Neurodegeneration from Mitochondrial Insufficiency: Nutrients, Stem Cells, Growth Factors, and Prospects for Brain Rebuilding Using Integrative Management

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Abstract

Degenerative brain disorders (neurodegeneration) can be frustrating for both conventional and alternative practitioners. A more comprehensive, integrative approach is urgently needed. One emerging focus for intervention is brain energetics. Specifically, mitochondrial insufficiency contributes to the etiopathology of many such disorders. Electron leakages inherent to mitochondrial energetics generate reactive oxygen "free radical" species that may place the ultimate limit on lifespan. Exogenous toxins, such as mercury and other environmental contaminants, exacerbate mitochondrial electron leakage, hastening their demise and that of their host cells. Studies of the brain in Alzheimer's and other dementias, Down syndrome, stroke, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Friedreich's ataxia, aging, and constitutive disorders demonstrate impairments of the mitochondrial citric acid cycle and oxidative phosphorylation (OXPHOS) enzymes. Imaging or metabolic assays frequently reveal energetic insufficiency and depleted energy reserve in brain tissue *in situ.* Orthomolecular nutrients involved in mitochondrial metabolism provide clinical benefit. Among these are the essential minerals and the B vitamin group; vitamins E and K; and the antioxidant and energetic cofactors alpha-lipoic acid (ALA), ubiquinone (coenzyme Q10; CoQ10), and nicotinamide adenine dinucleotide, reduced (NADH). Recent advances in the area of stem cells and growth factors encourage optimism regarding brain regeneration. The trophic nutrients acetyl L-carnitine (ALCAR), glycerophosphocholine (GPC), and phosphatidylserine (PS) provide mitochondrial support and conserve growth factor receptors; all three improved cognition in double-blind trials. The omega-3 fatty acid docosahexaenoic acid (DHA) is enzymatically combined with GPC and PS to form membrane phospholipids for nerve cell expansion. Practical recommendations are presented for integrating these safe and well-tolerated orthomolecular nutrients into a comprehensive dietary supplementation program for brain vitality and productive lifespan. (*Alternative Medicine Review* 2005;10(4):268-293)

Introduction

Degenerative brain disorders take away independence and individuality, strain the fabric of relationships, deplete family resources, and place an ever-increasing burden on society as a whole. Conventional medicine has made very little progress in treating these disorders. A more radical approach is called for, one that first identifies root causes (L. *radix*, root),[1] then brings to bear the widest range of modalities for prevention and medical management. One relatively unexplored modality is combination treatment with safe and potent dietary supplements, targeted at energetic support for the brain.

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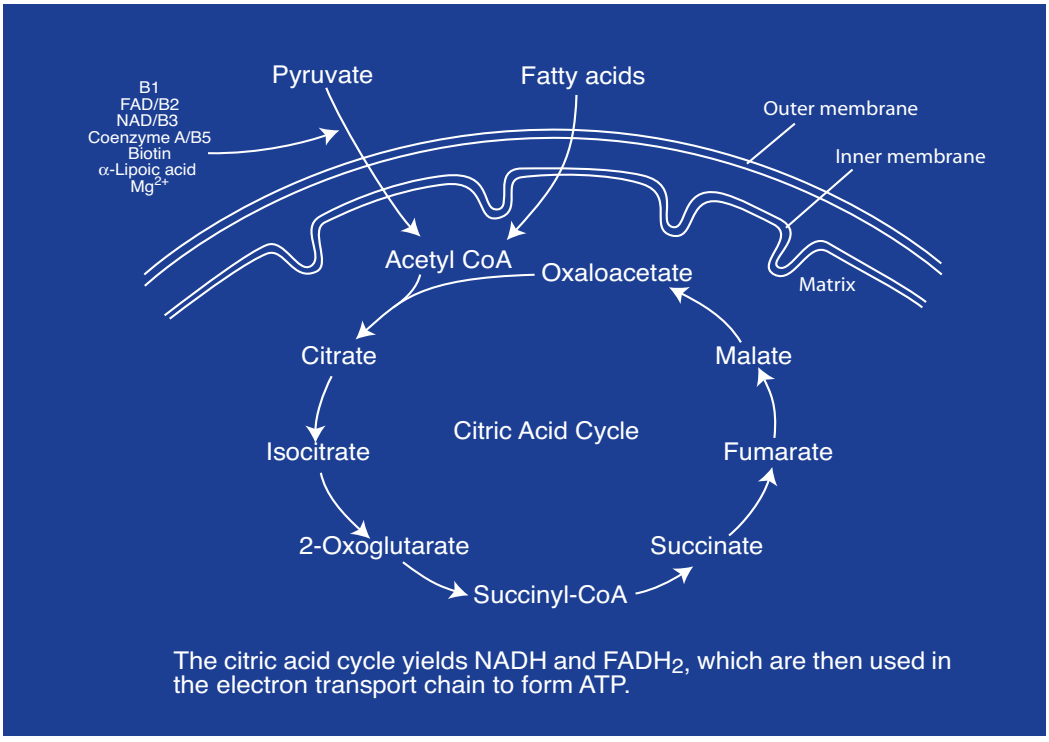
Oxidative "free radical" damage is generally viewed as an etiological factor in brain degeneration; the literature in this area of inquiry is extensive. A more focused variation on this theme is the importance of mitochondrial decline in energetic failure within nerve cells and the brain as whole. This article marshals the evidence for this phenomena, examines the brain's potential for recovery based on its inherent plasticity, and delves into the rapidly expanding area of stem cell and growth factor research, which has matured from futuristic speculation into new therapeutics transforming the medical paradigm.

Figure 1. The Citric Acid Cycle Occurs Exclusively in the Mitochondrial Matrix



The citric acid cycle yields NADH and $FADH_2$, which are then used in the electron transport chain to form ATP.

The Mitochondria: Cell Powerhouses with Design Flaws

Mitochondria are the energy powerhouses of the cells. Mitochondria are cellular organelles, inner compartments of the cell demarcated by membranes; they have an inner microenvironment and perform specialized functions.[2,3] The unique function of mitochondria is to generate life energy, some of it electrical but most as adenosine triphosphate (ATP), the main energy currency of the body. The mitochondria generate more than 90 percent of the cell's supply of ATP.

Pyruvate and fatty acids are transported from the cell cytosol into the mitochondria and processed through the citric acid cycle (tricarboxylic acid cycle; Kreb's cycle). The resulting high-energy intermediates – nicotinamide adenine dinucleotide, reduced (NADH) and flavin adenine dinucleotide, reduced ($FADH_2$) – are utilized in the inner membrane to make ATP.

Mitochondria are distinct from other cellular organelles in having separate genomes, structurally and functionally distinct from the chromosomal assemblies housed in the cell nucleus. The mitochondrial genome is circular, resembling that of bacteria and differing from that of the nuclear genome. Cells have multiple mitochondria, each of which is derived from a pre-existing mitochondrion; they divide by binary fission and propagate distinct "lineages" within each cell. In the living cell, mitochondria are dynamic, constantly changing shape, even fusing with one another and separating again.[2]

These and other unique features of mitochondria sparked the idea that they evolved from prokaryotic (very simple) cells that took up residence in other prokaryotic cells. Margulis hypothesized that mitochondria were originally endosymbionts, internal prokaryotic guest cells that generated energy for the host cell in return for shelter and a steady supply of food. Margulis suggested that over the eons these microscopic houseguests moved in for good.[4]

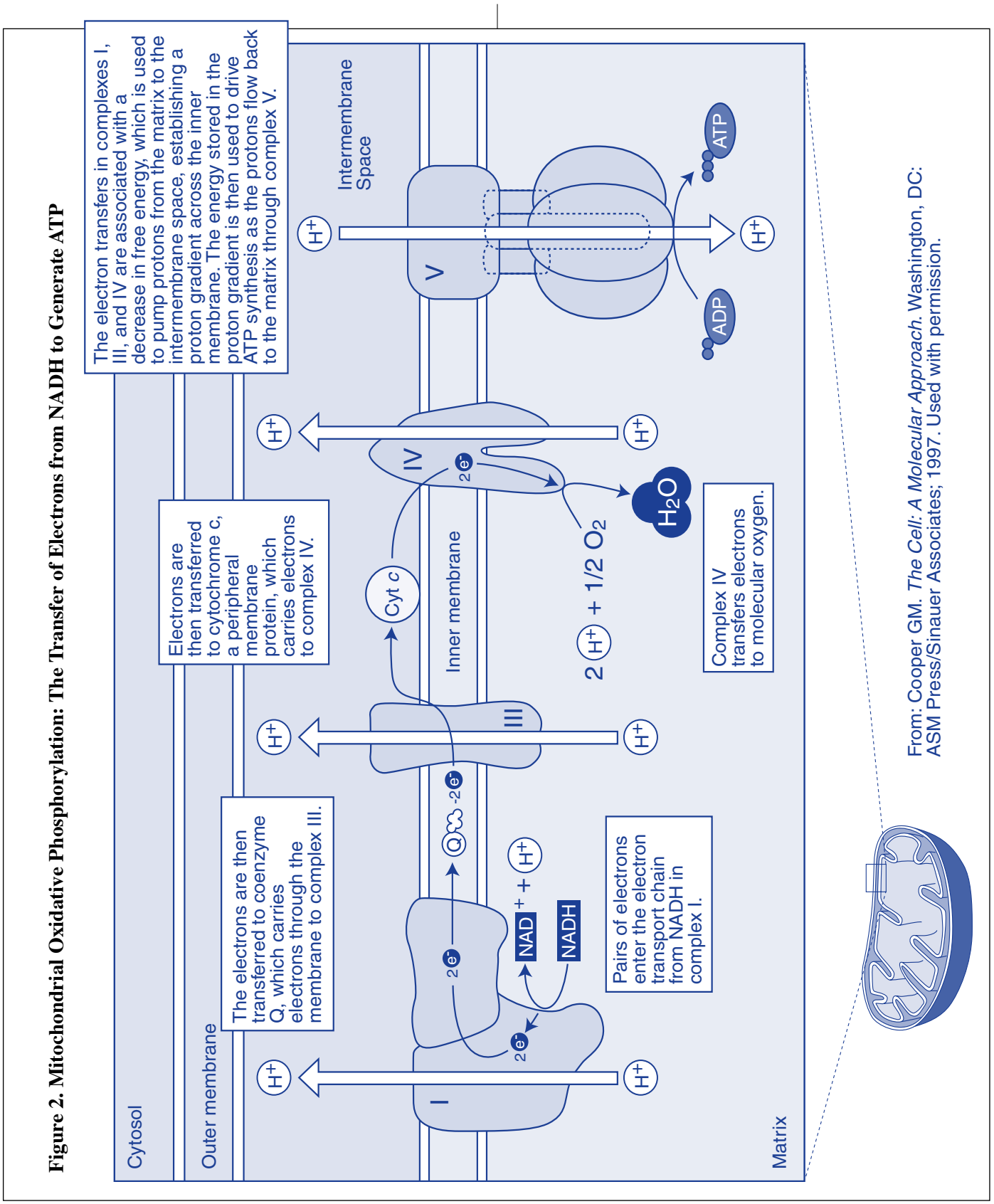


Figure 2. Mitochondrial Oxidative Phosphorylation: The Transfer of Electrons from NADH to Generate ATP

