

## Free radicals and low-level photon emission in human pathogenesis: State of the art

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Convincing evidence supports a role for oxidative stress in the pathogenesis of many chronic diseases. The model includes the formation of radical oxygen species (ROS) and the misassembly and aggregation of proteins when three tiers of cellular defence are insufficient: (a) direct antioxidative systems, (b) molecular damage repairing systems, and (c) compensatory chaperone synthesis. The aim of the present overview is to introduce (a) the basics of free radical and antioxidant metabolism, (b) the role of the protein quality control system in protecting cells from free radical damage and its relation to chronic diseases, (c) the basics of the ultraweak luminescence as marker of the oxidant status of biological systems, and (d) the research in human photon emission as a non-invasive marker of oxidant status in relation to chronic diseases. In considering the role of free radicals in disease, both their generation and their control by the antioxidant system are part of the story. Excessive free radical production leads to the production of heat shock proteins and chaperone proteins as a second line of protection against damage. Chaperones at the molecular level facilitate stress regulation vis-à-vis protein quality control mechanisms. The manifestation of misfolded proteins and aggregates is a hallmark of a range of neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, polyglutamine (polyQ) diseases, diabetes and many others. Each of these disorders exhibits aging-dependent onset and a progressive, usually fatal clinical course.

The second part reviews the current status of human photon emission techniques and protocols for recording the human oxidative status. Sensitive photomultiplier tubes may provide a tool for non-invasive and continuous monitoring of oxidative metabolism. In that respect, recording ultraweak luminescence has been favored compared to other indirect assays. Several biological models have been used to illustrate the technique in cell cultures and organs *in vivo*. This initiated practical applications addressing specific human pathological issues. Systematic studies on human emission have presented information on: (a) procedures for reliable measurements, and spectral analysis, (b) anatomic intensity of emission and left-right symmetries, (c) biological rhythms in emission, (d) physical and psychological influences on emission, (e) novel physical characteristics of emission, and (f) the identification of ultraweak photon emission with the staging of ROS-related damage and disease.

It is concluded that both patterns and physical properties of ultraweak photon emission hold considerable promise as measure for the oxidative status.

**Keywords:** Antioxidants, Chaperones, Chronic disease, Free radicals, Heat shock proteins, Photon count distribution, Ultraweak photon emission

### Introduction

In 1954 Gerschman and Gilbert proposed that most of the damaging effects of elevated oxygen concentrations in living organisms might be attributed to the formation of free radicals<sup>1</sup>. In 1956, Harman proposed the "free radical theory of aging" which suggested that free radical damage on cellular macromolecules is responsible for the aging process. However, this idea did not capture the interest of many biologists and clinicians until the discovery in 1969 of

the enzyme, superoxide dismutase (SOD) with the function of catalytically removing a specific free radical<sup>2,3</sup>. During the 70's and 80's, many scientists, unfamiliar with free radicals, regarded the field as highly specialized or irrelevant to mainstream biology, biochemistry and medicine. In fact, however, it is just the opposite.

Much experimental data emphasizes that aerobic life is connected with the continuous production of free radicals, particularly reactive oxygen species (ROS) that may be dangerous for the living organism<sup>4-12</sup>. The reactive species attack biomolecules producing alterations in DNA, proteins and lipids, and were implicated in the pathogenesis of age-related disease<sup>13</sup>.

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In recent years a wealth of experimental data was collected to clarify mechanisms that are critically involved in free radical damage resulting in pathologies. The data emphasize the role of heat stress proteins (HSP's) in protection against damage by free radicals. The HSP's are also named as to their function, such as "chaperone proteins", since they form complexes with proteinaceous structures in order to prevent deleterious interactions between proteins. Understanding the molecular mechanisms of cellular protection and recovery from damage in injured cells had steadily increased. In particular, how chaperones at the molecular level facilitate stress regulation vis-à-vis protein quality control mechanisms, and have become critical in development of a range of chronic diseases.

To record ROS, many techniques have been made available. Most of these techniques are invasive; they require the destruction of living cellular or tissue structures to estimate either specific ROS species or products derived from reactions between ROS and macromolecules, mostly lipids. Although these techniques are available to measure the progress of oxidation, none is applicable to all circumstances. In the present study, attention is drawn to the method of low-level chemiluminescence to detect electronically-excited states in biological systems. Low-level chemiluminescence was related to the direct utilization of molecular oxygen<sup>14,15</sup> and the production of electronically-excited states in biological systems<sup>16</sup>; in particular, the oxygen dependent chain reactions involving biological lipids<sup>17-19</sup>. This earlier research on low-level chemiluminescence was largely unnoticed in America and Europe, notwithstanding the reports by Stauff and Ostrowski on the chemiluminescence of mitochondria<sup>20</sup> as well as Howes and Steele on the chemiluminescence of microsomes<sup>21,22</sup>, both from rat liver. This hesitation probably evolved because of earlier reports of the so-called "mutagenic radiation"<sup>23,24</sup> from living tissue which could not be observed with the then contemporary photon counting equipment<sup>25</sup>.

In the meantime, data have demonstrated that spontaneous (natural) ultraweak photon emission originating from living organisms may be considered to reflect the state of oxidative stress *in vivo*. The aim of the present overview is to introduce (a) the basics of free radical and antioxidant metabolism, (b) the role of the protein quality control system in protecting cells from free radical damage and its relation to

chronic diseases, (c) the basics of the ultraweak luminescence as marker of the oxidant status of biological systems, and (d) the research in human photon emission as a non-invasive marker of oxidant status in relation to chronic diseases. Perspectives in future research is presented that allow the evaluation of ultraweak luminescence as a method for recording *in vivo* and noninvasively the state of oxidative stress in human subjects vis-à-vis the development of chronic disease.

### Free radicals and antioxidants

A "free radical" is defined as any atom, group of atoms or molecules containing one unpaired electron within an outer orbit. Molecular oxygen (O<sub>2</sub>) is a triplet in its ground state because it contains two unpaired electrons within its outer orbits having parallel or unpaired spins. Singlet oxygen, by definition, is not a free radical; both electrons occupy the same orbit and the electron spins are paired. In O<sub>2</sub>, parallel electron spin prevents the direct addition of electron pairs (this would include electron spins in both parallel and anti-parallel directions) unless an electron spin inversion occurs. A number of enzymatic systems have evolved that are capable of circumventing electron spin restriction by a one-electron reduction of O<sub>2</sub>. This intermediate univalent pathway is an essential biological process that provides the pairing electron. The cytochrome oxidase complex localized at the inner mitochondrial membrane tetravalently reduces the majority of O<sub>2</sub> used by aerobic cells. It appears to be a major intercellular source of both O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>.

Apart from the mitochondrial respiratory chain, all the monooxygenases, several dehydrogenases, cytochrome-P450, prostaglandin synthetase, leucotri-ene synthetase, vitamin K-dependent enzymes and many other enzymes normally generate radicals. The body not only produces radicals during normal metabolism but it also purposefully produces radicals, designed to be toxic, during immune and inflammatory responses. These radicals are deliberately generated during the respiratory burst of a macrophage in order to kill invading organisms.

In considering the role of free radicals in disease, their generation is only part of the story; the other part is their control, containment and safe disposal. Because radicals and their products are continuously generated and are so reactive chemically, they must physiologically be closely controlled and they must be released in an orderly fashion to avoid damage of vital

components. To maintain cell and tissue integrity, the “antioxidant system” maintains a “check and balance” with the production of reactive free radicals regarding their use in essential pathways and their effective clearance.

In their definition of antioxidant, Halliwell and Gutteridge<sup>26</sup> state, “any substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate”. The “antioxidant system” includes a number of enzymes and low molecular weight compounds, many dependent on essential nutrients including vitamin E (tocopherol), vitamin C (ascorbic acid), beta-carotene, zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and selenium (Se). The vitamins are not dependent on other factors that allow them to participate in free radical defence. However, the metals exert their action as antioxidants primarily via incorporation into specific enzymes. Most significant biologically reactive oxygen intermediates are superoxide radical, hydroxyl radical, lipid hydroperoxides and hydrogen peroxide. These oxygen intermediates are regularly discussed in the following paragraphs and, therefore, will be shortly introduced.

Superoxide radicals can be generated as part of many biological redox reactions. Approximately 1-4% of the total oxygen utilized by mitochondria is converted to superoxide and released from the mitochondria<sup>27</sup>. Thus, tissues such as muscle which increase their oxygen uptake during exercise generate larger amounts of superoxide<sup>28</sup>. The superoxide anion is also produced by several cellular redox systems including xanthine oxidase and membrane-associated NADPH oxidase. Phagocytic cells in particular demonstrate increased oxygen uptake and utilize NADPH oxidase to release large amounts of the superoxide anion into extracellular fluid<sup>29</sup>. Superoxide also appears to be produced during ischemia and reperfusion in tissues containing xanthine oxidase<sup>30</sup>.

The accumulation of the superoxide anion is prevented by enzymes called superoxide dismutases which contain manganese or copper-zinc at their active site<sup>31</sup>. The superoxide radical is not very reactive. It is capable of slowly inactivating a number of essential macromolecules (including catalase and glutathione peroxidase). Since hydroxyl radical scavengers are capable of protecting damage induced by superoxide generation systems, hydroxyl rather than superoxide radicals are responsible for the damaging effect. Transformation of superoxide

radical into a hydroxyl is possible because the superoxide radical is capable of diffusing throughout relatively large distances in the cell and undergoes, in the presence of iron or copper, a metal-catalysed Haber-Weiss reaction with the actual formation of the highly reactive hydroxyl radical (OH<sup>•</sup>)<sup>31,32</sup>.

The hydroxyl radical is very reactive<sup>33</sup>. It is the key radical species damaging tissue<sup>31</sup>. It readily reacts with almost every type of molecule (e.g., sugar, amino acid, phospholipids, nucleotides, and organic acids). On the other hand, hydroxyl radicals may be too reactive (see half life below) to survive collisions with compounds adjacent to the site of formation.

Lipid hydroperoxides are associated with the process of lipid peroxidation. In the presence of some transition metals, lipid hydroperoxides may also be cleaved homolytically to form more free radicals and thus accelerate peroxidation of membrane lipids. A variety of hydrophobic scavengers such as tocopherols, intercollated into cellular membranes, may prevent chain-propagating reactions<sup>33</sup>. Lipid hydroperoxides are injurious to cells; they may be detoxified and/or metabolized by glutathione peroxidase systems.

Hydrogen peroxide can be produced by (a) the enzymatic dismutating action of superoxide dismutase and (b) many other biological reactions involving molecular oxygen, including the divalent reduction of O<sub>2</sub> by enzymes such as urate oxidase, D-amino acid oxidase and xanthine oxidase. The majority of the divalent enzymes that result in H<sub>2</sub>O<sub>2</sub> generation are localized in specialized organelles called peroxisomes<sup>34</sup>. Mitochondria are major intracellular sources of H<sub>2</sub>O<sub>2</sub> generation although any intracellular source of O<sub>2</sub><sup>-</sup> can result in H<sub>2</sub>O<sub>2</sub> production. Hydrogen peroxide is decomposed to H<sub>2</sub>O by catalase and a variety of peroxidases. Glutathione peroxidase (GSH-Px) has been the most intensely studied enzyme of this group<sup>35</sup>. Hydrogen peroxide is a weak oxidizing agent. However, it can inactivate sulfhydryl enzymes. Whereas the peroxide is not very reactive, it can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, this property places hydrogen peroxide in a more prominent role to initiate cytotoxicity than its chemical reactivity indicates.

The half life times of the major reactive oxygen species are vastly different. The highest rate constant for the reaction with target molecules is correlated with the hydroxyl radical; its reactions are diffusion

limited; i.e., they take place practically at the site of generation<sup>36</sup>. In contrast, other radicals are relatively stable with enzyme dependent half lives in the range of seconds. Such molecules may diffuse away from their site of generation and transport the radical or oxidant function to other target sites<sup>37</sup>.

The repertoire of antioxidant protection constitutes antioxidants, protective enzymes, coenzymes and regenerating pathways. There are many essential nutrients involved. Table 1 overviews some of the antioxidants of biological interest<sup>38</sup>.

Properties of an ideal free radical scavenger can be easily summarized as:

- (a) it must be present in adequate amounts in the body;
- (b) it must accumulate within compartments where a need for protection exists;
- (c) it must be versatile in order to combine with a wide variety of free radicals. For example, a limitation of SOD in eliminating free radicals is its lack of versatility; it can interact with only one substrate;
- (d) if some organisms are devoid of synthetic capability (such as ascorbic acid in primates), the compound must be eaten; therefore, it must exist in plant products and be stable for periods of days or weeks after harvest; and
- (e) it might be suitable for regeneration. That is, the process of neutralizing a free radical results in the scavenger becoming oxidized. Thus, a scavenger would be particularly useful if it actually can be recycled. It must have a biologically convenient reducing mechanism, either a specific enzyme or a direct chemical reaction (Table 1).

### Free radicals and medical implications

The free radical "hype" often alluded to medical implications. Thus, based on research begun in the 80's, free radicals were implicated in ischemic-reperfusion damage and pathogenesis of cancer, atherosclerosis, and other chronic diseases. Some of the earlier experimental evidence will be shortly introduced.

### Hypoxia, ischemia and reperfusion

Oxygen free radicals are important mediators of hypoxic or anoxic cell death in heart, lung, kidney, gastrointestinal tract and brain<sup>9, 39-44</sup>. Hypoxic injury can occur during respiratory failure, systemic hypotension and regional hypoperfusion of organs.

A simple model of hypoxia utilizes *in vitro* cell cultures wherein ATP depletion and the stress of hypoxia is mimicked by exposing cells to inhibitors of mitochondrial respiration and glycolysis, cyanide and iodoacetate, respectively<sup>45-49</sup>. Hepatocytes under the impact of such metabolic inhibition generate hydroperoxides and other reactive oxygen species both during hypoxia and before the onset of cell death. In this model, the loss of viability was delayed by antioxidants in an oxygen-dependent manner<sup>50</sup>.

During severe hypoxia or ischemia, the reperfusion of the ischemic tissue can suffer additional injury. For instance, in the treatment of acute coronary thrombosis, reperfusion of ischemic myocardium tissue (a major therapeutic aim) can produce injury<sup>51</sup>. Such effects of temporary ischemia-reperfusion have also been documented during organ transplantation<sup>52,53</sup>. Direct and spin-trapping EPR (electron paramagnetic resonance) and other techniques including chemiluminescence<sup>54-56</sup> have demonstrated that there is a burst of oxygen free radical generation after post-ischemic reperfusion of the heart<sup>57-67</sup>.

During severe hypoxia or ischemia, oxidation-reduction components that are normally oxidized in the aerobic state become reduced. When oxygen is

Table 1—Condensed list of antioxidant compounds and enzymes<sup>38</sup>

Non-enzymic	Enzymic (direct)
$\alpha$ -Tocopherol (Vitamin E) (radical chain-breaking)	Superoxide dismutase (CuZn enzyme, Mn enzyme)
$\beta$ -Carotene (singlet oxygen quencher)	GSH peroxidases (GPx, PHGPx)
Lycopene (singlet oxygen quencher)	Catalase (heme protein, peroxisomes)
Ubiquinol-10 (radical scavenger)	Ancillary enzymes
Ascorbate (vitamin C) (diverse antioxidant function)	Conjugation enzymes (glutathione-S-transferases; UDP-glucuronosyl-transferases)
Glutathione (GSH) (diverse antioxidant function)	NADPH-quinone oxidoreductase (two-electron reduction)
Urate (radical scavenger)	GSSG reductase (maintaining GSH levels)
Bilirubin (plasma oxidant)	NADPH supply (NADPH for GSSG reductase)
Flavonoids (plant antioxidant e.g. rutin)	Transport systems (GSSG export; thioether (S-conjugate) export)
Plasma proteins (metal binding e.g. coeruloplasmin)	Repair systems (DNA repair systems; oxidized protein turnover; oxidized phospholipid turnover)
Chemical (food additives, drugs)	

restored, the components that are reduced may promote intracellular formation of ROS that can then attack lipids, thiols and other cellular components culminating in lethal cell injury<sup>28,53</sup>. Both oxygen-derived free radicals and radicals produced by xanthine oxidase (the other major source of such radicals) have been studied.

Many studies have focused on myocardium "reflow" injury producing cell death as well as mechanical dysfunction. Illustrative are *in vivo* studies of myocardium, either isolated or perfused. Reflow during reperfusion can cause either "stunning"<sup>68</sup> or arrhythmias<sup>69</sup>.

The myocardium possesses a number of free radical scavenging systems (superoxide dismutase, catalase and glutathione peroxidase) that protect against injury under normal cellular conditions<sup>70</sup>. However, in presence of excessive radical formation, these systems become saturated and the cells become vulnerable to oxidative injury. Supplementing scavengers or other antioxidant agents, therefore, may enhance cellular protection against free radical injury. The role of oxygen-free radicals has been demonstrated with this indirect approach, utilizing xanthine oxidase inhibitor and radical scavengers such as SOD and catalase<sup>68-70</sup>.

It is concluded from these earlier studies that: (a) hypoxia and ischemia followed by reperfusion results in free radical generation; (b) a variety of ROS sources exists, and (c) that the range of produced free radical species depends on the cellular or tissue complexity of the biological system.

#### **Cancer and cancerogenesis**

The metabolism of ROS in cancer cells is drastically altered. There is evidence favoring at least two mechanisms: (a) cancer cells produce larger amounts of ROS compared to non-neoplastic cells, and (b) suppression of the antioxidant system in cancer cells. Early evidence demonstrated diminished amounts of Mn superoxide dismutase of all tumors examined at that time<sup>71</sup>. Less Cu/Zn superoxide dismutase has also been documented in many, but not all tumors. Other studies have demonstrated that tumor cells frequently exhibit low catalase activity<sup>72</sup>. Therefore, the amount of superoxide or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) contained in tumor cells should also be elevated. Indeed, most, if not all, hepatic tumours that were evaluated *in vivo* did exhibit more peroxidation than normal livers. In fact, in several human carcinoma cells including colon, pancreatic, breast and ovarian plus malignant melanoma and neuroblastoma demonstrated large amounts of hydrogen peroxide produced *in vitro* without exogenous stimulation<sup>73</sup>.

However, the early studies with isolated cell fractions demonstrated that antioxidant systems are very complicated. Mitochondrial or microsomal suspensions prepared from cancer cells exhibited slow peroxidation<sup>74-78</sup> with some exceptions<sup>79</sup>. Data suggest that circuits might be differently regulated during tumor progression with a variety of patterns all characterized by persistent oxidative stress. The significance of such persistent oxidative stress in cancer has been debated. Perhaps, it may activate transcription factors<sup>80</sup> and induce expression of proto-oncogenes<sup>81,82</sup>. It may also induce damage such as modified base products and strand breaks that lead to further genomic instability<sup>83</sup>.

Much research has been directed at clarifying the relationship between ROS and the development of neoplasias. If one considers the three-stage model of carcinogenesis (initiation, promotion, progression), the first phase is ROS mediated induction of several types of DNA damage including strand breakage, base modification and DNA-protein cross-linkage. Oxidative DNA damage can also be indirect; e.g., the action of peroxy radicals freed by endogenous lipid peroxidation or derived from the metabolism of classical chemical carcinogens. Some chemicals are directly carcinogenic, but most require metabolic activation before they can react with genetic material. Free radicals are involved in these activation reactions. Metabolic activation of carcinogens in P450-mediated reactions is known to produce a variety of activated species. The formation of these free radicals is in the endoplasmic reticulum.

It is important to remember that highly reactive free radicals are essentially trapped in the immediate vicinity of their formation as a consequence of rapid interaction with neighbouring molecules. Therefore, their radius of diffusion is frequently small from cellular perspective. Reactive free radicals formed in the endoplasmic reticulum are unlikely to diffuse far enough to react with nuclear DNA. It has been postulated<sup>84</sup>, therefore, that metabolically activated free radicals must involve an intermediate chemical reactivity to directly impact DNA with covalent adducts.

Therefore, the issue of location has led to the hypothesis that most cancer may originate in the mitochondrion rather than in the cell nucleus<sup>85</sup>. Mitochondria are self-regulating and contain their own DNA that directs the synthesis of some of the mitochondrial proteins. Mitochondrial DNA is a

single, circular molecule, much less protected than the coiled and chromatin-packaged nuclear DNA<sup>86</sup>. Mutagens bind to mitochondrial DNA up to 1,000 times more strongly than to nuclear DNA<sup>87</sup>. Also, DNA repair mechanisms are much less efficient in the mitochondrion<sup>87,88</sup>. Thus, both mitochondrial DNA and the organelle's inner and outer membranes, high in polyunsaturated fatty acids, are susceptible to attack by free radicals and electrophilic metabolites despite the impressive multilayer free radical defence system<sup>87, 89,90</sup>. It has been suggested that the damage to the mitochondrion by oxygen free radicals leaking from the electron transport chain may cause a baseline level of cancer ("natural" cancer), whereas damage resulting from mutagenic metabolites of chemicals may account for the remainder<sup>91</sup>.

In the multi-step process of carcinogenesis, cell division is another critical factor<sup>92-94</sup>. When the cell divides, an unrepaired DNA lesion can give rise to a mutation. It is of interest that oxidants form one important class of agents that stimulate cell division<sup>95-97</sup>. This may be related to the stimulation of cell division that occurs during the inflammatory process, accompanying wound healing<sup>92</sup>. The idea of oxygen free radical involvement in tumor promotion is mostly supported by indirect evidence such as the ability of tumor promoters to induce the respiratory burst in phagocytic cells, the anti-promotor efficiency of antioxidants and free radical scavengers and the capacity of oxygen free radical generating compounds to promote tumors<sup>98-106</sup>.

The relationship between chronic infection, inflammation and cancer is also of interest.

Leukocytes and other phagocytic cells combat bacteria, parasites and virus-infected cells by destroying them with a powerful oxidant mixture of NO, O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, and OCl<sup>-</sup><sup>107,108</sup>. These oxidants protect humans from immediate death vis-à-vis infection and simultaneously cause oxidative damage to DNA plus mutation<sup>109,110</sup> thereby contributing to the carcinogenic process. It is estimated that chronic infections contribute to about one-third of the world's cancer. Hepatitis B and C viri infect about 500 million people and are a major cause of hepatocellular carcinoma<sup>111-113</sup>. A chronic parasitic infection, schistosomiasis, may lead to cancer. It is prevalent in China and Egypt. It lays eggs in the colon producing inflammation that often leads to colon cancer<sup>114</sup>. It can also promote bladder cancer<sup>115</sup>. *Helicobacter pylori* bacteria infecting the stomachs of over one-third of

the world population appear to be the major cause of gastritis, ulcers and stomach cancer<sup>116-121</sup>. Chronic inflammation resulting from non-infectious sources also contributes to various pathological conditions ultimately leading to cancer. For example, asbestos exposure causing chronic inflammation may be a significant risk factor in the development of lung cancer<sup>122,123</sup>.

