Mitochondrial Dysfunction and Cancer: Modulation by Palladium a-Lipoic Acid Complex

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1. Introduction

Cancer is the uncontrolled multiplication of subtly modified or mutated normal human cells. While surgery, radiation, and chemotherapy are all commonly used to treat cancer, chemotherapy is the only option if the cancer has metastasized and spread through the body. To minimize side effects, one needs cancer-specific cytotoxic drugs, including DNA-binding agents, alkylating agents, and antimetabolites that interfere with DNA replication.

The numerous side effects as well as the cost associated with modern medicine have driven cancer patients, to look for other treatments, collectively termed alternative or holistic medicine. Holistic medicine's ostensible aims include addressing the physical, mental, emotional, and spiritual problems of the body. The medical systems that make up holistic medicine include herbal medicine including Ayurveda, homeopathy, acupuncture, yoga, and others. There are different philosophies driving each of these diverging systems, with the tenets of each often conflicting. There is a general reluctance on the part of practitioners of modern medicine to accept the tenets of holistic medicine, partly due to lack of rigorous clinical investigation. But there is a modern trend to accept some of these treatments as a complementary or adjuvant therapy to attain a feeling of maximum wellness, one that goes beyond recovery. In that sense a symbiotic relationship is slowly developing between modern medicine and its less tested brethren.

Our research is based on a holistic approach of a different kind. It addresses the need for modern medicine to include the role played by the mitochondria in optimal cellular function. It is different from traditional holistic medicine in that the results presented in this chapter are based on substantial investigations of mitochondrial effects. Mitochondria are involved in energy metabolism, calcium regulation and apoptosis-signaling pathways. The number of mitochondria in a cell is decided by its energy requirements [1-3]. Cells that are metabolically more active, such as those in cardiac and skeletal muscles, the brain and the liver have the most mitochondria. All human cells, other than mature erythrocytes, have mitochondria. We believe strongly that medications targeting the mitochondria address the same issues that holistic medicine focuses on, because healthy mitochondria contribute substantially to the physical, mental, and emotional elements needed to complement the allopathic or modern medicine. Mitochondria are ubiquitous, and taking care of mitochondria is similar to taking care of all the parts leading to greater achievements than the sum of the parts. This is essentially the true slogan of holistic medicine.

A recent focus of research has been on a group of agents with anti-cancer activity, mitocans, that induce apoptosis by way of mitochondrial destabilization [4]. Natural compounds including fruits and vegetables that preferentially kill cancer cells with mitochondrial dysfunction are receiving closer scrutiny to understand the underlying mechanisms and therapeutic implications for cancer treatment and prevention [5-7].

We have tried to establish a symbiotic relationship with modern medicine as well as holistic medicine by selecting a metal complex, palladium a-lipoic acid, that is active in mitochondrial cellular metabolism as well as in cancer cell death.

A substantial fraction of the cytoplasm in almost all eukaryotic cells is occupied by mitochondria [3]. Mitochondria were first identified at the end of the 19^{th} century. The energy-converting organelles of eukaryotes were generally believed to be evolved from prokaryotes that were engulfed by primitive eukaryotic cells or aerobic bacteria. This symbiotic relationship started more than 1.5 billion years ago allowed the evolution of multicellular organisms with aerobic respiration [3]. Since the development of procedures for isolation of intact mitochondria in 1948, extensive studies have been carried out to understand their role in energy metabolism [1].

An animal cell without mitochondria would be dependent on anaerobic glycolysis to make adenosine triphosphate (ATP). The conversion of glucose to pyruvate by glycolysis produces only 2 molecules of ATP compared to 36 molecules of ATP produced by glucose oxidation. The pyruvate produced in the cytosol by glycolysis and the fatty acids are selectively transported into the mitochondrial matrix where they are broken down into the acetyl group on acetyl-CoA before being fed into the tricarboxylic acid cycle or citric acid cycle or Krebs cycle.

The ATP in a cell is being continuously hydrolyzed and regenerated, with a half-life from seconds to minutes depending on the cell. An average person at rest consumes and regenerates ATP at a rate of ~3mol (1.5 kg) $h^{-1}$and as much as an order of magnitude faster during strenuous activity [1]. The rapid deterioration of brain tissue by oxygen deprivation is due to the fact that brain cells have only a few seconds of ATP available [1]. It is clear that the cellular role of ATP is as a free energy transmitter rather than as a free energy reservoir. Phosphocreatine acts as a reservoir of ATP in muscles and nerve cells that have high ATP turnover rates.

$$\text{ATP} + \text{Creatine} = \text{Phosphocreatine} + \text{ADP} \quad (1)$$

Even though this is an endergonic reaction under standard conditions, it is close to equilibrium due to the prevailing intracellular concentrations of its reactants and products. This allows the equilibrium to shift to the right at resting state because of high ATP concentration and shift to the left at high metabolic activity because of low ATP.

The glycolytic product pyruvate is the immediate precursor to acetyl-CoA from carbohydrate sources.

$$\text{Glucose} + 2NAD^+ + 2ADP + 2Pi \rightleftharpoons 2NADH + 2\text{Pyruvate} + 2ATP + 2H_2O + 4H^+ \quad (2)$$

For glycolysis to continue, the NADH produced must be reoxidized to NAD^+ because of its limited availability in cells. Under anaerobic conditions, this is achieved by oxidation of NADH by pyruvate to yield NAD^+ and lactate with the aid of lactate dehydrogenase. Under aerobic conditions, each NADH oxidized by the mitochondrial electron transport chain produces one NAD^+ and 3ATP. The pyruvate, under aerobic conditions, undergoes a series of five sequential reactions and produces NADH and acetyl-CoA with the aid of the enzyme, pyruvate dehydrogenase.

$$\text{Pyruvate} + \text{CoA} + NAD^+ \rightarrow \text{acetyl-CoA} + CO_2 + \text{NADH} \quad (3)$$

The acetyl group of the common intermediate acetyl-coenzyme A (acetyl-CoA or acetyl SCoA), obtained from the breakdown of carbohydrates, lipids, and proteins, is then converted to CO_2 and H_2O through a series of consecutive enzymatic reactions of Krebs cycle, the electron transport chain, and oxidative phosphorylation.

Even though the role of mitochondrial defects had been recognized in the development of cancer more than 80 years ago, mitochondrial dysfunction and its restoration have started gaining momentum only recently. Our emphasis is on restoration from mitochondrial dysfunction. To minimize toxic side effects or to avoid toxic effects completely, we have selected a ligand, a-lipoic acid that plays a key role in the mitochondria. a-Lipoic acid is part of the multi enzyme complexes, pyruvate dehydrogenase as well as a-ketogluarate dehydrogenase, the latter being involved in the Krebs cycle. A third enzyme, also containing a-lipoic acid, is the branched-chain a-keto acid dehydrogenase. This enzyme participates in the degradation of isoleucine, leucine, and valine. The other unique properties of this ligand are discussed in a later section.

After selecting a ligand, a-lipoic acid, that plays a critical role in biological energy metabolism, we wanted to tweak the properties of the ligand by complexing it with a metal that has very high catalytic and electronic properties. After numerous investigations with a variety of metals, the final selection was made to use palladium, a transition metal. The properties of the resulting complex are spectacular in many ways. After a series of investigations over numerous years, we have established that palladium a-lipoic acid complex formulation

1) has practically no toxic effects and its aqueous solution is safe up to at least 40 mL per day.
2) repairs DNA damage resulting from radiation.
3) scavenges free radicals and lowers lipid peroxidation.
4) increases the levels of glutathione and glutathione peroxidase.
5) increases the levels of manganese superoxide dismutase, and catalase.
6) enhances the Krebs cycle enzymes: isocitrate dehydrogenase, a-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase.
7) enhances mitochondrial respiratory enzymes, complex I, complex II, complex III, and complex IV.

8) promotes cell death in a variety of cancer cell lines such as skin melanoma, human (SKMel-5); liver, hepatocellular carcinoma, human (Hep G2); lung, malignant melanoma, human (malme-3M); mammary gland, ductal carcinoma, human (MDA-MB 435); prostate, left supraclavicular lymph node carcinoma, human (LNCaP); colon, colorectal adenocarcinoma, human (HT-29); human brain, glioblastoma; astrocytoma (U87); and glioblastoma (U251MG).
9) acts as a prophylactic for neuronal protection from transient ischemic attack.
10) acts as a prophylactic for protection from radiation.
11) exhibits unique electronic properties corresponding to diode or tunnel diode behavior.

The influence of protein structure on the rate of electron transfer is beyond the scope of this chapter. It is known that reduced hemes in the mitochondria can transfer electrons at physiologically significant rates over a distance of 100-200nm [1]. The electron transfer taking place through space or through bonds and the role of protein structure are all active areas of current research. To understand mitochondria related electron transfer, we have taken a few, but new, small steps to elucidate the electronic character of some components of the proteins involved in the electron transfer process. Apart from our ligand and our complex we have also investigated cysteine, lysine, histidine, and FAD. Since reactive oxygen species is also a source of mitochondrial dysfunction, we have also looked at the electronic properties of H_2O_2. The technique we have utilized to investigate the electronic properties of these small molecules is impedance spectroscopy, a field with high potential for drug discovery, but used only to a limited extent by researchers in the pharmaceutical industry.

To obtain a fairly complete picture of this complex, it is essential to include, briefly at least, other topics such as mitochondria and its dysfunction, free radicals, and common antioxidants.

2. Platinum(II) and Palladium(II) Complexes

Active platinum(II) compounds such as cisplatin, carboplatin, and oxaliplatin are the cornerstones of solid tumor chemotherapy. Cis-platin, cis-diamminedichloro platinum(II), is a square planar d^8 platinum(II) complex. Since its approval in 1978 for clinical use, cis-platin has made significant contributions to the treatment of testicular and ovarian cancer [8]. However, cisplatin and other platinum drugs suffer from serious side effects such as tissue toxicity, and resistance to the treatment [8-10]. Cis-platin is highly toxic to kidneys, limiting its dose. The therapy gets complicated due to the nauseas and intense vomits, indicating gastrointestinal toxicity. Cisplatin is also not orally bioavailable. The molecular target for the platinum drugs is DNA. Recent advances have identified other molecular targets such as thiol-containing proteins and growth factor receptors [10].

Cis-platin reacts with water to give several aqua species, replacing the chloride ligands. The rate constants for the hydrolysis of the first chloride from cis- or trans- platins at 25°C are 2.5×10^{-5} and 9.8×10^{-5} s^{-1}, respectively [8].

$$[Pt(NH_3)_2Cl_2] + H_2O = [Pt(NH_3)_2Cl(OH_2)]^+ + Cl^- \quad (4)$$

$$[Pt(NH_3)_2Cl(OH_2)]^+ = [Pt(NH_3)_2Cl(OH)] + H^+ \quad (5)$$

$$[Pt(NH_3)_2Cl(OH_2)]^+ + H_2O = [Pt(NH_3)_2(OH_2)_2]^{2+} + Cl^- \quad (6)$$

$$[Pt(NH_3)_2(OH_2)_2]^{2+} = [Pt(NH_3)_2(OH_2)(OH)]^+ + H^+ \quad (7)$$

$$[Pt(NH_3)_2(OH_2)(OH)]^+ = [Pt(NH_3)_2(OH)_2] + H^+ \quad (8)$$

These reactions are dependent on pH and chloride concentrations. The higher (~ 0.1 M) Cl^- concentration in the plasma facilitates the passage across cell membranes as the neutral cis-platin. The lower (~ 0.004 M) Cl^- concentration inside the cell facilitates its hydrolysis. At the pH of the blood of 7.4, most of cis-platin is in the monohydroxo form. The pK_a for the deprotonation reaction of the monoaqua species is 6.41 [9].

Cis-platin interacts with DNA to form inter- and intra-strand cross-links. The intra-strand cross-link between adjacent guanine bases on the DNA strand causes cancer cell death.

The coordination chemistry of palladium (II) and platinum (II) compounds being similar, the antitumor activity of several palladium (II) complexes had been explored [11-14]. Mononuclear palladium(II) complexes with aromatic N-containing ligands, amino acid ligands, S-donor ligands, and P-containing ligands have respective qualities and properties due to the different structures as well as properties of the ligands [11]. It is interesting to note that the cis-platin analogue of palladium did not exhibit any antitumor activity probably due to its high reactivity and consequent inability to reach the DNA. Palladium(II) analogues of platinum(II) complexes are about 10^4 to 10^5 times more reactive [11]. To minimize the high lability and fast hydrolysis of palladium(II) complexes in biological environments, chelating ligands were used to synthesize the antitumor agents. An interesting observation was that the trans- palladium(II) complexes had better activity than the cis- palladium(II) or cis-platinum(II) complexes [12]. Advances involving palladium complexes mainly for cancer therapy have recently been reviewed [11-13]. Even though there are structural and thermodynamic similarities between platinum(II) and palladium(II) complexes, palladium(II) complexes seem to exhibit biological action very different from those of the toxic platinum complexes. While the main target of platinum based drugs is DNA, palladium based drugs show preferential targets such as enzymes and lysosomes [12].

It has been found that the antitumor activity of a ligand or metal was much less than the metal-ligand complex because the binding affinity of metals to proteins or enzymes will change their interaction process with DNA thereby affecting the DNA replication and cell proliferation [15]. These conclusions were drawn from studies using cell lines, human malignant melanoma G-361, human osteogenic sarcoma HOS, human chronic myelogenous leukemia K-562, and human breast adenocarcinoma MCF7 and iron (III) and copper (II) complexes of N^6-

benzylaminopurine derivatives. Similar results were also observed for palladium(II) –benzyl bis (thiosemicarbazonate) against cell lines, cervix epithelial human carcinoma (HeLa), transformed monkey kidney fibroblasts (Vero), normal murine keratinocytes (Pam 212), and murine keratinocytes transformed with the H-ras oncogene and resistant to cis-platin (Pam-ras) [16].

A recent review on palladium(II) complexes for the cancer therapy has lamented the lack of progress of palladium-based drugs. "In addition, it is important to note that, to the best of our knowledge, the palladium-based complex had not yet been tested in human beings due to the following factors: the success of platinum-based complexes in the cancer therapy, the enormous quantity of these complexes described in the literature, the high costs of the developmental phases in human clinical trials, the legal difficulties involving the drug assays in human beings and for the novelty of this subject" [12]. We must add that the palladium a-lipoic acid complex formulation has been in the market for more than fifteen years, without specifying any potential benefits for any cancer even though the cell line data presented here indicate its potential applications for a variety of cases.

3. Gene-based Therapy

Still in its infancy, serious attempts are being made in drug discovery based on pharmacogenomics. It is based on the proteins, enzymes, and RNA molecules associated with genes and specific diseases and based on a patient's genetic profile. Sorting out a few single nucleotide polymorphisms (SNPs) responsible for the disease from the millions of SNPs and their response for each specific drug remain a Herculean challenge. For example, a recent sequencing of 20,661 protein coding genes in 22 human glioblastoma multiforme (grade IV astrocytoma) samples revealed recurrent mutations in the active site of isocitrate dehydrogenase 1 (IDH1 gene on chromosome 2q33) [17]. The oxidative carboxylation of isocitrate to a-ketoglutarate resulting in the production of nicotinamide adenine dinucleotide phosphate (NADPH) is catalyzed by isocitrate dehydrogenase 1. These IDH1 mutations were found to occur preferentially in younger patients compared to the older patients with wild-type mutations in IDH1. A similar study of 20,661 protein-coding genes in 24 pancreatic cancers revealed 63 genetic alterations defining a core set of 12 cellular signaling pathways [18]. The complexity of gene-based therapy can be recognized easily by knowing the enormous number of mutated genes in some tumors such as pancreas (1007), brain (685), and breast (1026) [18].

4. Hallmarks of Cancer

Self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis seem to characterize the cancer cells [19]. Another added feature, first suggested by Warburg, seems to be ATP production in cancer cells by glycolysis even under aerobic conditions [20, 21]. A correlation is also observed between glycolytic ATP production and aggressiveness of tumor

cells [22]. Most anticancer drug development strategies are based on recent advances in the discovery of oncogenes and tumor suppressor genes. Recent studies on the role of mitochondria in signaling pathways for cell death and regulation of calcium homeostasis have generated renewed interest in investigating the role of antioxidants and nutraceutical supplements for induction of apoptosis and anticancer treatment [4-7, 23].

5. Mitochondria

Mitochondria or the "powerhouse of the cell", are about 0.5-1 μm in diameter and 7 μm in length [1]. Mitochondria exist in a variety of different shapes depending on the source from which they are derived. In electron micrographs they appear as spheres, rods or filamentous bodies. Twenty percent by volume of a typical eukaryotic cell is occupied by about 2000 mitochondria. These semi-autonomous, highly dynamic organelles, containing a double membrane structure, are involved in cellular respiration (aerobic metabolism), regulation of calcium homeostasis and cell death. Two membranes that have different sets of enzymes and biochemical functions surround the internal matrix. The matrix contains the enzymes of the Krebs cycle except succinate dehydrogenase (SDH). SDH is bound to the inner membrane. To increase the surface area, nature has cleverly convoluted or invaginated the inner membrane into the matrix of the mitochondrion to form cristae, its number varying with the respiratory activity of the type of cell. The components of the respiratory chain and the mechanism for ATP synthesis are part of the inner membrane. While the outer membrane is relatively permeable, the inner membrane is highly selective. This makes the contents of the intermembrane space and the matrix different. The enzymatic composition of the various mitochondrial sub compartments, outer membrane, intermembrane space, inner membrane and matrix, are easily available [1-3].

The transport of electrons through respiratory chain complexes I-IV in the inner mitochondrial membrane requires a series of coupled redox reactions. Within the inner motchondrial membrane, these complexes are all laterally mobile. The redox enzymes involved in the electron transfer process play a major part in the bioenergetic metabolism. The fate of the reducing equivalents from catabolic processes entering the respiratory chain as NADH and $FADH_2$ is briefly described [1, 2].

Complex I (NADH-ubiquinone oxidoreductase or NADH-CoQ reductase) catalyzes oxidation of NADH by coenzyme Q (also known as CoQ_{10} in mammals).

NADH + oxidized CoQ ? NAD^+ + reduced CoQ or $CoQH_2$, ? $E^{o'} = 0.360V$ (9)

Complex II (succinate-ubiquinone oxidoreductase or succinate-CoQ reductase) catalyzes oxidation of $FADH_2$ by coenzyme Q. This reaction does not produce enough energy to synthesize ATP. It serves as a conduit to inject electrons from $FADH_2$ to the electron transport chain.

$FADH_2$ + oxidized CoQ ? FAD + $CoQH_2$, ? $E^{o'} = 0.015V$ (10)

Complex III (coenzyme Q-cytochrome c reductase) catalyzes oxidation of $CoQH_2$ by cytochrome c

$CoQH_2$ + oxidized cytochrome c ? oxidized CoQ + reduced cytochrome c, ? $E^{o'}$ = 0.190V (11)

Complex IV (cytochrome c oxidase) catalyzes oxidation of reduced cytochrome c by O_2.

Reduced cytochrome c + ½ O_2 ? oxidized cytochrome c + H_2O. ? $E^{o'}$ = 0.580V (12)

Complex V (proton translocating ATP synthase) carries out energy coupling or energy transduction. The electron transport and ATP synthesis are coupled.

$2H^+$ + ½ O_2 ? H_2O, ? $E^{o'}$ = 0.815V (13)

Even though NADH can participate in a two electron transfer process only, the coenzymes FMN and CoQ of complex I are capable of receiving or donating one or two electrons because of their stable semiquinone radical forms. Cytochromes, on the other hand, allow passage of only one electron.

Part of the energy released during this passage of "high energy" electrons along a series of electron carriers embedded in the ion-impermeable membrane is harnessed to pump protons from one side to the other side of the membrane. Complexes I, III, and IV pump protons. This movement of protons from the mitochondrial matrix to the intermembrane space creates an electrochemical gradient. The redox enzymes in the respiratory chain build this proton gradient or pH gradient in a stepwise fashion. ATP-synthase, a nonredox enzyme, is responsible for keeping the ATP ratio far from equilibrium by catalyzing the phosphorylation of ADP. ATP-synthase uses the gradient of the electrochemical potential of the proton as a source of free energy.

The electronic properties (bias or rectifier or "ratchet") of some of these redox enzymes and the group responsible for these activities are described later in this chapter.

In 1963, it was discovered that mitochondria had their own DNA [24]. They contain their own genome with their own transcription, translation, and machinery for protein synthesis. Two genetic systems, the mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA) encode the mitochondrial electron transport chain complexes. The mtDNA codes for 13 different complexes are given in Table 1. The codes for nDNA are 36, 4, 10, 10 and 14 protein subunits for Complexes I, II, III, IV and V respectively [24]. Complex II is encoded by nDNA only. Mitochondrial DNA also contains genes encoding 2 ribosomal RNAs (12S rRNA and 16S rRNA) and all the necessary 22 transfer RNAs that are required for protein synthesis in mitochondria. The human mtDNA is a supercoiled, double-stranded molecule containing 16,569 base pairs. It is also known that the frequency of mtDNA migration to nDNA is much greater than nDNA migration to mtDNA [24].