Whether or not they were ever totally free-living cells, mitochondria cannot now survive independently since many of their proteins are made from host (nuclear) genes. The presence of mitochondria in human eukaryotic cells provides the capacity to make ATP 15 times more efficiently than anaerobic cells that rely on glycolysis alone.[3]

Mitochondrial Metabolism and Oxidative Phosphorylation (OXPHOS)[2]

The cell cytosol metabolizes dietary substances to pyruvate and fatty acids, which are then actively taken up by the mitochondria and processed by water-phase enzymes in the citric acid cycle (Figure 1). This cycle generates the high-energy intermediates NADH and $FADH_2$, which are essentially holding molecules for pairs of highly energized electrons.

The action then shifts from the water phase of the matrix to the inner mitochondrial membrane, which is densely packed with multiple sets of large catalytic proteins (Figure 2). Five discrete multiprotein complexes, known as complex I, II, III, IV, and V,[2,3] manage the process called oxidative phosphorylation. OXPHOS begins as complex I engages NADH and complex II engages $FADH_2$. Each enzyme strips away the pair of electrons from its substrate and passes them to coenzyme Q10 (CoQ10).

Coenzyme Q10 is a deceptively simple molecule. Its small size enables it to rapidly diffuse between the huge protein assemblies of the inner mitochondrial membrane. As CoQ10 moves through the phospholipid matrix it relays electrons to complex III. This complex extracts additional electron energy and passes electrons to cytochrome c, a mobile protein that transfers electrons to complex IV. At complex IV the last available energy is extracted from the electron pair and these electrons are combined with molecular oxygen to make water. The net electron transfer from complex I through to complex IV is "drawn" by the high electron-attracting potential of molecular oxygen.

Complexes I, III, and IV siphon electrochemical energy from the electrons they handle, and use it to transport positively charged hydrogen ions (protons) into the mitochondrial inter-membrane space, creating a strong proton diffusion potential that drives complex V. Also called ATP synthase, this complex is actually rotated by the protons passing through it. As it rotates it catalyzes the conversion of low-energy ADP to high-energy ATP. Complex V is a molecular turbine converting electrochemical energy to chemical bond energy.[2]

Reactive Oxygen Species: The Price of Sophisticated Life

The capability of their mitochondria to produce large quantities of ATP from the diet likely empowered the early single-celled eukaryotes to evolve within cellular communities as sophisticated as those of the human body. But with this advance came a challenge: the erosive effects of the highly reactive molecular oxygen on the metabolic apparatus. Dioxygen, O_2, has an avid affinity for electrons, a property that suits it well for use by the mitochondria.[5] It is this affinity that drives the entire electron transfer gradient of the OXPHOS system. Unfortunately, dioxygen has substantial free radical character, and as the mitochondria process oxygen, free radicals of oxygen are generated. This is intrinsic to the OXPHOS process and has potent potential to destroy cells, usually beginning with the mitochondria themselves.

Oxygen is said to have a Janus face: good on one side, bad on the other.[5] The good side includes numerous enzyme-catalyzed reactions that use molecular oxygen for normal metabolism. The bad side encompasses deleterious effects on structure and function from oxygen-derived free radicals, more accurately termed reactive oxygen species (ROS). Their ultimate source is the intense metabolism of oxygen by the mitochondria.[5]

Every living organism has antioxidant defense enzymes to cope with the ROS "sparks of metabolism" continually being generated within our cells, mostly by the mitochondria.[2,3,5] However, since no enzyme defense system is 100-percent efficient, the entire array of available endogenous antioxidant enzymes cannot fully neutralize the ROS being emitted from the mitochondria. Exogenous antioxidants derived from the diet help fill this gap, but inevitably some proportion of ROS escape control and damage the cell, including its mitochondria. Cumulative damage from ROS over decades may well be the final determinant of maximum lifespan.

In 1956, Harman proposed the free radical theory of aging: "Free radical reactions are involved in the aging changes associated with the environment, disease, and intrinsic aging processes."[6] Of the many existing theories for aging, this one remains the most rational and credible. In 1972, Harman wrote "The Biologic Clock: The Mitochondria?" in which he proposed that the mitochondria, by generating the bulk of the cells' free radicals, set the ultimate limit on lifespan.[7] Today, with subsequent contributions and refinements from Harman and many others, the "free radical" theory of aging has evolved into the "free radical-mitochondrial" theory of aging.[8]

The Free Radical-Mitochondrial (FRM) Theory of Aging

As articulated by Schipper, aging represents a progressive decline in the efficiency of physiological processes subsequent to the reproductive phase of life, and a tendency for diminished recovery following insult.[8] The FRM theory essentially asserts that cumulative oxidative injuries to the mitochondria, triggered by endogenous metabolic processes and/or by exogenous oxidative influences, cause the mitochondria to progressively become less efficient. They have more trouble handling the "hot" electrons traversing their membranes, become damaged, and consequently produce less energy. A vicious cycle develops as functional damage and impaired energy production feed on each other.

As the mitochondria progressively lose their functional integrity, ever-greater proportions of oxygen molecules reaching them are converted to ROS. Because free radical reactions self-propagate, additional reactive species are produced downstream, including reactive carbon species (RCS; e.g., from unsaturated fatty acids of cell membranes), reactive nitrogen species (RNS), and reactive sulfur species (RSS). Fatty acids and cell-membrane phospholipids, DNA, RNA, and the cell's entire metabolic apparatus are subject to attack. Antioxidant enzymes, as well as the DNA and protein repair enzymes themselves, can become damaged and no longer provide protection. Mutations accumulate along with malfunctioning molecules and other debris. Eventually the cell is either crippled, killed outright (necrosis), commits suicide (apoptosis), or loses growth control and becomes cancerous.

Besides modeling aging, the FRM theory also predicts degenerative disease. Exogenous factors that damage the mitochondria would tend to accelerate cellular deterioration. It is likely that exogenous oxidative stressors contribute markedly to this process of mitochondrial decline over time. Industrial chemicals, pharmaceuticals, other chemicals often assumed safe, and increasingly toxic food, air, and water supplies all have oxidative potential.[9] These exogenous oxidants exacerbate the endogenous oxidant burden from electron leakage by the mitochondria.

Numerous estimates concur that when mitochondria function optimally they convert up to 95 percent of the oxygen they handle into water (see Floyd and Hensley for a review[10]). The remaining five percent is converted to ROS, even under the most favorable conditions. Excluding other relatively minor metabolic sources, this is the minimum free radical production intrinsic to human metabolism. In a healthy individual this degree of oxygen leakage is manageable, at least for several decades. This endogenous ROS production is thought to interact with other oxidative susceptibilities of the living system to set the ultimate limit on lifespan for each species.[10]

In today's world a considerable oxidative burden from exogenous toxins undoubtedly accelerates the oxidative aging process. The cells progressively accumulate end products of biological oxidation, progressively depleting the organ reserves.[8] Animal experiments confirm that the greater the oxidative stress burden the more rapid is the loss of function; in no organ is this more evident than the brain.

The Brain is Highly Vulnerable to Oxidative Stress[8,10]

The human brain uses more oxygen and produces more energy per unit mass than any other organ. Both features of brain metabolism translate into extremely high OXPHOS activity, accompanied by correspondingly high electron leakage. Furthermore, the brain has high iron content (mostly in OXPHOS proteins) that can catalyze oxidation. The brain is also particularly loaded with unsaturated fatty acids in the myelin sheath, and long-chain fatty acids of the

cell membranes highly susceptible to peroxidation. Finally, the brain is poorly equipped with antioxidant enzyme defenses. These factors make this organ exceptionally vulnerable to oxidative degeneration.[10]

Evidence is mounting that the mitochondria are the most vulnerable functional subset of brain tissue. The mitochondria have antioxidant defenses inferior to the greater cell, making mitochondrial DNA 10-100 times more likely to become damaged than nuclear DNA.[10] Neurons also have constant calcium flux, and the mitochondria provide backup for calcium homeostasis.[11] Thus, mitochondrial insufficiency could tip the delicate intracellular calcium balance toward cell death.

For some neurodegenerative disorders (e.g., Alzheimer's disease and other dementias), the available evidence falls short of proving that mitochondrial abnormality is the primary etiologic mechanism. For others (e.g., Down syndrome and Parkinson's disease), the evidence is persuasive that mitochondrial inadequacies play a major role. In the case of stroke, the mitochondrial contribution may be overwhelming. The flood of ROS generated in the neuronal and glial mitochondria during hypoxic-hyperoxic ischemic insult can be acutely devastating to brain tissue.[12]

Whatever the extent of the mitochondrial contribution to the etiology of these disorders, current evidence indicates mitochondrial impairment is a universal contributor to neurodegeneration. Given the extreme impacts of these disorders on lifespan and life quality, the prospects for their successful medical management hinge on comprehensive brain support. Orthomolecular nutritional support of mitochondrial performance and structural integrity offers a nontoxic, well tolerated, and rational option.