#### Atherosclerosis

The predominant role of atherosclerosis in causing human disease and death justifies a short discussion of the possible role played by ROS in such pathogenesis (for review see refs. 124, 125). The view that peroxidative processes are involved in the etiology of cardio-vascular diseases, particularly atherosclerosis was suggested by early experimental and clinical data<sup>39,126-132</sup>. Epidemiological studies have demonstrated an association with low plasma concentrations of ascorbate, tocopherol, and B-carotene<sup>133-142</sup>. Within this context, the pathogenetic role of lipid peroxidation in myocardial infarction and stroke was repeatedly discussed. However, evidence also was considered at that time as circumstantial<sup>8,143-145</sup>. The strongest evidence in favour of this assumption was the protective effect of radical scavengers, particularly enzymes or drugs.

Different mechanisms have been postulated wherein lipid peroxidation is involved in the development of atherosclerotic plaques causing thrombotic events including stroke or myocardial ischemia<sup>146</sup>. Lipid peroxidation especially that achieved via the production of ROS by activated monocytes/macrophages adhering to the arterial endothelium<sup>147</sup> could make an early and significant contribution to the development of atherosclerotic plaques<sup>148</sup>.

It has been demonstrated that one of the earliest events, which occurs in atheroma formation is the accumulation of cholesterol-laden foam cells in the subendothelial space. Most of the cholesterol deposited in the cells is derived from low-density lipoproteins (LDL). Human LDL is not only rich in cholesterol but also in polyunsaturated fatty acids (PUFA) which are susceptible to lipid peroxidation

Free radical oxidation of LDL, is one of the biological modifications occurring *in vivo* that increases the rate at which LDLs are scavenged by macrophages; nonoxidized LDL is not scavenged at an increased rate<sup>149-157</sup>. Macrophages, the main precursors of the foam cells, do not take up low-

density lipoproteins at a rate rapid enough to cause lipid loading<sup>149,158,159</sup>. However, presence of Fe<sup>2+</sup> in plaques following entry of blood through plaque fissures and subsequent local hemolysis enhances the oxidation of LDL and thus promotes the accumulation of foam cells. In addition, toxic products of lipid peroxidation favour local necrosis, which may, in concert with other factors, initiate an inflammatory process. Furthermore, oxidative modifications of LDL can, in conjunction with cytokines promote the attachment of even more monocytes to the endothelium. In line with this thinking, SOD has been found to inhibit the oxidation of LDL suggesting that the superoxide radical is responsible for the process. However, metal ion chelators and other general free radical scavenger can also prevent this oxidation<sup>160-162</sup>.

#### Brain pathologies

A third field of early interest came from biochemical studies suggesting that ROS is important in a number of brain pathologies<sup>163-169</sup>. The brain consumes a disproportionate amount of the body's O<sub>2</sub>. It derives its energy, almost exclusively from the oxidative metabolism of the mitochondrial respiratory chain. Mitochondria are found in neuronal cell bodies but are also distributed throughout the neuritic structures.

Apart from high oxygen consumption, the brain is rich in oxidizable substrates, mainly unsaturated lipids and catecholamines. This initiated early interest regarding "oxygen radicals" as mediators of the action of certain neurotoxins, in the role of vitamin E in the nervous system and in the possible use of antioxidants in treating degenerative diseases of the nervous system as well as the consequences of ischemia.

The discovery of enzymes that specifically scavenge superoxide in aerobic cells (superoxide dismutases) led to the proposal that O<sub>2</sub> is a major agent of O<sub>2</sub> toxicity. This superoxide theory of O<sub>2</sub> toxicity<sup>170-173</sup> is based upon a mass of evidence demonstrating that superoxide dismutases are important for survival in the presence of O<sub>2</sub>. SOD enzymes co-operate with other enzymes such as catalase and glutathione peroxidase that destroy H<sub>2</sub>O<sub>2</sub><sup>173</sup>. Catalase decomposes H<sub>2</sub>O<sub>2</sub> directly. Very little catalase is present in brain as compared with liver, kidney and erythrocytes. Catalase in tissues is located in small subcellular particles known as "peroxisomes". The peroxisomes found in brain are very small as compared with liver peroxisomes and

are often called "microperoxisomes"<sup>174</sup>. Most H<sub>2</sub>O<sub>2</sub> generated in brain *in vivo* is probably disposed of by glutathione peroxidase<sup>175</sup>. This enzyme removes H<sub>2</sub>O<sub>2</sub> by using it to oxidize glutathione (GSH). Glutathione peroxidase requires selenium for its action. Oxidized GSH (GSSG) is reconverted to GSH by a glutathione reductase enzyme. Both glutathione peroxidase and reductase are present in all parts of the brain and nervous system. A role of GSH in neurodegeneration is suggested by the observation that inborn defects in the ability to synthesize GSH produce severe mental and motor retardation and seizures<sup>176</sup>. It was also suggested that GSH depletion is involved in the Parkinson's disease-like syndrome induced by the meperidine analogue, MPTP<sup>177</sup>.

Particular attention has been focused on a role of oxygen radicals in Alzheimer's disease. Alzheimer's disease is a progressive neurodegenerative disorder affecting >5% of the population over the age of 65. It is characterized pathologically by cortical atrophy, neuronal loss, glial proliferation, excessive neurofibrillary tangles, and deposition of B-amyloid in neuritic plaques<sup>178-181</sup>. One hypothesis is that cellular events involving oxidative stress may lead to neurodegeneration<sup>182-189</sup>. Indeed, ROS may be involved in the production, aggregation and toxicity of B-amyloid<sup>190</sup> which is thought to contribute to neuronal damage in Alzheimer's disease<sup>191</sup>.

Recently, attention has been focused on proteins exposed to reducing sugars. These proteins undergo nonenzymatic glycation and oxidation, which ultimately form irreversible advanced glycation end products (AGEs). AGEs-modified proteins form cross-links which result in aggregation and insolubility; they are also a continuing source of potentially damaging reactive oxygen species. The longstanding protein aggregates in Alzheimer's disease such as paired helical filament (PHF) tau and amyloid B-protein<sup>192-194</sup>, could form AGEs and contribute to the development of neuronal dysfunction. It has been demonstrated that PHF tau contains AGEs. Other evidence emanates from a study comparing the levels of oxidative damage to proteins, lipids and DNA bases from seven different brain areas of Alzheimer's disease along with matched control tissues. No differences in levels of lipid peroxidation were found in any of the brain regions by using two different assay systems. However, both protein carbonyl levels and oxidized DNA bases were increased in Alzheimer's in several

areas. The documentation of increased damage to protein and DNA strengthens the possibility that oxidative damage may play a role in the pathogenesis of Alzheimer's disease.

A few epidemiological studies are consistent regarding a protective effect by fruits, vegetables or antioxidants<sup>195-197</sup> in a number of neurological pathologies including cerebral ischemia, Parkinson disease, familial amyotrophic lateral sclerosis (a chronic motor neuron degenerative disorder)<sup>198,199</sup>.

The previous diseases are examples; data have demonstrated that many other diseases and clinical disturbances involve ROS reactions in mammalian systems. A list of such diseases is presented in Table 2<sup>200</sup>.

#### **Protein control quality and chronic disease development: Free radical damage and heat shock c.q. chaperone proteins**

In this section a survey is presented regarding the mechanisms underlying the defence reactions following increased oxygen radical production. Stress conditions, including excessive free radical production, lead to the production of heat shock proteins (HSPs), able to protect against damage. The HSP or stress proteins are also named as to their function, such as "chaperone proteins", since they form complexes with proteinaceous and other cellular structures in order to prevent deleterious interactions between proteins. Understanding the molecular mechanisms of cellular protection and recovery from damage in injured cells had increased greatly in recent years. In particular, how chaperones at the molecular level facilitate stress regulation vis-à-vis protein quality control mechanisms, and have become hallmarks of a range of chronic diseases including neurodegenerative disorders, diabetes, atherosclerosis and many others.

#### **ROS damage protected by heat stress**

The suggestion that heat stress provides myocardial protection against ischemic-reperfusion injury has

been extensively studied vis-à-vis cell cultures. When cells are exposed to a few degrees above their normal growth temperatures, inhibition of protein synthesis and cell death can occur<sup>201</sup>. However, when the treatment is sub-lethal, the cells exhibit a heat shock response<sup>202</sup>. The dramatic feature of this response is the massive and selective increase in synthesis of a small number of heat shock proteins<sup>203,204</sup>.

Lee *et al.*<sup>205</sup> observed that heat shock and oxidative stress share a common effect on cells. Heat shock can increase levels of lipid peroxidation as determined by the formation of TBA-products. The supporting evidence was obtained from studies on the induction of heat shock proteins and increased antioxidant enzyme activity by heat shock and oxidant stress<sup>206-208</sup>. Furthermore, it was observed that (a) inhibition of antioxidant defences induce the production of heat shock proteins and increase lethal susceptibility to heat shock<sup>209,210</sup>; and (b) augmenting antioxidant defences decrease tissue damage that occurs during reoxygenation following a period of hypoxia<sup>211</sup>.

Similar conclusions were derived from studies with lung slices exposed to oxidant and hyperthermic stresses. Heat and oxidants as well as reoxygenation following hypoxia at normal temperatures induced heat shock proteins. Heat shock protein synthesis was also induced in lung slices exposed to the Cu chelator diethyldithiocarbamate which decreases the activity of Cu/Zn superoxide dismutase<sup>212</sup>.

In isolated rat<sup>213</sup> and rabbit<sup>214</sup> hearts, heat stress can provide myocardial protection against ischemic-reperfusion injury, reducing infarct size. In addition, heat stress can lead to an increase in cardiac catalase activity in the rat<sup>213</sup> providing an important pathway for hydrogen peroxide detoxification<sup>160</sup>. Inhibition of catalase abolishes the protection against post-ischemic dysfunction afforded by prior heat stress<sup>215</sup>. It has,

Table 2—List of diseases and clinical disturbances that involve ROS reactions in mammalian systems<sup>200</sup>

Adult respiratory distress syndrome	Contact dermatitis	Myasthenia gravis
Aging	Dermatomyositis	Pancreatitis
Alcoholism	Emphysema	Parkinson disease
Allergic encephalomyelitis	Favism	Psoriasis
Alzheimer disease	Glomerulonephritis	Retrolental fibroplasias
Arteriosclerosis	Gout	Rheumatoid arthritis
Autoimmune vasculitis	Haemachromatosis	Senile dementia
Bronchopulmonary dysplasia	Ischemia-reperfusion injury	Sickle cell anemia
Cancer	Lypofuscinosis	Stroke
Cataract	Malaria	Systemic lupus erythematosus
Chronic autoimmune gastritis	Multiple sclerosis	Thalassemia
Cirrhosis	Muscular dystrophy	Ulcerative colitis

therefore, been proposed that the benefit afforded by heat stress is due to an enhancement of cardiac anti-oxidant status<sup>215</sup> and HSP in facilitating cellular repair<sup>216</sup>. It can be concluded that both the induction of the anti-oxidant enzymes and the induction of HSP's may be considered as part of the second tier of defense that takes place at the level of gene expression. Its significance has become very clear nowadays.

Earlier work was perplexing in the way that many different agents were able to lead to the so-called 'stress response' which started as a molecular curiosity in fruit flies in the early sixties<sup>217</sup>. Following the nomenclature first used for fruit flies, various heat shock proteins in animal cells are referred to on the basis of their mode of induction and apparent molecular mass in kDa. Hence their designation as HSP70 or *grp78* for example refers to heat shock proteins of 70kDa and glucose regulated proteins of 78kDa, respectively. Over the last 25 years, a number of observations provided support for the so-called abnormal protein or proteotoxicity hypothesis put forward to explain the induction of the heat shock response by a large variety of stress conditions<sup>218,219</sup>. When cells have been exposed to heat shock or to toxic substances such as ethanol, cadmium, arsenite or oxidative stress, the structure of many proteins is damaged. These abnormally shaped proteins become functionally inactive. Moreover, there is also a high risk that these abnormal protein molecules aggregate not only with other damaged proteins but also with still functional proteinaceous cellular structures.

Proteins, with their structural and functional complexity are fragile macromolecules. Already during their growth, when polypeptides mature stage by stage, the chains cannot fold correctly until a complete folding domain has been created raising the possibility that incomplete domains may misfold. These developments take place within highly crowded compartments. Such conditions compete with normal folding and may cause the phenomenon of misassembly. Misassembly is defined as the misguided association of two or more polypeptide chains to form nonfunctional structures<sup>220</sup>. These structures may be as small as dimers or large enough to be insoluble. The emphasis on function serves to distinguish misassembly from the formation of functional oligomers termed oligomerization. Misassembly should be distinguished from misfolding which is defined as the formation of a conformation

which cannot proceed to a functional level within a biologically relevant time scale. Misassemblies are by definition misfolded.

Each protein in the cell has its own intrinsic propensity to unfold and misfold spontaneously, a tendency which increases with variations of environmental conditions. Thus, a continuous flux of toxic, misfolded proteins is spontaneously formed during the lifetime of a cell. Depending on their cellular concentration, misfolded species tend to assemble into stable protein aggregates in the cytoplasm which is also extremely crowded and viscous. The term 'crowded' is preferred to 'concentrated' because, generally no single, macromolecular species occurs at a high concentration. However, taken together, macromolecules occupy approximately 8-40% of the total volume<sup>221</sup>. The cytoplasm is a space, in which densely crowded proteins, each with a different complementary function, must be able to move randomly to meet and timely interact with rare specific partners. Most proteins native to a living system contain repulsing, negative charges on their surfaces and thus refrain from exposing hydrophobic segments; these proteins can optimally maneuver and avoid each other in the highly promiscuous environment of the cytoplasm. In this context, the spontaneous conversion of a functional native protein into a misfolded one, exposing positive charges and new hydrophobic surfaces, will greatly increase both the friction between the macromolecules and the viscosity of the cytoplasm. Increased cytoplasmic viscosity reduces freedom of movement and consequently impairs the function of many cytoplasmic proteins in addition to the above-mentioned cytotoxic effects of aggregates<sup>222-224</sup>.

Bacteria and eukaryotes have developed defence mechanisms against "toxic" protein aggregation, utilizing two protein types: the molecular chaperones (typically HSP90, HSP70, HSP60, HSP27) and the ATP-dependent proteases (typically Lon, ClpC/X/P, FtsH, KslU/V, and the 26S proteasome)<sup>225</sup>. Laskey first proposed the term "molecular chaperone" for nucleoplasm<sup>226</sup>. Ellis expanded the definition of molecular chaperone: a fully developed (stable) protein that escorts still developing proteins to prevent improper associations<sup>227</sup>. Presently, this definition of a protein with a simple escorting role is still applicable regarding some simple, binding chaperones such as the small HSP's. However, it has since been

demonstrated that chaperones<sup>228</sup> possess many active functions<sup>229</sup>: they convert part of the energy of ATP hydrolysis to repair structural damages in stable, misfolded, dysfunctional proteins. These chaperones forcefully disentangle stable dysfunctional aggregated proteins, unfold, refold and re-stabilize them into “re-educated and born again” native, functional proteins in the cell.

When there is no appreciable stress, molecular chaperones and the proteases exist in cytoplasm at low concentrations. This is sufficient to perform physiologic housekeeping functions and to remove sporadically misfolded proteins. However, during extreme situations such as oxidative stress or heat shock, chaperone and protease systems become overloaded by toxic protein forms. Cells synthesize then massive amounts of molecular chaperones and proteases<sup>230</sup>. The stress-inducible nature of many molecular chaperones had led to early classification among the heat shock proteins (HSPs). They are categorized by molecular weight: HSP100, HSP90, HSP70 (HSP40, HSP20), HSP60 (HSP10) and HSP22/27 in eukaryotes (co-chaperones in brackets); and correspond to bacteria: ClpB, HtpG, DnaK (DnaJ, GrpE), GroEL (GroEs) and IbpA/B. Different chaperones display mutually non-exclusive properties. Some “binding” chaperones, e.g., HSP90, HSP70, HSP60, HSP40 and HSP22/27 can provide adhesive surfaces, which, upon interaction with partially denatured polypeptides or oligomerizing subunits, can passively reduce the extent of aggregation<sup>231,232</sup>. Unfolding chaperones, such as HSP100, HSP70 and HSP60 (possibly also HSP90) are involved in ATP-dependent unfolding (followed by the spontaneous native refolding) of denatured polypeptides<sup>233,234</sup>.

The literature regarding the roles of various chaperone types suggests two functionally different classes. Small chaperones (less than 20 kDa) bind transiently to short hydrophobic sequences on polypeptide chains and prevent them from both folding prematurely and misassembling by binding to these sequences for a period of time. Large chaperones, exemplified by GroEL, function basically by providing a molecular cage composed of one oligomer of GroEL capped by one oligomer of GroES<sup>235</sup>. Single, partly folded chains are encapsulated one at a time inside this cage. The enclosed chain continues to fold in the absence of other folded chains until the hydrophobic surfaces that cause misassembly are buried within the final folded structure. The time

of folding inside this cage is set by the slow ATPase activity of the GroEL subunits and results ultimately in the release of the folded chain into the cytosol<sup>220</sup>. These data demonstrate that during one’s lifetime, cells maintain a battery of defense that reduces the concentration of toxic, misfolded protein species, maintaining them below critical toxic concentrations.

As we age molecular chaperones and proteases are insufficiently produced. We may react poorly to environmental stress<sup>236</sup>. The levels of molecular chaperones and proteases are significantly decreased. Simultaneously, irreversibly damaged proteins accumulate<sup>237, 238</sup> due to decline in functional proteasomes and lysosomes. In addition to their general cytotoxic effect, irreversibly damaged proteins can inhibit the activity of the remaining minority of functional chaperones and proteases. At this stage, old cells often choose suicide, which may at times, be advantageous, for example with cancer cells. HSP70 has been shown to protect against cell death by directly interfering with the mitochondrial apoptosis pathway<sup>239</sup>.

The occurrence of protein damage as the origin of cellular disorder is increasingly recognized as a main biomedical focus of interest since its occurrence not only has been observed as a result of physical and chemical stress but also upon exposure to pathogens as bacteria and viruses, during ischemia, inflammation, transplantation and upon neurodegenerative and other chronic diseases (see further in this chapter). The overproduction of molecular chaperones following treatments with various non steroidal anti-inflammatory drugs (NSAIDs, e.g., sodium salicylate<sup>230</sup>, ibuprofen<sup>240</sup>), and less classical HSP-inducers such as celastrol<sup>241</sup>, resveratrol (french paradox)<sup>242</sup> and geranylgeranylacetone<sup>243</sup>, may be responsible for the reduction of damages related to reactive oxygen and induced programmed cell death in various damaging contexts. Examples include ARDS<sup>244</sup>, and post-ischemic reperfusion<sup>245</sup>.

#### Regulation at gene expression level

A simple model for the regulation of availability of protector proteins in defense following damage is regulated at cell’s DNA level. The quantity of free protector proteins available in the cell decreases under these adverse circumstances. As long as these essential proteins are available, damage is reduced to a minimum. However, when a shortage arises in the

case of an overload of damage, the originated abnormal protein molecules are capable of complexing with other cell structures. Cell damage can then only be avoided by production of new protector proteins. The replenishment of these protector proteins starts with activation of associated protector protein gene promoters on the cell's DNA. This highly specific event occurs by binding of specific DNA-binding factors, called heat shock transcription factors (HSF's) on specific (promoter) DNA-sites<sup>246</sup>. This binding constitutes the signal that triggers transfer of information from DNA into mRNA, leading eventually to synthesis of new protector proteins.

Whether or not these DNA-binding factors interact with the DNA depends on the existing quantity of protector proteins in the cell. The genome is only specifically activated to trigger this synthesis of additional protector proteins when their quantity falls below a certain threshold. Normally, at least one type of protective protein HSP70, forms a complex with HSF, which provides the basis for this regulation. If protector proteins are required to neutralize abnormal proteins, this complex dissociates, causing release of HSF which then binds to the promoters and induces mRNA production with the ensuing synthesis of new protector proteins. When sufficient new protector proteins have been produced, i.e. when their amount is raised above the threshold value, HSP70 will again form a complex with HSF molecules, uncoupling it from DNA, with a concomitant halt of mRNA production. This molecular reaction cycle can be indicated, in terms of systems theory, as the autoregulation loop which is the basis of damage-induced recovery processes.

However, cells do not use only one type of transcription factor (HSF) in response to stress conditions; they use multiple signalling pathways and transcription factors to fine-tune their response to specific circumstances. In addition to the heat shock factor, also nuclear factor-kB, nuclear factor erythroid-2 and activator protein-1 families have been recognized as important regulators of the cellular stress response. These different families of transcription factors are generally activated by different stress conditions. Although there is a functional overlap between these individual families and a given stimulus can activate members of more than one (and even all four) of these transcription

factors, they broadly regulate different aspects of the cellular stress response by modulating specific target genes. As was described above, HSF is activated under stress conditions characterized by significant intracellular accumulation of non-native proteins and consequently activates genes whose products are capable of alleviating this condition and restoring the integrity of damaged proteins. NF-kB is an important regulator of cytokines and other mediators of the immune and inflammatory response that provides protection against bacterial and viral infections. Nrf2 is activated by various xenobiotics and oxidants and therefore regulates genes encoding proteins with xenobiotic detoxification and antioxidant activities. Finally, AP-1 factors control cellular fate by regulating production of proteins that mediate cell growth or cell death, the latter being the most drastic decision by a cell under extreme stress. Various stimuli may simultaneously cause multiple types of 'molecular' stress and therefore may activate two or more of the transcription factors, leading to a differential stimulus-specific gene expression. It has indeed been observed that a unique pattern of stress proteins is induced when cells are exposed to different stress conditions<sup>247</sup>.

Heme oxygenase-1 (HO-1 or HSP32) appears to be the only protein which is induced by all four of the stress-responsive factors<sup>248</sup>. Upregulation of the HO-1 gene is associated with marked cytoprotection. Studies using HO-1 deficient cells and mice<sup>249</sup> have confirmed that the HO-1 system is indispensable to survival and, in particular, to protection from oxidant stress<sup>250-252</sup>. HO-1 is the rate-limiting enzyme in the breakdown of heme with bile pigments (biliverdin and bilirubin), iron and the gas CO as catalytic end products. Although initially viewed as obscure waste products with potential toxicological implications, they are currently seen as serving a critical physiological role in cytoprotection during cellular stress and organ pathology. Bilirubin is considered to be the most potent antioxidant molecule in serum<sup>253</sup>. CO also serves a clear physiological (hormetic) role in cellular defence ameliorating inflammatory and ischemic injuries<sup>254</sup>, whereas iron stimulates the upregulation of the iron-binding ferritin protein which helps to prevent Fenton reactions leading to the highly damaging hydroxyl radical. A number of review papers have emphasized the importance as well as the clinical relevance of heme oxygenase since an

upregulation of HO-1 leads to an enhanced resistance against a broad range of (oxidative) stress conditions and alleviates a number of pathological conditions including cardiovascular disease, neurodegenerative diseases and inflammation<sup>251,252,254,255</sup>.