Table 1. Subunits of Electron Transport Chain Complexes Encoded by Human Mitochondrial DNA [24]

Complex	Subunits	Number of Subunits Encoded by mtDNA
I	NADH dehydrogenase	7
II	Succinate dehydrogenase	0
III	Cytochrome b	1
IV	Cytochrome c oxidase	3
V	ATP synthase	2

Mitochondria are not self-replicating organelles in spite of their ability to transcribe their own DNA and translate the resulting mRNAs. Most of the mitochondrial proteins (more than 90%), synthesized in cytosol and imported into mitochondria, are encoded in nDNA.

6. Mitochondrial Dysfunction

Apart from ATP production, mitochondria also produce reactive oxygen species (ROS). Some electrons escaping or leaking from the electron transport complexes, mainly from complexes I and III, during respiration react with oxygen to form superoxide radicals. Oxygen undergoes a series of progressive reduction reactions producing $O_2^{\cdot -}$, H_2O_2, and finally $HO^{\cdot}$ along with OH^-. The cause of this leakage of electrons is not clearly understood. However, it may be possible for mtDNA mutations to disrupt the normal electron flow and seriously affect energy production.. Compared to nDNA, mtDNA is far more susceptible to mutations due to a lack of histone protection and limited repair capacity [24]. During the production of ATP in the cell, about 85% of oxygen is consumed by the mitochondria. Superoxide radical, $O_2^{\cdot -}$, may be produced from about 4% of all oxygen consumed [23]. Manganese superoxide dismutase or copper/zinc superoxide dismutase converts the superoxide radical into hydrogen peroxide. The active site of cytosolic and extracelllar forms of superoxide dismutase contains copper/zinc and the mitochondrial form contains manganese [2]. Glutathione peroxidase or peroxoredoxin III converts the peroxide into oxygen and water. Oxidative damage is due to the inadequacy of these detoxifying processes.

The iron-sulfur cluster in the aconitase enzyme, localized to the matrix space of mitochondria, is oxidized by superoxide and the exposed iron reacts with the peroxide to produce hydroxyl radicals [23]. Also the NO produced within mitochondria by mitochondrial NO synthase produces peroxynitrite radical ($ONOO^-$) by reaction with $O_2^{\cdot -}$. Superoxide and peroxinitrite radicals contribute to substantial mitochondrial damage.

Lipid peroxidation by the hydroxyl radical can alter the structural integrity of membranes. In patients with Parkinson's disease, the excess Fe^{2+} can reduce peroxide and

produce $HO^{\cdot}$. These radicals and their reactions cause oxidative stress and consequent mitochondrial damage resulting in mutations and probably cancer.

Since each cell contains many mitochondria with multiple copies of mtDNA, it is possible for wild-type and mutant mtDNA to coexist in a state called heteroplasmy. After numerous cell divisions over time, this status may change to predominantly wild-type or mutant , a state called homoplasmy.

Mitochondrial DNA depletion and deletion to cancer progression has been reviewed recently [25]. The mitochondrial function and energy metabolism in cancer cells as well as mitochondrial genetics have also been reviewed [26-28].

Mitochondrial defects in cancer have been reviewed recently [24, 28-30]. A general feature of malignant cells is alterations in respiratory chain activity and mtDNA abnormalities. Some gene mutations observed in some common cancers are included in Table 2.

Table 2. Mutated Genes in Different Cancers [24]

Type of Cancer	Mutated Gene/Region	Affected Respiratory Complex
Breast	16S rRNA	None
	ND1	Complex I
	ND2	Complex I
	ND4	Complex I
	ND5	Complex I
	Cyt b	Complex III
	ATPase 6	Complex V
Ovarian	12S rRNA	None
	16S rRNA	None
	Cyt b	Complex III
Pancreatic	12S rRNA	None
	16S rRNA	None
	ND1	Complex I
	ND2	Complex I
	ND4	Complex I
	ND4L	Complex I
	ND5	Complex I
	ND6	Complex I
	Cyt b	Complex III
	COXI	Complex IV

	COXII	Complex IV
	COXIII	Complex IV
	ATPase 6	Complex V
Prostate	16S rRNA	None
	ND4	Complex I
	ND4L	Complex I

6.1. Glioblastoma multiforme (GBM) and type - I meningioma (transitional meningioma, TM)

It has been found that mtDNA was highly amplified in most of the malignant glioma specimens [24]. The comparative amplification of nDNA was very low. The membrane phospholipids of the brain containing high amounts of unsaturated fatty acids are likely to be damaged by oxidation by oxygen radicals. The formation of these lipid peroxides affects the integrity and function of the membranes, proteins and DNA The natural defense mechanism against this adverse effect is by glutathione peroxidase (GPx), glutathione reductase (GRx), and superoxide dismutase. Superoxide dismutase converts $O_2^{\cdot -}$ into H_2O_2, which is eliminated by the actions of catalases and peroxidases. An analysis, given in Table 3, of 48 brain tumors obtained during surgery and 15 normal brain tissues collected during autopsy for GPx, GRx, and protein oxidation (POx) revealed that GPx and GRx activities were significantly lower in GBM and TM when compared to the controls [31]. The decrease in GPx and GRx were more obvious in GBM than in TM. Also the POx levels were much higher in both GBM and TM compared to controls.

Table 3. Comparison of glutathione peroxidase, glutathione reductase and protein oxidation levels in glioblastoma, transitional meningioma, and normal brain tissues [31].

Parameters	GPx (U/g wet tissue)	GRx (U/g wet tissue)	Pox (nmol/g wet tissue)
Glioblastoma	17.72±3.9	5.11±0.9	599.6±56
Transitional meningioma	19.04±2.0	5.66±0.9	588.3±49
Normal brain tissue	45.26±6.9	10.08±1.2	439.1±31

6.2. Breast cancer

The bulk of the mutations were identified in the D-loop region, the main non-coding area of the mtDNA. The others are given in Table 2. Lipid peroxidation, coenzyme Q10 levels and antioxidant status of breast cancer patients have been investigated [32-35]. It was observed that

coenzyme Q10 concentrations were significantly less in tumor tissues compared to normal surrounding tissues [34]. Also higher levels of malondialdehyde (MDA) (a measure of lipid peroxidation) were observed in tumor tissues. However, the activities of manganese superoxide dismutase, total superoxide dismutase, glutathione peroxidase, and catalase levels were also higher in tumor tissues compared to normal tissues [33, 34]. On the other hand, the superoxide dismutase and catalase levels, given in Table 4 and Table 5, reported in another recent study [32] indicate a trend that is consistent with data for other cancers. This discrepancy is attributed probably to the differences in the stage of the tumor selected for different studies. It was suggested, from a study of free radicals and antioxidants in stages I-IV of carcinoma breast in blood and tissue that at the early stages of cancer, the antioxidant levels may be higher to meet the challenge of carcinogenesis [32].

Table 4. Comparison of lipid peroxidation, catalase and superoxide dismutase levels in normal and breast cancer blood and tissue [32].

Parameters in blood	Control	Patients
Malondialdehyde (nmol/mL plasma)	2.27±0.36	4.52±0.78
Catalase (U/mL red blood cell)	15.42±0.59	10.60±0.99
Superoxide dismutase (U/mL red blood cell)	10.52 ±0.37	7.36±0.55
Parameters in tissue		
Malondialdehyde (nmol/mL plasma)	2.20±0.22	4.73±0.69
Catalase (U/mL red blood cell)	15.61±0.72	10.37±1.16
Superoxide dismutase (U/mL red blood cell)	10.61 ±0.36	7.24±0.26

Table 5. Comparison of lipid peroxidation, catalase and superoxide dismutase levels in different stages of breast carcinoma in blood and tissue [32].

Parameters in blood plasma	Stage I	Stage II	Stage III	Stage IV
Malondialdehyde (nmol/mL plasma)	3.38±0.44	3.99±0.60	4.52±0.58	5.12±0.49
Catalase (U/mL red blood cell)	10.76±0.95	11.23±1.24	11.03±0.55	9.90±0.65
Superoxide dismutase (U/mL red blood cell)	8.15 ±0.90	7.58±0.55	7.22 ±0.22	7.13±0.39

Parameters in tissue				
Malondialdehyde (nmol/mL plasma)	3.86±0.63	4.60±0.71	4.57±0.41	5.12±0.63
Catalase (U/mL red blood cell)	12.21±1.02	10.85±1.07	9.82±0.72	9.99±0.97
Superoxide dismutase (U/mL red blood cell)	7.08 ±0.32	7.43±0.26	7.23 ±0.21	7.19±0.32

6.3. Ovarian Cancer

Somatic mutations were mostly observed in 4 regions of the mitochondrial genome (Table 2), D-loop, 12S rRNA, 16S rRNA, and cytochrome b [24]. The levels of lipid peroxidation and conjugated dienes were much higher in ovarian cancer patients compared to controls [36]. The catalase and superoxide dismutase levels were lower in the ovarian cancer

Table 6. Comparison of lipid peroxidation, conjugated dienes, catalase, superoxide dismutase, vitamin C and vitamin E levels in blood in normal and ovarian cancer patients[36].

Parameters in blood	Control	Patients
Malondialdehyde (nmol/mL plasma)	2.13±0.18	5.64±0.52
Conjugated dienes (μmol/mL plasma)	0.71±0.06	1.71±0.13
Catalase (U/mg hemoglobin)	5.71±0.61	4.4±0.39
Superoxide dismutase (U/mg hemoglobin)	1.91 ±0.29	0.9±0.11
Vitamin C (mg/dL of plasma)	1.05 ±0.09	0.40±0.03
Vitamin E (mg/dL of plasma)	2.82 ±0.20	1.36±0.10

patients. The levels of antioxidant vitamins C and E were also lower in the ovarian cancer patients.

6.4. Prostate Cancer

Incidence of mutations in mitochondrial DNA of prostate cancer was infrequent [24]. A recent study of lipid peroxidation and antioxidant status in prostate cancer patients, shown in Table 7, revealed elevated levels of lipid peroxidation and decreased levels of vitamin C, vitamin E, reduced glutathione, glutathione peroxides, and superoxide dismutase in plasma, erythrocytes and erythrocyte membranes when compared with normal patients [37].

Table 7. Comparison of lipid peroxidation, catalase, reduced glutathione, glutathione peroxidase, vitamin C and vitamin E levels in plasma, in erythrocytes and erythrocyte membranes of normal and prostate cancer patients[37].

Parameters in plasma	Control	Patients
Malondialdehyde (nmol/mL plasma)	3.8±0.2	6.9±0.52
Conjugated dienes (μmol/mL plasma)	0.71±0.06	1.71±0.13
Catalase (U/mL plasma)	0.76±0.07	0.56±0.04
Vitamin C (mg/dL of plasma)	1.39 ±0.007	1.29±0.06
Vitamin E (mg/dL of plasma)	1.4 ±0.06	1.28±0.09
Reduced glutathione (mg/dL plasma)	52.7 ±4.2	42.8±2.9
Glutathione peroxidase (U/L)	189.8±23.4	160.1±12.7
Parameters in Erythrocyte		
Malondialdehyde (nmol/mg protein)	0.33±0.04	5.7±0.42
Reduced glutathione (mg/dL plasma)	54.9 ±3.8	44.8±2.7
Vitamin E (μg/mg protein)	2.31 ±0.09	1.76±0.09
Parameters in Erythrocyte Membrane		
Superoxide dismutase (U/mg hemoglobin)	4.71±0.52	4.32±0.34
Catalase (U/mg hemoglobin)	1.7±0.09	1.3±0.07
Glutathione peroxidase (U/g hemoglobin)	22.2 ±1.7	20.6±1.7

It is obvious from the examples given above that the main gateway for electrons to enter the respiratory chain, complex I, is affected in all these cancers.

7. Free Radicals and Antioxidants

The appearance of oxygen in the atmosphere is associated with a great expansion of the varieties and numbers of higher living forms. Oxygen is the source for the emergence of

respiratory metabolism and energy efficiency. It is also the source of free radicals such as hydroxyl and superoxide.

A free radical is a highly reactive species with an unpaired electron. It can be a neutral species such as hydroxyl, $HO^{\cdot}$, or a charged negative ion (anion) such as superoxide, $O_2^{\cdot -}$, or a charged positive ion (cation) such as guanine radical. An unpaired electron is shown as a dot after the symbol (example: $HO^{\cdot}$). Free radicals are, in general, good oxidizing agents. They can remove an electron from other materials and in that process the unpaired electron gets paired. They can also participate in chain reactions to produce new free radicals.

The free radicals produced during phagocytosis are beneficial [1, 23]. The primary purpose of leukocytes is phagocytosis, which is the engulfing and destruction of particulate matter and bacteria. Leukocytes contain the enzymes of the hexose-monophosphate shunt, glycolysis, citric acid cycle, and respiratory enzymes. Phagocytosis requires a lot of energy, which is obtained from glucose by glycolysis and also by the hexose-monophosphate shunt. The role of this shunt is to produce hydrogen peroxide from superoxide free radical, which is used in the phagocytotic process.

The beneficial aspect of hydrogen peroxide in cell signaling is emerging. Neurons and brain macrophages produce superoxide ions in pathological situations and the hydrogen peroxide produced from superoxide increases gap junctional communication in astrocytes [38]. Examples of signaling processes include the over oxidation of the cysteine in peroxiredoxins from the cysteine sulfenic acid to cysteine sulfinic acid, and the over oxidation of methionine residues in proteins to methionine sulfoxide [39]. The need for a certain amount of oxidative stress and the role of redox for embryonic and fetal growth has been exemplified recently [40].

Free radicals are also a liability because they produce DNA damage by easily oxidizing the guanine base in DNA. The altered form of guanine, 8-oxoguanine, has been the subject of much study. Another liability of free radicals is that oxyradicals allow lipid peroxidation.

Antioxidants are physiologic reducing agents. They donate electrons to free radicals and in that process become oxidized. Their specific reactions are a function of their redox potential, measured in volts.

Reduction or redox potentials predict the direction of a reaction. They cannot predict how fast the reaction will take place. Each oxidized species of a redox couple having a higher positive voltage is capable of extracting an electron from a reduced species of a redox couple having a less positive or higher negative voltage. Some examples of one-electron reduction potentials and two-electron reduction potentials of reactions of biological interest are given in Table 8 [41] and Table 9 [1]. Depending on the position of the redox couple in the redox table of potentials, free radicals can act as both oxidizing and reducing agents and produce other free radicals.

Table 8. One-electron reduction potentials at pH 7.0, 1 atm, and 1.0 M [41]

Couple	E/mV
$HO^{\cdot}, H^{+}/H_2O$	2310
$O_3^{\cdot-}, 2H^{+}/H_2O + O_2$	1800
$HOO^{\cdot}, H^{+}/H_2O_2$	1060
$PUFA^{\cdot}, H^{+}/PUFAH$ (Polyunsaturated fatty acid, bis-allylic-H)	600
a-Tocopheroxyl$^{\cdot}$, H^{+}/a-tocopherol (Vitamin E)	500
$H_2O_2, H^{+}/H_2O, HO^{\cdot}$	320
Ascorbate$^{\cdot-}$, H^{+}/ascorbate monoanion (Vitamin C)	282
Ferricytochrome c/Ferrocytochrome c	260
Semiubiquinone, H^{+}/ubiquinol ($CoQ^{\cdot-}, 2H^{+}/CoQH_2$)	200
Ubiquinone, H^{+}/semiubiquinone ($CoQ/CoQ^{\cdot-}$)	-36
Dehydroascorbic/ascorbate$^{\cdot-}$	-174
$O_2/O_2^{\cdot-}$	-330
$O_2, H^{+}/HO_2^{\cdot}$	-460
GSSG/GSSG$^{\cdot-}$ (Glutathione disulfide and its radical ion)	-1500

Table 9. Two-electron reduction potentials at pH 7.0, 1 atm, and 1.0 M [1]

Reaction	E/mV
$2H^{+} + 2e^{-} = H_2$	- 421
Cystine + $2H^{+} + 2e^{-}$ = Cysteine	-340
$NAD^{+} + H^{+} + 2e^{-}$ = NADH	-315
$NADP^{+} + H^{+} + 2e^{-}$ = NADPH	-320
Lipoic acid + $2H^{+} + 2e^{-}$ = Dihydrolipoic acid	-290
FAD + $2H^{+} + 2e^{-}$ = $FADH_2$ (free coenzyme)	-219
Pyruvate + $2H^{+} + 2e^{-}$ = Lactate	-190
FAD + $2H^{+} + 2e^{-}$ = $FADH_2$ (in flavoproteins)	~0.
Ubiquinone + $2H^{+} + 2e^{-}$ = Ubiquinol	45
½ O_2 + $2H^{+} + 2e^{-}$ = H_2O	820

Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase are known as preventive antioxidants because they eliminate the species involved in the initiation of free radical chain reactions. Hydrogen peroxide and organic hydroperoxides in the cytosol are destroyed by a selenium (a cofactor) containing metalloenzyme glutathione peroxidase. Superoxide dismutases exist in several varieties with copper, zinc, and manganese in the active center. The dismutation yields hydrogen peroxide, which can be removed by catalase and glutathione peroxidase to oxygen and water by different mechanisms. The highest concentration of catalase is present in peroxisomes and to a lesser extent in cytosol and mitochondria [2].

Food industry uses butylated hydroxyl toluene and butylated hydroxyl anisole for preservation. However their metabolic fate is not clearly understood.

Small molecules such as ascorbate (vitamin C), tocopherols (vitamin E), reduced coenzyme Q10 ($CoQH_2$), glutathione (?-glutamylcysteinylglycine), and a-lipoic acid "repair" oxidizing radicals directly and are known as chain breaking antioxidants.

The criteria often used to evaluate the antioxidant potential as well as preventive or therapeutic applications of a compound are 1) specificity of free radical quenching, 2) metal chelating ability, 3) interaction with other antioxidants, 4) effects on gene expression, 5) absorption and bioavailability, 6) concentration in tissues, cells, and extracellular fluid, and 7) location (in aqueous or membrane domains or in both) [42].

8. Free Radical production and Chain Reactions:

Normal (triplet state) oxygen, O_2, has two single parallel (spin) electrons in separate orbitals. A two electron interaction is not possible because it will result in parallel spins in the same orbital, which is not allowed. Thus the preferable interaction is reduction of oxygen by addition of one electron at a time. This process leads to the production of oxygen radicals that can cause cellular damage. The high energy singlet oxygen with two electrons and opposite spins in the two orbitals is capable of two-electron interactions.

Progressive one electron reduction of O_2 produces $O_2^{\cdot -}$, H_2O_2, and finally $HO^{\cdot}$ along with OH^-.

$$O_2 + e^- \rightarrow O_2^{\cdot -} \quad \text{(superoxide anion)} \qquad (14)$$

$$O_2^{\cdot -} + e^- + 2H^+ \rightarrow H_2O_2 \text{ (hydrogen peroxide)} \qquad (15)$$

$$H_2O_2 + e^- \rightarrow OH^- + HO^{\cdot} \text{ (hydroxyl radical)} \qquad (16)$$

$$HO^{\cdot} + e^- + H^+ \rightarrow H_2O \qquad (17)$$

The hydroxyl radical is undoubtedly the most dangerous. It is involved in lipid peroxidation and generation of other toxic radicals. While there is no enzyme to destroy it, there is enzymatic transfer of hydroxyl to the proline in procollagen. Because of its high reactivity, hydroxyl radical has a short life.

The superoxide ion can act both as a reductant (for Fe^{3+}) and as an oxidant for catecholamines. Free iron and copper present under physiological conditions are sequestered by

proteins (iron as transferrin and ferritin and copper by ceruloplasmin) to minimize production of free radical chain reactions such as reactions (18) and (19).

$$O_2^{\cdot-} + Fe^{3+} \rightarrow O_2 + Fe^{2+} \quad (18)$$

$$H_2O_2 + Fe^{2+} + H^+ \rightarrow HO^{\cdot} + Fe^{3+} + H_2O \text{ (classical Fenton reaction)} \quad (19)$$

Adding reactions (18) and (19) gives the Haber-Weiss reaction (20) which is catalyzed by metal ions.

$$H_2O_2 + O_2^{\cdot-} + H^+ \rightarrow HO^{\cdot} + O_2 + H_2O \quad (20)$$

The superoxide anion shown in reaction (14) is often released by mitochondria. Dismutation of superoxide anion, shown in reaction (21) produces hydrogen peroxide.

$$2H^+ + O_2^{\cdot-} + O_2^{\cdot-} \rightarrow H_2O_2 + O_2 \quad (21)$$

Damage to both mtDNA and nDNA may result in mutations. Nonspecific binding of Fe^{2+} to DNA may result in the formation of $HO^{\cdot}$ (reaction 19) that attack individual bases and cause strand breaks.

An example of a polyunsaturated fatty acid (PUFAH) peroxidation chain reaction is the following.

Initiation reaction: $PUFAH \rightarrow PUFA^{\cdot} + H^{\cdot}$ (22)

Propagation reaction: $PUFA^{\cdot} + O_2 \rightarrow PUFAOO^{\cdot}$ (23)

Propagation reaction: $PUFAOO^{\cdot} + PUFAH \rightarrow PUFAOOH + PUFA^{\cdot}$ (24)

Tocopherol (vitamin E) can break the propagation chain reaction by reacting with lipid peroxyl radical, $PUFAOO^{\cdot}$.