One integrative physician taking the lead is David Perlmutter, MD, Medical Director for the Perlmutter Health Center of Naples, Florida. In his practice and educational activities he convincingly justifies the rationale for using energizing and protective, orthomolecular nutrients.[13] His successful management of Parkinson's disease with a comprehensive protocol, including injected glutathione, is a major contribution to the management of this difficult disease.

Alzheimer's and Mitochondrial Failure – Strength of the Evidence

A consensus is emerging that Alzheimer's disease (AD) and the other dementias have multifactorial etiologies.[8,13,14] In contrast to normal aging, which features very little cell death, the extent of nerve cell and whole circuit dropout in AD is widespread and sometimes catastrophic. During normal aging, the brain suffers morphological and functional modifications affecting dendritic trees and synapses, neurotransmitters, tissue perfusion and metabolism, motor and sensory systems, sleep, memory and learning, and demonstrates lipofuscin accumulation with moderate amounts of amyloid. Many studies implicate ROS and mitochondrial decline as the basis for these changes (see Barja, 2004 for a comprehensive review[15]). AD manifests as an exaggeration of these changes – and more.

Progressive formation of neurofibrillary tangles and the secretion of beta-amyloid that condenses to form plaques characterize the pathology of AD. Amyloid formation has been convincingly linked to oxidative damage.[16] Energetic decline is one of the earliest changes evident in the AD brain, and mitochondrial abnormalities have been detected all across the brain cortical zones.

Energetic Impairments in Alzheimer's Disease

Some of the most direct evidence for mitochondrial abnormalities in AD comes from non-invasive, *in vivo*, positron emission tomography (PET) imaging. These findings were reviewed in 2005 by Sullivan and Brown.[16] In particular, the temporal and parietal cortical zones consistently exhibit metabolic abnormalities. Some PET reports document abnormally high oxygen utilization in comparison to the amounts of glucose utilized, indicating impairment of the OXPHOS process in the mitochondria.

The decrements in brain metabolism seen with non-invasive imaging tend to precede both the neuropsychological impairment and anatomical changes of AD, such as atrophy. The frontal cortex and middle temporal gyrus, areas that manifest the most prominent metabolic abnormalities via PET, are also the areas that most strongly exhibit synaptic

dysfunction and circuit loss seen in AD brains on morphological examination.

The brain's ongoing viability is dramatically dependent on energy from glucose. In both AD and the closely related vascular dementia (VD), bioenergetic impairment can appear early and progress rapidly, consistent with a primary defect.[17] A similar pattern is evident in Wernicke-Korsakoff syndrome, a dementia associated with thiamine depletion and often seen in alcoholics.[18]

Alterations in mitochondrial enzymes are consistently linked to dementia. As early as 1980, enzyme assays using tissue homogenates from autopsied AD brain revealed decreases of pyruvate dehydrogenase (PDH) activity in the frontal, temporal, and parietal cortex.[19] PDH, the enzyme crucial to driving the citric acid cycle (CAC), is markedly affected in vascular dementia as well as in Alzheimer's dementia.[17]

Alpha-ketoglutarate dehydrogenase (KGD) is the probable rate-limiting enzyme of the citric acid cycle. Gibson and coworkers found KGD significantly decreased in the AD temporal and parietal cortex.[20,21] Other CAC enzymes are also found impaired in AD, and the overall degree of metabolic impairment tends to correlate with clinical status.[22]

Beyond the water-phase CAC enzymes of the mitochondrial matrix, the enzymes that make up complexes I-V in the inner mitochondrial membrane are indispensable for ATP generation. Various studies have catalogued impairments of all five complexes in multiple zones of the AD brain.[16,23-26] Most widely involved was complex IV (cytochrome c oxidase), for which the enzyme activity was found significantly decreased in the frontal, temporal, parietal, and occipital cortex from AD brains compared to age-matched controls.[25,26] Complex I protein levels were significantly reduced in the temporal, parietal, and occipital zones;[24] complex III protein was significantly reduced in the temporal cortex;[25] and complex V proteins were significantly reduced in the hippocampus (reviewed in Kim et al[25]). Kim et al also demonstrated lowered biosynthesis of one subunit of complex I in the temporal and occipital cortices, and of a different complex I subunit in the parietal cortex.[24]

It is unclear to what extent these marked energetic impairments of AD could be constitutive, versus secondarily acquired (from heavy metal or other toxic cumulative damage, for example). After much investigation, no abnormal mitochondrial genes or gene clusters have been identified for later-onset AD, which represents the majority of cases. A recent study, however, reported mitochondrial DNA from the brain tissue of AD patients had significantly greater damage compared to controls.[27]

Although there still is no "smoking gun," it is tempting to speculate that exogenous oxidative toxins play a role in AD. Especially worthy of investigation is mercury. This toxic heavy metal has a continued presence in dental amalgams throughout the population, is still being added to vaccines and other injectable preparations, and has a ubiquitous environmental presence from industrial emissions. Mercury is uniquely toxic to nerve cells, with its accumulation in the mitochondria linked to cell destruction.[28] Elevated blood mercury levels in childhood correlate with lifelong cognitive impairment.[29] While still not providing definitive proof, a small German study found blood mercury levels were significantly elevated more than two-fold in AD patients compared to age-matched, non-AD controls.[30] Early-onset AD patients demonstrated a significant three-fold higher mercury elevation.

Down Syndrome May Model Mitochondrial Dementia

Down syndrome (DS) is a highly heritable disease that begins prior to birth. By the third decade, subjects with DS typically exhibit neuronal pathology resembling Alzheimer's;[31] many develop frank dementia.

DS subjects have inherited an extra copy of part or all of chromosome 21, resulting in an extra gene for copper-zinc superoxide dismutase (cytoplasmic SOD), often also associated with premature accumulation of beta-amyloid in the brain.[31] SOD metabolizes superoxide to hydrogen peroxide, which normally would then be detoxified by other antioxidant enzymes. However, DS subjects do not seem equipped to metabolize this elevated hydrogen peroxide flux. As a result, their tissues are abnormally vulnerable to oxidative damage from high ambient

peroxide levels and neuronal mitochondria likely fall victim to this hostile intracellular environment. Experiments with transgenic mice, engineered to carry extra human SOD genes, confirm this probable scenario.[32]

There is credible evidence for mitochondrial impairment in DS. When fibroblasts were cultured from DS subjects and exposed to an experimental mitochondrial toxin, they showed poor capacity for mitochondrial-DNA repair.[33] Platelets drawn from adult and juvenile DS subjects showed a one-third reduced activity in two mitochondrial enzymes.[34] Using new proteomic technology, Kim et al were able to track the biosynthesis of a large number of proteins in DS-brain homogenate and compare it with AD homogenate. They found protein levels for two subunits of complex I were reduced in DS brains – the very same subunits they found reduced in AD brains, albeit with a slightly different cortical distribution. Also different from AD, DS brains showed complex I reduction in the deeper thalamus and caudate nucleus zones,[24] and a complex V subunit was reduced in the DS frontal cortex.[25]

Mitochondrial dysfunction in DS likely begins prior to birth. When Kim et al cultured cerebral cortex from fetal DS brain, they found deficiencies of a complex I protein.[35] Lee et al cultured fetal amniotic cells from DS and non-DS fetuses and found low messenger RNA levels for yet another mitochondrial OXPHOS gene.[36] Busciglio et al cultured astrocytes from fetal DS brain and found abnormal beta-amyloid processing with premature accumulation of beta-amyloid aggregates.[37] They could duplicate this pattern in normal fetal astrocytes by adding OXPHOS mitochondrial inhibitors, suggesting that DS-mitochondrial impairment prior to birth increases the risk for its Alzheimer's-like, degenerative pathology that typically manifests by age 30.

The most obvious difference between DS and AD is that DS has earlier onset. DS is heavily genetically determined, while most AD is not, which might help explain why aging is the most consistent risk factor for AD. It may well be that in DS the systemic oxidative stress from elevated SOD activity is so high that mitochondrial performance is already compromised at birth. In AD, the genetic burden is seemingly less severe, but the passage of time allows for cumulative oxidative influences to initiate and drive a degenerative progression that eventually afflicts the mitochondria. Speculatively, mitochondrial destruction would promote a downward spiral of bioenergetic capacity that progressively contributes to disease progression.

Parkinson's Disease, Mitochondrial Compromise, and Neuronal Death

As reviewed by Eriksen, two of the four principal genes implicated in familial Parkinson's disease (PD) are of mitochondrial origin.[38] The gene DJ1 protects against mitochondrial damage and oxidative stress, and (in cell cultures) its experimental deletion sensitizes cells to these effects. The gene PINK1 likely has a role in mitochondrial homeostasis. Mutations in this gene increase cell susceptibility to stressful conditions, inducing mitochondrial dysfunction and cellular apoptosis. Still, only about 10 percent of all PD cases are familial. Investigators tend to concur that the large majority of non-familial cases require environmental triggers to clinically manifest.