The unique pattern of stress proteins induced in cells that are exposed to different stress conditions has other highly interesting consequences. A brief and moderate heat shock to Reuber H35 hepatoma cells causes a rapid increase in the synthesis of heat shock proteins (HSP) and initiates the development of thermotolerance, which results in an increased ability to survive exposure to otherwise lethal temperatures. Low doses of various chemical stressors [arsenite, cadmium, mercury, lead, copper, menadione and diethyldithiocarbamate (ddtc)], at concentrations that do not exert any effect in control cultures, are able to enhance the synthesis of HSP's and to stimulate the development of thermotolerance when applied to cultures which were pretreated with a mild heat shock<sup>256</sup>. The degree of stimulation appears to be stressor-specific, which is not only observed in the ensuing development of thermotolerance but also in the enhancement of the heat shock-induced synthesis of stress proteins. The different HSP's that show an enhanced induction when heat shocked cultures are exposed to the various secondary applied low doses of chemical stressors, were found to resemble the HSP pattern that is characteristic for the secondary stressor and not for the initial heat shock. In other words, the nature of the post-treatment determines the observed pattern of enhanced synthesis of HSP's. In order to analyze the origin of the stimulation of survival capacity by low doses of the mentioned stressors, it was studied whether the degree of stimulation is determined by the degree of similarity between the overall stress response to heat shock and to the second stress condition when applied singly. The degree in which low doses of chemical stressors stimulate tolerance development and enhance the synthesis of HSP's in cells that were previously heat shocked, appears to be related to the degree of similarity in the HSP pattern induced by both stressors. The results support the notion that low doses of toxic compounds may, under certain conditions, have beneficial effects related to a stimulation of endogenous cytoprotective mechanisms.

#### Misfolded proteins and aggregates in disease

Misfolded proteins and aggregates are hallmarks of a range of neurodegenerative disorders including

Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), polyglutamine (polyQ) diseases that include Huntington's disease and related ataxias<sup>257-259</sup> as well as diabetes<sup>260</sup>. Each of these disorders exhibits aging-dependent onset and a progressive, usually fatal clinical course. Despite differences in the underlying genes and clinical presentation, similarities observed have led to the proposal that cellular protein quality control is the underlying common denominator of these diseases<sup>261</sup>. In this section this is first illustrated for a clinical situation, type 2 diabetes mellitus, and subsequently illustrated with basic research utilizing a model for polyQ pathogenesis.

#### Type 2 Diabetes mellitus (T2DM)

One of the most important cellular stressors in T2DM that contribute to protein misfolding and aggregation is redox stress. ROS may impact disulfide bond formation<sup>262</sup> and subsequently influence the development of Islet amyloid polypeptide (IAPP) misfolding. IAPP oligomers precede islet amyloid deposition. Disulfide bonds formed in newly synthesized proteins are important for proper protein folding, protein structure, biological activity, and stability of many secreted and membrane proteins<sup>258,263,264</sup>. Protein folding in eukaryotes takes place in the ER with assistance from many redox-sensitive chaperones and oxidoreductases (e.g., protein disulfide isomerase, Erp44, Erp57, Erp72, GRP58, HSP33)<sup>264</sup>. Growing evidence implicates both ROS and RNS (radical nitrogen species, such as the reaction of superoxide anion ( $O_2^-$ ) with nitric oxide (NO) to form peroxynitrite and other RNS) could contribute to protein misfolding<sup>265</sup>, and are important in the development of diabetes<sup>266-270</sup>. When the protein quality control system is overwhelmed and IAPP is not capable of being correctly refolded, this protein can become a soluble toxic monomer. Soluble IAPP oligomers have been shown to be cytotoxic and possibly responsible for beta cell apoptosis in T2DM<sup>271-273</sup>. Accumulation of mature islet amyloid is responsible for the space-occupying lesion with associated secretory and absorptive defects within the islet.

Thus, type 2 diabetes mellitus (T2DM) is an example of a conformational disease featuring a protein that aggregates in beta-pleated sheets that are linked by hydrogen bonding between their aligned pleated structures<sup>260</sup>. The contribution of islet amyloidosis to disease pathogenesis has been

vigorously debated<sup>274</sup>. IAPP oligomers that precede islet amyloid deposition are likely more toxic to beta cells than islet amyloid itself. The misfolded, soluble oligomeric proteins promote apoptosis<sup>271,275</sup>. Clinically, it is clear that aggregates of misfolded IAPP are a prominent pathological feature in the development of T2DM (reviewed by Hayden and Tyagi<sup>276</sup>). Islet amyloid is present at autopsy in as many as 96% of patients with T2DM<sup>277</sup>. In case of T2DM, amyloid fibrils are formed with subsequent stabilization by accessory molecules, such as serum amyloid P, perlecan, and apolipoprotein E<sup>274</sup>.

An additional factor in disease development is that mitochondrial respiratory function has been demonstrated to decline in various human tissues during the aging process<sup>278,279</sup>. Mitochondria are the major intracellular source and primary target of ROS, which are generated under normal conditions as by-products of aerobic metabolism in animal and human cells. It has been established that defects in the respiratory chain lead to increased production of ROS and free radicals in mitochondria<sup>280-282</sup>. Mitochondrial biology is one of the fastest growing areas in molecular genetics and medicine. Mitochondrial diseases are very numerous and different. Apart from diseases definitely caused by abnormalities in mitochondrial DNA, many diseases are suspected to be caused in part by dysfunction of mitochondria, such as diabetes mellitus, forms of cancer and cardiovascular disease, lactic acidosis, specific forms of myopathy, osteoporosis, Alzheimer's disease, Parkinson's disease, stroke, and many more. The decline in functioning is caused, at least partly, by oxidative damage and mutation of mitochondrial DNA (mtDNA) and lipid peroxidation in somatic tissues of aged individuals<sup>279, 283-287</sup>. Recently, it was found that mtDNA copy number is increased in the tissues of elderly human subjects<sup>283</sup>. Taken together, these findings suggest that the increase in mitochondrial mass and mtDNA content are the early molecular events of human cells in response to endogenous or exogenous oxidative stress through cell cycle arrest and it was thought to compensate for respiratory function decline during the aging process<sup>288,289</sup>.

#### **PolyQ disease: *Caenorhabditis elegans* in basic research**

There is growing evidence for genes involved in protein folding and degradation that modulate onset, development and progression in models of multiple neurodegenerative disease<sup>290-292</sup>. Some of the disorders,

including the polyQ diseases, exhibit familial inheritance that facilitates the identification of single gene alterations underlying the disorders<sup>293-296</sup>. Other diseases are sporadic and yet, they too have helped to identify candidate genes that could reveal insights into pathology. These include mutations of amyloid precursor protein in Alzheimer's disease, parkin and alpha-synuclein in Parkinson's disease and superoxide dismutase in amyotrophic lateral sclerosis<sup>297-302</sup>. Identification of these genes has led to the development of transgenic mouse, cell culture models as well as models using *Drosophila* and *C. elegans* to study neurodegenerative disease<sup>303-307</sup>.

In a few animal models it can be demonstrated that aggregation is accompanied by cellular dysfunction and formation of polyQ aggregates visible by light microscopy<sup>308,309</sup>. An illustrative research line is the study of polyQ-length-dependent aggregation in neuronal dysfunction by Morimoto and colleagues utilizing *C. elegans*. Behavioral phenotypes of *C. elegans* were examined to test whether polyQ aggregation in neurons was accompanied by neurotoxicity. There was a polyQ length-dependent loss of coordinated movements leading to nearly complete paralysis. Animals with no visible polyQ aggregates (Q0 animals) demonstrated rapid movements similar to wild type animals. Animals with visible aggregates, Q67 and Q86, had limited capacity for coordinated movements. Animals with intermediate polyQ length, for instance Q19, showed an intermediate situation with slight decrease of movement. These data suggest that formation of visible polyQ aggregates correlates with neuronal dysfunction. Studies on the influences of aging regarding the threshold for polyQ aggregation and toxicity focused on the behavior of polyQ proteins<sup>309</sup>. Individual animals were examined daily for the appearance of protein aggregates and motility. Q40 and Q82 animals quickly accumulated aggregates of protein and exhibited a rapid decline in motility; Q33 and Q35 animals exhibited an initial lag prior to the gradual accumulation of aggregates demonstrating ultimately lower levels. This data reveal that the threshold for polyQ aggregation and toxicity is age-dependent<sup>309</sup>. The molecular link between these pathways is regulated, in part, by factors that detect and respond to misfolded proteins: namely, heat shock transcription factor (HSF) and molecular chaperones/heat shock proteins. For example, it has

been shown that inhibition of HSF-1 function leads to decreased lifespan and an accelerated aging phenotype in *C. elegans*<sup>310-312</sup>. Conversely, overexpression of HSF-1 in *C. elegans* extends lifespan<sup>311,312</sup>.

In summary, there is convincing evidence to support a role for oxidative stress in the pathogenesis of many chronic diseases. The model includes misassembly and aggregation of proteins when three major tiers of defense are insufficient: (a) direct antioxidative systems, (b) molecular damage repair systems (like chaperones), and (c) capacity of the compensatory chaperone synthesis. Aggregates of amyloid proteins are commonly novel producers of ROS.

This has resulted in the hypothesis that during life time depending on both, predisposition and stressful conditions, the defence system can fail and an increase of ROS occurs in the early period of development of chronic diseases. The implications of this hypothesis for diagnostic purposes has raised interest in the use of noninvasive procedures to record human oxidative in relation to development of pathology of chronic diseases (such as Alzheimer's disease and diabetes) that are supposed to be linked to non-linear progression in ROS production. A number of patients with these diseases are also taking antioxidant therapy on the recommendation of their caregivers or physicians in the belief that such therapy may offer some protective benefit. Along this line, the development of a noninvasive tool for detection of human emission and its validation is crucial.

The next section reviews the current status and future issues of the human photon emission techniques and protocols for recording the human oxidative status, i.e., recent knowledge regarding uniformity and variation in anatomical pattern of photon emission, its dynamics in vis-à-vis internal physiology and psychophysiology, and its relation to health and disease.

#### **Low level luminescence as marker of oxidant status of biological systems**

Many techniques are available to measure the progress of oxidation, but none is applicable to all circumstances. A summary of techniques as biomarkers of oxidative stress in tissue damage, focusing predominantly on the measurement of biological lipid peroxidation has been reviewed by Gutteridge and Halliwell<sup>313</sup> and summarized in Table 3. The table contains the techniques to detect

lipid peroxidation as evidence most frequently cited to support the involvement of free-radical reactions in toxicology and disease. However, no single method is adequate for all stages of lipid peroxidation in a biological system and few have the desired specificity. Although most techniques have focused on lipids, it is evident that proteins are also targets for oxidation in biological systems under oxidative stress. Both aspects of oxidative stress can be recorded with the chemiluminescence method. Both lipid<sup>314</sup> and protein oxidation<sup>315</sup> are accompanied by spontaneous light emission that may be easily detected with sensitive photomultipliers<sup>316</sup>.

Luminescence of living organisms has fascinated scientists since antiquity<sup>313,317,318</sup>. Until 1961 it was thought to be restricted to organisms with "light organs" containing luciferin-luciferase systems. At that time, Tarusov *et al.*<sup>17</sup> used photon counting to identify a weak blue-green light emission from mouse liver *in situ*. This observation was later extended to brain, muscle, intestine, tissue homogenates and lipid extracts<sup>17-19, 319-321</sup>. The existence of such light emission was labeled "low-level" chemiluminescence or "dark" chemiluminescence to differentiate it from the more effective photoemission of the luciferin/ luciferase systems which is  $10^3$ - $10^6$  times brighter<sup>16,19</sup>. In early studies, yeast cells<sup>322, 323</sup>, phagocytosing leukocytes/macrophages<sup>324-326</sup> and hepatocytes<sup>327</sup> exhibited "low level" luminescence<sup>16</sup>. This luminescence cannot be seen by the dark adapted human eye because retinal illumination of  $3 \times 10^3$  photons sec<sup>-1</sup> cm<sup>-2</sup> is required to perceive a luminous signal<sup>328</sup>.

#### **Low level chemiluminescence in production of electronically-excited states**

Low-level chemiluminescence was soon related to the direct utilization of molecular oxygen and the production of electronically-excited states in biological systems; in particular, the oxygen dependent chain reactions involving biological lipids<sup>14,15,17-19,321</sup>. This earlier research on low-level chemiluminescence was largely unnoticed in America and Europe, but reports by Nakano and colleagues on light emission during lipid peroxidation, both in isolated microsomes and during other oxidative reactions, revived the interest in chemiluminescence and suggested its use as a tool for the investigation of the radical reactions of lipid peroxidation under physiological conditions<sup>329-331</sup>. The simplified systems employed in these earlier studies demonstrated a remarkable rule: during the early stage of lipid

Table 3—Methods used to detect and measure biological lipid peroxidation.

What is measured	Method	Remarks
Loss of unsaturated fatty	Analysis of fatty acids by GC or HPLC	Very useful for assessing lipid peroxidation stimulated by different metal complexes that give different product distributions.
Uptake of oxygen by carbon-centered radicals	Oxygen electrode	Dissolved oxygen concentration is measured. Useful in vitro when spectrophometric interference occurs or when chemicals interfere. Not very sensitive.
Lipid peroxides	Iodine liberation	Lipid peroxides oxidize I <sup>-</sup> to I <sub>2</sub> for titration with thiosulfate. Useful for bulk lipids, e.g., foodstuffs. H <sub>2</sub> O <sub>2</sub> also oxidizes I <sup>-</sup> to I <sub>2</sub> . In the presence of excess iodine the triiodine anion (I <sub>3</sub> <sup>-</sup> ) can be measured at 353 nm.
Lipid peroxides	Heme degradation of peroxides	Heme moiety of proteins can decompose lipid peroxides with formation of reactive intermediates. Radicals produced can be reacted with isoluminol to produce light.
Lipid peroxides	GSHPx	GSHPx reacts with H <sub>2</sub> O <sub>2</sub> and hydroperoxide, oxidizing GSH to GSSG. Addition of glutathione reductase and NADPH to reduce GSSG back to GSH results in consumption of NADPH, which can be related to peroxide content. Cannot measure peroxides within membranes; they must first be cleaved out by phospholipases.
Lipid peroxides	Cyclooxygenase	Stimulation of cyclooxygenase activity can be used to measure trace amounts of peroxide in biological fluids. This assay cannot be used to identify specific peroxides.
Lipid peroxides/aldehydes	GC-MS	Extraction, reduction (e.g., by borohydride) to alcohols, separation by GC, identification by MS. Several variations of these methods exist.
Pentane and ethane	Hydrocarbon gases	GC measurement of gases formed during lipid peroxide decomposition. Only a minor reaction pathway but can be used as a noninvasive in vivo measure of peroxidation. Results in practice have been variable. Rigorous controls are required.
Excited carbonyls, singlet oxygen	Light emission	Reaction of peroxy radicals can produce excited-state carbonyls and singlet O <sub>2</sub> . Both species emit light as they decay to the ground state. Measurement of low-level chemiluminescence is a method for measuring generation of reactive oxygen species in whole organs, but the light appears to arise from several sources.
Aldehydes	Fluorescence	Aldehydes such as malondialdehyde can react with amino groups to form Schiff bases (at acid pH only). At neutral pH, fluorescent dihydropyridines may be formed. Formation of fluorescent products is a minor reaction pathway and has very complex chemistry. It should never be assumed, without detailed characterization, that fluorescent products accumulating in vivo are end products of lipid peroxidation.
TBARS	TBA test	The test material is heated at low pH with TBA, and the resulting pink chromogen is measured by absorbance at ~532 nm or by fluorescence at 553 nm. The chromogen can be extracted into butan-1-ol. Most of the aldehydes that react with TBA are derived from peroxides and unsaturated fatty acids during the test procedure. Simple and nonspecific assay, rigorous controls required.
Aldehydes	Antibody techniques, GC-MS, HPLC	Hydroxyalkenals such as 4-hydroxynonenal are products of lipid peroxidation that are cytotoxic at nanomolar concentrations. They can be measured by HPLC or GC-MS. Several techniques have been developed that involve antibodies to detect proteins modified by lipid peroxidation products.
Diene conjugation	UV spectrophotometry	Oxidation of unsaturated fatty acids is accompanied by an increase in UV absorbance at 230-235 nm. Useful for bulk lipids. Requires extraction or separation techniques for biological use. Serious problems can arise when used on human body fluids.
Octadeca-9,11-dienic acid	Linoleic acid isomer	This isomer accounts for most of the diene conjugation present in human plasma and tissues but has not been produced in oxidatively stressed animal and model lipid systems. A single isomer of one polyunsaturated fatty acid is more indicative of an enzymic reaction than random free radical attack.
Nitron adducts of reactive short-lived free radicals	Spin trapping	Spin traps allow the formation of stable nitroxides, which can be examined by electron spin resonance. Spin traps can be used in animal experiments in vivo to detect carbon-centered radicals as well as alkoxyl and peroxy radicals.
F <sub>2</sub> -isoprostanes	GC-MS HPLC	Peroxidation of polyunsaturated fatty acids produces a complex mixture of non-specific rostaglandin isomers.

GC, gas chromatography; MS, mass spectrometry; GSH, glutathione; GSHPx, glutathione peroxidase; GSSG, oxidized glutathione; UV, ultraviolet; TBARS, thiobarbituric acid-reactive substance.

peroxidation, the intensity of chemiluminescence is proportional to the square of the concentration of lipid peroxide, suggesting that singlet oxygen and a compound in the triplet state (probably a carbonyl compound) are both generated by a self-reaction of lipid peroxy radicals<sup>330</sup>.

Other reports on chemiluminescence directed attention to mitochondria as the second important contributor to cellular chemiluminescence. Data collected in experiments with perfused liver, isolated liver mitochondria and isolated submitochondrial particles suggested that (a) intensity of light emission by mitochondria was dependent on the metabolic state, (b) singlet molecular oxygen was mainly responsible, and (c) chemiluminescence integratively measures radical reactions involved in lipid peroxidation and related processes<sup>332</sup>.

In this sense, mitochondrial and microsomal fractions<sup>332, 333</sup> behave similarly with respect to light-emission: in both cases, singlet molecular oxygen appears mainly responsible for the observed chemiluminescence. The experimental evidence for the generation of singlet oxygen was obtained mainly through the effect of specific quenchers<sup>329,333,334</sup> or spectral analysis<sup>329</sup>. In mitochondria, microsomes and submitochondrial particles, optimization of light emission requires (a) a membrane-bound electron transfer system, (b) added hydroperoxide and (c) the presence of O<sub>2</sub><sup>332,333</sup>. Oxygen containing species (O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, HO<sup>-</sup>, RO<sup>-</sup>, ROO<sup>-</sup> and singlet oxygen) were generated either by an interaction of oxygen with the components of the respiratory chain or by the homolytic scission of hydroperoxides by heme proteins<sup>332,333,335-337</sup>. These species were able to initiate free radical reactions, primarily through increased HO<sup>-</sup> and RO<sup>-</sup> formation, ending in lipid peroxidation<sup>28,338-341</sup>.

Despite many experiments demonstrating that spontaneous chemiluminescence increases when using extracellular stimuli such as hydroperoxides or increased oxygen tension<sup>342</sup>, it took more time to understand how chemiluminescence is affected when changes in the intracellular steady-state concentration of hydroperoxides occur. However, this problem was eventually addressed with additional knowledge about inhibitors of enzymatic and nonenzymatic intracellular defences against partially reduced oxygen species that, as a consequence, could lead to an increased intracellular concentration of oxygen radicals. The intracellular steady-state concentrations

of hydrogen peroxide or the superoxide anion were increased by inhibiting either catalase, glutathione peroxidase or superoxide dismutase activities. This information explained the increased spontaneous chemiluminescence after inhibition of any antioxidant enzyme<sup>343</sup>.

The most important aspect of organ chemiluminescence is that it provides, on a non-invasive basis, a signal of oxidative metabolism and the (overall) free radical, steady-state concentration that is readily and continuously detectable. It is possible to continuously monitor the metabolism of organs *in vivo* with chemiluminescent techniques. In that respect, chemiluminescence has been favored compared to other indirect assays of lipid peroxidation such as glutathione release<sup>28</sup>, evolution of hydrocarbons<sup>344,345</sup> or malondialdehyde accumulation<sup>327,346</sup>.

Many comparative studies on different assays have been completed utilizing chemiluminescence compared to other assays regarding lipid peroxidation. One study focused on doxorubicin (DXR), a widely used antineoplastic agent that is known to induce cardiotoxicity. This toxicity is mediated by reactive free radicals produced by DXR. DXR undergoes NADH dehydrogenase-catalyzed one-electron reduction to a semiquinone free radical in mitochondria<sup>347,348</sup>. Subsequently, these free radicals participate in DXR induced lipid peroxidation of mitochondrial membranes. DXR induced lipid peroxidation is generally evaluated by TBARS formation. However, fluorescent substances and high molecular weight protein aggregates, both non-specific indicators of lipid peroxidation, have also been employed<sup>349</sup>. ESR measurements have been used to specify the molecular nature of reactive oxygen species generated during DXR redox cycling<sup>350,351</sup>. An additional chemiluminescence study confirmed an increased free radical generation utilizing noninvasive, continuous monitoring of chemiluminescence produced during DXR redox cycling and its analysis with chemiluminescence spectroscopy.

Both chemiluminescence and one other type of assays for detecting free radical generation were utilized to study ischemia and reoxygenation (reperfusion) in heart and liver.

In the late 80's and early 90's, the rapidly growing interest in oxygen toxicity and free radical reactions in biology and medicine led to the hypothesis that reoxygenation damage may be produced by increased free radical generation<sup>352-355</sup>. The possibility that myocardial ischemia followed by an attempt to

therapeutically reoxygenate, generates free radicals was, in fact, supported by the direct Electron Spin Resonance (EPR) technique<sup>59,356-358</sup>. An additional chemiluminescence study confirmed an increased free radical generation rate during post ischemic reoxygenation of the heart utilizing non-invasive, continuous monitoring of ultra-weak chemiluminescence at the surface of the heart<sup>359</sup>.

A similar involvement of oxygen radicals was proposed in the pathogenesis of hepatic ischemia-reperfusion injury<sup>360</sup>. One related potential practical clinical application is when a liver has been preserved for transplantation and in the process subjected to a prolonged period of anoxia<sup>361</sup>. Reperfusion (reoxygenation) is required for the graft to function; however, paradoxically, a sequence of events may occur during reimplantation that leads to increased injury (reperfusion injury). The major support for this theory was first based on experiments that demonstrated protective effects of superoxide dismutase, catalase or other oxygen radical scavengers<sup>362,363</sup>. However, a sensitive biochemical methodology to measure free radical formation in the liver is not easily available. The short half-lives and broad line widths of many of the oxygen radicals make direct measurement with ESR within physiologic conditions difficult, if not impossible. The use of spin traps such as 5,5-dimethyl-1-pyrroline N-oxide (DMPO) have been used in biological systems to study oxyradical formation, but they may alter the very processes that are being measured. Other commonly used parameters such as lipid peroxidation or efflux of oxidized glutathione in the bile<sup>316</sup> lack sensitivity; the latter situation is limited by a dearth of biliary effect during hypoxia / anoxia. Monitoring oxygen radicals in a continuous and non-invasive manner by the chemiluminescence technique in an isolated liver perfusion model has, in fact, provided the essential data<sup>361,364</sup>. It has clearly demonstrated superoxide radical formation during hepatic reperfusion.