The most reactive hydroxyl radical, $HO^{\cdot}$, is produced in biological systems by reductive cleavage of H_2O_2 by a reduced metal complex. The source of iron (II) complex may be ferrocytochrome c, iron(II) citrate, iron(II) transferrin, and iron(II) ADP. The source of H_2O_2 may be a direct two-electron reduction of O_2 or a one electron reduction of O_2 to produce superoxide ion.

Iron (II) complex + H_2O_2 → Iron (III) complex + OH^- + $HO^{\cdot}$ (25)

$HO_2^{\cdot}$ (the perhydroxyl radical) + $O_2^{\cdot-}$ (+ H^+) → $H_2O_2 + O_2$ (26)

The dangerous superoxide ion converts the iron (III) complex directly to the iron (II) complex.

$O_2^{\cdot-}$ + iron(III) complex → iron(II) complex + O_2 (27)

Production of $O_2^{\cdot-}$ is from the use of stronger reductants. Thus $O_2^{\cdot-}$ can produce both hydrogen peroxide and the iron (II) complex needed for the Fenton reaction.

9. Vitamin C, L-ascorbic Acid

Ascorbic acid is in the form of ascorbate anion at biological pH because it has pK values of 4.17 and 11.57. It is a cofactor in several biosynthetic pathways. It is also an antioxidant. Humans do not synthesize it due to lack of the enzyme L-gulono-?-lactone oxidase. This enzyme mediates the last step in the ascorbate biosynthetic pathway originating from glucose.

Ascorbic acid is a cofactor for the enzyme prolylhydroxylase, which modifies the polypeptide collagen precursor to facilitate the formation of collagen fibers. It also plays important roles in carnitine synthesis, catabolism of tyrosine, synthesis of norepinephrine by dopamine ß-monooxygenase or dopamine ß-hydroxylase , and the amidation of peptides with C-terminal glycine to activate hormone precursors.

Due to resonance, the ascorbate radical has a long half life of 1 second. Ascorbate can be oxidized in two successive one-electron steps to ascorbate free radical and dehydroascorbic acid respectively.

Ascorbate anion – e^- ? Ascorbate free radical (28)

Ascorbate free radical – e^- ? dehydroascorbic acid (29)

The unpaired electron in the ascorbate free radical is distributed over its ring structure. This electron distribution stabilizes the molecule. Ascorbate radical disproportionates to give ascorbate anion and dehydroascorbic acid. This acid is a strained molecule and is not stable. The strain is relieved by hydration and subsequent bicyclic structure formation, which is hydrolyzed to form a linear molecule 2,3-diketo-L-gulonic acid. This ring opening reaction is biologically irreversible and results in the loss of the vitamin. However the oxidation of ascorbate to the radical and dehydroascorbic acid can easily be reversed. Both ascorbate radical anion and dehydroascorbic acid can be reduced by enzyme systems that use NADH or NADPH as sources of reducing equivalents.

The redox chemistry of ascorbate is pH dependent. The ascorbate radical has pK values of 1.10 and 4.25 and is an anion at physiological pH.

Dehydroascorbic acid imported into the mitochondria via facilitative glucose transporter 1,GLUT1, is reduced by glutathione and protects the mitochondrial genome and membrane [43].

10. Vitamin E

Vitamin E includes eight different related homologues. Of these, a-tocopherol is the most abundant and active form in vivo. The dynamics of antioxidant action of vitamin E have been recently reviewed [44]. Vitamin E acts only in membrane or lipid domains. It quenches lipid peroxyl radicals. It has no activity in the aqueous phase.

Vitamin E, the primary lipid soluble small molecule antioxidant and vitamin C, the terminal water soluble small molecule antioxidant cooperate to protect lipids and lipid structures against peroxidation. Although vitamin E is located in membranes and vitamin C is located in aqueous phases, vitamin C is able to recycle vitamin E (See Table 8 and reaction (30). That is, vitamin C repairs the tocopheroxyl (chromanoxyl) radical of vitamin E thereby permitting vitamin E to function again as a free radical chain breaking antioxidant.

a-Tocopheroxyl$^{\cdot}$ + ascorbate monoanion ? ascorbate$^{\cdot -}$ + a-tocopherol, E = + 218 mV (30)

(Vitamin E) (Vitamin C)

The concentration of vitamin E is less than 0.1 nmol per mg of membrane protein which corresponds to about one molecule for every 1000 to 2000 membrane phospholipids molecules that are the target of oxidation [41]. Lipid peroxyl radicals are generated at the rate 1-5 nmol per mg of membrane protein per minute [42]. Since vitamin E is recycled by other antioxidants such as vitamin C, ubiquinols and glutathione, the membrane is not degraded and the vitamin E levels stay nearly the same. Recycling of vitamin E by dihydrolipoic acid seems weak. However, dihydrolipoic acid prevents lipid peroxidation by regenerating glutathione. Dihydrolipoic acid can recycle vitamin E via glutathione, vitamin C, ubiquinol, NADPH and NADH [42].

Each a-tocopherol (vitamin E) can donate 2 electrons as a cha in breaking antioxidant.

11. Glutathione

Glutathione or ?-glutamylcysteinylglycine, GSH, one of the body's major antioxidants, can react with various highly oxidizing species such as $HO^{\cdot}$, $RO^{\cdot}$ or $ROO^{\cdot}$ and make, H_2O, ROH, or ROOH and $GS^{\cdot}$ which is glutathiyl radical. This is less oxidizing. However, $GS^{\cdot}$ can react rapidly with GSH, most efficiently via GS^{-} to make $GSSG^{\cdot -}$, which is a very strong reducing species. It can produce $O_2^{\cdot -}$ and glutathione disulfide, GSSG, by reaction with oxygen.

$$GSSG^{\cdot -} + O_2 \; ? \quad O_2^{\cdot -} + GSSG \qquad (31)$$

Selenium (as selenocysteine) is a cofactor of glutathione proxidase [2]. One can see that superoxide dismutase and glutathione providing an excellent natural combination for cellular antioxidant defense by removing $O_2^{\cdot -}$ and $HO^{\cdot}$ respectively.

The $GS^{\cdot}$ reacts with oxygen to form $GSOO^{\cdot}$ and other free radicals such as GS^{-} sulfonylperoxyl radical ($GSO_2OO^{\cdot}$), GS-sulfonyl radical ($GSO_2^{\cdot}$), and GS-sulfinyl radical ($GSO^{\cdot}$). The stable end products of glutathione oxidation are glutathione disulfide, glutathione sulfinic acid (GSOOH), and glutathione sulfonic acid (GSO_3H).

The intracellular concentration of GSH is about 1 mM while the mitochondrial respiration keeps O_2 about 0 to 10 µM in the cell. Therefore, 99% of $GS^{\cdot}$ formed should react with GSH to make GSSG and $O_2^{\cdot -}$. Thus the importance of superoxide dismutase is obvious. The normal GSH to GSSG ratio in erythrocytes is 100:1 [2].

Glutathione is involved in reactions such as peroxide detoxification by glutathione peroxidase, NADPH dependent reductio n of GSSG to GSH by glutathione reductase, thiol transferase modulation of protein disulfide balance and leukotriene biosynthesis by glutathione-S-transferase [1]. It is also involved in the transport of amino acids across cell membranes. Glutathione reductase contains an electron-transfer prosthetic group FAD. The electronic properties of the cysteine and FAD groups are discussed in a later section.

12. Hydrogen Peroxide (H_2O_2)

Pure hydrogen peroxide is a pale blue syrupy liquid with a boiling point of 152.1°C and a freezing point of -0.89°C. The dielectric constant of 93 at 25°C for the pure liquid increases to

120 for a 65% aqueous solution [45]. In the pure liquid state, the hydrogen peroxide is more strongly associated by hydrogen bonding than pure water. The dipole moment of hydrogen peroxide is 2.1 Debye units compared to 1.84 Debye units for water. Thus ion-dipole interactions are stronger with hydrogen peroxide than with water. Its influence on ion solvation is discussed in a later section.

Hydrogen peroxide has a skew, chain structure. The O-H bond distance is 97 pm and O-O bond distance is 149 pm. A dilute aqueous solution of hydrogen peroxide is more acidic than water [45].

$$H_2O_2 = H^+ + HO_2^- \quad K_{20^oC} = 1.5 \times 10^{-12} \tag{32}$$

The potential given by the following equations indicate that hydrogen peroxide is a strong oxidizing agent in both acidic and basic solutions.

$$H_2O_2 + 2H^+ + 2e^- = 2H_2O \qquad E^o = 1.77V \tag{33}$$

$$O_2 + 2H^+ + 2e^- = H_2O_2 \qquad E^o = 0.68V \tag{34}$$

$$HO_2^- + H_2O + 2e^- = 3OH^- \qquad E^o = 0.87V \tag{35}$$

Hydrogen peroxide behaves as a reducing agent only in the presence of stronger oxidizing agents such as permanganate.

The enzymes, monoamine oxidases, located in the outer mitochondrial membrane of mammalian tissues catalyze the oxidation of biogenic amines and produce H_2O_2.

Hydrogen peroxide plays a dual role. In cancer cells, increased levels of H_2O_2 result in DNA alterations, cell proliferation, apoptosis resistance, metastasis, angiogenesis, and hypoxia inducible factor 1(HIF-1) activation. On the other hand, it also induces apoptosis in cancer cells selectively and the activity of many anticancer drugs is mediated, at least in part by H_2O_2 [46]. The mystery surrounding the dual role of hydrogen peroxide may be solved, at least partially, by looking at its electronic properties, which is described in a later section.

Oxyradical reactions catalyze the mitochondrial electron transfer chain to oxygen. While the energy advantage of oxygen metabolism favors selection of such developments, we have studied the contribution of periodic oscillation which peroxide brings to hydrated physico-chemical systems. Hydrogen peroxide exhibits a variety of oscillations [47, 48] under potentiostatic conditions. Spatiotemporal oscillation in a system allows the far reaching electronics of alternating current circuits, long range signals, and oscillation amplifier behavior. Peroxide may allow these reactions to occur in the thin film hydration double layers throughout the cell and organism.

To highlight the importance of mitochondrial dysfunction in other major diseases, other than cancer, we have included two brief sections, Parkinson's disease and Alzheimer's disease,

12.1 Parkinson's Disease

Oxidative stress is implicated in the pathogenesis of Parkinson's disease [49, 50]. Reduced activity of Complex I of the electron transport chain and the gene mutations in

Parkinson's disease have been discussed in great detail [51-54]. The hydroxyl radical can lead to lipid peroxidation and alter the structural integrity of neural membranes. Excess Fe^{2+}, also found in patients with Parkinson's disease, can reduce peroxide and produce $HO^{\cdot}$. Dopamine undergoes auto-oxidation, producing $HO^{\cdot}$, H_2O_2, semiquinone radical and finally a quinone [49]. Enzymatic metabolism of dopamine also produces H_2O_2.

$$\text{Dopamine} + O_2 \;? \quad \text{Semiquinone}^{\cdot} + O_2^{\cdot -} + H^+ \tag{36}$$

$$\text{Dopamine} + O_2^{\cdot -} + 2H^+ \;? \quad \text{Semiquinone}^{\cdot} + H_2O_2 \tag{37}$$

$$\text{Semiquinone}^{\cdot} + O_2 \;? \quad \text{Quinone} + O2^{\cdot -} + H^+ \tag{38}$$

Hydrogen peroxide is also produced when dopamine is metabolized enzymatically by monoamine oxidase [49].

$$\text{Dopamine} + O_2 + H_2O \rightarrow \text{3,4 Dihydroxyphenylacetaldehyde} + NH_3 + H_2O_2 \tag{39}$$

12.2. Alzheimer's Disease

Oxidative damage to both mtDNA and nDNA has been examined in several studies [55-58]. Significant three-fold increase in the amount of 8-hydroxy-2'-deoxyguanosine in parietal cortex of Alzheimer's patients in mtDNA and a small significant increase in oxidative damage to nDNA have been observed [55]. A deficiency in cytochrome c oxidase has been reported in Alzheimer's disease [56]. Significant decreases were observed in the activities of pyruvate dehydrogenase complex (-41%), isocitrate dehydrogenase (-27%), a-ketoglutarate dehydrogenase complex (-57%). There were good correlations between the diminished activity of these enzymes and the Clinical Dementia Rating [57, 58]. On the other hand the activities of succinate dehdrogenase (complex II) (+44%) and malate dehydrogenase (+54%) were increased. The activities of the other 4 Krebs cycle enzymes, citrate synthase, aconitase, succinate thiokinase, and fumarase were unchanged.

13. a-Lipoic Acid

Lipoic acid, shown in Fig. 1, is a very unique biological molecule. It has a carboxylic acid (pK_a 4.7) which is ionized at biological pH, and it has a cyclic disulfide or dithiolane ring [59, 60]. It exists intracellularly as the reduced form, dihydrolipoic acid. The redox property, the antioxidant capacity and the fatty acid properties of lipoic acid account for its biological effects. We will describe its electronic contributions later in this chapter.

The dihydrolipoic acid can regenerate or recycle the antioxidants CoQ (ubiquinol), vitamins C and E, and glutathione. Both lipoic acid and its reduced form are known to scavenge reactive oxygen and nitrogen species such as H_2O_2, $HO^{\cdot}$, hypochlorous acid (HOCl), and peroxynitrite ($ONOO^{-}$) [42, 59].

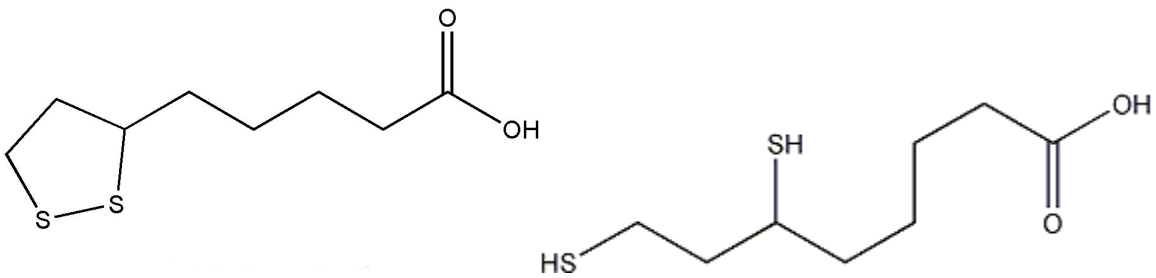


Figure 1. Alpha lipoic acid in its oxidized and reduced forms

Compared to the inefficient transport of disulfides such as cystine that is needed in modulating GSH levels in cells, the efficient transport of lipoic acid and dihydrolipoic acid in and out of the both mitochondria and cells as well as mitochondrial ß-oxidation have been attributed to its fatty acid properties, similar to that of octanoic acid [59]. Lipoic acid can also cross the blood-brain barrier. The ß-oxidation products of lipoic acid, the oxidized and reduced forms of bisnorlipoic acid and tetranorlipoic acid may also have important redox and antioxidant biological effects.

The a-lipoic acid/dihydrolipoic acid couple is called a "universal antioxidant" because it fulfills all the criteria mentioned earlier [42]. a-Lipoic acid absorbed from the diet is readily converted into dihydrolipoic acid in many tissues. There is ample evidence to indicate the usefulness of this redox couple as a therapeutic agent for diabetes, ischemia-reperfusion injury, and heavy metal poisoning. a-Lipoic acid was found to protect hematopoietic tissues in mice from radiation damage [61]. It was also found that a-lipoic acid offered protection from radiation for children affected by the Chernobyl nuclear accident [62], neurodegeneration, and HIV infection[42, 59]. a-Lipoic acid scavenges hydroxyl radicals but is not effective against hydrogen peroxide and superoxide radical. The reduction potential for the a-lipoic acid/dihydrolipoic acid couple of -320 mV [42] or -290 mV [1] and the GSSG/GSH) couple of -240 mV indicate that dihydrolipoic acid can react with glutathione disulfide and regenerate glutathione[42]. Thus lipoic acid helps to maintain GSH/GSSG ratio (about 100 to 10,000 times greater than other redox couples such as NAD^+/NADH, and $NADP^+$/NADPH), an estimate of redox state, in cells [59].

Treatment with lipoic acid increases the GSH levels in cells. This is explained by 1) facile transport of lipoic acid into cells, where it is reduced by NADH or NADPH dependent pathways to dihydrolipoic acid. 2) Dihydrolipoic acid is transported back into the extracellular media where it is oxidized by cystine regenerating lipoic acid and producing cysteine. 3) Compared to cystine, cysteine is more easily transported into the cell which aids the synthesis of GSH [59].

The pharmacokinetics of R-lipoic acid, reviewed recently, revealed a plasma level concentration, C_{max}, of 1.154 µg/mL from 1 g R-lipoic acid compared to the proposed therapeutic range of 10-20 µg/mL or 50-100 µM [63]. A dose of 600-800 mg sodium R-lipoate gave plasma

levels of 8-18 µg/mL, which is within the therapeutic range. The upper limit suggested for therapeutic action of 45 µg/mL or 225 µM is reached by a dose of about 1.2 g of racemic-a-lipoic acid. The no adverse observed effect level (NOAEL) of racemic lipoic acid is considered to be 60 mg/kg body mass/day.

The oxidized form a-lipoic acid can undergo further oxidation at sulfur or get reduced. Therapeutic and energy production applications of this powerful antioxidant have also been explored extensively [59].

Located within the mitochondrial matrix are lipoic acid requiring enzmes: three a-keto acid dehydrogenase complexes, that catalyze the oxidative decarboxylation of a-keto acids such as pyruvate, a-ketoglutarate, and branched chain a-ketoacids [1]. In organisms, hydrogen atom transfer and acyl group transfer take place in the oxidative decarboxylation of a-ketoacids with the aid of a-lipoic acid. The reversible redox reaction between a-lipoic acid and dihydrolipoic acid is thus a very important biochemical reaction. The reversible reduction to dihydrolipoic acid is favored by the presence of the ring strain in the 1,2-dithiolane ring of about 15-25 $kJmol^{-1}$[59].

The multienzyme complex, pyruvate dehydrogenase, consists of three enzymes, pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3) [1]. This enzyme complex participates in five sequential reactions during the conversion of pyruvate to acetyl-CoA. The lipoic acid is covalently linked to a e-amino group of lysine residue via an amide linkage. These lipoic acid containing enzymes participate in four out of the five reactions.

The multienzyme complex, a-ketoglutarate dehydrogenase, also consists of three enzymes, a-ketoglutarate dehydrogenase (E1), dihydrolipoyl transsuccinylase (E2), and dihydrolipoyl dehydrogenase (E3) [1].

The branched chain a-ketoacid dehydrogenase is also a multienzyme complex resembling the other two enzymes mentioned above. These three enzymes have the same dihydrolipoyl dehydrogenase and employ the coenzymes thiamine pyrophosphate, lipoamide, FAD and the terminal oxidizing agent NAD^+ [1]. The importance of lipoic acid in the energy metabolism is illustrated by these three enzymes.

14. Synthesis of Palladium a-Lipoic Acid Complex

The synthesis of copper, zinc, and arsenic complexes of a-lipoic acid have been reported [60, 64]. Palladium a-lipoic acid complexes with (1:2) [64] and 1;1 [65] stoichiometry have also been reported. Details of synthesis of palladium a-lipoic acid complex (1:1) using alkaline sodium lipoate and H_2PdCl_4 in basic conditions and its possible applications for treatment of tumors and psoriasis are also available [66-68].

15. Investigations Using Palladium a-Lipoic Acid Complex Formulation

The different characteristics of this complex in comparison with that of the ligand are described in this section. These include in vitro cell lines studies, animal mitochondria, radiation protection, animal glioblastoma studies, human safety studies, voltammetry, and impedance spectroscopic studies.

15.1. Voltammetric Studies of Palladium a-Lipoic Acid Complex Formulation

The anodic oxidation of a-lipoic acid at a glassy carbon electrode and palladium a-lipoic acid complex interaction with double stranded DNA have been investigated using atomic force microscopy and voltammetry at highly oriented pyrolytic graphite electrode [69, 70]. An important observation was the dissociation of the palladium a-lipoic acid complex at negative potentials and deposition of Pd(0) nanoparticle deposition.. The application of a positive potential induced the oxidation of the palladium a-lipoic acid complex and the formation of a mixed layer of lipoic acid and palladium oxides.

15.2 Oxygen Radical Absorbance Capacity or ORAC analysis of Palladium a-Lipoic Acid Complex Formulation

ORAC assay measures the oxygen radical absorbance capacity of a compound as compared to Trolox (vitamin E). These analyses carried out by Brunswick Labs, Inc., Wareham, Massachusetts gave the following normalized values as Trolox equivalent per gram: Vitamin A 1.6; Vitamin C 1.12; Vitamin E 1.0; a-Lipoic acid 1.4; and Palladium a-lipoic acid complex formulation 5.65. Thus the superior free radical scavenging capacity of the palladium a-lipoic acid complex formulation is obvious. This radical scavenging superiority of the metal complex compared to that of the ligand is similar to the antitumor activities observed for metal complexes [15,16].