In 2000, this reviewer summarized integrative management of PD.[39] At that time there was already compelling evidence for mitochondrial contributions to the initiation and progression of the disease. That review presented substantial evidence for the following:

- ▼ Constitutive mitochondrial abnormalities in the brain's substantia nigra (SN), but also in extra-cerebral tissues
- ▼ Mitochondrial complex I impairment in the SN, ranging from 16-54 percent
- ▼ The SN as a unique concentrator of iron (redox-active free radical generator), monoamine oxidase enzymes (also free-radical generating), and dopamine and melanin (both with an exquisite tendency to auto-oxidize to reactive free radicals)
- ▼ Progressive depletion (by 40-90%) of reduced glutathione in the SN as the disease progresses

- ▼ Elevation of lipid peroxides, malondialdehyde, and other oxidation byproducts in the SN
- ▼ Increased oxidative damage to DNA and proteins in the SN
- ▼ Substantial evidence that heavy metals (most specifically mercury), pesticides, and other exogenous oxidants contribute to the clinical emergence of PD

Since that review appeared, the evidence that mitochondrial failure and resultant oxidative stress contribute to the etiology of PD has been further strengthened. Activity of the CAC enzyme KGD was found reduced by about half in cerebellar tissue obtained from 19 PD patients and compared to 18 controls.[40]

PD is characterized pathologically by the presence of Lewy bodies, of which the protein alpha synucleain (ASN) is a prominent constituent. Mouse breeds are now available that lack the genes for ASN. New studies with these animal models are exploring the possibility that mitochondrial energetic failure causes aberrant metabolism of ASN and triggers Lewy body formation.[38]

Progressive supranuclear palsy (PSP) is a rare, neurodegenerative movement disorder often mistaken for PD. Various lines of evidence link its etiology to mitochondrial DNA aberrations.[41]

Multiple Sclerosis

Multiple sclerosis (MS) is generally considered to be an inflammatory disease with a substantial autoimmune contribution.[42] Current therapeutic strategies primarily target the immune system, with some success at down-regulating plaque formation and decreasing relapse rate.[43] Progress in the medical management of MS has been poor, characterized by therapies that were theoretically promising but failed in human application.[44]

Kalman et al proposed that mitochondrial abnormalities could drive the inflammatory processes in MS.[43] In 2000, her group demonstrated that impaired complex I activity in chronic active plaque zones was associated with oxidative damage to mitochondrial DNA.[45] One case report documented constitutive mitochondrial energy failure as a cause of the intermittent demyelination and profound central nervous system (CNS) symptoms that mimic MS.[46]

Kalman's group spent a decade searching for a genetic link to mitochondrial failure in MS. In 1995 they reported finding mitochondrial DNA mutations in 20 percent of patients studied[47] and by 1999 they had identified specific suspect gene groups (haplotypes).[48] Finally, in 2005 they definitively stated that mitochondrial complex I gene variants are associated with MS.[49] This series of studies appears to implicate complex I abnormalities in the degenerative processes downstream of CNS inflammatory injury in MS patients.

Amyotrophic Lateral Sclerosis (ALS)

Considerably more has been learned about ALS since it killed Lou Gehrig 60 years ago, although its medical management has not appreciably improved. Mitochondrial dysfunction is now known to be a primary feature of ALS, with mitochondrial pathology observed at an early stage in the degeneration of the motor neurons.[41] Muscle mitochondria from ALS patients exhibit impaired electron transport, elevated free radical generation, and inability to buffer intracellular calcium. Nuclear magnetic resonance (NMR), which can accurately measure mitochondrial metabolite levels *in vivo*, demonstrated a significant correlation between known mitochondrial pathology in ALS skeletal muscle and abnormal mitochondrial metabolite ratios in the cerebral cortex.[50]

This still-mysterious disease appears to exhibit many of the biochemical and morphological identifiers of a free radical-mitochondrial disease. One recorded case of constitutive mitochondriopathy so closely mimicked ALS that it was mistaken for it.[51]

Huntington's Disease

Huntington's disease (HD), a strongly heritable disease (autosomal dominant), follows a horrific course. It can emerge in early- or mid-life, but progression is generally inexorable and leads to death within 10-15 years. HD features "anticipation," where the age at onset tends to decrease in successive

generations. The mutant gene has been identified, producing a protein aptly christened huntingtin.[52] The mechanisms of action of this protein are being explored using transgenic mice that express the HD gene.

In addition to excitotoxicity, mitochondrial dysfunction has been identified as a pathologic factor in HD. Gardian and Vecsei summarized the evidence:

- ▼ Ultrastructural studies reveal structurally abnormal mitochondria in the cortex
- ▼ Mitochondria isolated from HD lymphoblast cells show poor membrane integrity
- ▼ Biochemical studies demonstrate impaired complex II and III in basal ganglia homogenates from HD patients.[52]

In 1997, Koroshetz et al used magnetic resonance spectroscopy (MRS) to confirm earlier reports of abnormally elevated lactate in the cerebral cortex and basal ganglia of HD patients; lactate builds up when the CAC fails. This indication of mitochondrial decline tends to appear early in the disease.[53] Whether the mitochondrial contribution to HD is primary or secondary, it is being investigated by leading HD researchers as a potentially treatable facet of this disease.

Friedreich's Ataxia

Friedreich's ataxia (FA) is an autosomal recessive disease with a relentlessly progressive course, characterized by limb ataxia and other CNS difficulties. The most marked pathological changes include loss of large sensory neurons in the dorsal root ganglia and degeneration of the dorsal columns of the spinal cord;[54] many non-CNS organ abnormalities are also present. Sophisticated investigation of cardiac and skeletal muscle has confirmed mitochondrial iron accumulation and mitochondrial OXPHOS abnormalities, likely resulting in increased free radical generation.[55] Therapeutic intervention in FA has so far been focused on antioxidant protection, mitochondrial energy enhancement, and iron chelation.

The Normal Aging Process

Ames et al have amassed a persuasive body of evidence linking mitochondrial oxidation to the aging process.[56,57] They discovered that, with age, cumulative oxidative damage to human enzymes causes loss of catalytic efficiency. Mitochondria, being more poorly equipped with antioxidant or DNA repair systems, are susceptible to such decline with age. Ames' group developed a convincing case that the mitochondria of aged animals perform more poorly than in the young.

In a mouse strain used to study aging, the activities of complex I, complex IV, and one other mitochondrial enzyme were found to decline with age.[58] Moderate exercise and supplementation with vitamin E increased lifespan, retarded the decline of these enzymes, and partially preserved brain function on cognitive tests. The Ames group documented that diets deficient in various micronutrients can accelerate this process of mitochondrial decay.[57]

Aging does not appear to be determined exclusively by genetic or other constitutive factors, or to be dominated by an "aging clock" that ticks away the days to one's demise. Rather, it seems that aging is a process, one that proceeds in fits and starts, the net outcome of myriad interacting factors. This type of aging pattern is consistent with the FRM theory, which also predicts that excessive exposure to exogenous oxidative stressors would tend to hasten aging, primarily by accelerating the cumulative free radical oxidative damage to tissues and organs.

Ames et al argue that the age-accelerating effect of oxidation can be offset (at least partially) by supplementing the diet with higher levels of micronutrients.[57] They catalogued an array of nutrients that support or boost mitochondrial energetic efficiency in human tissues. Of these, coenzyme Q10, lipoic acid, and acetyl L-carnitine will be discussed in a later section. The others are listed below:

- ▼ Iron: essential for the heme constituents of several of the mitochondrial OXPHOS proteins. Ames claims approximately two billion people worldwide may be iron deficient.[57] However, iron excess (>10x nutritional need) can exacerbate ROS production and oxidative damage.

▼ Zinc: essential for the SOD antioxidant enzyme of the cell cytoplasm. Ten percent of the U.S. population ingests less than half the RDA;[56] deficiency linked to increased DNA damage and decreased DNA repair; deficiency linked to cognitive deficit in humans.

▼ Copper: necessary for heme assembly and an essential structural cofactor ("prosthetic group") for mitochondrial complex IV.

▼ Thiamine (vitamin B1): cofactor for pyruvate dehydrogenase. This enzyme may require 300 mg/day or higher.[59]

▼ Riboflavin (vitamin B2): precursor of $FADH_2$; cofactor for electron transport by complexes I and II and the electron transfer flavoprotein of the OXPHOS array. A 100-mg/day intake can be helpful to this process.[59]

▼ Nicotinamide (niacinamide; vitamin B3): precursor of NADH, an energized electron carrier that delivers electrons to complex I of the electron transport chain in the mitochondria.

▼ Pantothenic acid (vitamin B5): precursor of coenzyme A, which produces acetyl-CoA to enter the citric acid cycle; deficiency lowers heme and may affect complex IV.

▼ Pyridoxine (vitamin B6): converted to the coenzyme pyridoxal-5' phosphate (P5P) that is directly involved in heme synthesis. As with zinc, as much as 10 percent of the U.S. population may ingest less than half the RDA.[56]

▼ Biotin: prosthetic group for four carboxylase enzymes, three of which are mitochondrial. These three help produce intermediates for the CAC. Deficiency is common in the U.S. population.

▼ Vitamin K: both K1 (phylloquinone, phytonadione) and K3 (menadione) have been administered in conjunction with ascorbate (vitamin C, 4 grams/day) to donate electrons and successfully improve mitochondrial myopathy.[59] Researchers recommend K1 at 10 mg/day and K3 at 25-35 mg/day.[59]

▼ Glycine: essential for an early step in heme synthesis; component of reduced glutathione, a pivotal water-phase antioxidant.

Mitochondrial Nutrients to Counter Neurodegeneration

Since elevated free radical generation and other mitochondrial dysfunction are involved in neurodegenerative pathology, nutrients that support mitochondrial function should be investigated for treatment of these disorders. The data from clinical trials and other lines of inquiry are generally inconclusive, but more than adequate to justify further investigation.