Summarizing these developments, 4 points can be made:

- (1) Substantial information has been gathered regarding what reactions lead to increased light emission. Several models have been used to characterize the nature of the emissive species in *in vitro* systems. *In vivo*, antioxidant enzymes are commonly utilized to manipulate spontaneous light emission.
- (2) Spontaneous light emission is useful as a non-invasive technique to monitor changes in the steady-state oxidative conditions of intact cells and organs without adding reagents that could interfere with the process<sup>343,365-368</sup>. Chemiluminescence seems to be one of the earliest physiologic responses to oxidative stress<sup>367</sup>. It precedes other parameters of oxidative stress, other parameters being based on metabolic end products of oxidative reactions (i.e. carbonyls, lipoperoxide derivatives, pentane and ethane release, capillary permeability, etc).
- (3) A few factors have limited the application of the technique. (a) The low intensity of emitted light limits the use of interference filters to better characterize emission wavelength and provide information on chemical nature of specific oxidized intermediates<sup>369</sup>; (b) Moreover, photomultipliers are not equally sensitive to light emitted at different wavelengths. Most sensitive photomultipliers detect light between approximately 350 and 850 nm, with maximal quantum efficiency around 24% at 400–500 nm. However, knowledge about species specificity is more interesting for scientific purposes than for diagnostic purposes. It is also interesting to observe the increasing interest to develop these methods as part of biophotonics research methodology<sup>370</sup>.
- (4) Taking the technical limitations into account, spontaneous (natural) ultraweak photon emission originating from living organisms offers significant information on physiological and functional conditions of vital systems and may be considered to reflect the state of oxidative stress *in vivo*.

#### Technical developments in low-level luminescence recording

The spontaneous chemiluminescence technique offers additional perspectives since such a system can also be developed for highly sensitive imaging and spatiotemporal analysis. A two-dimensional photon counting imaging of a rat's brain was technically achieved in 1999<sup>371</sup>. The equipment used in this first experiment consisted of a two-dimensional photon-counting tube with a photocathode measuring 40 mm in diameter, a highly efficient lens system, and an electronic device to record time series of a photoelectron train with spatial information. Utilizing the imaging system, regional time courses of emission intensity have been demonstrated, indicating the potential usefulness of spatiotemporal characterization

regarding physiological information on oxidative stress. Spontaneous photon emission was further imaged from a rat's cortex *in vivo* during cardiac arrest. The intensity after cardiac arrest was depressed to approximately 60%. This technology constitutes a novel method with the potential to extract pathophysiological information from the central nervous system<sup>372</sup>.

Another application has been in the field of transplanted tumors, both with photomultipliers and imaging equipment. Early interest focused on the liver of tumor-bearing animals. The liver of tumor-bearing animals is subjected, during the early phase after tumor implantation, to an increased oxidative stress. The increased steady-state levels of peroxy radicals are essentially responsible for the increased photoemission observed *in vivo*. Utilizing integrative studies on tumor-bearing animals, the *in situ* liver chemiluminescence<sup>316</sup> was measured simultaneously along with the activity of antioxidant enzymes and the content of endogenous antioxidants. The increased *in situ* liver chemiluminescence in the early phase after tumor implantation in tumor-bearing mice is associated with (a) decreased activity of the protective antioxidant enzymes in the liver and with (b) increased hydroperoxide initiated chemiluminescence in the homogenates and mitochondria of the liver<sup>373</sup>. Other data demonstrate that spontaneous light emission was not only enhanced within *in situ* liver but also within *in situ* brain in tumor bearing animals<sup>374</sup>, an observation that was reminiscent of the decrease in catalase activity found in most tumors<sup>375</sup>.

In the first report of a two-dimensional imaging and photon counting of ultraweak light emission from transplanted cancer, attention was focused on bladder cancer transplanted into the feet of nude mice. The photon emission of the developing cancer was followed. During the early log phase of cancer cell growth, necrosis, hemorrhage, leukocyte infiltration or crusta formation are not observed<sup>376</sup>. Observations in that early period suggested that increased photon emission was soon observed in the implanted tumor region indicating development of the actively proliferating cancer. In other studies, ultraweak photon intensity from different transplanted malignant tumors was recorded<sup>377-379</sup>.

Recently, ultraweak photon detection was reported utilizing a novel technique for cancer imaging<sup>380</sup>, a highly sensitive and ultra-low-noise charge-coupled device (CCD) camera system that records two-

dimensional biophoton images from tumors transplanted in mice. In addition, a procedure for whole body scanning of mice was developed utilizing a small, mobile and sensitive photomultiplier tube (PMT) operated at room temperature in a dark box. The investigations focused on scanning, ultraweak photon emission from mice that were transplanted with ovarian cancer cells. This scanning procedure is a potentially cost effective method for detecting tumors compared to the cooled CCD system. Data confirm the increased photon emission of tumors.

This, then, begins to initiate discussion regarding practical applications addressing specific pathological issues. Unsolved is the depth from which photons are able to penetrate tissue. In some papers it is suggested that a recorded value of 10 cps/cm<sup>2</sup> corresponds to approximately 20 cps/cm<sup>3</sup>.sec if the light originates through a 5 mm thick tissue. However, studies on transparency of tissue are required. Photon emission seems to transfer through rather large tissue distances as documented by studies utilizing paraquat, tumor transplants, and the recording of emissions through the skull. Furthermore, two-dimensional data show more gradient-like pictures without sharp boundaries. Data suggest chemiluminescence spreads from areas of high emission. These data might help to explain (a) the observed patterns of oxidative activity and (b) the quantitative evaluation of oxygen radical activity.

### Human photon emission

In recent years, there has been increasing interest in the use of human luminescence to record ROS in the development of pathology of chronic diseases (such as Alzheimer's disease and diabetes) that are supposed to be linked to non-linear progression in ROS production. The method is also interesting for recording the status of patients with these diseases that are taking antioxidant therapy. Along this line, in particular the development of a noninvasive tool for detection of human photon emission and its validation is crucial. This section reviews the current status of human photon emission recording techniques and protocols, the knowledge regarding both uniformity and variation in the anatomical pattern of photon emission, its dynamics in internal physiology and psychophysiology, and its relation to health and disease. Future perspectives deal with applications in physiologic and patho-physiologic conditions where the technique can be rapidly and easily implemented. Such conditions can be found in (a) extreme exercise (in sports physiology), (b) shift work that leads to

alteration of the circadian rhythm e.g., jet lag or alternating work schedules (in industrial/company medical office), and (c) during development of chronic diseases (in clinical practice).

#### Historical aspects

Research in human photon emission with single photon counting devices started about three decades ago<sup>381,382</sup>, utilizing a setup consisting of a photon detection system mounted in a darkroom in which subjects placed different anatomical part under the photomultiplier opening. Shortly after these reports, the first publications appeared utilizing more advanced devices to deal with large anatomic surfaces. In the Inaba Biophoton Project (funded by Research corporation of Japan (presently, Japan Science and Technology Corporation) human photon emission was investigated with two-dimensional photomultipliers in order to record the two-dimensional pattern of ultra weak photon emission (UPE) from human body surfaces. In Germany, Popp started pioneering research in human biophoton emission in 1993 by building a darkroom for the installment of a detector head that by hanging on runners, could be moved over the whole body of a subject lying on a bed underneath.

In the next 10 years, only a few systematic studies were performed and published. Cohen and Popp<sup>383</sup> considered long-term periodicity in a systematic study on photon emission from hands and forehead using the moveable photon detector. The authors examined both the palms of the hands and the forehead of one subject, daily, over a period of 9 months. Recordings demonstrate a clear preference for left and right hand correlation. Long-term biological rhythms of spontaneous emission of that subject became evident with Fourier analysis. Influence of age on photon emission of hands was investigated<sup>384</sup>. Spontaneous photon emission was increased in elderly subjects. A few studies have focused on photon emission from the hands in relation to disease. A study with 7 hemiparesis patients demonstrated that left and right differences of photon emission rates from the palm and the dorsum of the hands were large for 4 out of those 7 patients, compared to 20 healthy subjects<sup>385</sup>.

The limited number of studies did not allow hard conclusions about the implications and significance of biophotons in relation to health and disease or mental state. Still, the presented experimental data make clear that these aspects need attention and were the reason for a large systematic study that started in 2003 and

was supported by Samueli Institute for Information Biology. At present, the studies contain information on: (a) procedures for reliable measurements, and spectral analysis, (b) anatomic intensity of emission and left-right symmetries, (c) biological rhythms in emission, (d) physical characteristics of emission, (e) physical and psychological influences on emission, and (f) emission in health and disease.

#### Reliable multi-anatomical site recording of UPE and spectral analysis

The goal of the initial studies was to describe a protocol for management of subjects that (a) avoids interference with light-induced (long-term) delayed luminescence, and (b) includes the time slots for recording photon emission. The protocol was utilized to discriminate photon emissions from anatomical locations within a subject and to complete spectral analysis of emission from different body locations. The accuracy was sufficient: photon counts ranged between 6 and 40 counts per second (cps) wherein a difference of 1.1 cps is significant. The thorax-abdomen region demonstrates the lowest emission. The upper extremities and head region emit the highest levels<sup>386-389</sup>. Spectral analysis in the 200-650 nm range was possible with cut-off filters and repetitive measurements. Analysis documents major spontaneous emission at 470-570 nm. This indicates specific electron-excited states<sup>386</sup>.

In subsequent studies, a newly developed and highly sensitive charge-coupled device (CCD) imaging system was utilized in collaboration with Kobayashi and coworkers to fundamentally characterize spatial distribution of ultra-weak photon emission. The CCD images from the upper frontal torso, head and neck and upper extremities corresponded with the data of multi-site recordings utilizing the moveable photomultiplier system<sup>390-392</sup>. Systematic, multi-site recording with a group of 60 healthy males of ultra weak photon emission over high- and low anatomic emission locations presented evidence for a "common" human anatomic intensity emission pattern<sup>391,392</sup>. The common pattern opened a possibility to measure photon emission characteristics utilizing a few representative anatomic locations. Attention was focused on the hands. The hands emit high strength photon signals. CCD images of hand UPE were statistically analysed (manuscript in preparation). The study demonstrated that UPE was equally distributed over the palm and dorsum of the hand. To learn about the anatomic origin of UPE, the

patterns of UPE are currently compared with anatomic characteristics of the corresponding locations.

#### **(Diurnal) fluctuations**

The stability of UPE at a single anatomic location was studied. Recording of 29 selected, body sites demonstrated that emission over the body is systematically lower in the morning than in the evening at all locations<sup>386-388</sup>. Data registered during daytime hours demonstrated anatomic locations on the right side had higher photon strength than corresponding locations on the left. The right-left asymmetry was subsequently confirmed with a larger number of male (n=20) and female (n=20) subjects by recording over a smaller number of locations (manuscript in preparation).

In a subsequent study fluctuations in UPE were studied during 24 hr by recording ventral and dorsal sites of both right and left hands every 2 hr in 5 separate experiments. Data demonstrated that intensity as well as left-right symmetry varies diurnally. Emission intensity is low during the day, rises during the evening and is high at night. Time patterns for left and right hand are different. Although the fluctuations in UPE during the course of 24 hr were more over dorsal than ventral sites, all were highly significant. Correlations of fluctuations over ventral and dorsal sides are not apparent. During the 24 hr period, a change in left-right symmetry occurred at night. Photon emission over left locations was high at night, whereas the right sides emitted primarily during the day. Specific parameters have been developed to represent laterality<sup>393-395</sup>.

#### **Temperature and photon emission**

Human photon emission is a product of biochemical processes. Therefore, environmental temperature is expected to influence photon emission when it originates from anatomic layers that are not kept at 37°C and instead depends on environmental temperatures. Indeed, as temperature of hand declines, the intensity of UPE decreased, whereas UPE increased at increasing temperature<sup>396</sup>.

Another study led to a similar conclusion. In the study, subjects participated in cold exposure experiments after being dark-adapted. They lay in supine position to stabilize their body temperatures in a dark room at temperature of 17°–18°C for 15 min. Subsequently they disrobed for 30 min to only T-shirts, shorts and sport shoes. Both skin temperature

and photon emission were recorded from several anatomic locations. Temperature decrease during cooling roughly correlated with the photon emission of a specific location<sup>397</sup>.

Initially, the question was whether anatomic locations with different photon emission intensities reflected different skin temperature. Multi-site recordings of both photon emission intensities and skin temperature demonstrated that temperature at different anatomic locations ranged between 26° and 34°C. However, a relationship with photon emission was not observed<sup>397</sup>. In a second study, both UPE and temperature were recorded at the body location in different subjects in order to evaluate whether differences between subjects correlate with temperature. Between subjects, temperature of any of the recorded anatomic sites was not correlated with the UPE<sup>397</sup>.

It was concluded that differences between both anatomic locations and subjects could not be explained by differences in temperatures. At a given location, however, photon emission intensity is temperature-sensitive, suggesting that temperature recording at anatomic locations concomitant with photon emission is an improvement in comparative studies between different subjects and within a subject.

#### **Hypoxia, exercise and photon emission**

In three types of experiments the effect of hypoxia on photon emission was investigated. In the first study a tourniquet was placed around the upper arm to depress the supply of oxygen and nutrients to the hand. Photon emission of the hand was recorded during periods of increasing degree of tourniquet tightness. Data demonstrate that photon emission progressively decreased during blood flow limitation. After removing the tourniquet, photon emission returned to the former level within minutes. Data confirm that photon emission is oxygen dependent<sup>386,388,398</sup>. Direct exposure of the hand also resulted in some decreased photon emission<sup>396</sup>. Data suggest that generation mechanisms of photons emitted from the hand are both from interior sources and from skin.

#### **Daily tasks and photon emission**

Subjects engaged in cognitive tasks, e.g., filling out forms and addressing mathematical challenges, were also recorded for ultra weak photon emission. These tasks did not influence photon emission of hands. The results led to the selection of a new, more difficult

challenge in a second study. The more sophisticated mathematical challenges were addressed in the dark. Data demonstrated that cortisol levels were higher after the completion of the challenges, and decreased after relaxation. However, statistical significant changes in ultra weak photon emission during the challenges were not observed. It was concluded that common daily tasks had no statistically significant effect on photon strength and related parameters.

#### **Procedure for advanced photon count analysis of human ultra weak photon emission**

Spontaneous photon signals demonstrated that variance was higher than the mean suggesting that photocount distributions were not normal. Skewness was not zero implying a skewed distribution. Kurtosis was non-zero and large, thus also ruling out normal distribution of photon counts. However, this was true for both photon signals and background. To find the appropriate correction for background signals, a novel method to physically characterize UPE was utilized. The procedure was based on quantum optics<sup>399</sup> and has been applied in studies on non-human living systems<sup>400-402</sup>. The procedure describes the fluctuations in the signals by assuming the signal in a quantum squeezed state of photons. This state is specified by four real parameters (magnitude of displacement,  $|\alpha|$ ; magnitude of squeezing,  $r$ ; and phase angles  $\theta$  and  $\Phi$ )<sup>399</sup>. Utilizing this approach, a procedure for correcting background noise was developed.

A novel method was utilized to characterize human photon signals of low, intermediate and high intensities. Fluctuations in these signals are measured utilizing probabilities of detecting different numbers of photons in a bin, and establishing the optimal bin size. These measurements suggested that this set of parameters is quite useful. The subsequent study utilizing the measurements parameter compared additional anatomic locations between subjects. Photon count distribution over 12 different locations in 20 male subjects was examined. The fluctuation of each signal was characterized by the parameters  $|\alpha|$ ,  $r$ ,  $\theta$  and  $\Phi$ . The possibility of systematic differences of squeezed state parameters between different locations was studied. There were differences between the 12 locations for  $|\alpha|$ ,  $r$  and  $\Phi$ , but not for  $\theta$ . The question then arose for each parameter whether correlations existed between locations within subjects. Anatomical locations were grouped in three regions: (a) torso region, including abdomen right and left, stomach

region and heart; (b) head region, including throat, cheeks right and left and forehead and (c) hand regions, including ventral right and left and dorsum right and left. Data demonstrated for the parameters  $|\alpha|$  and  $r$  significant correlations (with a trend for  $\Phi$ ) between hand regions and the anatomic region of torso plus head<sup>403</sup>. It was concluded that the novel analysis was able to discover a squeezed state structure in the fluctuating photon emission; the squeezed state parameters can be expressed for each individual subject as mean values of measurements at all anatomic sites. Correlations in signals of different locations implied that squeezed states can differ between subjects. Differences between individuals in the fluctuations of the photon emission can thus be expressed by utilizing the combination of these parameters. The relationship between the structure in the fluctuating photon emission and the underlying elementary biochemical processes is part of the basic studies<sup>403</sup>.

#### **Application of photon emission in health research**

There is mounting evidence indicating that reactive free radical species are involved in initiation and development of many different forms of human pathologies, including psychiatric disorders. The utilization of ultraweak photon detection to evaluate the oxygen radical activity has increased the interest in a study on human photon emission in health and disease. In this section, preliminary data of two types of studies are presented:

- a. Comparative studies on subjects ranging from healthy to clinically diseased.
- b. Influence of long-term meditation on intensity and pattern of ultra weak photon emission.

#### **Ultraweak photon emission in disease**

Several studies suggest that the intensity of photon emission changes in a state of disease. Japanese studies of the two-dimensional pattern from the index and middle finger indicated that intensities could be used to differentiate hypothyroidism, lower state of metabolic activity<sup>404-406</sup>. Ultraweak photon emission in patients with hyperthyroidism was less intense than normal. The lower emission was also found in patients whose thyroid glands had been removed. Another study reported of several multiple sclerosis patients who emitted more photons than ordinary healthy subjects<sup>383,407,408</sup>. In this study, the authors introduced a second parameter for disease, e.g.,

percentage of difference in emission between left and right hand. They suggested that in certain diseases left-right symmetry was broken. In another study, left-right symmetry of photon emission from the palm and the dorsum of the hands of hemiparesis patients was compared with similar data from the hands of 20 self-reportedly healthy subjects. The variation in left-right symmetry among healthy subjects was not large. In hemiparesis patients though, the left and right differences were reported very large in 4 out of 7 patients both for the palm and dorsum of the hand. In the 3 other patients the differences were within normal range<sup>409</sup>.

Quantitative data on ultraweak photon emission of ROS-related diseased state require a study design with at least three defined stages from health to disease: (a) healthy state, (b) early state of dysregulation in which an impaired cell function is detectable, and (c) overt diseased state. The putative model is that accumulation of aggregates increased in subsequent stages with corresponding oxygen radical activity. Well-defined markers for most chronic diseases are available only for the stage that the disease is overt and the subject has contacted the medical circuit for specific symptoms. Relative few markers are available for earlier stages. An extra difficulty is that the many ROS-related chronic diseases are final manifestations of early stages of a common dysregulation (increased ROS activity).

As a first approach we have chosen for a descriptive, explorative study, in which no treatments are provided. The aim is to describe photon emission of 150 subjects divided into three groups. The two extreme groups are the (a) healthy young subject without severe disease history, and (b) chronic disease subject that ask for CAM after long-term disease history. The intermediate group are pre-diabetic subjects. Each group will include 50 subjects. The 50 healthy subjects participated in the study according to the inclusion criteria (a) healthy (assessed by questionnaires), (b) normal Dutch eating habits, (c) age > 20 and < 30 years.

The 50 chronic diseased subjects are included according to the criteria (a) chronically diseased with a history of medical events and having a general practitioner, (b) normal Dutch eating habits, (c) age > 30 and < 65 years. The inclusion criteria for the pre-diabetic group of 50 subjects are: (a) healthy (assessed by questionnaires and physical examination), (b) normal Dutch eating habits, (c) age

> 30 and < 65 years, (d) body mass index >26 and <35 kg/m<sup>2</sup>, and (e) pre-diabetic as established by fasting glucose blood values >6.0 to < 7.0.

For this study a new mobile device was constructed, since the original scientific equipment for human research is too large and bound to a specific research sites. The new equipment is smaller and more appropriate for laboratories and clinical practices. It is built in such manner that both hands can be recorded simultaneously, if required. Data collection protocol allows the analysis for: (a) strength of photon signal, (b) squeezed state parameters, and (c) left right symmetry. The selection for hand recordings was based on data discussed above. Such data have demonstrated that ventral and dorsal surfaces commonly demonstrate high emissions compared to other anatomical locations. A higher signal results in more reliable estimations of squeezed state parameters, whereas mean values for the four locations can be considered as representative for the subject.

The preliminary data support the hypothesis that photon strength ( $|\alpha|$ ) in chronic diseased subjects has increased compared to healthy subjects, whereas mean photon emission for the pre-disease group was intermediate. The squeezing parameter ( $r$ ) is small in chronic diseased subjects compared to healthy subjects. It has an intermediate value for the pre-disease group (manuscript in preparation).

#### **Long-term meditation and free radicals**

It is generally accepted that meditation, if practiced for a long time, induces a greater state of self-awareness and inner calm in its practitioners. Techniques of meditation include attention to one's breath, repeating a mantra and detaching from various thought processes in order to focus one's attention. The resulting "inner calm" implies reduction of stress which may have prophylactic and therapeutic health benefits. The hypothesis suggesting a possible link between meditation and its therapeutic effect utilizes the information about the initiating role of free radical-mediated oxidations in disease and proposes that oxidized lipids may reflect free radical induced damage that may contribute to pathophysiology<sup>410,411</sup>. The hypothesis has stimulated considerable curiosity in the scientific community. The measurement of serum lipid peroxide fluctuations indicates that chronic psychosocial stress probably does increase oxidative stress<sup>412,413</sup>. In addition, findings suggest the presence of lower lipid peroxide levels in the plasma

of practitioners of transcendental meditation (TM)<sup>414</sup>, Zen meditation<sup>415</sup> and yoga practitioners<sup>416</sup>.

Recent studies have focused on ultraweak photon emission of long-term practitioners of meditation. The studies utilized the system that is capable of multi-site recordings<sup>407</sup> according to a defined protocol<sup>386</sup>. The comparison between 10 TM practitioners and 10 subjects without experience in meditation indicated an intensity discrimination of ultraweak photon emission in meditation practitioners compared to control subjects<sup>417</sup>.

A follow up study examined the ultraweak photon emission from the hands of three groups of subjects: control group having no experience in meditation, TM group practicing Transcendental meditation, and a different group practicing a form of meditation other than TM (OTM). Each group consisted of 20 healthy, non-smoking subjects. Data demonstrated that the intensity of ultraweak photon emission by subjects of both meditation groups is lower by 15-33% for the TM group and 4-15% for the OTM group compared to the control group. All subjects demonstrated a high degree of symmetry<sup>418</sup>. Additionally, the photon signal was described according to a quantum optical approach utilizing the four parameters ( $|\alpha|$ ,  $\phi$ ,  $r$ ,  $\theta$ ) that determine the signal<sup>419</sup>. Both the squeezed state parameters and asymmetries suggest that the control group is different from both meditation groups. The difference between TM and control group is more than that between OTM and control group. The data support the conclusion that persistent meditation influence metabolic activities responsible for photon emission.