15.3. In Vitro Cell line Studies using Palladium a-Lipoic Acid Complex Formulation

The effects of the palladium a-lipoic acid complex formulation on the following 8 different cell lines were examined at K.G.K. Synergize Inc, Canada. 1) Skin melanoma, human (SKMel-5); 2) Liver, hepatocellular carcinoma, human (Hep G2); 3) Lung, malignant melanoma, human (malme-3M); 4) Mammary gland, ductal carcinoma, human (MDA-MB 435); 5) Prostate, left supraclavicular lymph node carcinoma, human (LNCaP): 6) Colon, colorectal adenocarcinoma, human (HT-29); 7) Human brain, glioblastoma; astrocytoma (U87); 8) Glioblastoma (U-251MG). Palladium a-lipoic acid formulation was administered at 3 different dosages and the cell growth was measured using [^{3}H]thymidine uptake after 24, 48, and 72 hours of culture. The data shown in Figure 2 is 48 hours after exposure. Palladium a-lipoic acid formulation was effective to varying degrees of cell death (statistically significant level of cell death), on the entire group of cell lines tested. The varying effectiveness appears to be a consequence of the particular cell lines used and their associated degree of anaplasia.

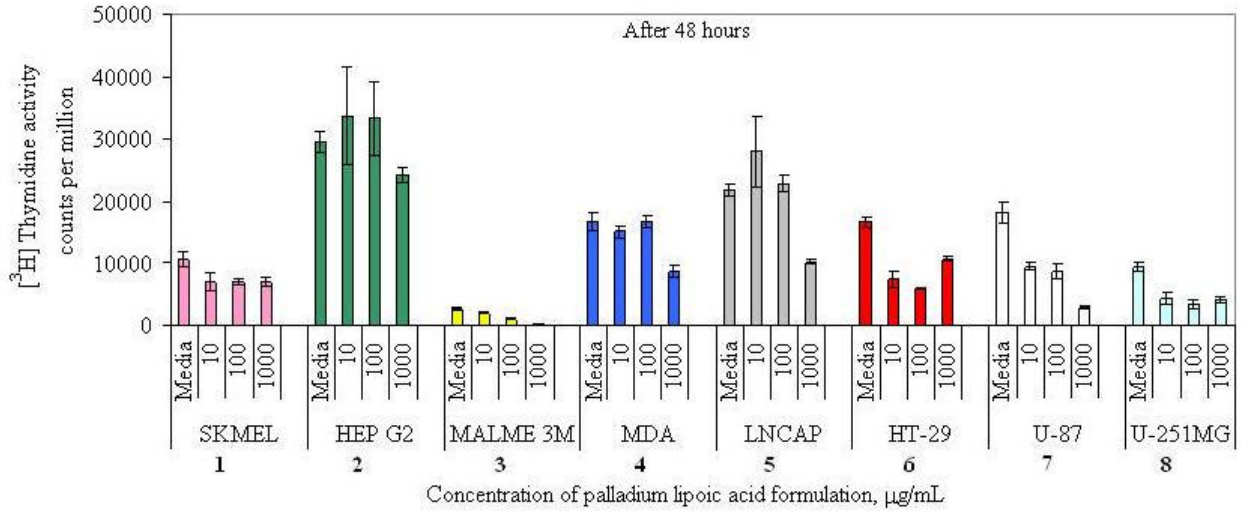


Figure 2. Effect of palladium a-lipoic acid complex formulation, after 48 hours, on 1) Skin melanoma, human (SKMel-5); 2) Liver, hepatocellular carcinoma, human (Hep G2); 3) Lung, malignant melanoma, human (malme-3M); 4) Mammary gland, ductal carcinoma, human (MDA-MB 435); 5) Prostate, left supraclavicular lymph node carcinoma, human (LNCaP): 6) Colon, colorectal adenocarcinoma, human (HT-29); 7) Human brain, glioblastoma; astrocytoma (U87); 8) Glioblastoma (U-251MG)

The effect of palladium lipoic acid complex formulation on the growth of canine osteosarcoma (CCL-183, D17) cells was also examined in vitro by a similar procedure. While the lowest dose did not have any significant effect, the higher doses of 100 and 1000 µg/mL inhibited the growth of the cells after 48 and 72 hours of culture.

We have examined the effects of palladium a-lipoic acid complex formulation on different cell lines from National Cancer Institute's (NCI) repository, breast cancer (adenocarcinoma, MCF-7), brain tumor (stage IV glioblastoma multiform, U-251Mg), lung (non-small cell carcinoma, A-549), and brain (astrocytoma, H-4), ovarian cancer (OVCAR-5) using NCI's cell screening protocol, sulforhodamine B assay. The results shown in Figure 3 indicate significant cell death.

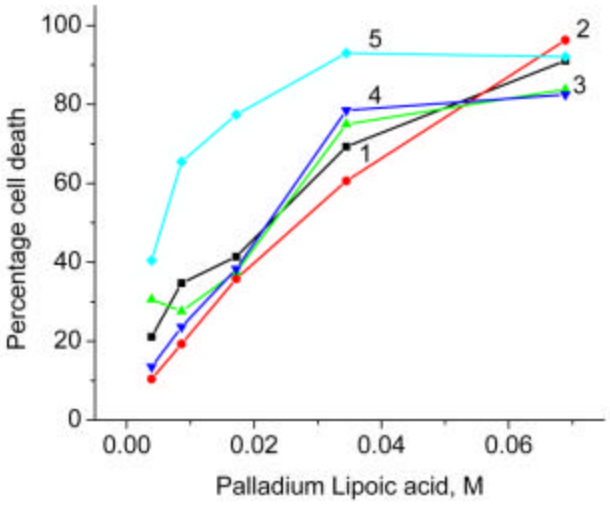


Figure 3. Effect of palladium a-lipoic acid complex formulation on 1) Breast cancer (adenocarcinoma, MCF-7), 2) brain tumor (stage IV glioblastoma multiform, U-251MG), 3) lung (non-small cell carcinoma, A-549), 4) brain (astrocytoma, H-4), and 5) ovarian cancer (OVCAR-5)

Whether we use [^{3}H]thymidine uptake assay or suforhodamine B assay, significant reduction in cell growth is observed in a variety of cell lines.

15.4. In Vivo Studies of Palladium a-Lipoic Acid Complex Formulation

These studies were carried out at Calvert Labs (previously known as Pharmakon USA), PA, USA.

The Ames/Salmonella Plate incorporation assay confirmed that the complex formulation is free of mutagenicity. Also acute oral toxicological studies showed no accumulation in or damage to any tissues. The median lethal dose, LD_{50}, in mice was found to be greater than the highest dosage tested, 5000mg kg^{-1}

The effectiveness of palladium lipoic acid formulation in halting the growth of glioblastoma cells in vivo was studied using nude mice. On day zero, 10 million U-87 MG tumor cells (glioblastoma-astrocytoma, human) from American Type Culture Collection (ATCC) were injected subcutaneously in the scruff of the neck of female Swiss nude mice (11 weeks old). When the tumors reached 200 to 400 mm3 in volume, the mice were divided into 8 groups of 10 mice. Four groups of mice were given daily intravenous (i.v.) doses of this formulation or placebo (0.9% saline); four groups were given orally by gavage (p.o.) doses of 0.5, 1.0, or 2.0 mg palladium lipoic acid complex per mouse for a total of 4 weeks or until the tumors became too large for the viability of the animal. Tumor volume was measured throughout the study, twice per week.

A reduction in tumor size compared to the placebo treated group was seen in all groups of mice treated orally with the palladium lipoic acid complex. However the only statistically significant reduction was in the group treated with 1 mg/mouse.

When mice were treated intravenously with the palladium lipoic acid complex, a statistically significant reduction was observed in all treatment groups compared to the placebo group.

15.5. Clinical Veterinary Studies

The largest integrative cancer investigation of palladium lipoic acid complex formulation was an open-label, veterinary oncology program at CVS Angel Care Cancer Center, San Diego, CA, USA, with over 900 dogs enrolled. The dogs received palladium lipoic acid complex formulation as part of their chemotherapy, radiation and/or surgical protocol at a dosage of 1mL/2.3kg. p.o. twice daily (equivalent human dose of approximately 40 mL per 70 kg.). The palladium lipoic acid formulation seemed most effective in the cases of solid tumors (such as soft tissue sarcoma, hemangiosarcoma, mast cell, transition cell carcinoma, lung, anal sac carcinoma, renal carcinoma, squamous cell carcinoma, fibrosarcoma, melanoma, meningioma, neuroblastoma, and mammary adenocarcinoma). Some of the most effective findings were apparent in the dogs suffering from osteosarcoma. The etiology of osteosarcoma in large dogs is considered identical to the disease progression in humans. While in canines the "standard of care" is limb amputation followed by chemotherapy, in human patients, limb–sparing surgery following tumor excision is performed [71]. The results summarized in Table 10 suggest the following. In this open labeled study, integrative palladium lipoic acid complex formulation support improved the animals' median survival time 62% (103 days more) compared to surgery alone (n= 11 and 162, respectively). When the palladium lipoic acid complex formulation was added to the chemotherapeutic regimen (carboplatin + doxorubicin) the dogs exhibited a 27% longer median survival (79 days more). Furthermore, there was no significant difference (p=0.30) in median survival time between dogs treated with amputation + palladium lipoic acid complex formulation versus those that were treated with amputation + the "standard of care" chemotherapy.

Table 10. Open label veterinary Oncology Study using Palladium a-Lipoic Acid Complex Formulation

Study	Median Survival, days
Amputation alone (n = 162)	165
Amputation with palladium lipoic acid complex formulation	

(n = 11)	268
Amputation with chemotherapy (n = 32)	288
Amputation with chemotherapy + palladium lipoic acid complex formulation (n = 17)	367

It was observed that following palladium a-lipoic acid complex formulation complementary support, chemotherapeutic animals' demonstrated improvements in various objective parameters (i.e. weight, anemia, liver and kidney function). In addition to these enhanced clinical parameters, a subjective owner quality of life survey resulted in an 86% improvement following the addition of palladium a-lipoic acid complex formulation adjunctive support.

15.6. Transient Ischemia Studies with Gerbils

Animal studies, carried out at Stony Brook University using adult male Mongolian gerbils (Charles River, Inc., New York), used as controls or treatment group, demonstrated that acute, post ischemic and prophylactic administration of palladium a-lipoic acid complex formulation limits ischemic damage. The animals were sacrificed after 72 hours after transient ischemia surgery (n =6 per surgical group; n = 6 per sham group, each trial in triplicate) [72]. The palladium a-lipoic acid complex formulation was administered intraperitoneally (IP) immediately after surgery, then once daily for 3 days. The control group received saline while the treatment group received 30, 50 or 70 mg/kg of palladium lipoic acid formulation.

Selective neural damage to hippocampal cornus ammon's field 1 of the hippocampus (CA 1) neurons takes place after transient ischemic attack. An activation of the pro-apoptotic protein, bax, results in a shift in the dimerization ratio between the anti-apoptotic protein bcl-xl and bax. The increase in bax results in the formation of pores in the mitochondrial membrane and these pores facilitate the passage of the electron transport chain protein, cytochrome c, into the cytosol along with the release of the pro-caspase 9. The initiator caspase 9 activates the caspase family of cysteine proteases and results in the destruction of the cell.

Following bilateral carotid artery occlusion in the Mongolian gerbil, palladium lipoic acid complex formulation treatment significantly protected CA1 hippocampal pyramidal cells from transient global ischemia at 30 ($p<0.05$), 50 ($p<0.01$), and 70 ($p<0.05$) mg/kg per 24 h.

A delayed application of the palladium a-lipoic acid complex formulation after 48 hours of ischemic attack had no significant effect in protecting CA 1 cells. On the other hand, a delayed administration of palladium a-lipoic acid complex formulation after 6 hours of ischemic attack was as good as giving it immediately after ischemic attack in minimizing cell death.

Nesting behavior is an inherent behavior in Mongolian gerbils. Five minutes of carotid artery occlusion was sufficient to hinder or impair nesting behavior for approximately 3 days. The nesting behavior of gerbils was observed to improve significantly after treatment with palladium lipoic acid complex formulation (50 mg/kg every 24 h ($P<0.05$) and 30 mg/kg /24h at 24 and 72 hours after ischemia. There were no significant differences after the 70 mg/kg/24h treatment (n= 6 per group, each experiment was conducted in triplicate). The 70 mg/kg-treated animals demonstrated excessive energy, thus ignoring the nesting material.

It was observed that preventive or prophylactic treatment with 10 mg/kg gerbil (based on allometric scaling from rodent to human, 10 mL-human dosage) offered significant behavioral and morphological improvement from transient global ischemia. While behavioral improvement was apparent with 3 days of pretreatment, approximately one week of pre-treatment was necessary for morphological rescue.

In summary, treatment with palladium a- lipoic acid complex formulation after a transient ischemic attack offers behavioral improvement as well as morphological protection of CA1 hippocampal pyramidal cells.

15.7. Clinical Human Studies

A Phase I, palladium a-lipoic acid complex formulation "dose escalation safety study in normal individuals" (DESSTINI) was carried out at Stony Brook University, New York, USA. This study was divided into three tiers, each consisting of five subjects. Tier I, Tier II, and Tier III received oral dosages of the formulation, 10, 20, and 40 mL/day respectively for a period of six weeks. Subjects were monitored for the washout of palladium by examining the concentration of palladium in both blood serum and urine. Washout periods ranged from three to seventeen weeks after cessation of the formulation. However, the washout period did not appear to be related to dosage. No serious adverse effects occurred.

The 15 subjects experienced a total of 24 AE (Adverse Events) during the study that were considered potentially, possibly or probably related to the study formulation. The events included: fatigue after cessation of oral dosage, diarrhea, worsening leg cramps, headache, increased urination, light-headedness, difficulty sleeping, and increased excitement. All AEs were adjudicated by the Data Safety and Monitoring Board (DSMB), with approximately 66% being anticipated or considered mild. Overall, the tolerability of all three tiers was 93.3% and the DSMB deemed the formulation to be safe. In addition, DSMB gave consent to continue with a subsequent, ongoing glioblastoma trial.

15.8. Mitochondrial Studies using Palladium a-Lipoic Acid Complex Formulation

The results with the transient ischemia studies with gerbils prompted us to investigate the influence of palladium lipoic acid complex on the activities of enzymes involved in energy production. In eukaryotes and prokaryotes, the most common mode of oxidative degradation of

carbohydrates, fatty acids, and amino acids is by the citric acid cycle or the tricarboxylic acid cycle or Krebs cycle. The net reaction is

$3NAD+ + FAD + GDP + Pi + acetyl\text{-}CoA \rightarrow 3NADH + FADH_2 + GTP + CoA + 2CO_2$ (40)

The liberated energy is used for ATP generation. The influence of palladium lipoic acid complex formulation has been investigated on the activities of four Krebs cycle enzymes, isocitrate dehydrogenase (ICDH), a-ketoglutarate dehydrogenase (a-KGDH), 6) succinate dehydrogenase (SDH), and 8) malate dehydrogenase (MDH). The other 4 enzymes of the Krebs cycle, citrate synthase, aconitase, succinyl-CoA synthetase, and fumarase as well as the enzyme, pyruvate dehydrogenase were not investigated. These investigations were carried out Amala Cancer Research Centre, Kerala, India [73, 74].

Male albino rats of Wistar strain and 24-26 months old were used to study their hearts. Each group had six rats and the animals were sacrificed at the end of 30 days of oral administration. The results for the Krebs cycle enzymes are given in Table 11.

Table 11. Effect of Palladium Lipoic Acid Complex on the Activity of Krebs Cycle Enzymes [73]

Groups	ICDH	a-KGDH	SDH	MDH
Aged control	702.8±133.4	63.0±15.1	42.4±14.2	260.9±26.1
DL-a-lipoic acid (5 mg/kg body mass)	3428.2±348.9	189.0±50.4	73.4±21.2	1386.1±265.5
Palladium lipoic acid complex, (0.38 mg/kg body mass)	3483.1±388.9	145.0±50.6	98.6±7.4	1305.7±56.4

Units: ICDH - µ moles of NAD+ reduced /min/mg protein; a-KGDH - µ moles of NAD+ reduced /min/mg protein; SDH - µ moles of 2,6-dichlorophenol indophenol sodium salt (DCPIP) reduced /min/mg protein; MDH - µ moles of NADH oxidized/min/mg protein

Table 12. Effect of Palladium Lipoic Acid Complex on the Activity of Respiratory Complexes [73]

Groups	Complex I	Complex II	Complex III	Complex IV
Aged control	23.34±2.12	26.75±2.09	13.57±3.89	30.85±1.31
DL-a-lipoic acid (5 mg/kg body mass)	62.04±11.90	45.55±28.25	21.16±8.36	47.36±7.54
Palladium lipoic acid	58.76±31.11	83.37±28.46	21.34 ±3.31	48.13±7.32

complex,
(0.38 mg/kg body mass)

Units: Complex I - µ moles of DCPIP reduced/min/mg protein; complex II - µ moles of DCPIP reduced/min/mg protein; Complex III - µ moles of ferricytochrome-C reduced/min/mg protein; Complex IV - µ moles of ferrocytochrome-C oxidized/min/mg protein

The activities of ICDH, a-KGDH, SDH and MDH, when compared to the aged control animals, indicate that administration of palladium lipoic acid complex formulation significantly increased the Krebs cycle enzyme activities. Both the a-lipoic acid and palladium lipoic acid complex increased the activities of the enzymes.

The results for the respiratory complexes I, II, III, and IV in aged rats is given in Table 11. The enhanced activities of complexes I, III, and IV were very similar for both the palladium lipoic acid formulation and the a-lipoic acid administered groups. The average values indicate a ~2.5, 1.6, and 1.6 fold increase for the activities of the complexes I, III, and IV when compared to the aged control group. In the case of complex II, the palladium lipoic acid complex formulation group had ~1.8 fold increases in the activity compared to the a-lipoic acid administered group.

However the superiority of the palladium lipoic acid complex is evident from the fact that the actual lipoic acid equivalent in the metal complex used for the oral administration is 13.2 times less than that of the ligand. The major question which is a still an unsolved puzzle is the source of the superiority of the metal complex compared to that of the ligand. Since we know from the pharmacokinetics of lipoic acid that the oral dose and the available plasma concentration are completely different, it is possible that the available plasma concentration for therapeutic effect in the presence of the palladium lipoic acid complex may be much higher than that of the lipoic acid only. Or somehow the chemistry of the transition metal is playing a dominant role in the enzymatic activity. It is interesting to note that the 2010 Noble Prize in chemistry was awarded to three Palladium chemists, Richard F. Heck, Ei-ichi Negishi, and Akira Suzuki for “palladium-catalyzed cross-couplings in organic synthesis”. While their methods are used by pharmaceutical industry for the synthesis of at least 25 % of drugs, it is ironic that no palladium-based drugs are available in the market today. However, a palladium lipoic acid complex formulation had been available in the market for more than 15 years as a dietary supplement. The starting material in the synthesis of the palladium lipoic acid complex is palladium(II). If the final complex is also palladium(II), the chances are it has no paramagnetism because almost all palladium (II) complexes are diamagnetic. An ESR/EPR spectrum would help solve this puzzle. It is also possible, that under physiological conditions, if dihydrolipoic acid is produced through one electron reduction processes, then it is possible to have reactive intermediates with unpaired electrons. In such a case the electron spin may be involved in the

enzymatic process. The impedance characteristics of the palladium lipoic acid as well as that of the lipoic acid, described later in this review, strongly suggest this possibility.

Male albino Wistar strain rats, both young and old, were also used to examine the declined mitochondrial antioxidant status in the myocardium of aged rats. The animals were administered orally for 30 days 0.38 mg lipoic acid and an equivalent dose of lipoic acid from the palladium lipoic acid complex formulation. The results for Mn SOD, CAT, and GP_x are given in Table . As expected the young group had higher levels of all the three enzymes than that of the aged control group. Also the levels of Mn SOD, CAT, and Gp_x were higher with the palladium lipoic acid treated group than with the a-lipoic acid group.

Table 13. Effect of Palladium Lipoic Acid Complex on the Activities of Enzymes in the Heart Mitochondria of Rats [74]

Groups	Mn SOD, U/mg protein	CAT U/mg protein	GP_X U/mg protein
Aged control	12.23±2.33	4.05±0.82	22.70±4.24
Young control	16.34±1.17	9.61±1.17	73.24±20.65
DL-a-lipoic acid (0.38mg/kg body mass)	15.59±5.31	8.26±1.48	168.58±63.74
Palladium lipoic acid complex, 0.38 mg/kg body mass)	12.72±5.94	4.81±1.34	64.19±15.50

Mn SOD = manganese superoxide dismutase; CAT = catalase; and GPx = glutathione peroxidase

The results of the lipid peroxidation levels measured as thiobarbituric acid reacting substance (TBARS) and expressed as equivalents of malondialdehyde (MDA) and the glutathione levels are given in Table

Table14. Lipid Peroxidation and GSH Level in the Heart Mitochondria of Aged Rats [74]

Groups	Lipid peroxidation, n moles of MDA formed/mg protein	GSH (moles/mg protein)
Aged control	1.94±0.27	5.08±0.39
Young control	0.88±0.06	6.78±0.45

DL-a-lipoic acid (0.38mg/kg body mass)	1.68±0.19	5.20±0.18
Palladium lipoic acid complex, (0.38 mg/kg body mass)	1.17±0.09	6.42±0.35

As expected, the lipid peroxidation level was less and the glutathione level was higher in the young control compared to that of the aged control. There was no significant different difference between the aged control group and the a-lipoic acid group for both the lipoic peroxidation level and glutathione level. However, the lipid peroxidation was less and glutathione levels were higher with the palladium lipoic acid complex formulation group when compared to the aged control group.