Vitamin E

Vitamin E is ubiquitous in cell membranes, serving to block free radical reactions from propagating within the membrane. Lab animals rendered vitamin E-deficient exhibit signs of premature aging at the cellular level.[60] Quantitatively, vitamin E is the major membrane-phase antioxidant in the brain. AD patients frequently demonstrate abnormally low levels in cerebrospinal fluid.[61] A U.S. epidemiological study concluded that dietary supplementation of vitamin E along with vitamin C, but not vitamin E alone, reduced AD incidence in an elderly population by one-half.[62]

In a double-blind trial investigating Alzheimer's disease, high intakes of vitamin E (2,000 IU/day) did not improve cognition but did delay progression of the disease.[63] However, in a recently published, double-blind trial on mild cognitive impairment, which can be a prodrome of AD, vitamin E (again at 2,000 IU/day) failed to improve cognition or slow progression to AD.[64]

The alpha-tocopherol form of vitamin E is the primary form used in research studies and nutritional supplements, even though seven other molecular species have vitamin E activity. Actually, the gamma-tocopherol form represents 70 percent of the vitamin E in the daily U.S. diet (refer to Jiang et al for an extensive review[65]). The body requires vitamin E for cell signaling and other regulatory functions that extend beyond strict antioxidant contributions often associated with alpha-tocopherol. Gamma-, but not alpha-tocopherol, is metabolized to a kidney-active substance ("natriuretic factor") that likely is physiologically important for regulating tissue water balance. Gamma-tocopherol also has significant anti-inflammatory activity that alpha-tocopherol lacks. As an antioxidant, gamma-tocopherol has higher blocking activity against the nitrogen free radical species known to be involved in neurodegenerative pathology.

Morris et al conducted a community dietary study using detailed food questionnaires.[66] Tracking 1,041 elderly people for six years, they found higher vitamin E intakes from food were associated with a significantly lowered incidence of progression to AD. The analyses indicated that a slower rate of cognitive decline was linked not just to alpha-tocopherol intake, but to gamma-tocopherol and total dietary vitamin E intake.

A telling difference between gamma- and alpha-tocopherol is that alpha-tocopherol in the diet tends to deplete gamma-tocopherol from the tissues, while the reverse is not true.[65] This is a compelling reason to include gamma-tocopherol in vitamin E dietary supplements. Future trials of vitamin E for the brain and circulation should utilize mixed tocopherol preparations that carry at least as much gamma- as alpha-tocopherol. Furthermore, considering how antioxidants work *in vivo*, and to conserve research funds, it would seem advisable that future clinical trials utilize vitamin E in combination with other antioxidants, including those discussed in this article.

alpha-Lipoic Acid

alpha-Lipoic acid (ALA; thioctic acid) is a highly effective antioxidant. ALA readily crosses the blood-brain barrier. Besides having potent electron-donating power, it is capable of conserving and regenerating reduced glutathione (GSH), the central cellular antioxidant. Like GSH, ALA is a redox regulator of enzymes in the cytoplasmic milieu. ALA is also an essential bound cofactor (prosthetic group) for two mitochondrial enzyme complexes centrally involved in bioenergetics.

The multi-enzyme assembly PDH is the largest protein complex in human cells. PDH is located in the mitochondrial matrix, where it processes the pyruvate coming from glycolysis into acetyl-CoA, which then enters the citric acid cycle.[2,3] Thus, PDH bridges the relatively inefficient, anaerobic glycolysis process that occurs outside the mitochondria, with the aerobic and OXPHOS processes that occur inside the mitochondria. ALA is an essential prosthetic cofactor for PDH and for another enzyme, KGD that is central to the CAC.

Upstream of the citric acid cycle, ALA supports insulin-regulated glucose utilization. In a placebo-controlled trial with 74 type 2 diabetes patients, ALA supplements at intakes of 600-1,800 mg/day over four weeks significantly improved insulin sensitivity, measured as glucose metabolic clearance rate.[67] This outcome confirmed similar benefits observed in earlier human studies.[68]

ALA for Neuropathies

The multi-faceted involvement of ALA in mitochondrial energetics – prosthetic group for two key enzymes, redox regulator of the mitochondrial matrix, versatile antioxidant helping to stem OXPHOS electron leakage – accounts for its impressive neurological benefits. Ziegler conducted a meta-analysis of seven randomized, controlled clinical trials and concluded that oral ALA (for 4-7 months) successfully treats both diabetic peripheral neuropathy and cardiac autonomic neuropathy.[69] ALA also improves burning mouth syndrome[70] and the related idiopathic dysgeusia – loss of taste.[71]

ALA May Help in Dementia

ALA can partially restore bioenergetic enzyme activity in the dementia brain. Froelich et al sampled brain tissue from recently deceased dementia patients (AD and VD), prepared homogenates, and assayed various enzymes.[17] They found PDH reduced by 53 percent in AD tissue and 46 percent in VD

tissue. By incubating ALA with the homogenates, the researchers were able to reactivate the enzyme in VD tissue, although not in AD tissue. Only the (R-) isomer of ALA was effective, consistent with it being the only isomer found in nature and with PDH normally using only this isomer as its prosthetic group.

In a pilot clinical trial of nine patients with AD or other dementias, ALA at 600 mg/day for one year appeared to improve cognition and slow disease progression.[72]

ALA in Multiple Sclerosis, Huntington's Disease, and Amyotrophic Lateral Sclerosis

ALA deserves further study for multiple sclerosis. In a 2002 summary report, a Russian group reported that ALA reduced relapse frequency and symptom intensity in relapsing-remitting MS patients.[73] In a 2005 pilot study, 37 MS subjects were randomly assigned to four groups: placebo, ALA 600 mg twice daily, ALA 1,200 mg once daily, and ALA 1,200 mg twice daily – all for 14 days.[74] ALA significantly lowered serum inflammatory indicators in a dose-related fashion and was well tolerated.

Experiments with the autoimmune encephalomyelitis animal model of MS,[75] and with cultured human T-lymphocytes,[76] further suggest ALA has marked anti-inflammatory activity. To date, there have been no clinical trials of ALA for ALS and HD, but in two transgenic mouse models carrying human genes that predispose to these diseases, ALA successfully improved survival rate.[17]

ALA Neuroprotection in Animal Models

In addition to the human and animal benefits discussed above, ALA has been a safe and effective neuroprotectant in many experimental animal models. In a thromboembolic stroke model in rats, ALA with vitamin E demonstrated anti-inflammatory and regenerative-trophic effects.[77] In aged rats, a combination of ALA and acetyl L-carnitine in the feed resulted in significant improvements of spatial and temporal memory.[78] Structural examination of the hippocampus showed reduced mitochondrial oxidative decay. ALA also benefits experimental excitotoxic amino acid injury and mitochondrial dysfunction.[79]

ALA has a longstanding experimental and clinical record of energetic, antioxidant, anti-inflammatory, and anti-excitotoxic support for the brain. This orthomolecule clearly deserves further investigation as a clinical neuroprotectant.

Coenzyme Q10 (CoQ10; ubiquinone)

Coenzyme Q10 resembles ALA in being both a potent antioxidant and a bioenergetic enzyme cofactor. This orthomolecule is central to mitochondrial oxidative phosphorylation, shuttling electrons from complexes I and II to complex III while also providing potent antioxidant protection for the inner membrane OXPHOS complex (Figure 2).[2,59] It is a conditionally essential nutrient, and (in skeletal muscle) CoQ10 deficiencies are invariably associated with subnormal or pathological mitochondrial performance. Coenzyme Q10 has also been shown to benefit symptoms of neurodegeneration.

Naini et al reviewed primary CoQ10 deficiency and the brain.[80] Abnormally low CoQ10 levels in skeletal muscle are associated not just with myopathy but often with CNS dysfunction, including ataxia, cerebellar dysfunction and atrophy, seizures, developmental delay, mental retardation, and pyramidal signs; patients usually respond to supplementation.[81]

A number of genetically conditioned mitochondrial diseases are known, many related to mutations or deletions that impair OXPHOS function; these invariably cause skeletal muscle pathology. Depending on severity of penetrance and other factors, the brain may also be affected. The clinical expression and management of these diseases have been reviewed in detail.[59,82] The mitochondriopathies attributed to nuclear DNA defects are poorly understood and often fatal,[82] while those linked to mitochondrial DNA abnormalities are better understood and more treatable.

Shoffner and Wallace report that many of the mitochondrial DNA OXPHOS syndromes respond to high-dose B vitamins and CoQ10.[59] Magnetic resonance spectroscopy found that many patients with "mitochondriopathy" had abnormally low brain energy reserve; in one case series CoQ10 (150 mg/day for six months) helped correct this deficit.[83]

CoQ10 in MELAS Syndrome

The MELAS syndrome (mitochondrial myopathy and encephalopathy with lactic acidosis and stroke-like episodes) produces intense headaches and sometimes an actual stroke. In MELAS, a minimum 300 mg/day CoQ10 appears to be required for benefit (Goda et al and Yamamoto et al, discussed in Shoffner and Wallace[59]). In a trial on chronic, progressive external ophthalmoplegias from mixed causes (only some subjects had mitochondrial DNA mutations), a relatively low intake of CoQ10 (2 mg/kg/day) raised blood levels only modestly, but improved tremor in patients with cerebellar involvement.[84] The synthetic, shorter chained CoQ analogue idebenone reportedly has shown some benefit for MELAS.[59]

Coenzyme Q10 for Parkinson's Disease

CoQ10 has been used with considerable success for PD. In these patients, achieving very high blood levels seems crucial. Shults et al randomized 80 PD patients to four groups: placebo, CoQ10 300 mg/day, CoQ10 600 mg/day, or CoQ10 1,200 mg/day.[85] Patients were followed for a maximum of 16 months or until disability necessitated levodopa treatment.

Scoring on the Unified Parkinson Disease Rating Scale (UPDRS) suggested all the CoQ10 groups progressed significantly less than the placebo group. The CoQ10 1,200-mg/day group demonstrated statistically significant improvement over placebo on all three segments of the rating scale – motor control; cognition, behavior, and mood; and activities of daily living. This relatively high CoQ10 dose was determined to be safe and well tolerated. Mitochondrial complex I activity was also monitored, and its activity in the 1,200-mg/day group was found to be 150-percent higher than the placebo group and 20-percent higher than the 600-mg/day group. Blood levels reached 4 mcg CoQ10/mL on 1,200 mg/day, about twice that of the 600-mg/day group.[85]

In a subsequent, open-label study, Shults et al assessed the safety and tolerability of still higher intakes of CoQ10.[86] They began with 17 PD subjects, 13 of whom completed the study. The design was to periodically escalate the dose of CoQ10 against a backdrop of high vitamin E intake. The patients received 1,200 IU/day alpha-tocopherol throughout the study, and were started on CoQ10 at 1,200 mg/day. Every two weeks the CoQ10 intake was increased by 600 mg – to daily doses of 1,800 mg, 2,400 mg, and finally 3,000 mg. Compliance became a problem at the highest dose; however, the plasma levels seemed to plateau at 2,400 mg/day (7.5 mcg/mL). One subject withdrew after suffering mild dyspepsia at the 2,400 mg/day intake. The investigators concluded that CoQ10 at 2,400 mg/day (in combination with 1,200 mg/day alpha-tocopherol) was safe and well tolerated. They recommended future PD trials use this CoQ10 dose, which appears to maximally saturate the blood.