### Conclusion and future perspectives

In the present review both the emergence and development of two separate lines of research and their convergence into one model have been discussed. The first line focused on biochemical hallmarks of ROS related chronic diseases: the biological defence tiers that, if overwhelmed, result in persistent conformational changes and the progression of ROS-related chronic diseases. The second line focused on ultraweak photon emission as an overall measure to monitor the oxidative status from enzyme level to man. The review illustrates the wealth of experimental data for both lines of research. The convergence of both lines has resulted in research on the application of ultraweak photon emission to monitor the oxidative status in human subjects both under physiologic and pathophysiologic conditions.

The wide array of recognized ROS related and conformational diseases have in common that they arise from secondary or tertiary structural changes within constituent proteins, with subsequent aggregation of those altered proteins. Examples are the systemic amyloidosis, neurodegenerative diseases and type 2 diabetes mellitus as discussed before. A human study focusing on the ultraweak photon emission in the development of these diseases can thus be performed with any of these diseases. Empirical foundations that make the application of ultraweak photon emission research in health and disease transitions possible have already been discussed and can be shortly summarized:

1. Components and origins of ultraweak photon emission. Variability in photon emission has been defined and parameters to describe the non-classical aspects of photon emission have been given. Technical requirements for recording have been established and both dynamic and steady state characteristics of the ultraweak photon emission have been studied. The significance of additional measures (e.g., temperature) has been discussed.
2. Guidelines, recommendations and caveats. Meaningful analysis of ultraweak photon emission is dependent on the integrity of the basic photon signal corrected for (technical) background. With modern methods the fluctuations in the signal is analyzed to identify the integrity of the signal, to identify abnormal peaks and for artefact editing. Artefacts from a variety of sources may contaminate the photon signal. Therefore, it is generally preferable to use a distribution-based artefact-detection algorithm. In practice, a combination of both automated and visual approaches is optimal. Detection and processing of abnormal gross fluctuations in the signal are more problematic in individuals. This happens not very often (less than 2% of the cases).
3. Deriving inferences from ultraweak photon emission. Current scientific interest in ultraweak photon emission emphasizes the potential relation of photon signal components to functional dimensions that presently cannot be measured directly in a non-invasive manner. Given this, it is essential to identify reference criteria against which these measures may be validated. The relevant functional dimension may vary from discipline to discipline. In biochemistry, reference criteria have included molecular reactions involving ROS. In

medicine, reference criteria have included organ (e.g., cardiovascular) damage and risk stratification for disease. In human health and sports, ultraweak photon emission has been proposed as markers of physical and psychological stress and workload. Whatever the focus of the study is, however, putative autonomic mechanisms are generally invoked as mediators of this relationship. Consequently, false interferences about ROS mediation, based on inadequate evaluation of validity, can hamper a valuable line of research. In fact, the pattern of ROS control is often the primary interest in many biomedical studies of ultraweak photon emission. It is at this most fundamental level that ultraweak photon emission measures must be further validated.

Unfortunately, most chemical measures of ROS activity have limited applicability and are associated with methodological and interpretative problems of their own. The invasiveness of the procedures, indirectness of the reactive processes, and limited applicability to broader functional contexts (from blood to tissues) restrict the utility of these approaches. Despite the technical difficulties of the non-invasive luminescence procedure, confirmatory approaches are now available and applicable to humans. These approaches can utilize the adequate recording and data processing procedures, control or correction for temperature, and the appropriate selection of squeezed state parameters, that provide a selective index of ROS.

As is with most (psycho-) physiological measures ultraweak photon emission is a more accurate index of a change than in absolute level. Consequently, within-subject differences among experimental conditions are likely to be more accurate than of absolute level of ROS. Potential contributions of age, sex, stress, and diet parameters and other individual characteristics need to be considered carefully in interpreting between-subject differences in ultraweak photon emission. In such aggregate, the findings suggest caution in inferring absolute levels across individuals. The identification of ultraweak photon emission with the staging of ROS-related damage and disease fosters the development of different experimental approaches. This convergence must offer perspectives for (a) basic research utilizing appropriate models that increase our understanding, and (b) applied research including possibilities and limitations of the application of human ultraweak

photon emission as a tool. To illustrate this, several lines of research are proposed.

#### **Basic research: *Caenorhabditis elegans***

The *C. elegans* model for studying loss of coordination in neurodegenerative diseases is a promising tool. Both coordination in movement and ultraweak photon emission can be monitored. In a previous paragraph the utilization of *C. elegans* in polyQ aggregation is discussed. PolyQ diversity and corresponding loss of coordination of movement (from normal movement to nearly complete paralysis) can be studied using a variety of strains. The model allows a parallel study in time (e.g., duration of lag period and rate of development) of loss of coordination and ultraweak photon emission. The resulting data allow the testing (or present crucial arguments) on non-linear development of ROS production. The model can be further used to study the influence of ROS scavenging system.

#### **Biophoton emission in health and disease (Type 2 diabetes)**

Diabetes mellitus is a complex syndrome of hyperglycaemia in association with metabolic and vascular abnormalities. Despite problems identifying the cause of these diseases, the concept that free radicals mediate pancreatic B-cell destruction and retinal vascular damage was already debated since the early 1980's. Several lines of evidence suggested that plasma lipid peroxide levels are significantly higher in diabetic patients than in control subjects and that the levels in diabetic patients with vascular complications were markedly raised as compared to diabetics without angiopathy<sup>420-423</sup>. Interestingly, studies on ultraweak photon emission of plasma of Type 2 diabetes patients showed that emission increased with the duration of overt diabetes<sup>424</sup>. In the meantime hardly any additional data were collected utilizing ultraweak photon emission.

In diabetic patients, the observed risk for complications in the vascular system exceeds that expected from the classic risk factors, which are known also as metabolic syndrome. Experimental and clinical findings have suggested that enhanced levels of free radicals found in Type 1 as well as in Type 2 diabetic patients could be the risk factor explaining the excess of mortality in these individuals. The disease can be considered as a good model to explore the role of oxidative stress in the development of late diabetic complications and the implications for therapy<sup>425</sup>.

We propose a subject (patient)-oriented research in which not the fulfilments of inclusion criteria are central. All patients are considered to provide valuable extra information and patients enrol in the study by the basic criteria for diabetes 2. Patients are not too old or too young, too illiterate, or suffer from co morbidity or concurrent psychiatric disturbances. Normally, these subjects are excluded from the study if it is disease-oriented in random clinical trials (RCT). Without wanting to undermine the enormous relevance RTC has for scientific development, the major drawback is that it does not allow maximum insight in the ROS production under conditions that the disease pattern becomes more and more complicated.

The patient-centered approach means that health care providers are directed to the illness, rather than to the disease, and have to explore and value – predominantly by questionnaires – the patients' relevant history (age, duration of diabetes, development of complications), biopsychosocial context in which biological (exercise, food, etc), psychological (stress coping, etc.) and social elements as important as the strictly biomedical (blood glucose, fructosamine, HbA1c, lipid peroxide, etc) elements.

#### **Exercise in sport and revalidation medicine**

There are limited data in literature concerning oxidative stress in hypokinesia and hyperkinesia. Extreme hypokinesia occurs with spaceflight, chronic bed rest, and immobilization. Extreme hyperkinesia occurs with extreme, long-duration, exercises.

Non-damaging habitual exercise using resistive or endurance regimens provides some protection against age-related contractile function and risk of muscle injury<sup>426-428</sup>.

A potential mechanism that would trigger increased protein degradation and atrophy in skeletal muscle is oxidative stress, where antioxidant proteins and scavenger protection are overwhelmed by oxidant production. The problem is particular interesting in relation with heart failure and corresponding anoxia.

Growing evidence indicates that impaired stress protein (e.g., antioxidant enzymes, heat shock proteins and other chaperones, IGF-1) may play a role in regulating muscle dysfunction that occurs in heart disease and chronic heart failure (CHF)<sup>429-431</sup>. Exercise training improves work capacity, tolerance to fatigue, reduces risk of myocardial infarction in cardiac patients, and reduces the risk of heart disease in healthy adults<sup>432-434</sup>. In contrast with healthy peers,

heart disease patients respond to endurance exercise training with primarily peripheral adaptations, rather than changes in central (i.e., cardiac) function. In healthy adults, exercise training increases protective stress proteins in skeletal muscle including antioxidant enzymes and chaperones<sup>435,436</sup>. Other data indicate that exercise results in a partial reversal of the reduction in antioxidant activity in heart failure patients<sup>430</sup>. Therefore, an interesting focus of application of non-invasive ultraweak recording to test free radical status is research in hypo- and hyperkinesias in healthy and heart patients, in particular a study of the combined effects of coronary ischemia and subsequent exercise training on free radical levels. It is evident that such measurements will be conducted in combination with the common techniques (e.g., heart beat rate measurements, oxygen consumption, body temperature).

In summary, patterns of ultraweak photon emission hold considerable promise as measure for the oxidative status. Further developments in measurement and analysis and advances in concepts and metrics of ROS-related diseases would foster further biomedical applications of ultraweak photon emission. A multifactorial interdisciplinary approach, at both biomedical and psychosocial levels, would undoubtedly contribute to this development of the field.

#### **Acknowledgement**

This work was supported by an independent research grant from the Samuelli Institute of Information Biology and the Rockefeller-Samuelli Center for Research in Mind-Body Energy. The authors thank Dr. Fritz-Albert Popp and Dr. Yan Yu for their scientific support in ultraweak photon emission studies; Mr. Jan Eikelenboom and Dr. John Ackerman for library and editorial work.

#### **References**

- 1 Gerschman R, Historical introduction to the "free radical theory" of oxygen toxicity, in: *Oxygen and living processes, an interdisciplinary approach*, edited by DL Gilbert (Springer, New York, USA) 1981, 44.
- 2 Harman D. Aging, a theory based on free radical and radiation chemistry, *J.Geronto*, 11 (1956) 298.
- 3 McCord J M & Fridovich I, Superoxide dismutase. An enzymic function for erythrocyte protein (hemocuprein), *J Biol Chem*, 224 (1969) 6049.
- 4 Freeman B A & Crapo J D, Biology of disease: free radicals and tissue injury, *Lab Invest*, 47 (1982) 412.
- 5 Pryor W A, Free radical biology: xenobiotics, cancer and aging, *Ann NY Acad Sci*, 393 (1982) 1.

- 6 Sies H, *Oxidative stress* (Academic Press, New York, USA) 1985.
- 7 Cross D E B, Halliwell E T, Borish W A, Pryor B A, Ames R S, Saul J M, McCord & Harman D, Oxygen radicals and human disease, *Ann Intern Med*, 107 (1987) 526.
- 8 Koster J F, Biemand P & Stam H, Lipid peroxidation and myocardial ischaemic damage: cause or consequence? in *Lipid metabolism in the normoxic and alchemic heart*, edited by H Stam H, G J Vusse (Steinkhoff Verlag, Darmstadt) 1986, 253.
- 9 Cerutti P A, Fridovich I & McCord J M, *Oxy-radicals in molecular and cellular biology*, in New Series 82, edited by R Alan (Liss Inc, New York) 1988.
- 10 Dormandi T L, Free radical pathology and medicine: a review, *J R Coll Physic (London)*, 23 (1989) 221.
- 11 Poli G, Cheeseman K H, Dianzani M U & Slater T F, *Free radicals in the pathogenesis of liver injury* (Pergamon Press, Oxford) 1989.
- 12 Packer L, Glazer, *Methods in enzymology: oxygen radicals in biological systems, Part B: Oxygen radicals and antioxidants*, (Academic Press, New York, NY), Vol. 186, 1990.
- 13 Dargel R, Lipid peroxidation – a common pathogenetic mechanism? *Exp. Toxic. Pathol*, 44 (1992) 169.
- 14 McElroy W D & Seliger H H, Origin and evolution of bioluminescence, in *Horizons in biochemistry*, edited by M Kasha and B Pullman (Academic Press, New York) 1962, 91.
- 15 McElroy W D & Seliger H H, The chemistry of light emission, *Adv Enzymol Relat Areas Mol Biol*, 25 (1963) 119.
- 16 Seliger H H, The origin of bioluminescence, *Photochem.Photobiol*, 21 (1975) 355.
- 17 Tarusov B N, Polidova A I & Zhuravlev A I, Study on ultra-weak spontaneous luminescence of animal cells, *Biofizika*, 6 (1961) 490.
- 18 Tarusov B N, Polidova N A I, Zhuralev A I, & Sekamova E N, Ultraweak spontaneous luminescence in animal tissue, *Tsitologiya*, 4 (1962) 696.
- 19 Barenboim G M, Domanski A N & Taroverov K K, *Luminescence of biopolymers and cells* (Plenum, New York) 1969.
- 20 Stauff J & Ostrowski J, Chemoluminescence of mitochondria, *Z. Naturforsch B*, 22 (1967) 743.
- 21 Howes R M & Steele R H, Microsomal ( S) chemiluminescence (CL) induced by NADPH and its relation to lipid peroxidation, *Res. Commun. Chem. Pathol. Pharmacol*, 2 (1971) 619.
- 22 Howes R M & Steele R H, Microsomal (muS) chemiluminescence (CL) induced by NADPH and its relation to aryl-hydroxylations, *Res. Commun. Chem. Pathol. Pharmacol*, 3 (1972) 349.
- 23 Gurvitsch A A, Eremeyev V F & Karabchievsky Y A, Ultra-weak emission in the visible and ultra-violet regions in oxidation of solutions of glycine by hydrogen peroxide, *Nature*, 3 (1965) 206.
- 24 Gurvitsch A G & Gurvitsch L D, *Die Mitogenische Strahlung* (Fischer, Jena) 1959.
- 25 Metcalf W S & Quickenden T I, Mitogenetic radiation, *Nature*, 216(5111) (1967) 169.
- 26 Halliwell B & Gutteridge J M C, The importance of free radicals and catalytic metal ions in human disease, *Mol asp Med*, 8 (1985) 89.
- 27 Forman H J & Boveris A, Superoxide radical and hydrogen peroxide in mitochondria, in *Free radicals in biology* (Academic Press, Inc., New York) 1982, 65.
- 28 Chance B, Sies H & Boveris A, Hydroperoxide metabolism in mammalian organs, *Physiol Rev*, 59 (1979) 527.
- 29 Kellogg E W & Fridovich I, Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system, *J Biol Chem*, 250 (1975) 8812.
- 30 McCord J M, Oxygen-derived free radicals in postischemic tissue injury, *N Engl J Med*, 312 (1985) 159.
- 31 Halliwell B & Gutteridge J M, The importance of free radicals and catalytic metal ions in human diseases, *Mol Aspects Med*, 8 (1985) 89.
- 32 Haber F & Weiss J, The catalytic decomposition of hydrogen peroxide by iron salts, *Proc R Soc London A*, (1934) 332.
- 33 Wilson R B, Middleton C C & Sun G Y, Vitamin E, antioxidants and lipid peroxidation in experimental atherosclerosis of rabbits, *J Nutr*, 108 (1978) 1858.
- 34 Del Rio L A, Sandalio L M, Palma J M, Bueno P & Corpas F J, Metabolism of oxygen radicals in peroxisomes and cellular implications, *Free Radic Biol Med*, 13 (1992) 557.
- 35 Gebicki S, Gill K H, Dean R T & Gebicki J M, Action of peroxidases on protein hydroperoxides, *Redox Rep*, 7 (2002) 235.
- 36 Autor A P, *Pathology of oxygen*, (Academic Press, New York) 1982.
- 37 Sies H, Strategies of antioxidant defense, *Eur J Biochem*, 215 (1993) 213
- 38 Rose R & Bode A, Biology of free radical scavengers, *FASEB Journal*, 7 (1993) 1135.
- 39 Goto Y, *Lipid peroxides as a cause of vascular disease. Lipid peroxides in biology and medicine*, (Academic Press, New York) 1982, 295.
- 40 Taylor A.E., Matalon S & Ward P.A, *Physiology of oxygen radicals* (Americ Physiol Society, Bethesda) 1986.
- 41 DeGroot H & Littauer A, Hypoxia, reactive oxygen and cell injury, *Free Radical Biol Med*, 6 (1989) 541.
- 42 Braughler J M & Hall E D, Central nervous system trauma and stroke: I. Biochemical considerations for oxygen radical formation and lipid peroxidation, *Free Radic Biol Med*, 6 (1989) 289.
- 43 Hall E D & Braughler J M, Central nervous system trauma and stroke. II. Physiological and pharmacological evidence for involvement of oxygen radicals and lipid peroxidation, *Free Radic Biol Med*, 9 (1989) 303.
- 44 Van der Kraaij A M, Schoonderwood K & Koster J F *et al*, Lipid peroxidation and its significance for (post) ischemic cardiovascular injury, *Prog Clin Biol Res*, 301 (1989) 61.
- 45 Gores G J, Flrsheim C E, Dawson T L, Nieminen A-L, Herman B & Lemasters J J, Swelling, reductive stress, and cell death in hepatocytes, *Am. J. Physiol.* 257, (Cell Physiol 26) (1989) C347.
- 46 Gores G J, Nieminen A-L, Fleishman K E, Dawson T L, Herman B & Lemasters J J, Extracellular acidosis delays the onset of cell death in ATP-depleted hepatocytes, *Am. J. Physiol* 255, (Cell Physiol 24) (1988) C315.
- 47 Gores, G K, Nieminen A-L, Wray B E, Herman B & Lemasters J J, Intracellular pH during “chemical hypoxia” in cultured rat hepatocytes by intracellular acidosis against the

- onset of cell death, *J Clin Invest*, 83 (1989) 386.
- 48 Kawanishi, Nieminen A-L, Herman B & Lemasters J J, Suppression of  $\text{Ca}^{2+}$  oscillations in cultured rat hepatocytes by chemical hypoxia, *J Biol Chem*, 266 (1991) 20062.
- 49 Lemasters J J, DiGuiseppi J, Nieminen A-L & B. Herman B, Blebbing, free  $\text{Ca}^{2+}$  and mitochondrial membrane potential preceding celldeath in hepatocytes, *Nature*, 325 (1987) 78.
- 50 Dawson T L, Gores G J, Nieminen A-L, Herman B & Lemasters J J, Mitochondria as a source of reactive oxygen species during reductive stress in rat hepatocytes, *Am J Physiol*, 264 (1993) 961.
- 51 Cairns J A, Collins R, Fuster V & Paasamani E R, Coronary thrombolysis, *Chest* 95 (1989) 73S.
- 52 Starzl T E, Transplantation, *JAMA*, 261(19) (1989) 2894.
- 53 Jones D P, The role of oxygen concentration in oxidative stress: hypoxic and hyperoxic models. In: *Oxidative Stress*, edited by H. Sies (Academic Press, London) 1985, 152.
- 54 Maupoil V & Rochette L, Evaluation of free radical and lipid peroxide formation during global ischemia and reperfusion in isolated perfused rat heart. *Cardiovasc, Drugs Ther*, 2 (1988) 615.
- 55 Brown J M, Terada LS, Grosso M A, Whitmann G J, Velasco S E, Patt A, Harken A H & Repine J E, Xanthine oxidase produces hydrogen peroxide which contributes to reperfusion injury of ischemic, isolated, perfused rat hearts, *J Clin Invest*, 81 (1988) 1297.
- 56 Ferrari R, Cargnoni A, Curello S, Boffa G M & Ceconi C, Effects of iloprost (ZK 36374) on glutathione status during ischaemia and reperfusion of rabbit isolated hearts, *Br J Pharmacol*, 98 (1989) 678.
- 57 Cerutti, P A, Fridovich, I & McCord, J. M. *Oxy-radicals in molecular biology and pathology* (Alan R. Liss, New York) 1988.
- 57A Nauta R J, Tsimoyiannis E, Uribe M, Walsh D B, Miller D & Butterfield A, Oxygen-derived free radicals in hepatic ischemia and reperfusion injury in the rat, *Gynecol Obstet*, 171 (1990) 120.
- 58 Taylor AE, Matalon S & Ward PA, *Physiology of oxygen radicals* (Williams and Wilkins, Baltimore) 1986.
- 59 Zweier J L, Flaherty J T & Weisfeldt ML, Direct measurement of free radical generation following reperfusion of ischemic myocardium, *Proc Natl Acad Sci USA*, 84 (1987) 1404.
- 60 Zweier J L, Rayburn B K, Flaherty JT & Weisfeldt M L, Recombinant superoxide dismutase reduces oxygen free radical concentrations in reperfused myocardium, *J Clin Invest*, 80 (1987) 1728.
- 61 Zweier J L, Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury, *J Biol Chem*, 263 (1988) 1353.
- 62 Ambrosio G, Zweier J L, Jacobus W E, Weisfeldt M L & Flaherty J T, Improvement of postischemic myocardial function and metabolism induced by administration of deferoxamine at the time of reflow: the role of iron in the pathogenesis of reperfusion injury, *Circulation* 76, (1987) 906.
- 63 Blasig I E, Bor P, Tosaki A, Szekeres L & Löwe H, Effect of activated oxygen species on mitochondria isolated from myocardium after reperfusion injury, *Gen Physiol Biophys*, 5(6) (1986 Dec) 655.
- 64 Arroyo C M, Kramer J H, Dickens B F & Weglicki W B, Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitron DMPO, *FEBS Lett*, 221 (1987) 101.
- 65 Zweier J L, Kuppusamy P, Williams R, Rayburn B K, Smith D, Weisfeldt M L & Flaherty J T, Measurement and characterization of post ischemic free radical generation in the isolated perfused heart, *J Biol Chem*, 264 (1989) 18890.
- 66 Thompson-Gorman S L & Zweier J L, Evaluation of the role of xanthine oxidase in myocardial reperfusion injury, *J Biol Chem*, 265 (1990) 6656.
- 67 Grill H P, Flaherty J T, Zweier J L, Kuppusamy P & Weisfeldt M L, Direct measurement of myocardial free radical generation in an in vivo model: effects of postischemic reperfusion and treatment with human recombinant superoxide dismutase, *J Am Coll Cardiol*, 20 (1992) 1604.
- 68 Bolli, R, Post ischemic myocardial "stunning", in *Pathogenesis. pathophysiology and clinical relevance*, edited by D M Yellon & R B Jennings (Raven Press, New York) 1992.
- 69 Hearse D J, Reperfusion-induced injury: A possible role of oxidant stress and its manipulation, *Cardiovasc Drugs Ther*, 5 (1991) 225.
- 70 Halliwell B, Reactive oxygen species in living systems: Source, biochemistry and role in human disease, *Am. J Med*, 91 (3C) (1991) 13S.
- 71 Oberley L W & Buettner G R, Role of superoxide dismutase in cancer: a review, *Cancer Research*, 39 (1979) 1141.
- 72 Sato K, Ito K, Kohara H, Yamaguchi Y, Adachi K & Endo H, Negative regulation of catalase gene expression in hepatoma cells, *Mol Cell Biol*, 12 (1992) 2525.
- 73 Sztatowski T P & Nathan C F, Production of large amounts of hydrogen peroxide by human tumor cells, *Cancer Res*, 51 (1991) 794.
- 74 Ahmed S M. & Slater, T F, Recent, in *Recent Advances in lipid peroxidation and tissue injury*, edited by T F Slater & A Garner (Brunel University, Uxbridge, U.K) 1981, 177
- 75 Cheeseman, K H, *Carbon tetrachloride metabolism and lipid peroxidation in rat liver microsomes* (Brunel University, Uxbridge, U.K.) 1982.
- 76 Burlokova E B, Bioantioxidants and synthetic inhibitors of radical processes, *Russian Chemical Reviews*, 44 (1975) 871.
- 77 Ugazio G., Gabriel L & Burdino E, Research on the lipid peroxidase inhibitors present in the cells of Yoshida ascites hepatoma, *Boll Soc Ital Biol Sper*, 44 (1968) 30.
- 78 Utsumi K, Yamamoto G & Inaba K, Failure of  $\text{Fe}^{2+}$ -induced lipid peroxidation and swelling in the mitochondria isolated from ascites tumor cells, *Biochim Biophys Acta*, 105 (1965) 368.
- 79 Dionisi D, Galeotti T, Terranova T & Azzi A, Superoxide radicals and hydrogen peroxide formation in mitochondria from normal and neoplastic tissues, *Biochim Biophys Acta*, 403 (1975) 292.
- 80 Schreck R, Rieber P & Baeuerle P A, Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1, *EMBO J*, 10 (1991) 2247.
- 81 Crawford D, Zbinden I, Amstad P & Cerutti P, Oxidant stress induces the protooncogenes c-fos and c-myc in mouse epidermal cells, *Oncogene*, 3 (1988) 27.