The Krebs cycle and mitochondrial respiratory chain enzymatic studies data also indicate that the palladium lipoic acid complex is catalytically more active than that of the ligand, a-lipoic acid. This is similar to the observations of antitumor activity of iron (III) and copper (II) complexes of N^6-benzylaminopurine derivatives and palladium (II) –benzyl bis (thiosemicarbazonate) where the complex had more activity than the ligand [15, 16].

Figure 4 summarizes the influence of palladium a-lipoic acid complex formulation on the activities of some of the Krebs cycle enzymes and mitochondrial respiratory enzymes. The percentage increases in enzymatic activities are indicated by an upward arrow. The CoQ is pictorially shown twice for convenience to show that the electron transfer from complex I and complex II are to CoQ and then to complex III.

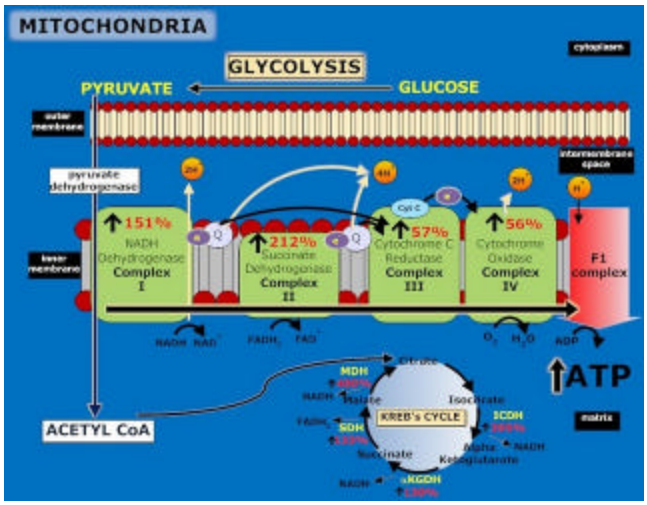


Figure 4. Influence of palladium a-lipoic acid complex formulation on Krebs cycle enzymes and mitochondrial electron transport chain complexes

15.9. Protection from Radiation using Palladium a-Lipoic Acid Complex Formulation

These studies were also carried out at Amala Cancer Research Centre, Kerala, India [75, 76].

In vivo radioprotection of cellular DNA was investigated using six to eight weeks old Swiss albino mice exposed to 8 Gy radiation from ^{60}Co at a dose rate of 1.88 Gy per min. The palladium lipoic acid complex formulation dose (oral), administered one hour prior to radiation exposure, was 1mL/kg and 2 mL/kg body mass for two different groups. After one hour, the animals were sacrificed and their blood leukocytes and bone marrow were examined for DNA damage using alkaline single cell gel electrophoresis (alkaline comet assay) and compared with those with sham irradiation and with distilled water as control. The comet parameters, DNA in tail, tail length, tail moment and olive tail moment were analyzed. All the comet parameters were significantly reduced in animals administered with the palladium lipoic acid complex formulation one hour prior to the radiation exposure. This significant reduction in DNA damage in mice receiving palladium lipoic acid complex formulation with doses of 1mL/kg and 2 mL/kg body mass demonstrates the in vivo radioprotection ability of the palladium lipoic acid complex.

15.10. Antioxidant Activity, Prophylactic Effects of Palladium a-Lipoic Acid Complex Formulation Determined from Radiation Experiments

The antioxidant activity of palladium lipoic acid formulation was examined using 4 groups of Swiss albino mice, 6-8 weeks old, one group receiving distilled water and the other group palladium lipoic acid complex formulation, 2 mL/kg body mass. Two other groups similar to the earlier ones received radiation exposure, 6Gy at the rate of 1.88 Gy per minute. The animals were sacrificed after 7 days of administration, and then radiation to two groups. Liver, kidney and brain were examined for lipid peroxidation, glutathione, super oxide dismutase, and glutathione peroxidase.

Table 15. Antioxidant Activity of Palladium a-Lipoic Acid Complex Formulation in Liver, Kidney and Brain of Mice [75]

Groups	Lipid peroxidation, nano moles of MDA formed/mg protein	GSH (nanomoles/ mg protein	Super oxide dismutase U/mg protein	Gutathione Peroxidase U/mg protein
1) Liver				
With radiation	4.65±0.86	15.28± 1.73	8.36±0.63	14.07±2.87
With complex & with radiation	1.82± 0.36	21.46±3.30	10.90±0.50	22.44± 2.40

2) Kidney				
With radiation	8.62±0.76	21.19±7.25	0.45±0.09	24.48±2.30
With complex & with radiation	5.44±0.98	42.61±4.61	0.98±0.29	39.28±10.28
3) Brain				
With radiation	16.94±2.04	55.60±14.58	0.68±0.10	32.68±2.90
With complex & with radiation	11.42±0.79	126.81±9.43	1.08±0.09	44.10±2.90

6 Gy radiation, 7 days palladium lipoic acid complex formulation dose before irradiation, 2 mL/kg body mass

The differences between the distilled water group and the palladium lipoic acid treated group were not statistically significant for all the enzymes studied. On the other hand the data given in table for the group that received water and the group that received 2mL/kg body mass palladium lipoic acid complex formulation for 7 days prior to 6 Gy irradiation indicate remarkable differences in each case. The glutathione, glutathione peroxidase, and superoxide dismutase levels were higher and the lipid peroxidation levels measured as thiobarbituric acid reacting substance (TBARS) and expressed as equivalents of malondialdehyde (MDA) were lower in liver, kidney and brain for the group that received palladium lipoic acid complex formulation for 7 days prior to 6 Gy irradiation. This clearly indicates the prophylactic protective effect of palladium lipoic acid complex formulation from radiation. It was also observed that 6 Gy radiation significantly reduced GSH , GPx, and SOD levels and increased the lipid peroxidation levels compared to the controls that received no radiation.

Radiation induced significant lowering of antioxidant levels. Administration of palladium lipoic acid complex formulation for 7 days, at a dose rate of 2 mL/kg body mass, kept nearly the same levels of antioxidants as the ones that received no radiation. It is not clear at this time whether this observation is the due to the ability of the tissues to counteract the reactive oxygen species generated from radiation injury or the ability of the tissue to regenerate the cellular antioxidants in response to radiation injury.

Oral administration of palladium lipoic acid complex formulation to male Balb/C mice, 6-8 weeks old, exposed to sub lethal 6 Gy ?-radiation enhanced endogenous spleen colony formation. Also alkaline comet assay showed that nuclear DNA comet parameters such as percent DNA tail, tail length, tail moment, and olive tail moment of the bone marrow and spleen cells increased after the whole body radiation of 8 Gy. These DNA damages as well as mortality rates were reduced by the administration of palladium lipoic acid complex formulation. Also it aided in the recovery from radiation induced weight loss in mice surviving after 8 Gy radiation.

These studies, carried out at Amala Cancer Centre coupled with the toxicity and cell line studies suggest the following. Unlike toxic platinum chemotherapy agents, the commercially available palladium a-lipoic acid complex formulation is safe and nontoxic. Its oral administration can be continued indefinitely. It is specially designed to provide energy for compromised body systems and promote overall health. It facilitates aerobic metabolism much more than that of a-lipoic acid, by significantly enhancing enzymatic activity of isocitrate dehydrogenase, a-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase at the Krebs Cycle and mitochondrial complexes I, II, III and IV of the electron transport chain in the heart of aged rats [73]. It also enhances the activities of catalase and glutathione peroxidase more than that of a-lipoic acid. The level of glutathione also was significantly improved and the level of lipid peroxidation was decreased in the heart mitochondria of aged rats [74]. It also protects DNA from radiation.

It must be pointed out that preventive or prophylactic effects observed in these radiation experiments are consistent with the observations of similar effects in gerbils after induction of transient global ischemia [72].

16. Diode or Tunnel-Diode Behavior in Biological Systems

To gain an understanding of the electronic aspects of biological functions such as cell signaling, long-range electron transfer, and biochemical oscillations in ATP production [77], one needs at least a rudimentary knowledge of the solid state electronics. A very brief attempt is made here to introduce the concepts.

A diode material is normally doped with one impurity atom per ten-million semiconductor atoms. This results in a relatively wide depletion region. When the potential applied is large enough to overcome the potential barrier of the junction, conduction takes place[78]. In a tunnel diode, on the other hand, the doping level is about thousand impurity atoms per ten million semiconductor atoms. This results in an extremely narrow depletion region. Compared to a normal junction diode, the tunnel diode exhibits an unusual current-voltage characteristic curve, a negative differential resistance region[78].

Protein film voltammetry data[79-86] have indicated that the catalytic activity of enzymes, especially electron transport enzymes involved in the respiratory chains, may be optimized at certain electrochemical potentials as well as pH. This technique allows the rate of catalysis to be measured accurately as a function of the applied potential (the driving force). Interesting current-potential curves were observed for several enzymes in which the optimum rate occurs at a particular potential and the rate thereafter drops in spite of the increase in the thermodynamic driving force. We must keep in mind that the active site, such as in flavins and Mo-bismolybdopterin guanine dinucleotide cofactors, may be the oxidized state, intermediate state or reduced state and these states may have their own characteristic affinities for the substrate before the reaction and for the product after the reaction. This offers the possibility for

catalytic pathway by several different routes [79]. The electrochemical potential controls the rate and thermodynamics of electron supply for the different states. A unique current-potential curve, often observed for respiratory enzymes such as succinate:ubiquinone oxidoreductase (complex II of mitochondria, with active site flavin adenine dinucleotide (FAD) and three Fe-S clusters)) and molybdoenzyme nitrate reductases, is interpreted in terms of a "potential dependent gate that bars catalysis of the reverse process" [79]. This intrinsic property of the enzyme is similar to the behavior of a tunnel diode with a characteristic negative differential resistance region observed in the current potential curves. In SDH, the group responsible for this behavior is attributed to the active site FAD. Similar to the electrochemical potential, the membrane provides a variable potential in the form of the ratio of quinone to hydroquinol (Q/QH_2). This potential can be further tuned depending on the nature of the quinone present. At lease three different quinones (ubiquinone being the dominant redox carrier during aerobic growth and menaquinone and demethylmenaquinone dominating under anaerobic conditions) are synthesized depending on the aerobicity.

An attenuation of the reductive activity at low potential was also observed for the mitochondrial enzyme, the "Fp" subcomplex of Complex I. This is understandable because, mitochondrial complex I (NADH-Ubiquinone oxidoreductase) also houses a flavin at the site of NADH oxidation and nine iron-sulfur clusters.

Our reason for including this section on tunnel diode behavior in biological systems is to indicate the "ratchet" or biased nature of enzymes systems depending on the potential and pH allowing possible feedback fine control of respiratory rates. We have demonstrated the ability of an enzyme to "rectify" electron flow at potentials close to electrochemical reversibility by studying impedance characteristics of simple biological molecules that are an integral part of the enzyme. For example, some of these enzymes have FAD, and Mo and we had shown in impedance studies the negative differential resistance characteristics (NDR) of FAD as well as Mo-peroxo complexes [87].

17. Impedance Spectroscopy

We have utilized the technique of impedance spectroscopy for understanding solute-solvent interactions, 'p –way' conduction, ion pair formation, water-structure enforced ion pair formation, potential induced and solvent mediated ion pair formation at the double layer, and semiconduction characteristics of simple biological molecules [87-94]. Simple molecules such as arginine, histidine, lysine, flavin adenine dinucleotide, riboflavin, cysteine, lidocaine hydrochloride, a-lipoic acid, and hydrogen peroxide exhibit negative differential resistance, which is a characteristic of diode or tunnel diode behavior. This technique has not been utilized extensively for discovery of drug molecules. Our technique of exploring drug discovery is based on impedance characteristics and self assembly and differs from the conventional drug discovery techniques. A brief outline of this technique [95, 96] is given below.

In impedance measurements, a perturbing sinusoidal voltage $E = E_o \sin(\omega t)$ is applied at angular frequency ω ($2\pi f$, where f in the conventional frequency in Hz) to the electrode system. The response is analyzed in terms of the resultant current $I = I_o \sin(\omega t + \phi)$, where ϕ represents a characteristic phase angle shift. The corresponding complex impedance spectrum $Z(\omega)$, obtained by varying the signal frequency ω, is expressed in terms of the displacement of the vector $Z(\omega)$. In the plane of Cartesian coordinates, an impedance is expressed by its real (Z') and imaginary (Z'') parts, i.e. $Z(\omega) = Z' - jZ''$. The modulus $|Z|$ and phase angle ϕ of $Z(\omega)$ can be obtained from $|Z| = [Z'^2 + Z''^2]^{1/2}$ and $\phi = \tan^{-1} [Z''/Z']$, respectively. Over a frequency bandwidth of interest, the impedance spectrum can be represented in various ways; typically in the well known Nyquist or Cole-Cole plot (Z'' as the Y-axis and Z' as the X-axis for the range of frequencies explored at a fixed potential) or Bode plots ($|Z|$ and ϕ vs. $\log\omega$). The impedance spectrum reflects dialectic behavior, oxidation-reduction reactions and mass migration across the electrochemical interface, which are determined by the electrical and chemical properties of the corrosive medium, and the electrode materials. The impedance spectrum can also be considered as a 'fingerprint', which is related to the transient behavior of a specific electrochemical interface. In simple terms, impedance is like a frequency dependent generalized resistance. In electrochemistry, the imaginary impedance is almost always capacitive and therefore negative. Phase angle is a balance between capacitive and resistive components. For a pure resistance $\phi = 0$ and for pure capacitance $\phi = \pi/2$.

The electrochemical impedance measurements reported in this chapter were made using and EG & G PARC Model 303A SMDE trielectrode system (mercury working electrode, platinum counter electrode and Ag/AgCl saturated KCl reference electrode) along with Autolab ecochemie. The measurements were carried out in the range 1000Hz to 30 mHz. The amplitude of the sinusoidal perturbation was 10 mV.

In our present studies we have explored the behavior of mercury in both the negative and positive range of potentials because in natural biochemical systems we have both positively and negatively charged surfaces at close distances where water molecules will be subjected to competing influences from the electric field of these charged centers as well as the charges from the electrolytes. We have used mercury as the working electrode because it allows us to get reproducible surface for our studies by using a fresh drop each time. Compared to using any other metal, fresh mercury drops allow repetitions and reproducibility better and easy. For example if we corrode a metal, it is not easy to clean and get the noncorroded surface again and again for each experiment.

17.1. Electronic Properties of H_2O_2

All living systems exhibit dynamical spatio-temporal periodicities [77]. The dynamical oscillations observed in the electrochemical passivation of metals and used as models for biological oscillations are attributed to the negative Faradaic impedance of the electrode [47].

Potential or current oscillations of different types have been observed in hydrogen peroxide systems at high concentrations and high acidities [48, 97]. The coupling of two or more chemical oscillations occurring at different locations, an important aspect for signal transport or communication in biological systems has also been duplicated in an electrochemical system involving hydrogen peroxide (300 -400 mM in 0.5 M H_2SO_4) [98]. To simulate biological systems, we had focused our studies on oscillatory behavior at low concentrations of peroxide and at low acidities. We had established and reported [90] the concentration range needed to exhibit oscillations in the presence of NaCl. Here we report, as an example, the impedance behavior of 88 mM H_2O_2 in 0.10 M KCl at pH 4.5. It is obvious that negative differential resistance (NDR), a characteristic of tunnel diode behavior is observed at the potential shown in Figure 5. It was observed that NDR occurred at 3.72 Hz, 2.90 Hz, and 2.24 Hz at potentials of -0.36 V, -0.37 V and -0.38 V.

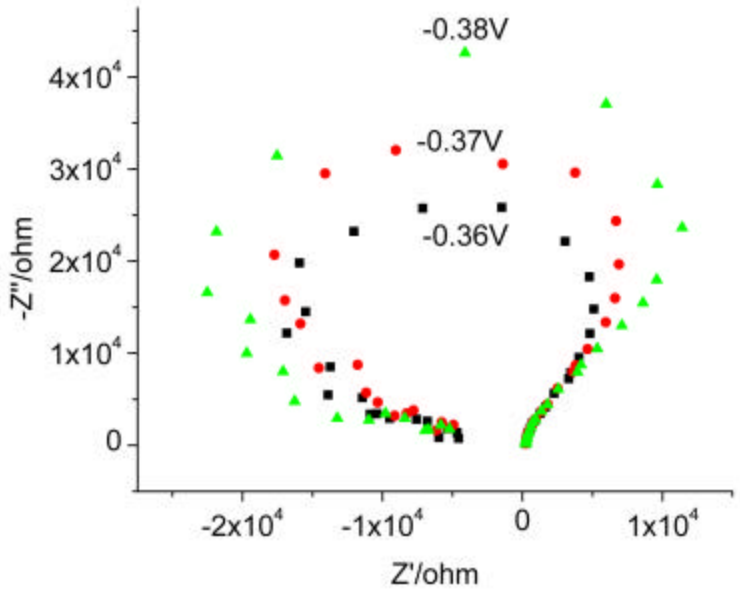


Figure 5. Nyquist plot for 0.10 M KCl, 88 mM hydrogen peroxide at pH 4.5

We had carried out detailed cyclic voltammetric and impedance investigations of aqueous solutions of H_2O_2 in the presence of NaCl to understand Parkinson's disease [90, 99]. Some relevant points from these studies of hydrogen peroxide in aqueous sodium chloride are illustrated here.

The negative impedance observed in Figure 5 is also seen at hydrogen peroxide concentrations as small as 10 mM and is sensitive to pH. At higher acidities, the potential at which NDR occurred shifted to more anodic potentials. Also, as the hydrogen peroxide concentration is decreased, the potential at which NDR occurred shifted to more anodic

potentials. The frequency at which NDR occurred also depended on the potential and concentration of hydrogen peroxide. We have also observed a sensitivity for chloride.

The importance of impedance measurements in understanding solute-solvent interactions is vividly illustrated in Figure 6. By comparing the admittance measurements of NaCl in the presence and absence of H_2O_2 at 3 different frequencies, it is obvious that the sodium and chloride ions are forming ion pairs under the influence of H_2O_2 at potentials in the range -0.25 to 0.0V.

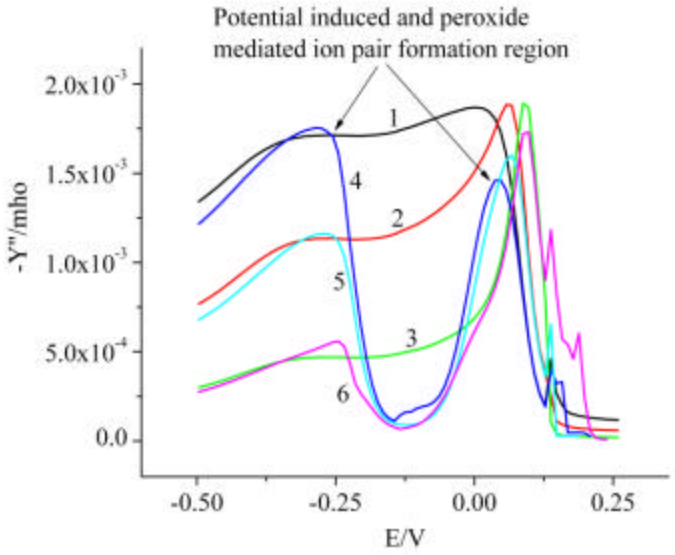


Figure 6. Admittance comparison of 0.10 M NaCl, pH 5.25 1) 500 Hz 2) 250 Hz 3) 100 Hz and 0.10 M NaCl and 88 mM H_2O_2, pH ~ 6.0 4) 500 Hz 5) 250 Hz 6) 100 Hz

To understand the admittance behavior of small amounts of H_2O_2 in 100mM NaCl, it is worthwhile to look at its relevant aqueous solution properties, The activity coefficient of hydrogen peroxide in sodium chloride and sodium sulfate solutions, determined using partition experiments with iso-amyl alcohol [100], was found to be less than unity. This salting in effect was later confirmed for several electrolytes [101]. These results suggest that water molecules surrounding the sodium ions were displaced by peroxide. This was attributed to the higher dipole moment of hydrogen peroxide compared to that of water. On the other hand, it was concluded from solubility measurements in hydrogen peroxide-water mixtures that smaller ions such as Li^+ and Na^+ were solvated by water and K^+, Rb^+ and Cs^+ were preferentially solvated by hydrogen peroxide [102].

The properties such as 1) deviations from Raoult's law, 2) finite heat of mixing and 3) finite volume changes on mixing for hydrogen peroxide-water mixtures indicate an enhancement or either the number or the force of attractions between the two molecules on forming the

solutions [103]. The decrease in conductance of alkali chlorides in hydrogen peroxide-water mixtures correlated well with the changes in viscosity. These results could not give any indication as to when the solvation of an ion changes from water to hydrogen peroxide [103].

It should be mentioned that the studies mentioned above were not at low hydrogen peroxide concentrations. The low concentrations employed in admittance measurements are comparatively low and closer to biological concentrations. However, the data are more complicated because of the double layer and changing potentials as well as frequencies. The results do suggest an ion-dipole orientations with peroxide preferentially, especially when the applied potential is about to change from negative to positive. We had explained these results using the concept of "potential induced and peroxide mediated ion pair formation" [90].