Another smaller, double-blind, placebo-controlled trial found CoQ10 might improve PD symptoms at lower doses. Mueller et al randomly assigned 28 PD patients to two groups, CoQ10 (360 mg/day) or placebo for four weeks.[87] The CoQ10 group demonstrated improvement compared to placebo on a test of color vision, although no significant improvement of motor symptoms or total UPDRS was noted compared to placebo. It is possible either the 360 mg/day CoQ10 intake was too low and/or the four-week dosing period was too short to demonstrate significant benefit. An earlier, small, open-label trial with 200 mg/day CoQ10 for three months also failed to show benefit in PD.[88]

Coenzyme Q10 in Huntington's Disease

CoQ10 may offer benefit for HD patients (reviewed in Beal and Shults[89]). In 1996 Feigin et al conducted a six-month, open-label trial with 10 HD patients, who received CoQ10 at doses of 600-1,200 mg/day.[90] All completed the trial, although four reported mild adverse effects. Using HD rating scales and neuropsychological measures, the researchers could find no significant clinical benefit.

In 1997, Koroshetz et al reported using MRS to document abnormally elevated lactate in the cerebral cortex and basal ganglia of HD patients.[53] Eighteen HD patients were treated with CoQ10 (360 mg/day) for 1-2 months and cortical lactate was markedly reduced. Occipital cortex lactate levels fell 37 percent and increased when CoQ10 was discontinued. This encouraging outcome led to a large controlled, clinical trial by the Huntington Study Group.

As described by Beal and Shults, the Huntington Study Group recruited 360 HD patients and

randomized them to treatment with CoQ10 (600 mg/day), remacemide (600 mg/day), or the combination, for 30 months.[89] Remacemide is a drug that blocks a receptor for glutamate, a neurotransmitter implicated in nerve cell death. According to Huntington rating scales, CoQ10 slowed total functional decline by 14 percent; remacemide had no effect. On neuropsychological tests, CoQ10 significantly benefited color naming, word reading, and attention, yet it failed to attain significance on the overall ratings. Beal and Shults criticized the design of the trial, pointing out that the statistical design allowed for anything less than a 40-percent functional improvement to elude statistical significance.[89] Thus, a potentially meaningful and measurable degree of clinical benefit by CoQ10 for HD may have been obscured by poor clinical trial design.

Coenzyme Q10 in other Neurodegenerative Disorders

CoQ10 has been applied to Friedreich's ataxia. Deficiencies in either CoQ10 or vitamin E can result in ataxia. Hart et al treated 10 FA patients with a combination of vitamin E (2,100 IU/day) and CoQ10 (400 mg/day) for three months and observed significant improvements in muscle energy metabolism.[55] Using an internationally approved rating scale, they performed follow-up assessments over the subsequent 44 months and found neurologically-related kinetic symptoms were improved in six of 10 subjects; however, posture and gait symptoms continued to decline. This long-term outcome, although modest, was judged by the researchers to be a meaningful slowing of FA progression.

Coenzyme Q10 has been tried as part of a combination nutrient therapy for the treatment of Alzheimer's disease. In 1992, Imagawa et al reported improvement after administration of CoQ10 (60 mg/day), vitamin B6 (180 mg/day), and ferrous citrate (150 mg/day) to 27 AD patients.[91]

Acetyl L-carnitine

Acetyl L-carnitine (acetylcarnitine; ALCAR) is integral to mitochondrial function. ALCAR and L-carnitine (CAR), its non-acetylated derivative, facilitate the transport of fatty acids into the mitochondria to become OXPHOS substrates. ALCAR is highly bioavailable, is thought to penetrate the brain better than CAR, and is readily converted to CAR as needed. A great deal of experimental evidence suggests ALCAR boosts mitochondrial ATP production and helps protect mitochondria against oxidative attack.[92,93]

ALCAR for Alzheimer's Disease

ALCAR has proven beneficial for Alzheimer's disease. A number of double-blind trials have been conducted, and a 2003 meta-analysis of 15 trials concluded ALCAR was beneficial for mild AD, according to clinical and psychometric tests and clinicians' global assessments.[94] Effective and well-tolerated oral intakes ranged from 1.5-3.0 g daily.

One double-blind trial of ALCAR versus placebo for AD included noninvasive bioenergetic monitoring using MRS.[95] At the onset of the trial, MRS detected probable abnormal cell membrane breakdown and abnormally low reserves of high-energy phosphate. After six months, the ALCAR group was significantly improved over the placebo group, with slowed progression of the disease and improvement of the metabolic measures on MRS. The researchers suggested ALCAR was facilitating neuronal membrane renewal and, through that process, restoring energy stores in the cortex.

ALCAR for Parkinson's Disease

ALCAR has potential to benefit patients with PD. In one clinical trial conducted in Italy, two groups of 10 patients received 1 or 2 g ALCAR intravenously for seven days.[96] Reactions to flickering light and sleep-wake patterns were studied by electroencephalography and ALCAR was found to improve both the sleep-wake patterns and abnormal sensitivity to light.

In a primate model that closely resembles human PD – MPTP-induced parkinsonism in macaque monkeys – ALCAR partially blocked the MPTP (1-methyl, 4-phenyl-1,2,3,6-tetrahydrophyridine) effect.[97] MPTP emerged as a mitochondrial toxin when it accidentally contaminated drugs of abuse and precipitated severe parkinsonism in humans (see Kidd for a discussion[39]). Since then it has been extensively used in animal studies related to PD.

In the above-mentioned macaque study, when seven monkeys were pretreated with ALCAR (50

mg/kg or 20 mg/kg intramuscularly beginning two weeks prior to MPTP administration), six of them remained symptom free during the study period (up to four years). One monkey on the low ALCAR dose developed a moderate parkinsonism that lasted two weeks. Of the 12 monkeys treated with MPTP and no ALCAR, 11 developed full-fledged parkinsonian symptoms that lasted longer than one year. Brain histology revealed that ALCAR markedly protected the dopaminergic neurons in the substantia nigra, which otherwise were devastated by the MPTP attack.[97]

ALCAR for Multiple Sclerosis

Calabrese et al studied ALCAR for treatment of MS.[98] The researchers had noted abnormally high levels of nitrogen-centered free radicals in cerebrospinal fluid (CSF) from MS patients. The enzyme nitric oxide synthase, one source of these potent oxidants, was also significantly elevated in the CSF, while GSH was abnormally low. When these researchers treated MS patients for six months with ALCAR, comparing them to untreated MS subjects or controls (patients with non-inflammatory neurological conditions), CSF nitrosated end-products decreased in the ALCAR group while GSH increased.

After ALCAR was found to improve the extreme fatigue of chronic fatigue syndrome, it was tried for the fatigue of MS.[99] Thirty-six MS patients with fatigue were randomized in a double-blinded trial to receive oral doses of the drug amantadine (200 mg/day) or ALCAR (2 g/day) for three months. This was followed by a three-month washout period, after which they were crossed over to the alternating treatment for another three months. ALCAR was better tolerated and significantly more effective at reducing fatigue than amantadine.

ALCAR and Stroke

ALCAR may be indicated for hypoxic stroke damage. Corbucci et al conducted a randomized, placebo-controlled, double-blind trial on carefully selected stroke patients who had both cerebral normoxic (perifocal) and hypoxic (focal) areas.[100] The hypoxic areas, when compared to the normoxic areas, showed significant functional damage to mitochondrial enzymes: PDH, succinate dehydrogenase (which generates $FADH_2$ for complex II), and cytochrome oxidase (complex IV). ALCAR partially countered this hypoxic damage and appeared to enhance cell survival in the hypoxic zones.

Nicotinamide Adenine Dinucleotide, Reduced

NADH receives energized electrons via the citric acid cycle and surrenders them to complex I of the mitochondria for OXPHOS processing. NADH has been used in exploratory trials for AD and PD, with some indications of benefit.[101,102]

In 1993, Birkmayer et al reported on their results with NADH for 885 PD patients.[101] Half the patients received NADH intravenously, the other half orally, for several years. The researchers claimed 80 percent of the patients showed 10-percent reduction in disability. The younger patients and patients with shorter disease duration seemed to respond better, and efficacy of the oral form of NADH was comparable to the intravenous form. In animal studies, NADH helped protect against focal cerebral ischemia and mitochondrial damage from MPTP.[92]

In 1996, Birkmayer reported on an open-label trial of NADH with 17 AD patients.[102] He claimed all his patients improved on the Mini Mental State Examination and the Global Deterioration Scale, although the trial lasted only three months.

Summary: Nutrients Support Mitochondrial Performance in the Brain

The clinical evidence available to date provides optimism that nutrients, preferably used in combinations, can significantly counter mitochondrial-based neurodegeneration. Reactive molecules produced in the mitochondria are at the root of these degenerative mechanisms. The effect of endogenous toxins is amplified by exogenous toxins, such as mercury and MPTP. The combination of endogenous antioxidants and exogenous orthomolecular dietary supplements offers real potential to counter neurodegenerative states. Vitamin E, ALA, CoQ10, ALCAR, and NADH combine to protect the brain cells, boost the brain's energy, and support its regenerative potential.