- 82 Shibanuma M, Mashimo J, Mita A, Kuroki T & Nose K, Cloning from a mouse osteoblastic cell line of a set of transforming growth factor  $\beta$ 1-regulated genes, one of which seems to encode a follistatin-related polypeptide, *Eur J Biochem*, 217 (1993) 13.
- 83 Jaruga P, Zastawny T H, Skokowski J, Dizdaroglu M & Olinski R, Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer, *FEBS Lett*, 341 (1994) 59.
- 84 Slater T F, Biochemical Pathology in microtime, *Panminerva Medica*, 18 (1976) 381.
- 85 Harman D, The biologic clock: The mitochondria? *J Amer Geriat Soc*, 20 (1972) 145.
- 86 Miquel J & Fleming J, Theoretical and experimental support for an "oxygen radical-mitochondrial injury" hypothesis of cell aging, in *Free radicals, ageing and degenerative diseases*, edited by J E Johnson, R Walford, D Harman & J Miquel (Alan R. Liss, New York) 1986, 51.
- 87 Fleming J E, Miquel J, Cottrell S F, Yengoyan L S & Economos A C, Is cell aging caused by respiration-dependent injury to the mitochondrial genome? *Gerontology*, 28(1) (1982) 44.
- 88 Stauber J L & Florence T M, Mechanism of toxicity of ionic copper and copper complexes to algae, *Mar Biol*, 94 (1987) 511.
- 89 Fleming J E, Miquel J & Bensch K G, Age dependent changes in mitochondria, *Basic Life Sci*, 35, (1985) 143.
- 90 Marcus D L, Ibrahim N G & L. Freedman L, Age-related decline in the biosynthesis of mitochondrial inner membrane proteins, *Exp Gerontol*, 17 (1982) 333.
- 91 Totter J R, Spontaneous cancer and its possible relationship to oxygen metabolism, *Proc Natl Acad Sci USA* 77 (1980) 1763.
- 92 Cohen S M, Purtilo D T & Ellwein L B, Pivotal role of increased cell proliferation in human carcinogenesis, *Mod Pathol*, 4 (1991) 371.
- 93 Ames B N, Shigenaga M K & Gold L S, DNA lesions, inducible DNA repair, and cell division: three key factors in mutagenesis and carcinogenesis, *Environ Health Perspect*, Dec 101 Suppl 5 (1993) 35. Review
- 94 Ames B N, Profet M & Gold L S, Dietary Pesticides (99.99% All Natural), *Proc Natl Acad Sci USA*, 87 (1990) 7772.
- 95 Cerutti P A, Oxidant stress and carcinogenesis, *Eur J Clin Invest*, 21(1) (1991) 1.
- 96 Amstad P A, Krupitza G & Cerutti P A, Mechanism of c-fos induction by active oxygen, *Cancer Res*, 52 (1992) 3952.
- 97 Orrenius S, Burkitt M J, Kass G E, Dypbukt J M & Nicotera P, Calcium ions and oxidative cell injury, *Ann Neurol*, 32 (1992) S33.
- 98 Cerutti P A, Prooxidant states and tumor promotion, *Science*, 227 (1985) 375.
- 99 Jamieson D, Oxygen toxicity and reactive oxygen metabolites in mammals, *Free Rad Biol Med*, 7 (1989) 87.
- 100 Sun Y, Free radicals, antioxidant enzymes, and carcinogenesis, *Free Rad Biol Med*, 8, (1990) 583.
- 101 Troll W & Wiesner R., The role of oxygen radicals as a possible mechanism of tumor promotion, *Annu Rev Pharmacol Toxicol*, 25 (1985) 509.
- 102 Weitzman S A, & Stossel T P, Effects of oxygen scavengers and antioxidants on phagocyte-induced mutagenesis, *J Immunol*, 182 (1982) 2770.
- 103 Salim A S, The permissive role of oxygen-derived in the development of colonic cancer in the rat, *Int J Cancer*, (1993) 1031.
- 104 Salim A S, Oxygen-derived free-radical scavengers prolong survival in colonic cancer, *Chemotherapy*, 38 (1992) 127.
- 105 Salim A S, Oxygen-derived free -radical scavengers prolong survival in gastric cancer, *Chemotherapy*, 38 (1992) 135.
- 106 Salim A S, Removing oxygen-derived free radicals delays hepatic metastases and prolongs survival in colonic cancer, *Oncology*, 49 (1992) 58.
- 107 Stamle, J, Singel D J & Loscalz, J, Biochemistry of nitric oxide and its redox-activated forms, *Science*, 251 (1992) 1898.
- 108 Ischiropoulos H, Zhu L & Beckman J S, Peroxynitrite formation from macrophage-derived nitric oxide, *Arch Biochem Biophys*, 298 (1992) 446.
- 109 Shacter E, Beecham E J, Covey J M, Kohn K W & Potter M, Activated neutrophils induce prolonged DNA damage in neighboring cells, *Carcinogenesis*, 9 (1988) 2297.
- 110 Yamashina K, Miller B E & Heppner G H, Macrophage-mediated induction of drug-resistant variants in a mouse mammary tumor cell line, *Cancer Res*, 46 (1986) 2396.
- 110A. Yamauchi T *et al*, Globular adiponectin protected ob/ob mice from diabetes and apoE-deficient mice from atherosclerosis, *J Biol Chem*, 278 (2003) 2461.
- 111 Beasley R P, Hepatitis B virus. The major etiology of hepatocellular carcinoma, *Cancer*, 61 (1988) 1942.
- 112 Tabor E & Kobayashi K, Hepatitis C virus, a causative infectious agent of non-A, non-B hepatitis: prevalence and structure--summary of a conference on hepatitis C virus as a cause of hepatocellular carcinoma, *J Natl Cancer Inst*, 84 (1992) 86.
- 113 Yu M-W, You S-L, Chang, A-S, Lu S-N, Liaw Y-F & Chen C-J, Association between hepatitis C virus antibodies and hepatocellular carcinoma in Taiwan, *Cancer Res*, 51 (1991) 5621.
- 114 Chen M & Mott K, Progress in assessment of morbidity due to *Schistosoma mansoni* infection, *Trop Dis Bull*, 85 (1988) 2.
- 115 Chen M & Mott K, Progress in assessment of morbidity due to *Schistosoma haematobium* infection. A review of recent literature, *Trop Dis Bull*, 86 (1989) 1.
- 116 Sobala G M, Pignatelli B, Schorah C J, Bartsch H, Sanderson M, Dixon M F, Shires S, King R F G & Axon A T R, Levels of nitrite, nitrate, N-nitroso compounds, ascorbic acid and total bile acids in gastric juice of patients with and without precancerous conditions of the stomach, *Carcinogenesis*, 12 (1991) 193.
- 117 Kneller R W, Guo W-D, Hsing A W, Chen J-S, Blot W J, Li J-Y, Forman D & Fraumeni Jr. F J, Risk factors for stomach cancer in sixty-five Chinese counties, *Cancer Epidemiol Biomarkers Prev*, 1(2) (1992) 113.
- 118 Parsonnet J, Friedman G D, Vandersteen D P, Chang Y, Vogelstein J H, Orentreich N & Sibley R K, *Helicobacter pylori* infection and the risk of gastric carcinoma, *N Engl J Med*, 325 (1991) 1127.
- 119 Dooley C P, Cohen H, Fitzgibbons P L, Bauer M, Appleman M D, Perez-Perez G I & Blaser, M J, Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons, *N Engl J Med*, 321 (1989) 1562.

- 120 Cover T L & Blaser M J, Helicobacter pylori and gastroduodenal disease, *Annu Rev Med*, 43 (1992) 135.
- 121 The Eurogast Study Group An international association between Helicobacter pylori infection and gastric cancer, *Lancet*, 341 (1993) 1359.
- 122 Marsh J P & Mossman B T, Role of asbestos and active oxygen species in activation and expression of ornithine decarboxylase in hamster tracheal epithelial cells, *Cancer Res*, 51 (1991) 167.
- 123 Korkina L G, Durnev A D, Suslova T B, Cheremisina ZP, Dauge-Dauge N O & Afanas'ev I B, Oxygen radical-mediated mutagenic effect of asbestos on human lymphocytes: suppression by oxygen radical scavengers, *Mutat Res*, 265 (1992) 245.
- 124 Bankson D D, Kestin M & Rifia N, Role of free radicals in cancer and arterioclerosis, *Clin Lab Med*, 13 (1993) 463.
- 125 Halliwell B, The role of oxygen radicals in human disease, *Haemostasis*, 23 (suppl) (1993) S118.
- 126 Lankin V Z, Vikhert A M, Tikhaze A K, *et al*, The role of lipid peroxidation in the etiology and pathogenesis of arteriosclerosis (review), *Vopr Med Khim*, 35 (1989) 18 – 24
- 127 Stringer M D, Gorog P G, Freeman A, *et al.*, Lipid peroxides and arteriosclerosis, *Brit Med J*, 298 (1989) 281.
- 128 Duthie G G & Wahle K J, Smoking, antioxidants, essential fatty acids and coronary heart disease, *Biochem Soc Transact*, 18 (1990) 1051.
- 129 Esterbauer H, Dieber-Rotheneder M, Waeg G, Puhl H & Tatzber F, Endogenous antioxidants and lipoprotein oxidation, *Biochem Soc Transact*, 18 (1990) 1059.
- 130 Gey K F, The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms, *Biochem Soc Transact*, 18 (1990) 1041.
- 131 Oliver M F, Linoleic acid, antioxidants and coronary heart disease, *Biochem Soc Trans*, 18 (1990) 1049.
- 132 Steinberg D, Antioxidants in the prevention of human arteriosclerosis, *Circulation*, 85 (1992) 2337.
- 133 Gaziano J M, Manson J E, Buring J E & Hennekens C H, Dietary antioxidants and cardiovascular disease, *Ann N Y Acad Sci*, 669 (1992) 249.
- 134 Gaziano J M & Hennekens C H, Update on dietary antioxidants and cancer, *Pathol Biol (Paris)*, 44(1) (1996) 42. Review
- 135 Enstrom J E, Kanim L E & Klein M A, Vitamin C intake and mortality among a sample of the United States population, *Epidemiology*, 3 (1992) 194.
- 136 Gey K F, Puska P & Moser U K, Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology, *Am J Clin Nutr*, 53 (1991) 326S.
- 137 Kaliora A C & Dedoussis GV, Natural antioxidant compounds in risk factors for CVD, *Pharmacol Res*, 56 (2007) 99.
- 138 Manson J E, Nathan D M, Krolewski A S, Stampfer M J, Willett W C & Hennekens C H, A prospective study of exercise and incidence of diabetes among US male physicians, *JAMA*, 268(1) (1992) 63.
- 139 Riemersma R A, Wood D A, Macintyre C C A, Elton R A, Gey K F & Oliver M F, Anti-oxidants and pro-oxidants in coronary heart disease, *Lancet*, 337 (1991) 1.
- 140 Stampfer M J, Hennekens C H, Manson J E, Colditz G A, Rosner B & Willett W C, Vitamin E consumption and the risk of coronary disease in women, *N Eng J Med*, 328 (1993) 1444.
- 141 Rimm E B, Stampfer M J, Aschrio A, Giovannucci E, Colditz G A & Willett W C, Vitamin E consumption and the risk of coronary heart disease in men, *N Eng J Med*, 328 (1993) 1450.
- 142 Steinberg D, Antioxidant vitamins and coronary heart disease, *N Eng J Med*, 328 (1993) 1487.
- 143 Golikovn A P, Polumiskov V J, Davydov B V *et al*, Lipid peroxidation and the major factors of its activation in patients with myocardial infarction, *Cardiology*, 29 (1989) 53.
- 144 Oldroyd K G, Chopra M, Rankin A C, Belch J J & Cobbe S M, Lipid peroxidation during myocard ischaemia induced by pacing, *Brit Heart J*, 63(2) (1990) 88.
- 145 Roth E, Torok B, Kellemen D *et al*, Free radical mediated injuries alter coronary artery occlusion, *Basic Res Cardiol*, 84(4) (1989) 388.
- 146 Hennig B & Chow C K, Lipid peroxidation and endothelial cell injury: Implications in atherosclerosis, *Free Radic Biol Med*, 4 (1988) 99.
- 147 Ward P A,; Mechanisms of endothelial cell injury, *J Lab Clin Med*, 118 (1991) 421.
- 148 Halliwell B & Chirico S, Lipid peroxidation: Its mechanism, measurement, and significance, *Am J Clin Nutr*, 1993(suppl) 715S.
- 149 Steinbrecher U P, Zhang H & Longheed M, Role of oxidatively modified LDL in arteriosclerosis, *Free Radic Biol Med*, 9 (1990) 155.
- 150 Brown M S, Basu S K, Palck J R, Ho Y K & Goldstein J L, The scavenger cell pathway for lipoprotein degradation; Specificity of the binding site that mediates the uptake of negatively charged LDL by macrophage, *J Supramol Struct*, 13 (1980) 67.
- 151 Brown M S & Goldstein J L, Lipoprotein metabolism in the macrophage; Implications for cholesterol deposition in stherosclerosis, *Annu Rev Biochem*, 52 (1983) 233.
- 152 Lehr H, Becker M, Marklund S L, Hübner C, Arfors K E, Kohlsch"tter A & Messmer K, Superoxide-dependent of leukocyte adhesion by oxidatively modified LDL *in vivo*, *Arterioscler Thromb*, 12 (1992) 824.
- 153 Palinski W, Rosenfeld M E, Ylä-Herttua S, Gurtner G C, Sicher S A, Btuler S W , Parthasarathy S, Carew T E & Steinberg D, Low density lipoprotein undergoes oxidative modification *in vivo*, *Proc Natl Acad Sci USA*, 86 (1989) 1372.
- 154 Parthasarathy S & Steinberg D, Cell-induced oxidation of LDL, *Curr Opin lipidol*, 3 (1992) 313.
- 155 Sato K, Niki E & Shimasaki H, Free radical-mediated chain oxidation of low density lipoprotein and its synergistic inhibition by vitamin E and vitamin C, *Arch biochem Biophys*, 279 (1990) 402.
- 156 56. Ylä-Herttua S, Jaakkola O, Solakivi T, Kuivaniemi H & Nikkari T, The effect of proteoglycans, collagen and lysyl oxidase on the metabolism of low density lipoprotein by macrophages, *Atherosclerosis*, 62 (1986) 73.
- 157 Lyons T J, Glycation and oxidation: a role in the pathogenesis of atherosclerosis, *Am J Cardiol*, 71 (1993) 26B.
- 158 Goldstein J L, Ho Y K, Basu S K & Brown M S, Binding site on macrophages that mediates the uptake and degradation of acetylated low density lipoprotein producing massive

- cholesterol deposition, *Proc Natl Acad Sci USA*, 76 (1979) 333.
- 159 Parthasarathy S, Wieland E & Steinberg D, A role for endothelial cell lipoxygenase in the oxidative modification of low density lipoprotein, *Proc Natl Acad Sci USA*, 86 (1989) 1046.
- 160 Frei B, Ascorbic acid protects lipids in human plasma and the low-density lipoprotein against oxidative damage, *Am J Clin Nutr*, 54 (1991) 1113.
- 161 Esterbauer H, Dieber-Rotheneder M, Striegl G & Waeg G, Role of vitamin E in preventing the oxidation of low-density lipoprotein, *Am J Clin Nutr*, 53 (1991) S314.
- 162 Hiramatsu K, Rosen J W, Heincke K, Wolfbauer G & Chait A, Superoxide initiates oxidation of low density lipoprotein by human monocytes, *Arteriosclerosis*, 7 (1987) 55.
- 163 Favit A, Nicoletti F, Scapagnini U & Canonico P L, Ubiquinone protects cultured neurons against spontaneous and excitotoxin-induced degeneration, *J Cereb Blood Flow Metab*, 12. (1992) 638.
- 164 Nisticò G, Ciriolo M R, Fiskin K, Iannone M, De Martino A & Rotilio G, NGF restores decrease in catalase activity and increases superoxide dismutase and glutathione peroxidase activity in the brain of aged rats, *Free Rad Biol Med*, 12 (1992) 177.
- 165 Adams Jr. J D & Odunze I N, Oxygen free radicals and Parkinson's disease, *Free Rad Biol. Med*, 10 (1991) 161.
- 166 Behl C, Davis J, Cole G M & Schubert D Vitamin E protects nerve cells from amyloid beta protein toxicity, *Biochem Biophys Res Commun*, 186 (1992) 944.
- 167 Halliwell B, Oxygen radicals as key mediators in neurological disease: fact or fiction? *Ann Neurol*, 32 (1992) S10.
- 168 Floyd R A & Carney J M, Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress, *Ann Neurol*, 32 (1992) S22.
- 169 Bindoli A, Rigobello M P & Deebler D J, Biochemical and toxicological properties of the oxidation products of catecholamines, *Free Radicals Biol Med*, 13 (1992) 391.
- 170 Fridovich I, Superoxide radical: an endogenous toxicant, *Annu Rev Pharmacol Toxicol*, 23 (1983) 239.
- 171 Halliwell B & Gutteridge J M C, (1984) Oxygen toxicity, oxygen radicals, transition metals and disease, *Biochem J*, 219 (1984) 11.
- 172 DiGuisepp J & Fridovich I, (1984) The toxicology of molecular oxygen, *Critical Rev Toxicol*, 12 (1984) 315.
- 173 Halliwell B, Free radicals, oxygen toxicity and aging, in *Age pigments*, edited by R S Sohal (Elsevier, Amsterdam) 1981.
- 174 Cohen G, Catalase, glutathione peroxidase, superoxide dismutase and cytochrome P-450 in the nervous system, in *Handbook of neurochemistry*, edited by A Lajtha (New York, Plenum Publishing) 1983.
- 175 Sinet P M, Heikkila R E & Cohen G, Hydrogen peroxide production by rat brain *in vivo*, *J Neurochem*, 34 (1980) 1421.
- 176 Sgaravatti A M, Sgarbi M B, Testa C G, Durigon K, Pederzoli C D, Prestes C C, Wyse A T, Wannmacher C M, Wajner M & Dutra-Filho C S, Gamma-hydroxybutyric acid induces oxidative stress in cerebral cortex of young rats, *Neurochem Int*, 50 (2007) 564.
- 177 Bannon M J, Goedert M & Williams B, The possible relation of glutathione, melanin and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) to Parkinson's disease, *Biochem Pharmacol*, 33(17) (1984) 2697.
- 178 Davies C A, Mann D M A, Sumpter P Q & Yates P.O, A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with AD, *J Neurol Sci*. 78 (1987) 151.
- 179 DeKosky S T & Scheff S W, Synapse loss in frontal cortex biopsies in AD correlation with cognitive severity, *Ann Neurol*, 27 (1990) 457.
- 180 Pearson R C A, Esiri M M, Hiorns RW, Wilcock G K & Powell T P S, Anatomical correlates of the distribution of the neocortex in AD, *Proc Natl Acad Sci USA*, 82 (1985) 1.
- 181 Hardy J & Allsop D, Amyloid deposition as the central event in the aetiology of AD, *Trends Neurosci*, 12 (1991) 383.
- 182 Martins R N, Harper C G, Stokes G B & Masters C L, Increased cerebral glucose-6-phosphate dehydrogenase activity in Alzheimer's disease may reflect oxidative stress, *J Neurochem*, 46 (1986) 1042.
- 183 Smith C D, Carney J M, Starke-Reed P E, Oliver C N, Stadtman E R, Floyd R A. & Markesbury W R, Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer's disease, *Proc Natl Acad Sci USA*, 88 (1991) 10540.
- 184 Halliwell B, Reactive oxygen species and the central nervous system, *J Neurochem*, 59 (1992) 1609.
- 185 Harman D, Free radical theory of aging: a hypothesis on pathogenesis of senile dementia of the Alzheimer's type, *Age*, 16 (1993) 23.
- 186 Gotz M E, Kunig G, Riederer P, & Youdim M B H, Oxidative stress: free radical production in neuronal degeneration, *Pharmacol Ther*, 63 (1994) 37.
- 187 Mecocci P, MacGarvey U & Beal M F, Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease, *Ann Neurol*, 36 (1994) 747.
- 188 Froelich L & Riederer P, Free radical mechanisms in dementia of Alzheimer type and the potential for anti-oxidative treatment, *Arzneimittelforschung Drug Res*, 45 (1995) 443.
- 189 Gsell W, Conrad R, Hicketier M, Sofic E, Froelich L, Wichart I, Jellinger K, Moll G, Raansmaayr G, Beckmann H & Riederer P, Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of alzheimer type, *J Neurochem*, 64 (1995) 1216.
- 190 Friedlich A L & Butcher L L, Involvement of free oxygen radicals in  $\beta$ -amyloidosis: an hypothesis, *Neurobiol Aging*, 15 (1993) 443.
- 191 Nixon R A & Cataldo A M, Free radicals, proteolysis and the degeneration of neurons in Alzheimer disease: how essential is the  $\beta$ -amyloid link? *Neurobiol Aging*, 15 (1994) 463.
- 192 Goedert M, Wischik C, Crowther R, Walker J & Klug A, Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau, *Proc Natl Acad Sci USA*, 85 (1988) 4051.
- 193 Kosik K, Alzheimer's disease: a cell biological perspective, *Science*, 256 (1993) 780.
- 194 Mukaetova-Ladinska E, Harrington C, Roth M & Wischik C, Biochemical and anatomical redistribution of tau protein in Alzheimer's disease, *Am J Pathol*, 143 (1993) 565.