These indicate the role of peroxide not only in neuron degeneration but also in controlling of electronic circuits involved in neuronal communications. The role of the stimulator implants seems to be to counteract the role of the new circuits caused by neuronal degeneration. Also the variability of the electronic circuit or signaling produced by hydrogen peroxide depending on the concentration, the ion-dipole interaction with water and/or peroxide, and the potential available may partially explain the multiple roles of hydrogen peroxide in biological systems.

Before closing this section, we want to add a comment on another related topic, "mechanisms for DNA charge transport". This hot subject has been investigated extensively during the last 15-20 years [104]. Numerous arguments regarding the nature of DNA as to whether it is a wire, a semiconductor or an insulator have been proposed and discussed. The role of the p-stacked base pairs in DNA has also been investigated in detail. In our impedance measurements with aqueous lidocaine hydrochloride, we could see the influence of "p-way" conduction [92]. This technique has not been explored in detain in the case of DNA. More importantly our results with H_2O_2 indicate its unique electronic properties. These properties are very sensitive to its concentration, electrolyte, frequency and available potential. Our results along with that of salting in effect of peroxide by electrolytes suggest a mechanism by which an electrolyte can pass though membranes as an ion pair solvated by peroxide. The solvation effect of peroxide on DNA bases, and the DNA charge transport in the presence of small amounts of peroxide need to be investigated to get a deeper understanding of this important field. Unfortunately the varying and tremendous influences of peroxide on these processes such as ion pair formation, preferential solvation by peroxide and its unique electronic properties have been ignored or neglected so far. We hope physicists, chemists and biologists will take a serous look at these properties of hydrogen peroxide in relation to their investigations.

17.2. Modulation of Electronic Properties of Simple Molecules by Transition Metals

The target of most metal based drugs is DNA [8, 9]. Targets other than DNA that have recently been reviewed include a gold(I) carbene complex interacting with mitochondrial

membrane [10]. However, the focus of our work was to improve mitochondrial enzyme activity by selecting a simple molecule involved heavily in mitochondrial enzyme activities and modulate its enzymatic activity by complexing with the metal palladium. Palladium (II), Platinum(II) and gold(III) have the same number of d^8 electrons. We believe strongly that by improving the mitochondrial enzyme activities, we can improve the quality of life, can induce apoptosis, and thus ward off many diseases including cancer.

The technique we have utilized to investigate the modulation of properties of simple molecules by transition metals is the electrochemical impedance technique. While we have investigated many molecules using this technique [87-94, 99], the electronic properties of hydrogen peroxide are briefly included in this chapter because of its unique electronic properties and importance in biological systems.

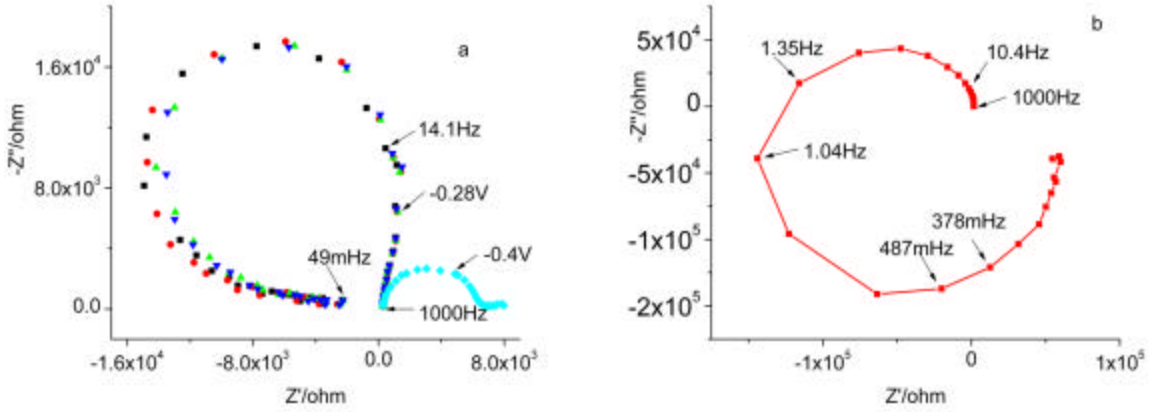


Figure 7 a. Nyquist plot for 88mM H_2O_2 in 0.10M NaCl pH 5.2; Four sets of data at -0.28V to indicate reproducibility. b. Modulation of peroxide impedance by molybdenum. Nyquist plot for 0.005M $H_2Mo_2O_3(O_2)_4$ (obtained by dissolving Mo metal in peroxide), pH 1.87, 0.15V.

Figure 7a gives the Nyquist plot for 88 mM H_2O_2 in 0.10 M NaCl. This may be compared with that in Figure 5. The results are similar in that they both are characterized by NDR. However, the electronic circuits are subtly different because of their own unique shapes. Also the frequencies at which the NDR takes place as well the potentials are slightly different. The four sets of data indicate the reproducibility of the data. The data at -0.4V indicate double capacitance and are different from the NDR behavior at -0.28 V. The modulation by molybdenum in the peroxo complex at a higher acidity is spectacular (Figure 7 b). We had discussed earlier the diode like behavior of the molybdoenzyme nitrate reductases [86].

Similarly the impedance spectra for a-lipoic acid and its modulation by complexing with palladium are shown in Figure 8.

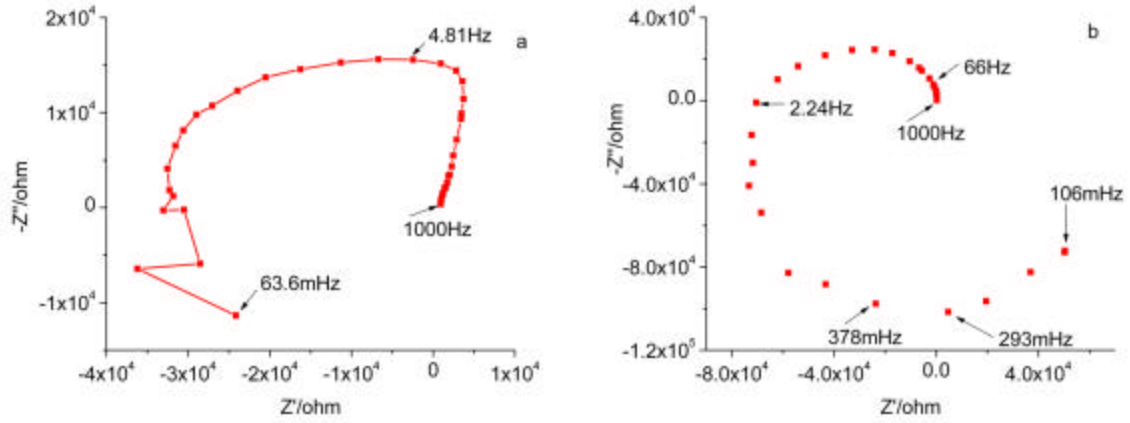


Figure 8. a. Nyquist plot for 0.0373 M sodium lipoate, -1.15V, pH 7.79, NDR at 4.81Hz. b. Modulation of lipoate impedance by palladium in 0.0373M palladium a-lipoic acid (1:1 complex) in 0.1792 M NaCl, -1.18V, pH 7.78, NDR at 66Hz.

Before concluding this chapter, we want to include the electronic properties of a few other simple biological molecules such as lysine, flavin adenine dinucleotide, cysteine, and histidine. Their involvement in the mitochondrial electron transport chain are included in a later section of this chapter. The data in Figures 9-12 are intended to demonstrate the usefulness of the impedance technique to understand the electronic character of simple biological molecules. These also illustrate the sensitivity of the electronic character for concentration, pH, surface area , applied potential, and the frequency at which the NDR is observed.

L-lysine, a dibasic amino acid with a butyl ammonium side chain has $pK_1(\alpha\text{-COOH})$, $pK_2(\alpha\text{-}NH_3^+$, and $pK_3(\varepsilon\text{-}NH_3^+)$ values of 2.16, 9.06 and 10.54 respectively so that it is positively charged at physiological pH. Elevated levels of lysine in blood and urine have been linked to mental and physical retardation. Histones have a large number of lysine residues and its positive charge promotes interaction with negatively charged phosphodiester linkages of DNA. Acetylation of lysine weakens this electrostatic interaction and loosens the chromatin structure allowing gene expression. The reversible histone acetylation and deacetylation reactions control the activation and inactivation of gene expression.

The Nyquist plots for L-lysine as well its modulation by molybdenum are shown in Figure 9.

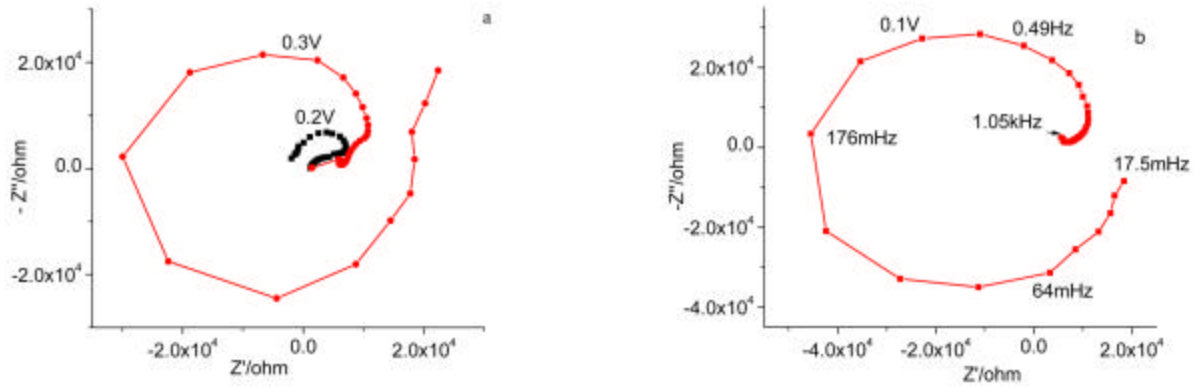


Figure 9. Nyquist plot for a) 0.10 M lysine, 0.021 M HCl, pH 9.6; b) 0.095 M Na_2MoO_4, 0.19M lysine, 0.12 M HCl, pH 8.9

Another important aspect of this system is the fact a-lipoic acid is linked to lysine by an amide bond in the multienzyme complexes of pyruvate dehydrogenase, a-ketoglutarate dehydrogenase and branched chain a-ketoglutarate dehydrogenase. Thus both a-lipoic acid as well as lysine have heavy involvement in the electronic aspects of the enzymatic process.

The impedance data on the interaction between sodium molybdate and flavin adenine dinucleotide (FAD) are shown in Figure 10. FAD, a cofactor in a number of enzymes, when bound to a protein can exist as it's fully oxidized flavoquinone form, its one-electron reduced flavosemiquinone form or its two-electron reduced flavohydroquinone form.

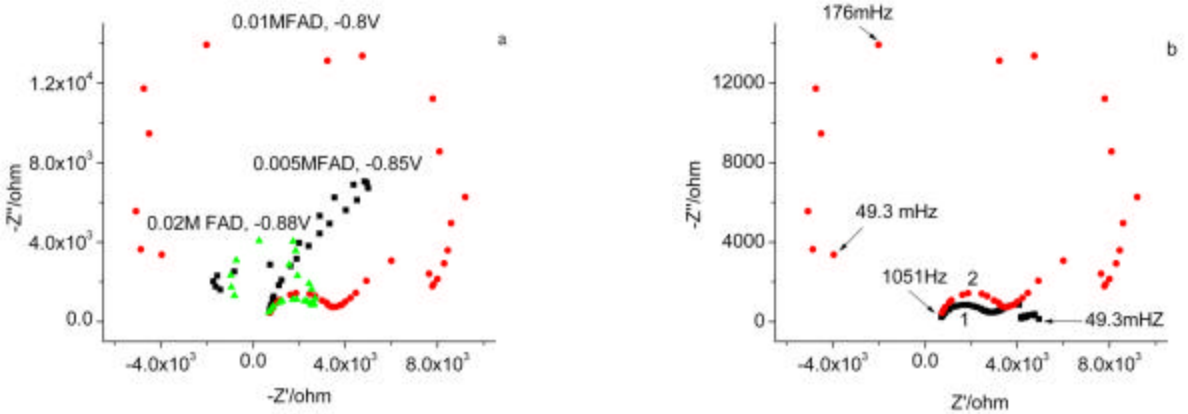


Figure 10. Nyquist plot for a) molybdate-FAD system, 0.02 M molybdate, pH 6.5; b) 0.02 M Na_2MoO_4, 0.01 M FAD, 1) -0.7 V, 2) -0.8V

It is well known that the orientation of the flavin and adenine groups at the electrode surface depends on the concentration of FAD [105]. The cyclic voltammogram of FAD is highly concentration dependent. The same behavior is reflected in the impedance data shown in Figure

10a. The data in Figure 10b indicate the potential dependence of its electronic character. The NDR is observed only at select potentials.

Another important aspect of this system is the fact FAD is also an integral part of the multienzyme complexes of pyruvate dehydrogenase, a-ketoglutarate dehydrogenase and branched chain a-ketoglutarate dehydrogenase.

Another important group present in complex I, II, and III is the cysteine group. We have carried out extensive investigation of this in the presence and absence of molybdate [74, 75]. A typical example is shown in Figure 11. The pH dependence on the observed NDR is vividly demonstrated in Figure 11a. The data in Figure 12 demonstrate that either the adsorbed molecule is regenerated for a repeat cycle or that the double layer near the electrode surface remains intact. In all our experiments a fresh mercury drop is used at the start of every impedance measurement. One of our major concerns was that the applied potential was very near the passivation of mercury. Repeat use of mercury giving nearly identical impedance curve seems to validate the procedure.

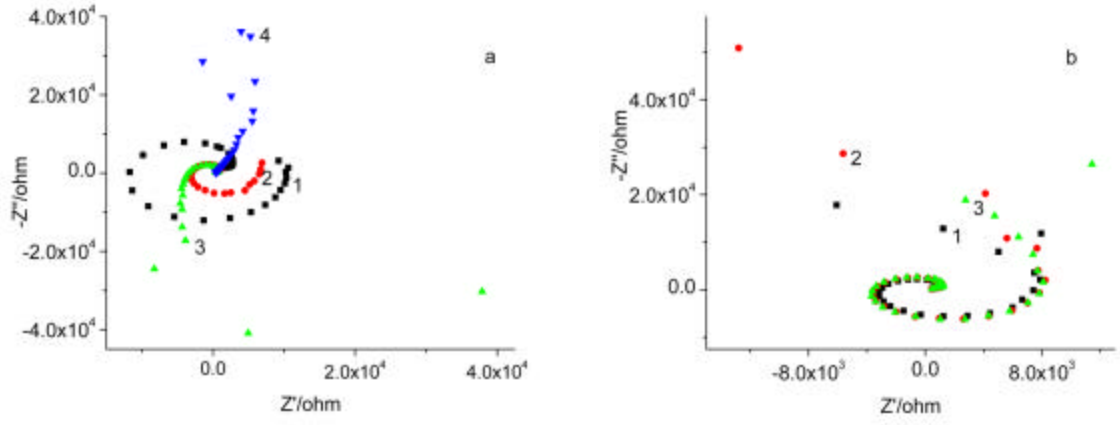


Figure 11. Nyquist plot for 0.1M cysteine-sodium molybdate, a) 0.3 V, pH 1) 5.22 2) 5.79 3) 6.91 4) 9.34 ; b) 0.25 V 1) Fresh Hg drop 2) Repeat with the used Hg drop 3) Repeat second time with the used Hg drop.

L-Histidine, an essential amino acid, has an aromatic nitrogen-heterocyclic imidazole side chain with pK_1(α-COOH), pK_2(α-NH_3^+), and pK_3(imidazole) values of 1.78, 8.97 and 5.97 respectively. Its isoelectric point (pH) is 7.47. Decarboxylation of histidine yields the neurotransmitter histamine. Histamine occurs in mast cells and basophils of blood. Histamine binds to H_1 receptors of the smooth muscle of bronchi which contracts leading to breathing difficulties (as in Asthma). Histidine is also an important component of enzymes such as carbonic anhydrase. The histidine residues in cytochromes a, b, c stretch both on the cytosolic side as well as on the matrix side. The electronic character of this important molecule is exemplified in Figure 12.

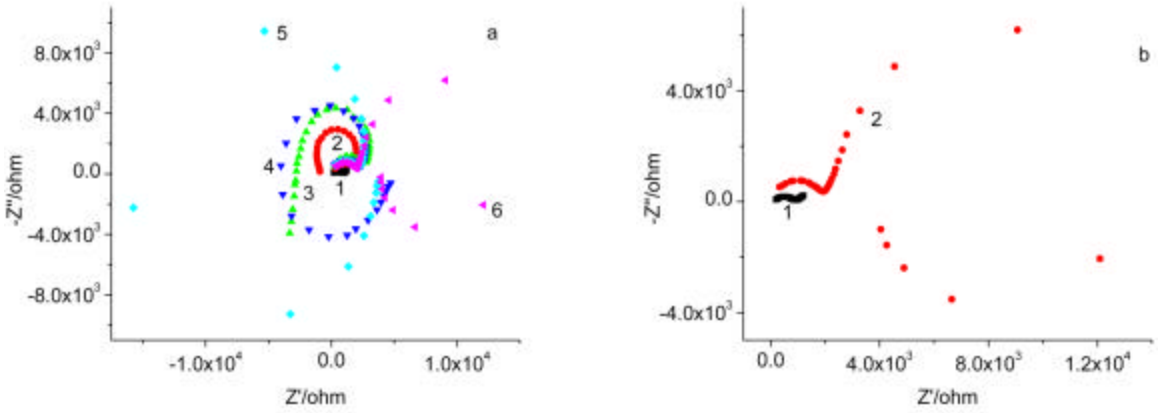


Figure 12. Nyquist plot for 0.177 M histidine-sodium molybdate, 0.067 M NaOH, pH 9.60, a) 1) -0.1 V 2) 0.0V 3) 0.03 V 4) 0.05 V 5) 0.07 V 6) 0.09 V b) expanded scale for 1) -0.1 V 2) 0.09 V

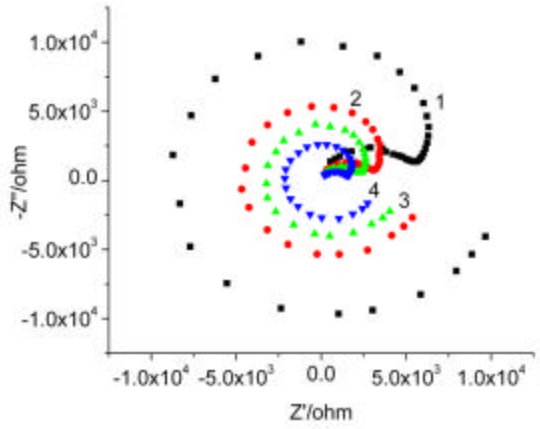


Figure 13. Nyquist plot for 0.177 M histidine-sodium molybdate, 0.067 M NaOH, pH 9.60, 0.05 V, influence of surface area of mercury drop, mm^2 1) 0.011 2) 0.017 3) 0.022 4) 0.031

These impedance data provide information on the instabilities or bifurcations and distinguish between saddle-node and Hopf bifurcations. However these aspects are not included in this chapter. Only the importance of the electronic properties of these molecules and its modulation by molybdenum to demonstrate the diode or tunnel diode characteristics of this system. The unique impedance behavior reflects periodicities and suggest global electronic coupling. Our data suggest that we have to include the electronic properties of these molecules to complement the enzymatic process.

Thus we have made a brief attempt to demonstrate the resourcefulness of the impedance technique as illustrated in 5-12, to understand the electronic properties of biological molecules. The negative differential resistance characteristics is demonstrated vividly and support the conclusions drawn from protein film voltammetry regarding the tunnel diode characteristics of some enzymes. Our data presented here raise the possibility that some of these simple molecules

that are present in the enzymes may be the ones that are exhibiting the unique electronic properties in biological systems.

18. Self-Assembly of Palladium a-Lipoic Acid Complex

The phase microscopy pictures (300X) of $1.34x10^{-2}$ M and $2.7x10^{-4}$ M palladium a-lipoic acid complex (1:1) are shown in Figure. The self-assembly of the complex even at very dilute solutions is remarkable. No self-assembly was observed for sodium lipoate even at concentrations as high as 0.20M.

A long flexible arm that can oscillate a distance of ~200 Å produced by the binding of a lysine residue in the protein to the lipoyl group of E2 in 2-oxoacid dehydrogenases is utilized during the catalytic cycle [59]. It is obvious that the self assembled palladium a-lipoic acid complex can make this process more facile.