The section that follows is a brief overview of the prospects for replacing lost brain tissue, stemming from research spurred by an emergent new brain paradigm. Certain nutrients seem to provide direct support for brain regeneration via trophic mechanisms.

The Brain Can Repair Itself: Stem Cells, Growth Factors, and Nutrients

Within the past decade, an important paradigm advance in brain research has occurred – the realization that the human brain can markedly repair and even regenerate itself. The old dogma that the brain has only postmitotic cells and therefore cannot make new reparative cells is no longer supportable.

Plasticity, Synapses, Growth Factors

The new paradigm views the adult human brain as highly adaptable, capable even of regeneration. When existing circuits become damaged, other circuits can reorganize and replace the lost circuitry. This phenomenon, plasticity, has been recognized for some time, but only recently has its scope become fully apparent.

Synapses Mediate Brain Plasticity

Synapses – the zones where one nerve cell contacts another – appear to be the structures that largely mediate brain plasticity. Most are chemical synapses that release and detect chemical transmitters.[103] A single brain neuron (Purkinje cell) can have more than 100,000 synapses, most of them chemical in nature. Each of the billions of chemical synapses in the brain has the same general task – to transmit a signal by releasing neurotransmitters at the presynaptic neuron, which cross over to the postsynaptic neuron in response to a presynaptic action potential. Successful synaptic transmission requires precise alignment of the two involved cells, yet synapses are found to be highly changeable, dynamic structures – the prerequisite for plasticity.

Synapses can undergo structural rearrangements on a time scale of minutes. These rearrangements are largely driven by the cumulative electrochemical activity passing through the particular synapse.[104] Relatively high stimulus activity in a synaptic circuit produces a reinforcing effect on the system, leading to proliferation of the synapses in that circuit. Conversely, low activity in the circuit is associated with a relative lack of synaptic proliferation.[104]

Long-lasting electrochemical activity at a synapse, such as the long-term potentiation that leads to memory consolidations, tends to increase the size of the individual synapse and increase the number of release sites for chemical transmitters. In this way positive reinforcement establishes the sequential synaptic pathways that carry the extended stimulus.[105] Evidence supporting the new plasticity paradigm predicts that major changes in whole-brain physiology can result from stimulative reinforcement at the quantal level of the individual synapse.[103-105]

Synaptic dynamics appear to require the presence of brain tissue growth factors (also called neurotrophins). Growth factors are small proteins present in every healthy tissue and are trophic (literally, nourishing) for their respective tissues. They stimulate tissue renewal, including the transformation of immature, relatively undifferentiated stem cells into mature, differentiated cells. Recent advances in brain research implicate growth factors as central enablers of brain plasticity via synaptic remodeling.

Brain Growth Factors Stimulate Plasticity

The brain carries a number of growth factors, including nerve growth factor (NGF), the first growth factor to be discovered; brain-derived neurotrophic factor (BDNF); glial derived neurotrophic factor (GDNF); ciliary neurotrophic factor (CNTF); neurotrophins generically labeled NT-3, NT-4/5, and NT-6; erythropoietin; and others.[105] Most growth factors operate by binding to one or both of two receptors. Each receptor is subject to activation specifically by one or more growth factor. The receptors are also subject to cell-level up- and down-regulation. As a result, the trophic milieu of brain tissue is a net outcome of growth factor production, spread, and ongoing impact on the available receptors.

The growth factor status of brain tissue differs from one zone to the next; this helps explain why some brain zones are more plastic than others. Stimulus input also affects growth factor status. In animal experiments, seizure activity induces a rapid increase

of NGF and BDNF in the hippocampus and cortex.[105] Blocking visual input down-regulates BDNF in the visual cortex, and exposing dark-reared animals to light reverses this change. Other physiological manipulations also modulate growth factor levels, reinforcing the old adage, "use it or lose it."

Individual growth factors also vary in the pattern of effects at the cellular level. NGF seems especially relevant to AD, since it specifically targets the cholinergic neurons that typically are the first to be destroyed in AD.[106] The cholinergic circuits degenerate en masse, thus profoundly contributing to the marked cognitive decline characteristic of AD.

Nerve Growth Factor as Alzheimer's Therapy

The administration of exogenous-NGF was developed out of extensive work on primates and other laboratory animals.[106] Primate studies established that autologous fibroblasts fitted with the NGF gene could survive transplantation into the brain and produce NGF for at least 18 months. Furthermore, the implanted cells did not form tumors, migrate within the brain, or cause any other discernible damage. In the primate brain, the NGF these cells produce can diffuse outward for 2-5 millimeters. The NGF-cell injection protocol successfully stimulated cholinergic function and improved cognitive performance in test animals.

The putative benefits of exogenous NGF for AD were explored in a pioneering phase I clinical trial conducted at the University of California at San Diego by Tusynski et al.[106] Eight subjects with early-stage probable AD were enrolled and NGF was administered to their brains using *ex vivo* gene delivery.

The investigators chose this novel method of delivery because NGF apparently does not cross the blood-brain barrier when administered peripherally. Also, when infused into the brain ventricular system, NGF apparently causes intolerable side effects, including pain. These limitations were circumvented by harvesting autologous (self-) fibroblasts from the skin of each subject, modifying them to carry the NGF gene using retroviral agents, then (in a single surgical session using precise stereotaxis) targeting injection of these NGF-fibroblasts into the basal forebrain. This cortical zone was chosen because it is the most cholinergic, and is the most predictably degraded by Alzheimer's disease progression.

The human trial had some success, although not without severe cost.[106] Two subjects moved abruptly during the surgery and sustained hemorrhages; one died five weeks later. The remaining six subjects successfully completed the surgery and, after a mean 22-month follow-up, no adverse effects of NGF were evident. The mean rate of decline on two cognition tests was reduced by about 50 percent in the six subjects. PET scanning of four of these subjects revealed significant increases of glucose consumption in the cerebellum and cholinergic areas of the cortex. An autopsy examination of the subject who died at five weeks revealed that some implanted cells had survived and produced NGF, and that cholinergic axons had robustly sprouted in these delivery sites. The investigators commented that the extent of the sprouting resembled that seen in young and aged monkeys receiving NGF.

Stem Cells Produce New Nerve Cells

Arguably the most exciting facet of the new brain paradigm is that the adult human brain carries a population of resident stem cells that can divide and make new cells;[107] the stem cell progeny can migrate and differentiate into neurons. In adult humans, the differentiation of stem cells into nerve cells (called neurogenesis) occurs in at least two zones – the hippocampus and the subventricular zone;[108] other brain regions likely carry smaller numbers of stem cells.

Brain stem cell activation and subsequent differentiation, although guided by the growth factor environment within the brain tissue, responds selectively to external stimuli. In learning experiments with rats, hippocampal stem cells were made to differentiate by subjecting the animal to learning experiences that required the hippocampus, such as spatial and temporal memory exercises. Non-hippocampal learning experiences, such as eyeblink conditioning, did not work. These early findings presage the first tangible clinical applications for endogenous stem cell activation.

An Ethical Basis for Stem Cell Transplantation

The term "stem cell" denotes cells that can give rise to a variety of cell types, while retaining great versatility in their potential to differentiate.[109] Stem cells are distinguishable by their behavior rather than by their appearance. The most versatile are the embryonic stem cells, harvested from the inner cell mass of the very early-stage embryo. These can produce any cell type in the body, but are politically (and ethically) controversial, since harvesting them requires destroying an embryo.

Stem cells that are somewhat restricted in potency, but less controversial to produce, may prove adequate for many applications. All adult human tissues appear to carry stem cells, but they are rare (less than 1 cell in 10,000) and, because of unassuming morphology, are hard to locate. Once isolated, they are notoriously slow and labor-intensive to grow.[109] Nonetheless, once inserted into the body, they can migrate great distances and "home" to tissue that resembles the original tissue source, where these cells (or at least some of them) can differentiate and engage in specialized activity.

Stem Cell Transplantation is a Clinical Reality

Stem cells have long been valuable in hematology, particularly in bone marrow transplantation. However, with the increasing perfection of stem cell technology comes further technical and ethical challenges. For cases of neurodegeneration, key questions are: whether to attempt to revitalize damaged or rapidly declining brain tissue by transplanting exogenous stem cells, or whether to try to activate endogenous stem cells already present in that particular tissue (or at least harvested from that same individual).

Endogenous (self-, autologous) transplantation, using the patient's own stem cells to repair an organ defect, has been successfully accomplished. In a study on patients with significant heart damage following severe myocardial infarction, each patient's own heart progenitor cells were used to repair damaged cardiac arteries. Four months after the cells were infused into the artery, the size of the damaged tissue had decreased by an average of 36 percent, and heart function had increased by 10 percent.[109]

Exogenous transplantation of stem cells was applied for PD in two controlled clinical trials.[110] In both trials, primary dopaminergic neuroblasts were obtained from the brains of aborted fetuses and subsequently implanted into the brains of PD patients, with disappointing results. Immune reactions developed against the grafts, spawning inflammatory responses that threatened graft survival.

The use of stem cells from aborted fetuses carries not just the technical problem of heterologous transplantation, but the ethical dilemma associated with using human fetal tissue. Research attention has reverted to exploring endogenous stem cell populations.[111] One immediate option is that human bone marrow or umbilical cord blood stem cells can be induced to express neural features.[112]

Human Umbilical Cord Stem Cells Hold Promise

The cell populations harvested from human umbilical cord blood (HUCB) are not ethically controversial, are easily obtained, carry little risk of blood-borne pathogen transmission, and are rich in stem cells (technically, progenitor cells since their potency is somewhat restricted).[112] HUCB cells are already approaching routine application for bone marrow repopulation. Under suitable culture conditions they can be made to differentiate into hepatocytes, myofibroblasts (muscle cells), osteoblasts, or a variety of neural cell types (see Walczak et al for a discussion and references[112]).

Using an animal model of stroke (middle cerebral artery occlusion in the mouse), Taguchi et al injected HUCB cells within 48 hours after inducing stroke.[113] They observed enhanced neurogenesis, angiogenesis, and structural and functional recovery in the damage zone; the extent of recovery was modest but unequivocal.