- 195 Bendich A & Butterworth Jr. C E, *Micronutrients in health and in disease prevention* (Marcel Dekker, New York) 1991.
- 196 Gaby, S K, Bendich, A, Singh, V N & Machlin, L J, *Vitamin intake and health* (Marcel Dekker, New York) 1991.
- 197 Zaman Z, Roche S, Fielden P, Frost P G, Niriella D C & Cayley A C D, Plasma concentrations of vitamins A and E and carotenoids in Alzheimer's disease, *Age Ageing*, 21 (1992) 91.
- 198 Jesberger J A & Richardson J S, Oxygen free radicals and brain dysfunction, *Int J Neurosci*, 57 (1991) 1.
- 199 Rosen D R, Siddique T, Patterson D, Figlewicz D A, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan J P, Deng H-X *et al*, Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, *Nature*, (1993) 59.
- 200 Martinez-Cayuela M, Oxygen free radicals and human disease, *Biochimie*, 77 (1995) 147.
- 201 Henle K J & Dethlefsen L A, Time-temperature relationships for heat-induced killing of mammalian cells, *Ann N Y Acad Sci*, 335 (1980) 234.
- 202 Craig E A, The heat shock response, *CRC Crit Rev Biochem*, 18(3) (1985) 239.
- 203 Slater A, Cato A C B, Sillar G M, Kiuoussis J & Burdon R H, The pattern of protein synthesis induced by heat shock of HeLa cells, *Eur J Biochem*, 117 (1981) 341.
- 204 Burdon R H in *Hyperthermie oncology*, edited by J Overgaard (Taylor & Francis, London) 1984, 223
- 205 Lee P C, Bochner B R & Ames B N, AppA, heat-shock stress, and cell oxidation, *Proc Natl Acad Sci USA*, 80 (1983) 7496.
- 206 Morgan R W, Chritsman M F, Jacobson F S, Story G & Ames B N, Hydrogen peroxide-inducible proteins in Salmonella typhimurium overlap with heat shock and other stress proteins, *Proc Natl Acad Sci USA*, 83 (1986) 8063.
- 207 Christman M F, Morgan R W, Jacobson F S & Ames B N, Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in Salmonella typhimurium, *Cell*, 41 (1985) 753.
- 208 Loven D P, Leeper D B & Oberley LW, Superoxide dismutase levels in Chinese hamster ovary cells and ovarian carcinoma cells after hyperthermia or exposure to cycloheximide, *Cancer Res*, 45 (1985) 3029.
- 209 Levinson W, Mikelens P, Oppermann H & Jackson J, Effect of antabuse (disulfiram) on Rous sarcoma virus and on eukaryotic cells, *Biochem Biophys Acta*, 519 (1978) 65.
- 210 Omar R A, Yano S & Kikkawa Y, Antioxidant enzymes and survival of normal and simian virus 40-transformed mouse embryo cells after hyperthermia, *Cancer Res*, 47(13) (1987) 3473.
- 211 McCord J M, Superoxide dismutase: rationale for use in reperfusion injury and inflammation, *J Free Radic Biol Med*, 2 (1986) 325.
- 212 Hass M A & Massaro D, Regulation of the synthesis of superoxide dismutases in rat lungs during oxidant and hyperthermic stresses, *J Biol Chem*, 263 (1989) 776.
- 213 Currie R W, Karmazyn M, Kloc M & Mailer K, Heat shock response is associated with enhanced postischemic ventricular recovery, *Circ Res*, 63 (1988) 543.
- 214 Nohl H & Jordan W, The metabolic fate of mitochondrial hydrogen peroxide, *Int J Biochem*, 111 (1980) 203.
- 214A. Yellon D M, Pasini E, Cargnoni A, Marber M, Latchman, D S & Ferrari R, The protective role of heat stress in the ischemic and reperfused rabbit myocardium, *J Mol Cell Cardiol*, 24 (1992) 895.
- 215 Karmazyn M, Mailer K & Vurrie R W, Acquisition and decay of heat shock enhanced post ischemic ventricular recovery, *Am J Physiol*, 259 (1990) H424.
- 215A. Walker D M, Pasini E, Kuckoglu S, Marber M, Iliodromitis E, Ferrari R & Yellon D M, Heat stress limit infarct size in the isolated perfused rabbit heart, *Cardiovasc Res*, 27 (1993) 962.
- 216 Donnelly T J, Sievers R E, Vissern F L J, Welch W J & Wolfe C L, Heat shock protein induction in rat hearts, *Circulation*, 85 (1992) 769.
- 217 Ritossa F, A new puffing pattern induced by temperature shock and DNP in Drosophila, *Experientia*, 18 (1962) 571.
- 218 Georgopoulos C & Welch W J, Role of the major heat shock proteins as molecular chaperones, *Annu Rev Cell Biol*, 9 (1993) 601.
- 219 Hightower L E, Heat shock, stress proteins, chaperones proteotoxicity, *Cell*, 66 (1991) 191.
- 220 Ellis R J, Protein misassembly: macromolecular crowding and molecular chaperones, *Adv Exp Med Biol*, 594 (2007) 1.
- 221 Ellis R J, Macromolecular crowding: Obvious but underappreciated, *Trends Biochem Sci*, 26 (2001) 597.
- 222 Lashuel H A, Hartley D, Petre B M, *et al*, Neurodegenerative disease: Amyloid pores from pathogenic mutations, *Nature*, 418 (2002) 291.
- 223 Satyal S H, Schmidt E, Kitagawa K, *et al*, Polyglutamine aggregates alter protein folding homeostasis in Caenorhabditis elegans, *Proc. Natl Acad Sci USA*, 97 (2000) 5750.
- 224 Ben-Zvi A P & Goloubinoff P, Proteinaceous infectious behavior in nonpathogenic proteins is controlled by molecular chaperones, *J Biol Chem*, 277 (2002) 49422.
- 225 Tomoyasu T, Mogk A, Laangen H, *et al*, Genetic dissection of the roles of chaperones and proteases in protein folding and degradation in the Escherichia coli cytosol, *Mol Microbiol*, 40 (2001) 397.
- 226 Dingwall C & Lasky R A, Nucleoplasm: The archetypal molecular chaperone, *Semin Cell Biol*, 1 (1990) 11.
- 227 Ellis R J, Discovery of molecular chaperones, *Cell Stress Chaperones*, 1 (1996) 155.
- 228 Shitlerman M, Lorimer G H & Englander S W, Chaperonin function: Folding by forced unfolding, *Science*, 284 (1999) 822.
- 229 Ben-zvi A P & Goloubinoff P, Review: Mechanisms of disaggregation and refolding of stable protein aggregation by molecular chaperones, *J Struct Biol*, 135 (2001) 84.
- 230 Westerheide S D & Morimoto R I, Heat shock response modulators as therapeutic tools for diseases of protein conformation, *J Biol Chem*, 280 (2005) 33097.
- 231 Chatellier J, Hill F & Fersht A.R, From minichaperone to GroEL 2: Importance of avidity of the multisite ring structure, *J Mol Biol*, 304 (2000) 883.
- 232 Mogk A, Tomoyasu T, Goloubinoff P, Rüdiger S, Röder D, Langen H & Bukau B, Identification of thermolabile Escherichia coli proteins, Prevention and reversion of aggregation by DnaK and ClpB, *EMBO J*, 18 (1999) 6934.
- 233 Goloubinoff P, Christeller J T, Gatennby A A, *et al*, Reconstitution of active dimeric ribulose biphosphate

- carboxylase from an unfoiled state depends on two chaperonin proteins and Mg-ATP, *Nature*, 342 (1989) 884.
- 234 Goloubinoff P, Mogk A, Zvi A P, *et al*, Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaaperone network, *Proc Natl Acad Sci USA*, 96 (1999) 13732.
- 235 Ellis R J, Revisiting the Anfinsen cage, *Folding and Design*, 1 (1996) R9.
- 236 Hinault M P & Goloubinoff P, Molecular chaperones and proteases: Cellular fold-controlling factors of proteins in neurodegenerative diseases and aging, *J Mol Neurosci*, (2006) (In Press).
- 237 Heydari A R, You S, Takahashi R, *et al*, Age-related alterations in the activation of heat shock transcription factor 1 in rat hepatocytes, *Exp Cell Res*, 256 (2000) 83.
- 238 Soti C & Csermely P, Aging and molecular chaperones, *Exp Gerontol*, 38 (2003) 1037.
- 239 Saleh A, Srinivasula S M, Balkir L, *et al*, Negative regulation of the Apaf-1 apoptosome by HSP70, *Natl Cell Biol*, 2 (2000) 476.
- 240 Waang Q, Mosser D D & Bag J, Induction of HSP70 expression and recruitment of HSC70 and HSP70 in the nucleus reduce aggregation of a polyalanine expansion mutant of PABPN1 in HeLa cells, *Hum Mol Genet*, 14 (2005) 3673.
- 241 Westerheide S D, Bosman J D, Mbadugha B N, *et al*, Chaperones as inducers of the heat shock response and cytoprotection, *J Biol Chem*, 279 (2004) 566053.
- 242 Delmas D, Jannin B & Latruffe N, Resveratrol: Preventing properties against vascular alterations and ageing, *Mol Nutr Food Res*, 49 (2005) 377.
- 243 Ooie T, Takahashi N & Saikawa T, Single oral dose of genanylgeranylacetone induces heat-shock protein 72 and renders protection against ischemia/reperfusion injury in rat heart, *Circulation*, 1104 (2001) 1837.
- 244 Haddad J J, Science review: Redox and oxygen-sensitive transcription factors in the regulation of oxidant-mediated lung injury: Role for nuclear factor-kappaB, *Crit Care*, 6 (2002) 481.
- 245 Lopez-Neblina F, Toledo A.H & Toledo-Pereyra L H, Molecular biology of apoptosis in ischemia and reperfusion, *J Invest Surg*, 18 (2005) 335.
- 246 Peper A, Grimbergen C A, Spaan J A E, Souren J E M & Van Wijk R, A mathematical model of the HSP70 regulation in the cell, *Int J Hyperthermia* 14 (1998) 97.
- 247 Alam J & Cook J L, How many transcription factors does it take to turn on the heme oxygenase-1 gene? *Am J Respir Cell Mol Biol*, 36 (2007) 166.
- 248 Wiegant F A C, Souren J E M, Van Rijn J & Van Wijk R, Stressor-specific induction of heat shock proteins in rat hepatoma cells, *Toxicology* 94 (1994) 143.
- 249 Poss K D & Tonegawa S, Reduced stress defense in heme oxygenase 1-deficient cells, *Proc Natl Acad Sci USA*, 94 (1997) 10925.
- 250 Maines M D, The heme oxygenase system: past, present and future, *Antioxid Redox Signal*, 6 (2004) 797.
- 251 Ryter S, Alam J & Choi A M, Heme oxygenase-1 / carbon monoxide: from basic science to therapeutic application, *Physiol Rev*, 85 (2006) 583.
- 252 Vitek L & Schwertner H A, The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases, *Adv Clin Chem*, 43 (2007) 1.
- 253 Stocker R, Yamamoto Y, McDonagh A F, Glazer A N & Ames B N, Bilirubin is an antioxidant of possible physiological importance, *Science*, 235 (1987) 1043.
- 254 Scott J R, Chin B Y, Bilban M H & Otterbein L E, Restoring Homeostasis. Is heme oxygenase-1 ready for the clinic? *Trends Pharmacol Sci*, 28 (2007) 200.
- 255 Schipper H M, Heme oxygenase expression in human central nervous disorders, *Free Radical Biol Med*, 37 (2004) 1995.
- 256 Wiegant F A C, Souren J E M & Van Wijk R, Stimulation of survival capacity in heat shocked cells by subsequent exposure to minute amounts of chemical stressors; role of similarity in HSP-inducing effects, *Hum Exp Toxicol*, 18 (1999) 460.
- 257 Kakizuka A, Protein precipitation: A common etiology in neurodegenerative disorders? *Trends Genet*, 14 (1998) 396.
- 258 Kopito R R & Ron D, Conformational disease, *Nat Cell Biol*, 2 (2000) E207.
- 259 Stefani M & Dobson C M, Protein aggregation and aggregate toxicity: New insights into protein folding, misfolding diseases and biological evolution, *J Mol Med*, 81 (2003) 678.
- 260 Hayden M R, Tyagi S C, Kerklo M M & Nicolls M R, Type 2 diabetes Mellitus as a conformational disease, *JOP J Pancreas*, 6 (2005) 287.
- 261 Dobson C.M, Protein folding and its links with human disease, *Biochem Soc Symp*, 68 (2001) 1.
- 262 Cumming R C, Andon N L, Haynes P A, Park M, Fischer W H & Schubert D, Protein disulfide bond formation in the cytoplasm during oxidative stress, *J Biol Chem*, 279 (2004) 21749.
- 263 Anelli T, Alessio M, Mezghrani A, Simmen T, Talamo F, Bachi A & Sitia R, ERp44, a novel endoplasmic reticulum folding assistant of the thioredoxin family, *EMBO J*, 21 (2002) 835.
- 264 Fassio A & Sitia R, Formation, isomerisation and reduction of disulphide bonds during protein quality control in the endoplasmic reticulum, *Histochem Cell Biol*, 117 (2002) 151.
- 265 Zou M H, Cohen R & Ullrich V, Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. Endothelium 2004; 11:89-9Adeghate E, Parvez SH. Nitric oxide and neuronal and pancreatic beta cell death, *Toxicology*, 153 (2000) 143.
- 266 Adeghate E & Parvez S H, Nitric oxide and neuronal pancreatic beta cell death, *Toxicology*, 153 (2000) 143.
- 267 Turko I V, Marcondes S & Murad F, Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase, *Am J Physiol Heart Circ Physiol*, 281 (2001) H2289.
- 268 Hayden M R & Tyagi S C, Islet redox stress: the manifold toxicities of insulin resistance, metabolic syndrome and amylin derived islet amyloid in type 2 diabetes mellitus, *JOP J Pancreas*, 3 (2002) 86.
- 269 Adeghate E, Molecular and cellular basis of the aetiology and management of diabetic cardiomyopathy: a short review, *Mol Cell Biochem*, 261 (2004) 187.
- 270 Cowell R M & Russell J W, Nitrosative injury and antioxidant therapy in the management of diabetic neuropathy, *J Investig Med*, 52 (2004) 33.
- 271 Butler A E, Janson J, Soeller W C & Butler P C, Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of

- islet amyloid formation rather than direct action of amyloid, *Diabetes*, 52 (2003) 2304.
- 272 Janson J, Ashley R H, Harrison D, McIntyre S & Butler P C, The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles, *Diabetes*, 48 (1999) 491.
- 273 Ritzel R A & Butler P C, Replication increases beta-cell vulnerability to human islet amyloid polypeptide-induced apoptosis, *Diabetes*, 52 (2003) 1701.
- 274 Kahn S E, Andrikopoulos S & Verchere C B, Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes, *Diabetes*, 48 (1999) 241.
- 275 Butler A E, Jang J, Gurlo T, Carty M D, Soeller W C & Butler P C, Diabetes due to a progressive defect in beta-cell mass in rats transgenic for human islet amyloid polypeptide (HIP Rat): a new model for type 2, *Diabetes*, 53 (2004) 1509.
- 276 Hayden M R & Tyagi S C, 'A' is for amylin and amyloid in type 2 diabetes mellitus, *JOP J Pancreas*, 2 (2001) 124-39
- 277 Clark A, de Koning E J, Hattersley A T, Hansen B C, Yajnik C S & Poulton J, Pancreatic pathology in non-insulin dependent diabetes (NIDDM), *Diabetes Res Clin Pract*, 28(Suppl) (1995) S39.
- 278 Papa S, Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and physiopathological implications, *Biochim Biophys Acta*, 1276 (1996) 87.
- 279 Wei Y H, Oxidative stress and mitochondrial DNA mutations in human aging, *Proc Soc Exp Biol Med*, 217 (1998) 53.
- 280 Sohal R S & Sohal B H, Hydrogen peroxide release by mitochondria increases during aging, *Mech Aging Dev*, 57 (1991) 187.
- 281 Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schnizer M, Suter M, Walter P & Yaffee M, Oxidants in mitochondria: from physiology to disease, *Biochim Biophys Acta*, 1271 (1995) 67.
- 282 Sohal R S, Sohal B H. & Orr W C, Mitochondrial superoxide and hydrogen peroxide generation, protein oxidative damage and longevity in different species of flies, *Free Radical Biol. Med*, 19 (1995) 499.
- 283 Lee H C, Lu C Y, Fahn H J & Wei Y H, Aging- and smoking-associated alteration in the relative content in human lung, *FEBS Lett*, 441 (1998) 292.
- 284 Fahn H J, Wang L S, Hsieh R H, Chang S C, Kao S H, Huang M H & Wei Y H, Age-related 4,977 bp deletion in human lung mitochondrial DNA, *Am J Respir Crit Care Med*, 154 (1996) 1141.
- 285 Lee H C, Lim M L R, Lu C Y, Liu V W S, Fahn H J, Zhang C, Nagley P & Wei Y H, Concurrent increase of oxidative DNA damage and lipid peroxidation together with mitochondrial DNA mutation in human lung tissues during aging-smoking enhances oxidative stress on the aged tissues, *Arch Biochem Biophys*, 362 (1999) 309.
- 286 Wei Y H, Kao S H & Lee H C, Simultaneous increase of mitochondrial DNA deletions and lipid peroxidation in human aging, *Ann NY Acad Sci*, 786 (1996) 24.
- 287 Lu C Y, Lee H C, Fahn H J & Wei Y H, Oxidative damage elicited by imbalance of free radical scavenging enzymes is associated with large-scale mtDNA deletions in aging human skin, *Mutat Res*, 423 (1991) 11.
- 288 Ames B N, Shigenaga K & Hagen T M, Mitochondrial decay in aging, *Biochim Biophys Acta*, 1271 (1995) 165.
- 289 Lee H C, Yin P H, Lu C Y, Chi C W & Wei Y H, Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells, *Biochem J*, 348 (2000) 425.
- 290 Bonini N M, Chaperoning brain degeneration, *Proc Natl Acad Sci USA*, 99 (2002) 16407.
- 291 Chan H Y, Warrick J M, Andriola I, *et al*, Genetic modulation of polyglutamine toxicity by protein conjugation pathways in Drosophila, *Hum Mol Genet*, 11 (2002) 2895.
- 292 Cummings C J, Mancini M A, Antalffy B, *et al*, Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1, *Nat Genet*, (1998) 148.
- 293 Kawaguchi T, Okamoto T, Taniwaki M, *et al*, CAC expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1, *Nat Genet*, 8 (1994) 221.
- 294 Koide R, Ikeuchi T, Onodera O, *et al*, Unstable expansion of CAC repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA), *Nat Genet*, 6 (1994) 9.
- 295 La Spada A R, Wilson E M, Lubahn D R, *et al*, Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy, *Nature*, 352 (1991) 77.
- 296 Orr H T, Chung M Y, Banfi, *et al*, Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1, *Nat Genet*, 4 (1993) 221.
- 297 Kamino K, Orr H T, Payami H, *et al*, Linkage and mutational analysis of familial Alzheimer disease kindreds for the APP gene region, *Am J Hum Genet*, 51 (1992) 998.
- 298 Laing N G & Siddique T, Cu/Zn superoxide dismutase gene mutations in amyotrophic lateral sclerosis: Correlation between genotype and clinical features, *J Neurosurg Psychiatry*, 63 (1997) 815.
- 299 Lucking CB, Durr A, Bonifati V, *et al*, Association between early-onset Parkinson's disease and mutations in the parkin gene, French Parkinson's Disease Genetics Study Group, *N Engl J Med*, 342 (2000) 1560.
- 300 Mizuno Y, Hattori N, Kitada T, *et al*, Familial Parkinson's disease, Alpha-synuclein and parkin, *Adv Neurol*, 86 (2001) 13.
- 301 Polymeropoulos M H, Lavedan C, Leroy E, *et al*, Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, *Science*, 2276 (1997) 2045.
- 302 Rosen D R, Siddique T, Patterson D, *et al*, Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, *Nature*, 362 (1993) 59.
- 303 Driscoll M & Gerstbrein B, Dying for a cause: Invertebrate genetics takes on human neurodegeneration, *Nat Rev Genet*, 4 (2003) 181.
- 304 Link C D, Transgenic invertebrate models of age-associated neurodegenerative diseases, *Mech Ageing Dev*, 122 (2001) 1639.
- 305 Thompson L M & Marsh J L, Invertebrate models of neurologic disease: Insights into pathogenesis and therapy, *Curr Neurol Neurosci Rep*, 3 (2003) 442.
- 306 Westlund B, Stilwell G & Sluder A, Invertebrate disease models in neurotherapeutic discovery, *Curr Opin Drug Discov Devel*, 7 (2004) 169.
- 307 Merry D E, Animal models of Kennedy disease, *NeuroRx*, 2 (2005) 471.
- 308 Ross C A & Poirier M A, Protein aggregation and neurodegenerative disease, *Nat Med*, 10 (2004) S10.
- 309 Brignull, H.R., Morley J.F., Morimoto R.I. The stress of