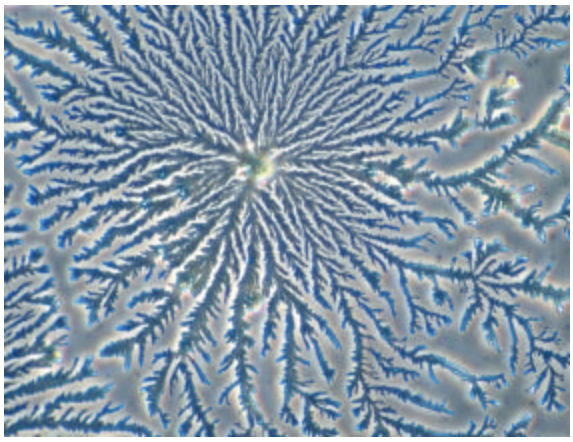
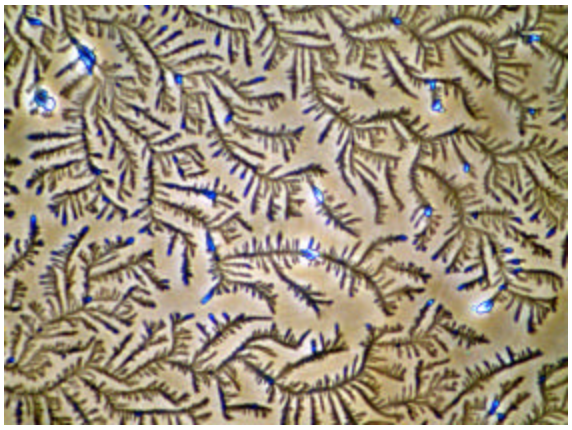
a.  b. 

Figure 13. Phase microscopy 300X (a) $1.34x10^{-2}$ M and b) $2.7x10^{-4}$ M palladium a-lipoic acid complex

It must be pointed out that in homogeneous systems, autocatalytic reactions and diffusion resulting from chemical instabilities lead to the formation of spiral waves and other concentration patterns of spatiotemporal phenomena. Our data suggest that the propagation of electrical signaling among the packing units and extending to long distances is viable by such self-assembled systems. Thus the self-assembly of biological molecules facilitates local disturbances to be felt at long distances by global coupling.

The physics and chemistry of non-equilibrium systems have been utilized to understand some of the spatial patterns and temporal patterning observed in biological processes such as bacterial colonies shaped by diffusive instabilities and calcium waves governed by nonlinear amplification during intracellular signaling [106]. We believe that the self assembled patterns of palladium lipoic acid may help electron transfer processes by extending it into the bulk from the membrane. In other words it may provide a spatial extension of the membrane with much more surface area with much less need for a bulky multi enzyme complex.

This is similar to the coupling that takes places in large multi-enzyme systems such as complex I where the Fe-S clusters help the signaling process during electron transfer.

19. Spin Coupling in Electron Transfer

Numerous electron paramagnetic resonance (EPR) or electron spin resonance (ESR) measurements have been carried out in biological systems [107]. This technique allows detection of unpaired electrons in any phase and the large magnetic dipole of the electron results in very-long range effects on the line shapes on responses to pulses and electron-electron spin relaxation times. It also allows to measure distances between spins based on the dipolar interactions.

The most complicated mitochondrial complex I, with a molar mass greater than 850 kD and at least 26 subunits, has been studied thoroughly by this technique to understand its structural aspects, the location of the NADH binding site, flavin, and most of the iron-sulfur clusters located in the hydrophilic electron entry domain of complex I. Also from spin-spin coupling interactions, it has been possible to identify the locations of cluster $[4Fe\text{-}4S]_{N2}$ and two complexes I associated species of semiquinone [108]. The nature of spin and orbitals states in [4Fe-4S] complexes have been reviewed recently [109]. Vectorial translocation of 4 or 5 protons across the mitochondrial inner membrane is coupled to this electron transfer process from NADH to quinone.

$$NADH + CoQ + H^+ + n(H^+)_{matrix} \; ? \; NAD^+ + CoQH_2 + n(H^+)_{intermembrane\ space} \qquad (41)$$

The electron transfer takes place from NADH to Complex I by passing through flavin mononucleotide, FMN, to a series of redox active iron-sulfur clusters such as $[2Fe\text{-}2S]_{N1a}$, $[2Fe\text{-}2S]_{N1b}$, $[4Fe\text{-}4S]_{N3}$, $[4Fe\text{-}4S]_{N4}$, $[4Fe\text{-}4S]_{N5}$, and $[4Fe\text{-}4S]_{N2}$, and two protein bound species of quinone, Q_{Nf} and Q_{Ns}.

Complex II, which is much less complicated than Complex I has a molar mass of about 127 kD and 5 subunits. The electrons pass through FAD to iron-sulfur clusters, $[2Fe\text{-}2S]_{S1}$, $[4Fe\text{-}4S]_{S2}$, and $[3Fe\text{-}4S]_{S3}$ as well as cytochrome b_{560}.

Complex III has a molar mass of about 280 kD and 10 subunits. The electrons pass through cytochrome b_L (b_{566}), cytochrome b_H (b_{562}), [2Fe-2S], and cytochrome c_1.

Complex IV with a molar mass of about 200 kD has 6-13 subunits. The electrons pass through cytochrome a, Cu_A, Cu_B and cytochrome a_3.

The iron-sulfur clusters as well as semiquinone radicals in complex I are all EPR detectable. The oxidized forms of these clusters are diamagnetic and reduced forms are paramagnetic. The iron atoms are bridged by acid-labile inorganic sulfides. Each iron-sulfur cluster has four protein cysteinyl sulfur bonds. The iron, with oxidation state varying between +2 and +3, in each cluster is tetrahedrally bonded to the sulfur.

Cytochromes a, b, and c have heme proteins compared to the nonheme proteins in the above mentioned iron-sulfur clusters. The heme group of c cytochromes has added cysteinyl sulfhydryl groups across their double bonds to forming thioether linkages to the protein. The

heme iron of the cytochromes a, b, and c also have one or two histidine residues as axial ligands. The histidine residues are on the cytoplasmic side as well as the matrix side. Cytochrome c also has several invariant lysine residues that lie in a ring around the exposed edge of it's otherwise buried heme group [1].

Apart from understanding the electron transfer pathways, topology of iron-sulfur clusters, and site of coupling in NADH-ubiquinone reductase, Complex I investigations have also been instrumental in understanding the mechanism of superoxide generation at the flavin site of Complex I [110-112].

Since complex I dysfunction is implicated in many human neurodegenerative diseases as well as cancer, it is critical to understand its function thoroughly. We must point out a missing link here. We have briefly indicated the contributions from the electronic character of cysteine from our impedance studies. Since it is bonded to the iron in the iron-sulfur clusters, it is important to investigate its electronic contributions to the electron transfer process. A recent X-ray investigation confirms our suggestion. "Cluster N2 is the electron donor to quinone and is coordinated by unique motif involving two consecutive (tandem) cysteines. An unprecedented "on /off switch" (disconnection) of coordinating bonds between the tandem cysteines was observed upon reduction" [109]. This is also true of FAD and histidine in Complex II. Since the palladium lipoic acid complex formulation enhances the Complex I and Complex II activities by 151% and 212% more than that a-lipoic acid, we have reason to believe that the self-assembled structure of the complex, by providing a spatial extension of the membrane with much more surface area, may be catalyzing the electron transfer process by enhancing the spin coupling.

Some supporting evidence for the probable electron spin coupling, even though not directly from the data of palladium a-lipoic acid complex, is given by the free radical reaction mechansim for the reaction of dihydrolipoyl dehydrogenase [113], studies on the one-electron reduction of the disulfide linkages [114] and studies on the lipoic acid free radical [115]. Sulfhydryl free radicals of monothiol compounds tend to interact with their parent compounds.

$$R\text{-}S^{\cdot} + RS^{-} \; ? \quad [R\text{-}S\text{-}S\text{-}R]^{-} \tag{42}$$

There was no similar reaction for between the lipoic acid radical and dihydrolipoi acid. The pK_a of lopoyl radical ($RS^{\cdot}S(H)R$ of 5.85 compared to 4.7 of the carboxyl group implied that the negative charge is on the sulfur of the radical [114, 115]. A direct electron transfer from the lipoic acid radical to FAD forming FADH. Was suggested [113] and confirmed by pulse radiolysis studies [115]. The

$$[RS\text{-}SR]^{\cdot -} + FAD + H^{+} \; ? \quad RS\text{-}SR + FADH^{\cdot} \tag{43}$$

The $FADH^{\cdot}$ radicals disproportionates eventually forming FAD and $FADH_2$. Similar radical formation with the lone electron in the sulfur or palladium or an oscillation between the two can couple the electron transfer process and enhance the catalytic process.

20. Oxidative Stress in HIV Infection

HIV infected patients were found to have lower level of intracellular glutathione, plasma cystine, and cysteine [116]. Increased lipid peroxidation was also evident from the increased plasma malondialdehyde and plasma lipid peroxides. Plasma concentrations of vitamin C and ß-carotene/vitamin A were also significantly reduced. Increased levels of oxidized 8-hydroxyguanine in DNA as well as thiobarbituric acid reactive substances (TBARS) along with decreases in the levels of superoxide dismutase and catalase in HIV infected patients have also been reported [42, 116]. Supplementation for 6 months with vitamin A or vitamin C or vitamin E decreased the level of DNA damage, along with a reduction of TBARS and restoration of the activity of the enzymes [117]. An a-lipoic acid supplementation study, 150 mg of lipoate three times daily for a period of 14 days, of HIV positive (classified CDC IV) patients showed increased levels of plasma ascorbate and glutathione and decreased plasma malondialdehyde in most patients. Also in a majority of patients, the T-helper cells increased and the T-helper/T-suppressor ratio improved [42].

A small study to probe the effectiveness of palladium a-lipoic acid complex formulation was also carried out with 5 HIV/AIDS patients suffering from chronic fatigue. This work was done at CIRCLE Medical LLC, Norwalk, CT, USA. It was observed that the palladium a-lipoic acid complex formulation was generally well-tolerated in all subjects; 4/5 patients reported sustained improvements in energy/fatigue through week 4 (1/5 patients noted improvements through week 2 with "decrease" by week 4); all subjects reported decrease energy/increased fatigue during the 2-week "wash-out" period; mean MOS-HIV Energy/Fatigue scores increased significantly through week 4, with a significant decrease in scores during the "wash-out" period; decreases were observed in TC, TC/HDL, and TG throughout the study period; and increased CD4%, decreased CD8%, and increased CD4:CD8 were observed. Of course these results are only preliminary and a comparative study with a-lipoic acid and many patients have to be carried out to arrive at meaningful conclusions. But the results do indeed are very promising.

21. Amelioration of Drug Induced Toxicity

A recent review has detailed the medication-induced mitochondrial damage and disease and suggested mitochondrial toxicity testing as part of the pre-approval process for medications to protect the public by identifying the most toxic medications before they are allowed to reach the market [118]. Recent studies indicate effectiveness of, natural antioxidants against adriamycin-induced toxicity in cancer patients [119], lipoic acid against methotrexate-induced oxidative stress when treating leukemia and autoimmune diseases [120], and lipoic acid against isoniazid-rifampicin-induced hepatotoxicity when treating tuberculosis [121]. Since all studies with palladium lipoic acid formulation have indicated that it is much more effective than lipoic acid, there is no reason to doubt its effectiveness in using it as an adjuvant for treating tuberculosis, leukemia, other cancers and autoimmune diseases. Of course confirmation of these statements needs experimental verification.

22. Conclusions

We have suggested a new way of looking at the holistic medicine or alternative medicine. Mitochondria are ubiquitous. By developing new ways of treating mitochondrial dysfunction, a symbiotic or bridging relationship between the allopathic medicine and a generally neglected part in allopathic medicine, the mitochondria, can be generated for improving the mental, emotional, and spiritual elements of the body. Medications targeting the mitochondria are much closer to a real holistic medicine because if you have healthy mitochondria, they will contribute substantially to the physical, mental, and emotional elements needed to complement the allopathic medicine.

Unlike toxic platinum chemotherapy agents, palladium a-lipoic acid complex formulation is safe and nontoxic. Its oral administration can be continued indefinitely. Palladium a-lipoic acid complex formulation is designed to provide energy for compromised body systems and promote overall health. It facilitates aerobic metabolism much more than that of a-lipoic acid, by significantly enhancing the enzymatic activity of isocitrate dehydrogenase, a-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase at the Krebs Cycle and mitochondrial complexes I, II, III and IV of the electron transport chain. Of œurse further investigations are needed to understand the mechanism of action of palladium a-lipoic acid complex formulation on some of these activities because the enzymes containing lipoamide are not direct participants in some of these activities.

Prior ischemia studies in gerbils demonstrated this energy benefit provided by palladium a-lipoic acid complex formulation to maintain the integrity of the electron transport chain following an ischemic insult.

Preliminary studies of HIV/AIDS patients under various cocktail treatment protocols demonstrated an almost immediate improvement in patient quality of life. Benefits included less depression and lethargy, more daily energy and increased appetite. Patients demonstrated significantly improved MOS-HIV Energy/Fatigue scores, increases in CD4, decreases in CD8, as well as improved lipid levels.

The unique electronic properties of palladium modulating the properties of a-lipoic acid appear to be a key to this physiological effectiveness. This is exemplified in our electrochemical impedance spectroscopic studies of a-lipoic acid and palladium a-lipoic acid.

The electronic properties of palladium also appear to modulate the antioxidant properties of a-lipoic acid in that palladium a-lipoic acid complex formulation enhances the activities of catalase and glutathione peroxidase more than that of a-lipoic acid. The level of glutathione also was significantly improved and the level of lipid peroxidation was decreased in the heart mitochondria of aged rats. Oral administration of palladium a-lipoic acid complex formulation showed an increase in glutathione and glutathione peroxidase levels and a decrease in malondialdehyde (a secondary product of lipid peroxidation) in the kidney and liver. Also

palladium a-lipoic acid complex formulation offered protection to cellular DNA from whole body radiation (8 Gy). It decreased the radiation–induced hematopoietic injury as revealed by the bone marrow cellularity, hemoglobin level, and endogenous spleen colony formation in irradiated animals.

Palladium a-lipoic acid complex formulation is similar to a multi-spectrum drug in that it carries out several functions such as combating age related as well as disease associated fatigue, and minimizes the effects of ischemic injury. It acts as a prophylactic for neuronal regeneration from transient ischemic attack and also for protection from radiation. Apart from being a powerful free radical scavenger, it is also highly effective against various cancer cells such as glioblastoma, breast, ovarian, osteosarcoma, and lung.

Finally, we believe that the aim of the slogan for holistic medicine or alternative medicine, "the whole is much more than the sum of the parts", is accomplished readily by targeting mitochondria, a ubiquitous organelle in the human body. This is achieved with the judicious choice of a naturally present ligand in the human body, a-lipoic acid, that plays a crucial role in the mitochondrial energy metabolism because of its unique chemical structure and consequent redox properties, and complexing it with a metal with very high catalytic and electronic properties, palladium. What we lack in its continuing saga is many more challenging investigations to probe more deeply to understand the underlying mechanisms of some of the spectacular observations.

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References

1. D. Voet, J.G. Voet, "Biochemistry", 2nd Edition, John Wiley & Sons, Inc., New York, (1995)
2. D.S. Beattie, "Bioenergetics and Oxidative Metabolism", in "Textbook of Biochemistry with Clinical Correlations" 5th Edition, Edited by T.M. Devlin, Wiley-Liss, A John Wiley & Sons, Inc., Publication, New York (2002)
3. B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J.D. Watson, "Molecular Biology of The Cell", Garland Publishing Inc., New York (1989)
4. L. Biasutto, L.F. Dong, M. Zoratti, J. Neuzil, "Mitochondrially targeted anti-cancer agents", Mitochondrion, 10 (6) (2010) 670-681

5. B.B. Aggarwal, S. Shishodia, “Molecular targets of dietary agents for prevention and therapy of cancer”, Biochemical Pharmacology, 71 (2006) 1397-1421
6. G. Chen, F. Wang, D. Trachootham, P. Huang, “Preferential killing of cancer cells with mitochondrial dysfunction by natural compounds”, Mitochondrion, 10 (6) (2010) 614-625
7. F.H. Sarkar, Y. Li, Z. Wang, D. Kong, “Cellular signaling perturbation by natural products”, Cellular signaling, 21 (2009) 1541-1547
8. S.E. Sherman, S.J. Lippard, “Structural Aspects of Platinum Anticancer Drug Interactions with DNA”, Chem. Rev., 87 (1987) 1153-1181
9. E J.C. Dabrowiak, “Metals in Medicine”, John Wiley & Sons Ltd, (2009)
10. S.P. Fricker, "Metal based drugs: from serendipity to design”, Dalton Trans.,(2007) 4903-4917
11. Gao, C. Liu, M. Zhu, H. Lin, Q. Wu, L. Liu, “Current Development of Pd(II) Complexes as Potential Antitumor Agents”, Anti-Cancer Agents in Medicinal Chemistry, 9 (2009) 356-368
12. A.C.F. Caires, “Recent Advances Involving Palladium(II) Complexes for the Cancer Therapy”, Anti-Cancer Agents in Medicinal Chemistry, 7 (2007) 484-491
13. A.S. Abu-Surrah, H.H. Al-Sa’doni, M.Y. Abdalla, “ Palladium-based chemotherapeutic agents: Routes toward complexes with good antitumor activity”, Cancer Therapy, 6 (2008) 1-10
14. T. Rau, R. Alsfasser, A. Zahl, R. van Eldik, “Structural and Kinetic Studies on the Formation of Platinum(II) and Palladium(II) Complexes with L-Cysteine-Derived Ligands”, Inorg. Chem., 37 (1998) 4223-4230
15. M. Malon, Z. Trávnícek, M. Maryško, R. Zboril, M. Mašlán, J. Marek, K. Doležal, J. Rolcík, V. Kryštof, M. Strnad, “Metal complexes as anticancer agents 2. Iron(III) and copper(II) bio-active complexes with N6-benzylaminopurine derivatives”, Inorg. Chim. Acta, 323 (2001) 119-129
16. A.I. Matesanz, J.M. Perez, P. Navarro, J.M. Moreno, E. Colacio, P. Souza, “Synthesis and characterization of novel palladium (II) complexes of bis (thiosemicarbazone). Structure, cytotoxic activity and DNA biding of Pd (II)-benzyl bis (thiosemicarbazonate)”, J. Inorg. Biochem., 76 (1999) 29-37
17. D.W. Parsons, S. Jones, X. Zhang, J.C.H. Lin, R.J. Leary, P. Angenendt, P. Mankoo, H. Carter, I.M. Siu, G.L. Gallia, A. Olivi, R. McLendon, B.A. Rasheed, S. Keir, T. Nikolskaya, Y. Nikolsky, D.A. Busam, H. Tekleab, L.A. Diaz Jr, J. Hartigan, D.R. Smith, R.L. Strausberg, S.K.N. Marie, S.M.O.Shinjo, H. Yan, G.J. Riggins, D.D. Bigner, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V.E. Velculescu, K.W. Kinzler, “An Integrated Genomic Analysis of Human Glioblastoma Multiforme”, Science, 321 (2008) 1807-1812

18. S. Jones, X. Zhang, D.W. Parsons, J.C.H. Lin, R.J. Leary, P. Angenendt, P. Mankoo, H. Carter, H. Kamiyama, A. Jimeno, S.M. Hong, B. Fu, M.T. Lin, E.S. Calhoun, M. Kamiyama, K. Walter, T. Nikolskaya, Y. Nikolsky, J. Hartigan, D.R. Smith, M. Hidalgo, S.D. Leach, A.P. Klein, E.M. Jaffee, M. Goggins, A. Maitra, C.L. Donahue, J.R. Eshleman, S.E. Kern, R.H. Hruban, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V.E. Velculescu, K.W. Kinzler, "Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses", Science, 321 (2008) 1801-1806
19. D. Hanahan, R.A. Weinberg, "The Hallmarks of Cancer", Cell, 100 (2000) 57-70
20. O. Warburg, "On the Origin of Cancer Cells", Science, 123 (1956) 309-314
21. M.G.V. Heiden, L.C. Cantley, C.B. Thompson, "Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation", Science, 324 (2009) 1029-1033
22. H. Simonnet, N. Alazard, K. Pfeiffer, C. Gallour, C. Béroud, J. Demont, R. Bouvier, H. Schägger, C. Godinot, "Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma", Carcinogenesis, 23 (2002) 759-768
23. K.K. Singh, "Oxidative Stress, Disease and Cancer", Imperial College Press, London, WC2H 9HE (2006)
24. J.S. Carew, P. Huang, "Mitochondrial defects in cancer", Molecular Cancer, 1:9 (2002); http://www.molecular-cancer.com/content/1/1/9
25. M. Higuchi, "Regulation of mitochondrial DNA content and cancer", Mitochondrion, 7 (2007) 53-57
26. D.C. Wallace, W. Fan, "Energetics, epigenetics, mitochondrial genetics", Mitochondrion, 10 (2010) 12-31
27. A. Mayevsky, "Mitochondrial function and energy metabolism in cancer cells: Past Overview and future perspectives", Mitochondrion, 9 (2009) 165-179
28. G. Kroemer, J. Pouyssegur, "Tumor Cell Metabolism: Cancer's Achilles Heel", Cancer Cell, 13 (2008) 472-482
29. C. Frezza, E. Gottlieb, " Mitochondria in cancer: Not just innocent bystanders", Seminars in Cancer Biology, 19 (2009) 4-11
30. V. Gogvadze, S. Orrenius, B. Zhivotovsky, "Mitochondria in cancer cells: What is so special about them?", Trends in Cell Biology, 18 (4) (2008) 165-173
31. T. Tanriverdi, H. Hanimoglu, T. Kacira, G.Z. Sanus, R. Kemerdere, P. Atukeren, K. Gumustas, B. Canbaz, M.Y. Kaynar, "Glutathione peroxidase, glutathione reductase and protein oxidation in patients with glioblastoma multiforme and transitional meningioma", J. Cancer Res Clin Oncol., 133 (2007) 627-633
32. R.J. Sinha, R. Singh, S. Mehrotra, R.K. Singh, "Implications of free radicals and antioxidant levels in carcinoma of the breast: A never-ending battle for survival", Indian Journal of Cancer, 46 (2) (2009) 146-150