These types of experiments are still in the early stages, but the early findings provide optimism that stem cells, or at least progenitor cells, can be successfully transplanted into a living brain, survive, and make new circuits. The ability of the host brain to produce supportive factors may define the limits of this approach. Growth factors are one category of support for stem cell activation and differentiation.

Growth Factors Plus Stem Cells Equal Neurogenesis

In rat experiments, the infusion of the growth factor BDNF into the lateral ventricle of the adult brain led to new neurons forming in several zones of the brain.[114] Similar effects have been seen with erythropoietin and a few other factors.

Erythropoietin (EPO), Model Growth Factor

Originally described from the kidneys and now commercially established as a hematopoietic growth factor, EPO has taken on new allure as an orthomolecular brain protectant.[108] EPO and its receptors are only weakly expressed in the healthy adult human brain. The system is established early during brain development and functions during embryogenesis and through to the postnatal stage. Then it becomes inactive, but can become dramatically reactivated following hypoxic or other metabolic insult to the tissue. Ehrenreich et al summarized the substantial evidence for EPO's various neuroprotective effects, including a profound influence on stem cell differentiation.[108]

By 1997, Ehrenreich et al had decided to test erythropoietin as a neuroprotectant for humans. Their decision was facilitated by safety validation and drug approval for treatment of anemia. They designed the Goettingen EPO-Stroke pilot study to establish "proof of concept."[108]

For this study they recruited only patients who had suffered a middle cerebral artery stroke (as confirmed by MRI), to optimize comparability. The protocol had two phases – first a safety study (13 patients), followed by a double-blind trial (40 patients). EPO was given intravenously and markedly increased in the CSF. The procedure proved safe and the double-blind trial phase yielded a significantly better outcome from EPO compared to placebo. The EPO patients experienced significantly less neurological deficit, better restitution of brain functions, and significantly smaller lesion size than the controls. Levels of a blood biomarker for glial cell damage were also significantly lowered in the EPO group, compared to placebo.

Ehrenreich's team also tested EPO for schizophrenia (SCZ), believing that "continuous neurodegenerative processes are involved in the pathogenesis of this disease."[108] In this proof-of-concept trial on SCZ, the researchers established that EPO receptors were more strongly expressed in the SCZ brain. Currently, a larger trial of EPO for SCZ is being conducted in Germany. Further trials with EPO in brain trauma, Parkinson's disease, stroke, and multiple sclerosis, perhaps in combination with other neuroprotectants, are in the planning stages.

Nutrients Likely To Improve Growth Factor Effectiveness

Brain cells are the natural source of brain growth factors. When healthy, they secrete various growth factors and produce receptor molecules that allow them to respond to growth factors. Consistent with the new brain dogma are the observations that healthy nerve cells produce substantial amounts of the various factors along with their receptors. This raises the question whether there are nutrients proven to support the brain's endogenous growth factor activity. Such nutrients would presumably have supportive trophic effects for the declining or aging brain.

Three nutrients – ALCAR, GPC, and PS – were found to enhance brain growth factor receptors for NGF in experimental animals. NGF receptors, currently the most studied, are known to decline with age. Each of the three nutrients enhanced either the receptor-binding capacities of the aging tissue (related to receptor density) or receptor-binding affinities (related to the strength of the binding of NGF molecules to the receptors).

Acetyl L-carnitine and NGF Receptors

Angelucci et al studied NGF-binding capacity in young (four-month old) and aged (26-month old) rats, and found marked declines of NGF-binding capacity with age in the hippocampus and the basal forebrain.[115] Binding affinity was unchanged, suggesting that the densities of receptors had declined in these zones. After treating the aging rats with ALCAR (75 mg/kg/day for one year), they discovered that NGF-binding capacity was conserved in the hippocampus and the basal forebrain, compared to age-matched controls. Although binding capacity under ALCAR treatment remained lower than in the young animals, these levels were more than double those of the aging, non-ALCAR controls.

Glycerophosphocholine and NGF Receptors

GPC is an orthomolecular water-phase, non-membrane phospholipid. It has been clinically validated in 21 clinical trials for attention, immediate recall, and other cognitive functions, as well as for Alzheimer's and vascular dementia, stroke recovery, and recovery from brain trauma.[116] Although not directly incorporated into cell membranes, GPC is readily coupled with the omega-3 fatty acid docosahexaenoic acid (DHA) by enzymes specialized for this task. The resulting phosphatidylcholine molecules containing DHA are highly fluidizing for the new nerve cell membrane. Little energy is required for this conversion, making it highly facile in the brain.

Rat studies indicating GPC counteracts age-related structural decline in the cerebellar cortex[116] led Vega et al to investigate whether GPC could up-regulate NGF receptors in that zone.[117] The researchers fed GPC orally (100 mg/kg/day, for six months) to aged (18-month old) rats, and compared them to adult (12-month old) rats and aged rats not treated with GPC. The number of cerebellar Purkinje cells carrying NGF receptors declined by 40 percent between the adult and the aged rats; GPC treatment maintained this cell population significantly higher in the aged rats than in the age-matched controls. The average receptor density on the Purkinje cells declined by 30 percent, and GPC treatment halved this loss to 15 percent.

Phosphatidylserine Conserves NGF Receptors

PS has been proven after 21 double-blind trials to be effective for improvement of memory, learning, mood, and stress.[118] Like GPC, PS also has a synergistic functional relationship with omega-3 DHA (see Chapter 8 in Kidd for details[118]). In rat experiments, PS protected against loss of water-maze performance skills and conserved neuron density normally lost to aging. Nunzi et al[119] performed a similar study to that of Vega et al with GPC,[117] but looked at the septal area of the rat brain rather than the cerebellum.

Comparing young-adult (seven-month old) rats with aged (21-24 month old) rats, Nunzi's team found that some of the older rats had retained navigation skills on the Morris water-maze test (spatial memory). These they labeled "old, non-impaired," in contrast to the "old impaired" rats. In the old, non-impaired rats, NGF-receptor density did not fall significantly; whereas, significant receptor loss was seen in the old, impaired rats. The old, impaired group, when given PS (50 mg/kg/day, for 12 weeks), exhibited a two-fold recovery in NGF-receptor density and significant improvement on the water-maze test.

Phosphatidylserine is directly linked to mitochondrial support by another route. Phosphatidylethanolamine (PE) is a phospholipid abundant in mitochondrial cell membranes and is produced *in vivo* almost exclusively from PS (reviewed in Kidd[118]). PE is a major phospholipid building block for mitochondrial membranes, and the demands placed on PS reserves to supply mitochondrial PE could possibly create PS shortages elsewhere in the brain cell.

Summary: Nutrients and NGF Receptors

The abilities of ALCAR, GPC, and PS (all nutrients known to clinically benefit the brain) to enhance receptors for nerve growth factor invite further research to establish: (1) whether these nutrients actually enhance NGF's trophic activities, (2) whether they enhance trophic actions by other growth factors, and (3) to what degree brain restoration is possible from the concurrent use of all three as high potency dietary supplements.

Century of the Brain: Expanding Integrative Brain Management

Although minimal progress has been achieved to date in managing neurodegenerative disorders, promising clinical advances that are linked to the new paradigm of brain plasticity are on the horizon.

Rapidly progressing research on stem cells and growth factors raise hopes for eventual success against neurodegenerative disease. However, while exciting, they are unlikely by themselves to halt or reverse progressive brain decline. Certain commercial interests continue to pursue individual growth factors or growth factor regulators as potential monotherapies, while integrative practitioners have ready access to a range of nutrients proven to protect, energize, and renew nerve cell mitochondria and the cells that carry them.

Premier Brain Nutrients For Renewal

Integrative practitioners can provide their patients with important nutrients while they await progress on technologically sophisticated therapies. Stem cells provide real promise for rebuilding damaged or lost tissue, conjuring images of the fountain of youth. Considering nutrients are available that not only support mitochondria but also coax stem cells into activity, both the integrative physician and the thoughtful "health consumer" may be tempted to stock up on these nutrients; all are naturally occurring in the body and feature highly favorable benefit:risk profiles. Table 1 illustrates clinically validated dosages of these premier brain nutrients, ranging from the lowest found clinically effective to the highest intake proven safe.

For prevention, lower dose nutrient intakes may be useful, especially when the individual is supplementing with more than one nutrient. But for the subject with a diagnosed condition, intakes within the indicated ranges are more likely to produce noticeable benefit. Optimal metabolic processing of these nutrients will benefit from generous daily intakes of vitamins and essential minerals. Under the guidance of experienced integrative physicians, neurotransmitter precursors or pharmaceuticals could be judiciously added to the regimen.

A Future Free of Toxins

With popular magazines increasingly touting micro bionics, smart chips, and pharmaceutical cocktails for mental performance, society has entered "the century of the brain." The coming era of stem cell transplantation and growth factor support strategies will undoubtedly advance progress toward ameliorating brain disorders and extending productive lifespan. But progress could be frustrated by the ubiquitous oxidative burden from exogenous toxins. Unless these are eliminated our brain mitochondria will continue to be challenged.

Decisive action is needed to eliminate, not just restrict, such toxins as mercury, lead, other heavy metals, PCBs, dioxins, and a plethora of aromatic ring compounds from the environment. For the present, the evidence is clear that an active and socially integrated lifestyle, including a social network, leisure activity, and physical exercise, together with a healthy diet and the premier brain nutrients, provide some protection against premature brain deterioration.[120]

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Table 1. Premier Brain Nutrients

Nutrient	Dosage Range
Alpha-lipoic acid	300-2,400 mg
Acetyl-L-carnitine	1-3 g
Coenzyme Q10	360-2,400 mg
DHA + EPA, omega-3 fatty acids	800-3,000 mg (1:1 ratio)
Glycerophosphocholine	600-1,200 mg
Phosphatidylserine	100-500 mg
NADH	5-15 mg

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