten, B. K., Janoski, A. H. 1969. Effect of high altitude and diet on lipid components of human serum. *Fed. Proc.* 28:983–86
124. Wieland, O. H. 1983. The mammalian pyruvate dehydrogenase complex structure and regulation. *Rev. Physiol. Biochem. Pharmacol.* 96:123–70
125. Williams, R. S. 1986. Regulation of mitochondrial biogenesis by contractile activity in skeletal muscle: recent advances and directions for future research. See Ref. 10, pp. 171–80
126. Wilson, D. F., Erecinska, M., Drown, C., Silver, I. A. 1979. The oxygen de-

pendence of cellular energy metabolism. *Arch. Biochem. Biophys.* 195:485–93

127. Yamada, E. W., Shiffman, F. H., Huzel, N. J. 1980. Ca^{2+}-regulated release of an ATPase inhibitor protein from sub-mitochondrial particles derived from skeletal-muscles of the rat. *J. Biol. Chem.* 255:267–73

128. Yamamoto, M., Sato, T., Anno, M., Ujike, H., Takemoto, M. 1987. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes with recurrent abdominal symptoms and coenzyme Q_{10} administration. *J. Neurol. Neurosurg. Psychiatry* 50: 1475–81