33. C.P. Rajneesh, A. Manimaran, K.R. Sasikala, P. Adaikappan, “Lipid peroxidation and antioxidant status in patients with breast cancer”, Singapore Med J, 49(8) (2008) 640-643
34. O. Portakal, O. Özkaya, M.E. inal, B. Bozan, M. Kosan, I. Sayek, “Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients”, Clinical Biochemistry, 33 (4) (2000) 279-284
35. K. Punnonen, M. Ahotupa, K. Asaishi, M. Hyöty, R. Kudo, R. Punnonen, “Antioxidant enzyme activities and oxidative stress in human breast cancer”, Journal of cancer Research and Clinical Oncology, 120 (6) (1994) 374-377
36. K. Senthil, S. Aranganathan, N. Nalini, “Evidence of oxidative stress in the circulation of ovarian cancer patients”, Clinica Chimica Acta, 339 (2004) 27-32
37. B. Sandhya, S. Manoharan, G.S. Lavanya, Ch.R. Manmohan, “Lipid peroxidation and antioxidant status in prostate cancer patients”, Indian Journal of Science and Technology, 3 (1) (2010) 83-86
38. N. Rouach, C. Calvo, H. Duquennoy, J. Glowinski, C. Giaume, “Hydrogen Peroxide Increases Gap Junctional Communication and Induces Astrocyte Toxicity: Regulation by Brain Macrophages”, Glia 45 (2004) 28-38
39. Z.A. Wood, L.B. Poole, P.A. Karplus, “Peroxoredoxin Evolution and the Regulation of Hydrogen Peroxide”, Science, 300 (2003) 650-653.
40. P.A. Dennery, “Oxidative Stress in Development: Nature or nurture?”, Free radical Biology & Medicine, 49 (2010) 1147-1151
41. G.R. Buettner, “The Pecking Order of Free Radicals and Antioxidants: Lipid Peroxidation, a-Tocopherol, and Ascorbate”, Arch. Biochem. Biophys, 300 (1993) 535-543.
42. L. Packer, E.H. Witt, H.J. Tritschler, “Alpha Lipoic Acid as a Biological Antioxidant”, Free Radical Biology and Medicine, 19, No.2 (1995) 227-250
43. F. Nualart, C. Rivas, V. Montecinos, A. Godoy, V. Guaiquil, D. Golde, J. Vera, “Recycling of vitamin C by a bystander effect”, J. Bio. Chem., 278 (12) (2003) 10128-10133
44. E. Niki, N. Noguchi, “Dynamics of Antioxidant Action of Vitamin E”, Acc. Chem. Res., 37 (2004) 45-51
45. F. A. Cotton and G. Wilkinson, “Advanced Inorganic Chemistry”, 3rd Edition, p. 415, Interscience Publishers, New York, (1972)
46. M. L. Lázaro, Dual role of hydrogen peroxide in cancer: “Possible relevance to cancer chemoprevention and therapy”, Cancer Letters, 252 (2007) 1-8
47. M.T.M. Koper, “Oscillations and Complex Dynamical Bifurcations in Electrochemical systems”, in “Advances in Chemical Physics”, I. Prigogine, S.A. Rice Editors, John Wiley & Sons, New York, 92 (1996) 161-297

48. Y. Mukouyama, S. Nakanishi, T. Chiba, K. Murakoshi, Y. Nakato, “Mechanisms of Two Electrochemical Oscillations of Different Types, Observed for H_2O_2 Reduction on a Pt Electrode in the Presence of Small Amount of Halide Ions”, J. Phys. Chem. B, 105 (2001) 7246-7253
49. C.W. Olanow and A.N. Lieberman Editors in, “The Scientific Basis for the Treatment of Parkinson’s Disease”, The Parthenon Publishing Group, New Jersey, 07656, USA, (1992)
50. W.J. Weiner, L.M. Shulman, A.E. Lang, “Parkinson’s Disease”, 2nd ed., The John Hopkins University , Maryland, USA (2007)
51. J.T. Greenamyre, G. MacKenzie, T. Peng, S.E. Stephans, “Mitochondrial dysfunction in Parkinson’s disease”, Biochem Soc Symp. 66 (1999) 85-97
52. K.F. Winklhofer, C. Haass, “Mitochondrial dysfunction in Parkinson’s disease”, Biochim Bipophys Acta (BBA) Molecular Basis of Disease, 1802 (2010) 29-44
53. A.H.V. Schapira, “Mitochondrial dysfunction in Parkinson’s disease”, Cell Death and Differentiation, 14 (2004) 1261-1266
54. W.D. Parker Jr, S.J. Boyson, J.K. Parks, “Abnormalities of the Electron Transport Chain in Idiopathic Parkinson’s Disease”, Ann. Neurol, 26 (1989) 719-723
55. P. Mecocci, U. MacGarvey, M.F. Beal, “Oxidative Damage to mitochondrial DNA is increased in Alzheimer’s disease”, Annals of Neurology, 36 (5) (1994) 747-751
56. R. Castellani, K. Hirai, G. Aliev, K.L. Drew, A. Nunomura, A. Takeda, A.D. Cash, M.E. Obrenovich, G. Perry, M.A. Smith, “Role of mitochondrial dysfunction in Alzheimer’s disease”, Journal of Neuroscience Research, 70 (3) (2002) 357-360
57. G.E. Gibson, V. Haroutunian, H. Zhang, L.C.H. Park, Q. Shi, M. Lesser, R.C. Mohs, R.K.F. Sheu, J.P. Blass, “Mitochondrial Damage in Alzheimer’s Disease Varies With Apolipoprotein E Genotype”, Ann Neurol, 48 (2000) 297-303
58. P. Bubber, V. Haroutunian, G. Fisch, J.P. Blass, G.E. Gibson, “Mitochondrial Abnormalities in Alzheimer Brain: Mechanistic Implications, Ann Neurol, 57 (2005) 695-703
59. M.S. Patel, L. Packer, “Lipoic Acid: Energy Production, Antioxidant Activity and Health Effects”, CRC Press, Taylor & Francis Group, New York, (2008)
60. M.R. Baumgartner, H. Schmalle, E. Dubler, “The Interaction of transition metals with the coenzyme a-lipoic acid: synthesis, structure and characterization of copper and zinc complexes”, Inorg. Chim. Acta, 252 (1996) 319-331
61. N. Ramakrishnan, W.W. Wolfe, G.N. Catravas, “Radioprotection of Hematopoietic Tissues in Mice by Lipoic Acid”, Radiation Research, 130 (1992) 360-365
62. L.G. Korkina, I.B. Afanas’ef, A.T. Diplock, “Antioxidant therapy in children affected by irradiation from the Chernobyl nuclear accident”, Biochemical Society Transactions, 314S (1993) 21

63. D.A. Carlson, K.L. Young, S.J. Fischer, H. Ulrich, " An Evaluation of the Stability and Pharmacokinetics of R-Lipoic Acid and R-Dihydrolipoic Acid Dosage Forms in Human Plasma from Healthy Subjects", p. 255 in "Lipoic Acid: Energy Production, Antioxidant Activity and Health Effects" Edited by M.S. Patel and L. Packer, CRC press, Taylor & Francis Group, Florida (2008)
64. H. Strasdeit, A. von Dollen, A.K. Duhme, "Metal Complexes of the Drug and Coenzyme a-Lipoic acid and Related Ligands", Contents of 7th EBIC Conference Abstracts Issue, C 45, J. Inorg. Biochem, 59 (1995) 228
65. M. Garnett, "Synthetic DNA Reductase", Contents of 7th EBIC Conference Abstracts Issue, C 48, J. Inorg. Biochem, 59 (1995) 231
66. M. Garnett, Palladium Complexes and Methods for Using Same in the Treatment of Tumors or Psoriasis, U.S.Patent, No. 5,463,093, Oct.31(1995)
67. M. Garnett, Palladium Complexes and Methods for Using same in the Treatment of Tumors, U.S.Patent, No. 5,679,697, Oct.21, (1997)
68. M. Garnett, Palladium Complexes and Methods for Using same in the Treatment of Psoriasis, U.S.Patent, No. 5,776,973, July7, (1998)
69. O. Corduneanu, A.M.C. Paquim, M. Garnett, A.M.Oliveira Brett, "Lipoic acid-palladium complex interaction with DNA, voltammetric and AFM characterization", Talanta, 77 (2009)1843-1853
70. O. Corduneanu, M. Garnett, A.M.Oliveira Brett, "Anodic oxidation of a-lipoic acid at a glassy carbon electrode and its determination in dietary supplements", Anal Letters 40 (2007)1763-1778
71. G.K. Ogilvie, A.S. Moore, "Tumors of Bone" in "Managing the Canine Cancer Patient: A practical Guide to Compassionate Care", R.A. Henry, Editor, Yardley, PA, Veterinary Learning Systems, (2000) 565-589
72. F. J. Antonawich, S.M. Fiore, L.M. Welicky, "Regulation of ischemic cell death by the lipoic acid-palladium complex, Poly MVA, in gerbils", Experimental Neurology, 189 (2004) 10-15
73. N.P. Sudheesh, T.A. Ajith, K.K. Janardhanan, C.V. Krishnan, "Palladium a-lipoic acid complex formulation enhances activities of Krebs cycle dehydrogenases and respiratory complexes I-IV in the heart of aged rats", Food and Chemical Toxicology, 47 (2009) 2124-2128
74. N.P. Sudheesh, T.A. Ajith, K.K. Janardhanan, C.V. Krishnan, "Effect of POLY-MVA, a palladium a-lipoic acid complex formulation against declined mitochondrial antioxidant status in the myocardium of aged rats", Food and Chemical Toxicology, 48 (2010)1858-1862

75. A. Menon, C.V. Krishnan, C.K.K. Nair, “Protection from gamma radiation insult to antioxidant defense and cellular DNA by POLY-MVA, a dietary supplement containing palladium lipoic acid formulation”, Int. J. Low Radiation, 6 (2009) 248-262
76. L. Ramachandran, C.V. Krishnan, C.K.K. Nair, “Radioprotection by a-Lipoic Acid Palladium Complex Formulation (POLY-MVA) in Mice”, Cancer Biotherapy and Radiopharmaceuticals, 25, No. 4 (2010) 395-399
77. J. Field and L. Gyorgyi, Editors in “Chaos in Chemistry and Biochemistry”, World Scientific Publishing Co., NJ 07661, USA, (1993)
78. D. G. Fink (Editor), “ Electronic Engineers Handbook”, McGraw Hill, New York, (1975)
79. C. Léger, P. Bertrand, “Direct Electrochemistry of Redox Enzymes as a Tool for Mechanistic Studies”, Chem. Rev., 108 (2008) 2379-2438
80. J.D. Gwyer, D.J. Richardson, J.N. Butt, “Diode or Tunnel-Diode Characteristics? Resolving the Catalytic Consequences of Proton Coupled Electron Transfer in a Multi-Centered Oxidoreductase”, J. Am. Chem. Soc., 127 (43) (2005) 14964-14965
81. S.J. Elliott, C. Léger, H.R. Pershad, J. Hirst, K. Heffron, N. Ginet, F. Blasco, R.A. Rothery, J.H. Weiner, F.A. Armstrong, “Detection and interpretation of redox potential optima in the catalytic activity of enzymes”, Biochimica et Biophysica Acta (BBA) – Bioenergetics, 1555, (2002) 54-59
82. H.R. Pershad, J. Hirst, B. Cochran, B.A.C. Ackrell, F. A. Armstrong, “Voltammetric studies of bidirectional catalytic electron transport in Escherichia coli succinate dehydrogenase: comparison with the enzyme from beef heart mitochondria”, Biochimica et Biophysica Acta (BBA) – Bioenergetics, 1412, (3) (1999) 262-272
83. J. Hurst, A. Sucheta, B.A. Ackrell, F.A. Armstrong, “Electrocatalytic Voltammetry of Succinate Dehydrogenase: Direct Quantification of the Catalytic Properties of a Complex Electron Transport Enzyme”, J. Am. Chem. Soc., 118 (21) (1996) 5031-5038
84. J.J. Van Hellemond, M. Klockiewicz, C.P.H. Gaasenbeek, M.H. Roos, A.G.M. Tielens, “Rhodoquinone and Complex II of the Electron Transport Chain in Anaerobically Functioning Eukaryotes”, Journal of Biological Chemistry, 270 (1995) 31065-31070
85. B.A.C. Ackrell, F.A. Armstrong, B. Cochran, A. Sucheta, T. Yu, “Classification of fumarate reductases and succinate dehydrogenases based upon their contrasting behavior in the reduced benzylviologen/fumarate”, Federation of European Biochemical Societies, 326 (1,2,3) (1993) 92-94
86. A. Sucheta, B.A.C. Ackrell, B. Cochran, F.A. Armstrong, “Diode-like behavior of a mitochondrial electron transport enzyme”, Nature, 356 (1992) 361-362
87. C. V. Krishnan, M. Garnett, “Liquid crystal behavior in solutions, electrode passivation, and impedance loci in four quadrants”, in “Passivation of Metals and Semiconductors, and Properties of Thin Oxide Layers”, P. Marcus and V. Maurice (Editors), Elsevier, Amsterdam, (2006), 389-394

88. C.V. Krishnan, M. Garnett, B. Chu, "Solute-solvent interactions in biological molecules: L-Cysteine", Int. J. Electrochem. Sci., 3 (2008) 854-872
89. C.V. Krishnan, M. Garnett, B. Chu, "Spatiotemporal oscillations in biological molecules: Molybdate-L-Cysteine", Int. J. Electrochem. Sci., 3 (2008) 873-890
90. C.V. Krishnan, M. Garnett, B. Chu, "Spatiotemporal oscillations in biological molecules: Hydrogen peroxide and Parkinson's disease", Int. J. Electrochem. Sci., 3(2008) 1364-1385
91. C.V. Krishnan, M. Garnett, B. Chu, "Admittance as a useful tool for investigation of solute-solvent interactions at the double layer", Electrochem. Communications 11 (2009) 2229-2232
92. C.V. Krishnan, M. Garnett, B.Hsiao, B. Chu, "Solute-Solvent Interactions from Impedance Measurements: 'p –way' Conduction and Water Structure-Enforced Ion Pair Formation in Aqueous Lidocaine Hydrochloride", Int. J. Electrochem. Soc., 4 (2009) 1085-1099
93. C. V. Krishnan, M. Garnett, B. Chu, "Spatiotemporal Oscillation in Peroxo-Molybdate Complexes in Acidic Solutions: 1. Impedance Measurements of $H_2Mo_2O_3(O\text{-}O)_4(H_2O)_2^{"}$, Int. J. Electrochem Sci., 2 (2007) 444-461
94. C.V. Krishnan, M. Garnett, B. Chu, "Solute-Solvent Interactions from Admittance Measurements: Potential Induced and Water Structure-Enforced Ion-Pair Formation", Int. J. Electrochem. Sci., 2 (2007) 958-972
95. J. R. Macdonald, W. B. Johnson, "Fundamentals of Impedance Spectroscopy" in Impedance Spectroscopy: Theory, Experiment, and Applications" 2nd Edition, E. Barsoukov, J. R. Macdonald Editors, Wiley Interscience, New Jersey, (2005)
96. A. Lasia, "Electrochemical Impedance Spectroscopy and its Applications", in "Modern Aspects of Electrochemistry, No 32, B.E. Coway, J.O'M. Bockris, R.E. White Editors, Kluwer Academic/Plenum Publishers, New York, (1999)
97. Y. Mukouyama, H. Hommura, S. Nakanishi, T. Nishimura, H. Konishi, Y. Nakato, "Mechanism and Simulation of Electrochemical Current Oscillations Observed in the H_2O_2-Reduction Reaction on Platinum Electrodes in Acidic Solutions", Bull. Chem. Soc. Jpn., 72 (1999) 1247-1254
98. S. Fukushima, S. Nakanishi, K. Fukami, S. Sakai, T. Nagai, T. Tada, and Y. Nakato, "Observation of synchronized spatiotemporal reaction waves in coupled electrochemical oscillations of an NDR type", Electrochemistry Communications, 7 (2005) 411-415

99. C.V. Krishnan, M. Garnett, B. Chu, Oxidative Stress and Parkinson's Disease: Electrochemical Behavior of Hydrogen Peroxide in Aqueous Sodium Chloride, Int. J. Electrochem. Sci., 3 (2008) 1348-1363
100. R. Livingston, "The Activity of Hydrogen Peroxide in Sodium Chloride and Sodium Sulfate Solutions", J. Am. Chem. Soc., 50 (1928) 3206
101. M.H. Gorin, "The "Salting-in" of Hydrogen Peroxide by Electrolytes", J. Am. Chem. Soc., 57 (1935) 1975-1978
102. M.E. Everhard, P.M. Gross Jr, J.W. Turner, "Properties of Electrolytes in Hydrogen Peroxide-Water Solutions. 1. Solvation of Alkali Nitrates", J. Phys. Chem., 66 (5) (1962) 923-926
103. D.K. Thomas, O. Maass, "Electrolytic Conductance in Hydrogen Peroxide-Water Mixtures", Can. J. Chem., 36 (1958) 449-455
104. J.C. Genereux, J.K. Barton, "Mechanisms for DNA Charge Transport", Chem. Rev., 110 (2010) 1642-1662
105. S.G. Roscoe, "Electrochemical Investigations of the Interfacial Behavior of Proteins", in "Modern Aspects of Electrochemistry", B.E. Conway, J.O'M. Bockris, Editors, Plenum Press, New York, 29 (1996) 319-399
106. H. Levine, E.B. Jacob, "Physical schemata underlying biological pattern formation-examples, issues and strategies", Physical Biology, 1 (1-2) (2004) P14-P22
107. L.J. Berliner, S.S. Eaton. G.R. Eaton Editors, "Biological Magnetic Resonance, 19: Distance Measurements in Biological Systems by EPR", Kluwer Academic Press/Plenum Publishers, New York (2000)
108. T. Ohnishi, "Iron-sulfur clusters/semiquinones in Complex I", Biochim. Biophys. Acta, 1364 (1998) 186-206.
109. L. Noodleman, C.Y. Peng, D.A. Case, J.-M. Mouesca, "Orbital interactions, electron delocalization and spin coupling in iron-sulfur clusters", Coord. Chem. Rev., 144 (1995) 199-244
110. L. Kussmaul, J. Hirst, "The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria", Proc. Natl. Acad. Sci., USA 103 (2006) 7607-7612
111. A. Galkin, U. Brandt, "Superoxide Radical Formation by Pure Complex I (NADH:Ubiquinone Oxidoreductase) from Yarrowia lipolytica", J. Biol. Chem., 280 (2005) 3019-10135.
112. J. M. Berrisford, L.A. Sazanov, "Structural Basis for the Mechanism of Respiratory Complex ", J. Biol. Chem., 284 (2009) 29773-29783
113. V. Massey, Q.H. Gibson, C. Veeger, "Intermediates in the Catalytic Action of Lipoyl Dehydrogenase (Diaphorase)", Biochem. J. 77 (1960) 341-351
114. M.Z. Hoffman, E. Hayon, "One-Electron Reduction of the Disulfide Linakge in Aqueous

Solution. Formation, Protonation, and Decay Kinetics of $RSSR^-$ Radical", J. Am. Chem. Soc., 94 (1972) 795-7957

115. S.W. Ghan, P.C. Chan, B.H.J. Bielski, "Studies on the Lipoic Acid Free Radical", Biochim. Biophys. Acta, General Subjects, 338 (1974) 323
116. W. Dröge, "Oxidative Stress in HIV Infection", in "Oxidative Stress, Disease and Cancer", K.K. Singh, Editor, Imperial College Press, London, WC2H 9HE (2006) 885-895
117. P. Jaruga, B. Jaruga, D. Gackowski, A. Olczak, W. Halota, M. Pawlowska, R. Olinski, "Supplementation with antioxidant vitamins prevents oxidative modification of DNA in lymphocytes of HIV-infected patients", Free Radical Biology & Medicine, 32 (5) (2002) 414-420
118. J. Neustadt, S.R. Pieczenik, "Medication-induced mitochondrial damage and disease", Mol. Nutr. Food res., 52 (2008) 780-788
119. S.G. Principal, J.L. Quiles, C.L.R. Tortosa, P.S. Rovira, M.R. Tortosa, "New advances in molecular mechanisms and the prevention of adriamycin toxicity by antioxidant nutrients", Food and Chemical Toxicology, 48 (2010) 1425-1438
120. H. Tabassum, S. Parvez, S.T. Pasha, B.D. Banerjee, S. Raisuddin, "Protective effect of lipoic acid against methotrexate-induced oxidative stress in liver mitochondria", Food and Chemical Toxicology, 48 (2010) 1973-1979
121. E. I. Saad, S.M.E. Gowilly, M.O. Sherhaa, A.E. Bistawroos, "Role of oxidative stress and nitric oxide in the protective effects of a-lipoic acid and aminoguanidine against isoniazid-rifampicin-induced hepatotoxicity in rats", Food and Chemical Toxicology, 48 (2010) 1869-1875