- misfolded proteins: *C. elegans* models for neurodegenerative disease and aging, in *Molecular aspects of the stress response: Chaperones, Membranes and Networks*, edited by P Csermely & L Vigh (Springer, New York) 2006, 167.
- 310 Garigan D, Hsu A L, Fraser A G, *et al*, Genetic analysis of tissue aging in *Caenorhabditis elegans*; A role for heat-shock factor and bacterial proliferation, *Genetics*, 161 (2002) 1101.
- 311 Hsu A L, Murphy C T & Kenyon C, Regulation of aging and age-related disease by DAF-16 and heat-shock factor, *Science*, 300 (2003) 1142.
- 312 Morley J F & Morimoto R I, Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones, *Mol Biol Cell*, 15 (2004) 657.
- 313 Gutteridge J M C & Halliwell B, The measurement and mechanisms of lipid peroxidation in biological systems, *Trends Biochem Sci*, 15 (1990) 129.
- 314 Boveris A, Cadenas E & Chance B, Low level chemiluminescence of the lipoxigenase reaction, *Photobiochem Photobiophys*, 1 (1980) 175.
- 315 Slawinski J, Luminescence research and its relation to ultra weak cell radiation, *Experientia*, 44 (1988) 559.
- 316 Boveris A, Cadenas E, Reiter R, Filipkowski M, Nakase Y & Chance B, Organ chemiluminescence: Non-invasive assay for oxidative radical reactions, *Proc Natl Acad Sci USA*, 77 (1980) 347.
- 317 Harvey E N, *Living light* (Princeton University Press, Princeton) 1940.
- 318 Harvey E N, *A History of luminescence* (American Philosophical Society, Philadelphia, Pennsylvania) 1957
- 319 Troitskii N A, Konev S V & Katibnikov M A, Investigation of the ultraviolet hemi luminescence of biological systems, *Biofizika*, 6(2) (1961) 80.
- 320 Vladimirov IuA, Litvin F F & T'an M C, On the problem of the role of the nature of ultraweak luminescence in biological systems, *Biofizika*, 7 (1962) 675.
- 321 Seliger H H & McElroy W D, Spectral emission and quantum yield of firefly bioluminescence, *Arch Biochem Biophys*, 88 (1960) 136.
- 322 Stauff J & Reske G, Model studies on chemical carcinogenesis and on the photodynamic effect of 3,4 – Benzopyrene and UV light in aqueous protein solutions with different SH group reactivity, *Z Naturforsch B*, 19 (1964) 716.
- 323 Quickenden T I & Quee Hee S S, Weak luminescence from the yeast *Saccharomyces cerevisiae* and the existence of mitogenetic radiation, *Biochem Biophys Res Commun*, 60 (1974) 764.
- 324 Allen R C, Stjernholm R L & Steele R H, Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity, *Biochem Biophys Res Commun*, 47 (1972) 79.
- 325 Kakinuma K, Cadenas E, Boveris A & Chance B, Low level chemiluminescence of intact polymorphonuclear leukocytes, *FEBS Lett*, 102 (1979) 38.
- 326 Cadenas E, Daniele R P & Chance B, Low level chemiluminescence of alveolar macrophages: spectral evidence for singlet oxygen generation, *FEBS Lett*, 123 (1981) 225.
- 327 Cadenas E, Wefers H & Sies H, Low-level chemiluminescence of isolated hepatocytes, *Eur J Biochem* 119, (1981) 531.
- 328 Pirenne M H & Denton E J, Accuracy and sensitivity of the human eye, *Nature*, 170 (1952) 1039.
- 329 Nakano M, Noguchi T, Sugioka K, Fukuyama H, Sato M, Shimizu Y, Tsuji Y & Inaba H, *J Biol Chem*, 250 (1975) 2404.
- 330 Sugioka K & Nakano M, A possible mechanism of the generation of singlet molecular oxygen in nadph-dependent microsomal lipid peroxidation, *Biochim Biophys Acta*, 423 (1976) 203.
- 331 Hamman J P, Gorby D R & Selige. H H, A new type of biological chemiluminescence: the microsomal chemiluminescence of benzo[a]pyrene arises from the diol epoxide product of the 7,8-dihydrodiol, *Biochem Biophys Res Commun*, 75, (1977) 793.
- 332 Scarpa A Dutton P L & Leigh J S, *Frontiers of biological energetics: From electron to tissues* (Academic Press, New York) 1978.
- 333 Cadenas E, Boveris A & Chance B, Low-level chemiluminescence of bovine heart submitochondrial particles, *Biochem J*, 186 (1980) 659.
- 334 King M M, Lai E K & McCay P B, Singlet oxygen production associated with enzyme-catalyzed lipid peroxidation in liver microsomes, *J Biochem*, 250 (1975) 6496.
- 335 Hatefi Y & Hanstein W G, Lipid oxidation in biological membranes. I. Lipid oxidation in submitochondrial particles and microsomes induced by chaotropic agents, *Arch Biochem Biophys*, 138 (1970) 73.
- 335A. Hanstein W G & Hatefi Y, Lipid oxidation in biological membranes. II. Kinetics and mechanism of lipid oxidation in submitochondrial particles, *Arch Biochem Biophys*, 138(1) (1970) 87.
- 336 Tappel A L, Studies of the mechanism of vitamin E action. III. In vitro copolymerization of oxidized fats with protein, *Arch Biochem Biophys*, 54 (1955) 266.
- 337 Misra H & Fridovich I, A peroxide-dependent reduction of cytochrome c by NADH, *Biochem Biophys Acta*, 292 (1973) 815.
- 338 McCord J M & Day jr. E D, Superoxide-dependent production of hydroxyl radical catalyzed by iron-EDTA complex, *FEBS Lett*, 86 (1978) 139.
- 339 Halliwell B, Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates: is it a mechanism for hydroxyl radical production in biochemical systems? *FEBS Lett*, 92 (1978) 321.
- 340 Cohen G. in *Superoxide and superoxide dismutases*, edited by A M Michelson, J M McCord & I. Fridovich (Academic Press, New York) 1977, 317.
- 341 Peters J W & Foote C S, Chemistry of superoxide ion. II. Reaction with hydroperoxides, *J Am Chem Soc*, 98 (1976) 873.
- 342 Cadenas E, Vasavsky A I, Boveris A & Chance B, Oxygen- or organic hydroperoxide-induced chemiluminescence of brain and liver homogenase, *Biochem J*, 198 (1981) 645.
- 343 Turrens J F, Giulivi C & Boveris A, Increased spontaneous chemiluminescence from liver homogenates and isolated hepatocytes upon inhibition of O<sub>2</sub>- and H<sub>2</sub>O<sub>2</sub> utilization, *Free Rad Biol Med*, 2 (1986) 135.
- 344 Riely C A, Cohen G & Lieberman M, Ethane evolution: a new index of lipid peroxidation, *Science*, 183 (1974) 208.

- 345 Dillard C J, Dumelin E E & Tappel A L, Effect of dietary vitamin E on expiration of pentane and ethane by the rat, *Lipids*, 12 (1977) 109.
- 346 Okuda M, Lee H C, Kumar C & Chance, Oxygen radical generation during ischemia-reperfusion in the isolated perfused rat liver monitored by enhanced chemiluminescence, *Circulatory Shock*, 38 (1992) 228.
- 347 Bachmann E, Weber E & Zbinden G, Effects of seven anthracycline antibiotics on electrocardiogram and mitochondrial function of rat hearts, *Agents Actions*, 5 (1975) 383.
- 348 Barnard M L, Gurdian S & Turrens J F, Activated polymorphonuclear leukocytes increase low-level chemiluminescence of isolated perfused rat lungs, *J Appl Physiol*, 75 (1993) 933.
- 349 Fukuda F, Kitada M, Horie T & Awazu S, Evaluation of adriamycin-induced lipid peroxidation, *Biochem Pharmacol*, 44 (1992) 755.
- 350 Doroshov J H & Davies K J A, Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide and hydroxyl radical, *J Biol Chem*, 261 (1986) 3068.
- 351 Sugioka K, Nakano H, Nakano M, Tero-Kubota S & Ikegami Y., Generation of hydroxyl radicals during the enzymatic reductions of the Fe<sup>2+</sup>-ADP-EDTA systems, Less involvement of hydroxyl radical and a great importance of proposed ferryl ion complexes in lipid peroxidation, *Biochim Biophys Acta*, 753 (1983) 411.
- 352 Meerson F Z, Kagan V E, Kozlov Yu P, Belinka L M & Arkhipenko Yu V, The role of lipid peroxidation in pathogenesis of ischemic damage and the antioxidant protection of the heart, *Basic Res Cardiol*, 77 (1982) 465.
- 353 Meerson F Z, Belkina L M, Sazontova T G, Saltykova V A & Arkhipenko Yu V, The role of lipid peroxidation in the pathogenesis of arrhythmias and prevention of cardiac fibrillation with antioxidant, *Basic Res. Cardiol*, 82 (1987) 123.
- 354 Guarnieri C, Flamigni F & Calderara C M, Role of oxygen in the cellular damage induced by reoxygenation of hypoxic heart, *J Mol Cell Cardiol*, 12 (1980) 797.
- 355 Chambers D E, Parks D A, Patterson G, Yoshida S, Burton K, Parmley L F, McCord J & Downey J M, Role of oxygen derived radicals in myocardial ischemia, *Feder Proc*, 42 (1983) 1093.
- 356 Kramer J H, Arroyo C M, Dickens B F & Weglicki W B, Spin-trapping evidence that graded myocardial ischemia alters post-ischemic superoxide production, *Free Radic Biol Med*, 3 (1987) 153.
- 357 Garlick P B, Davies M J, Hearse D J & Slater T S, Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy, *Circul Res*, 61 (1987) 757.
- 358 Arroyo C M, Kramer J H, Leiboff R H, Mergner G W, Dickens B F & Weglicki W B, Spin trapping of oxygen and carbon centered free radicals in ischemic canine myocardium, *Free Rad Biol Med*, 5 (1987) 313.
- 359 Barsacchi R, Coassin M, Maiorino M, Pelosi G, Simonelli C & Ursini F, Increased ultra weak chemiluminescence from rat heart at postischemic reoxygenation: protective role of vitamin E, *Free Rad Biol & Medic*, 5 (1989) 573.
- 360 Parks D A & Granger D N, Ischemia-reperfusion injury: A radical view, *Hepatology*, 8 (1988) 680.
- 361 Nunes F A, Kumar C, Chance B & Brass C A, Chemiluminescent measurement of increased free radical formation after ischemia/reperfusion, *Digestive Diseases and Sciences*, 40(5) (1995) 1045.
- 362 Adkinson D, Hollwarth M E, Benoit J N, Parks D A, McCord J M & Granger D N, Role of free radicals in ischemia-reperfusion in jury to the liver, *Acta Physiol Scand*, (Suppl) 548 (1986) 101.
- 363 Atalla S L, Tolodo-Pereyra L H, MacKenzie G H & Cederna J P, Influence of oxygen-derived free radical scavengers on ischemic livers, *Transplantation*, 40 (1985) 584.
- 364 Cadenas E, Boveris A, & Chance B, Low-level chemiluminescence of biological systems, in *Free radical in biology*, edited by W A Pryor (New York, Academic Press) 1984, 211.
- 365 Cadenas E, Arad I D, Boveris A, Fisher A B & Chance B, Partial spectral analysis of the hydroperoxide-induced chemiluminescence of the perfused lung, *FEBS Lett*, 111 (1980) 413.
- 366 Turrens J F, Giulivi C, Pinus C R, Lavagno C & Boveris A, Spontaneous lung chemiluminescence upon paraquat administration, *Free Rad Biol Med*, 5 (1988) 319.
- 367 Barnard M L, Gurdian S & Turrens J F, Activated polymorphonuclear leukocytes increase low-level chemiluminescence of isolated perfused rat lungs, *J Appl Physiol*, 75 (1993) 933.
- 368 Peralta J G, Barnard M L & Turrens J K, Characteristics of neutrophil influx in rat lungs following fecal peritonitis, *Inflammation*, 17 (1993) 263.
- 369 Cilento G, Photobiochemistry without light, *Experientia*, 44 (1988) 572.
- 370 Ntziachristos V, Ripoll J, Wang L V, Weissleder R, Looking and listening to light: the evolution of whole body photonic imaging, *Nature Biotechnol*, 23 (2005) 313.
- 371 Kobayashi M, Takeda M, Ito, K-I, Kato H & Inaba H, Two-dimensional photon counting imaging and spatiotemporal characterization of ultraweak photon emission from a rat's brain in vivo, *J Neurosci Methods*, 93 (1999) 163.
- 372 Kobayashi M, Two-dimensional imaging and spatiotemporal analysis of biophoton, in *Biophotonics: optical science and engineering for the 21<sup>st</sup> century*, edited by X Shen & R Van Wijk (Springer, New York) 2005, 155.
- 373 Boveris A, Llesuy S F & Fraga C G, Increased liver chemiluminescence in tumor-bearing mice, *J Free Rad Biol Med*, 1(2) (1985) 131.
- 374 Neifakh E A, Free-radical mechanism of ultraweak chemiluminescence accompanying the peroxidation of unsaturated fatty acids, *Biofizika*, 16 (1971) 560.
- 375 Greenstein J P, *Biochemistry of cancer*, (Academic Press, New York) 1947, 202
- 376 Amano T, Kunimi K, Nakashima K, Uchibayashi T & Hisazumi H, A combined therapy of hyperthermia and tumor necrosis factor for nude mice bearing KK-47 bladder cancer, *J Urol*, (1990) 144.
- 377 Amano T, Kobayashi M, Devaraj B, Inaba H, Ultraweak biophoton emission imaging of transplanted bladder cancer, *Urol Res*, 23 (1995) 315.
- 378 Kim J, Choi C, Lim J, You H, Sim S, Yom Y, Kim E & Soh K, Measurements of spontaneous photon emission and delayed luminescence from human cancer tissues, *J Altern Complement Med*, 11 (2005) 879.

- 379 Shimizu Y, Inaba H, Kumaki K, Mizuno K, Hata S & Tomita S, Measuring methods for ultra-low light intensity and their application to extra-weak spontaneous bioluminescence from living tissues, *IEEE Trans Instrum Meas*, IM-22 (1973) 153.
- 380 Takeda M, Kobayashi M, Takayama M, Suzuki S, Ishida T, Ohnuki K, Moriya T & Ohuchi N, Biophoton detection as a novel technique for cancer imaging, *Cancer Sci*, 95 (2004) 656.
- 381 Dobrin R, Kirsch C, Kirsch S, Pierrakos J, Schwartz E, Wolff T & Zeira Y, Experimental measurements of the human energy field, in *Psychoenergetic systems: The interface of consciousness, energy and matter*, edited by S Krippner (Gordon & Breach, New York) 1979, 227.
- 382 Dobrin R, Kirsch C, Kirsch S, Pierrakos J, Schwartz E, Wolff T Zeira, Y, Experimental measurements of the human energy field, in *The Energies of Consciousness*, edited by S Krippner & D Rubin (Gordon and Breach New York), 1975.
- 383 Cohen S & Popp F A, Whole-body counting of biophotons and its relation to biological rhythms, in *Biophotons*, edited by J J Chang, J Fisch & F A Popp (Kluwer Academic, Dordrecht) 1998, 183.
- 384 Saueremann G, Mei W P, Hoppe U & Stáb F, Ultraweak photon emission of human skin *in vivo*: Influence of topically applied antioxidants on human skin, *Methods Enzymol*, 300 (1999) 419.
- 385 Choi C, Woo W M, Lee M B, Yang J S, Soh K S, Yang J S, Yoon G, Kim M, Zaslavsky & Chang J J, Biophoton emission from the hands, *J Korean Physical Soc*, 41 (2002) 275.
- 386 Van Wijk E P A. & Van Wijk R, Multi-site registration and spectral analysis of spontaneous emission from human body, *Res in Complimentary Classical Natural Med*, 12 (2005) 96.
- 387 Van Wijk E P A & Van Wijk R, Ultra weak photon emission of human body, in *Biophotonics – Optical science and engineering for the 21st Century*, edited by X Shen & R. Van Wijk (Kluwer, New York) 2006.
- 388 Van Wijk R & Van Wijk E P A, Human photon emission, *Rec Res Dev Photochem Photobiol*, 7 (2004) 139.
- 389 Van Wijk R & Van Wijk E P A, An introduction to human biophoton emission, *Res Complementary Classical Natural Med*, 12 (2005) 77.
- 390 Kobayashi M, Modern technology on physical analysis of biophoton emission and its potential extracting the physiological information, in *Energy and information transfer in biological systems*, edited by F Musumeci, L S Brizhik & M W Ho (World Scientific Publishers, New Jersey, London) 2003, 157.
- 391 Van Wijk E, Kobayashi M & Van Wijk R, Spatial characterization of human ultra-weak photon emission, in *Biophotons and coherent systems in biology, biophysics and biotechnology*, edited by L Belousov, V L Voeikov & V S Martynyuk (Kluwer, New York) 2006.
- 392 Van Wijk R, Kobayashi M & Van Wijk E P A, Spatial characterization of human ultra-weak photon emission, *J Photochem Photobiol, B* 83 (2006) 69.
- 393 Cifra M, Van Wijk E P A, Koch H, Bosman S & Van Wijk R, Measurement of spontaneous ultra-weak photon emission from human body: spontaneous ultra-weak photon emission varies diurnally, *Measurement* (2007).
- 394 Cifra M, Van Wijk E P A, Koch H, Bosman S & Van Wijk R, Spontaneous ultra-weak photon emission from human hands is time dependent, *Radioengineering*, 15 (2007) 1.
- 395 Van Wijk E P A, Van Wijk R & Cifra M, Spontaneous ultra-weak photon emission from human hands varies diurnally, in *Biophotonics 2007: Optics in life science* (SPIE, Bellingham) 2007, 66331J1.
- 396 Nakamura K & Hiramatsu M, Ultra-weak photon emission from human hand: influence of temperature and oxygen concentration on emission, *J Photochem Photobiol B*, 80 (2005) 156.
- 397 Cifra M, *Measurements of spontaneous photon emission from the human body: technical aspects, parameters, time and temperature dependent fluctuations of photon emission*, Ph.D. thesis, University of Zilina, Zilina, Slovenia, 2006.
- 398 Yang J M, Lee C, Yi S H, Yang J S & Soh K S, Biophoton emission and blood flow in human hand, *ISLIS*, 22 (2004) 344.
- 399 Orszag M, *Quantum Optics* (Springer Berlin) 2000, 29.
- 400 Bajpai R J, Quantum coherence of biophotons and living systems, *Indian J Exp Biol*, 41 (2003) 514.
- 401 Bajpai R J, Biophotons emission in a squeezed state from a sample of *Parmelia tinctorum*, *Phys Lett A*, 322 (2004) 131.
- 402 Bajpai R P, Squeezed state description of the spectral decomposition of a biophoton signal, *Phys Lett A*, 337 (2005) 265.
- 403 Van Wijk R, Van Wijk E & Bajpai RP, Photon count distribution of photons emitted from three sites of a human body, *J Photochem Photobiol B*, 84 (2006) 46.
- 404 Usa M, Devaraj B, Kobayashi M, Takeda M, Ito H, Jin M & Inaba H, Detection and characterization of ultraweak biophotons from life processes, in *Optical methods in biomedical and environmental sciences*, edited by H Ohzu & S Komatsu (Elsevier Sciences, Amsterdam) 1994, 3.
- 405 Usa M & Inaba H, Spontaneous photon emission from human body, *Med. Imaging Technol*, 13 (1995) 47.
- 406 Usa M, Kobayashi M, Suzuki S, Ito H & Inaba H, *ITEJ Technical Report*, 15 (1991).
- 407 Cohen S & Popp F A, Biophoton emission of the human body, *J Photochem Photobiol B*, 40 (1997) 187.
- 408 Cohen S & Popp F A, Biophoton emission of the human body, *Indian J Exp Biol*, 41 (2003) 440.
- 409 Jung H H, Woo W M, Yang J M, Choi C, Lee J, Yoon G, Yang J S, Lee S & Soh K S, Left-right asymmetry of biophoton emission from hemiparesis patients, *Indian J Exp Biol*, 41 (2003) 452.
- 410 Berliner J, Navab M, Fogelman A, Frank J S, Dewer L L, Edwards P A, Watson A D & Lulis A J, Atherosclerosis: Basic mechanisms, Oxidation, Inflammation and Genetics, *Circulation*, 91 (1995) 2488.
- 411 Naito C, Kawamura M & Yamamoto Y, Lipid peroxides as the initiating factor of atherosclerosis, *Ann N Y Acad Sci*, 676 (1993) 27.
- 412 Scarpellini F, Sbracia M & Scarpellini L, Psychological stress and lipoperoxidation in miscarriage, *Ann N Y Sci*, 709 (1994) 210.
- 413 Adachi S, Kawamura K & Takemoto K, Oxidative damage of nuclear DNA in liver of rats exposed to psychological stress, *Cancer Res*, 53 (1993) 4153.
- 414 Schneider R H, Nidisch S I, Salerno J W, Sharma H M, Robinson C E, Nidich R J & Alexander C N, Lower lipid peroxide levels in practitioners of the Transcendental Meditation Program, *Psychosom Med*, 60 (1998) 38.

- 415 Kim D H, Moon Y S, Kim H S, Jung J S, Park H M, Suh H W, Kim Y H & Song D K, Effect of Zen Meditation on serum nitric oxide activity and lipid peroxidation. *Prog Neuropsychopharmacol Biol Psychiatry*, 29 (2005) 327.
- 416 Yadav R K, Ray R B, Vempati R & Bijlani R L, Effect of comprehensive yoga-based life style modification program on lipid peroxidation, *Indian J Physiol Pharmacol*, 49 (2005) 358.
- 417 Van Wijk E P A, Koch H, Bosman S, Van Wijk R, Spatial characterization of human ultra-weak photon emission in TM practitioners and control subjects, *J Altern Complement Med*, 12 (2006) 31.
- 418 Van Wijk E P A, Ludtke R & Van Wijk R, Differential effects of relaxation techniques on ultra-weak photon emission, *J Altern Complement Med*, (2008)
- 419 Van Wijk R, Van Wijk E P A & Bajpai R P, Quantum squeezed state analysis of spontaneous ultra-weak photon emission of practitioners of meditation and control subjects, *Indian J Exp Biol*, 46 (2008) 345.
- 420 Sato Y, Hotta N, Sakamoto N, Matsuoka S, Oshini N & Yagi K, Lipid peroxide level in plasma of diabetic patients, *Biochem Med*, 21 (1979) 104.
- 421 Murata R, Nishida T, Eto S, & Mukai N, Lipid peroxidation in diabetic rat retina, *Metab Pediat Ophthalmol*, 5 (1981) 83.
- 422 Nishigaki I, Hagihara M, Tsunekawa H, Maseki M & Yagi K, Lipid peroxide levels of serum lipoprotein fractions of diabetic patients, *Biochem Med*, 25 (1981) 373.
- 423 Stringer M D, Gorog P G, Freeman A & Kakkar V V, Lipid peroxides and atherosclerosis, *Br Med J*, 298 (1989) 281.
- 424 Van Wijk R, Wiegant F A C, Popp F A & Storms G E M G, Biophoton emission from blood serum of diabetic patients, *Prog Biomedical Optics*, 2328 (1994) 212.
- 425 Packer L, The role of anti-oxidative treatment in diabetes mellitus, *Diabetologia*, 36 (1993) 1212.
- 426 Brown M, Ross T P & Holloszy J O, Effects of ageing and exercise on soleus and extensor digitorum longus muscles of female rats, *Mech Ageing Dev*, 63 (1992) 69.
- 427 Song W, Kwak H B & Lawler J M, Effect of exercise training on iNOS and pro-apoptotic signaling in aging skeletal muscle, *FASEB J*, 18 (2004) A753.
- 428 Song W, Kwak H B & Lawler J M, Exercise training attenuates age-induced changes in apoptotic signaling in rat skeletal muscle, *Antioxidants Redox Signaling*, 8 (2006) 517.
- 429 Delp M D, Duan C, Mattson J P & Musch T I, Changes in skeletal muscle biochemistry and histology relative to fiber-type in rats with heart failure, *J Appl Physiol*, 83 (1997) 1291.
- 430 Linke A, Adams V, Schulze P C, Erbs S, Gielen S, Fiehn E, Mobius-Winkler S, Schubert G & Hambrecht R, Antioxidant effects of exercise training in patients with chronic failure, *Circulation*, 111 (2005) 1763.
- 431 Song Y H, Li Y, Du J, Mitch W E, Rosenthal N & Delafontaine P, Muscle-specific expression of OGF-1 blocks angiotensin II-induced skeletal muscle wasting, *J Clin Invest*, 115 (2005) 451.
- 432 Ades P A & Coello C E, Effects of exercise and cardiac rehabilitation on cardiovascular outcomes. *Med Clin North Am*, 84 (2000) 251.
- 433 Lavie C J & Milani R V, Cardiac rehabilitation and preventive cardiology in the elderly, *Cardiol Clin*, 17 (1999) 233.
- 434 Tikkanen H O, Hamalainen E & Harkonen M, Significance of skeletal muscle properties on fitness, long-term physical training and serum lipids, *Atherosclerosis*, 142 (1999) 367.
- 435 Hammeren J, Powers S, Lawler J, Criswell D, Martin D, Lowenthal D & Pollock M, Exercise training-induced alterations in skeletal muscle oxidative and antioxidant enzyme activity in senescent rats, *Int J Sports Med*, 13 (1992) 412.
- 436 Helwig B, Schreurs K M, Haansen J, Hagerman K S, Zbrowski M G, McAllister R M, Mitchell K E & Musch T L, Training-induced changes in skeletal muscle Na<sup>+</sup>-K<sup>+</sup> pump number and isoform expression in rats with chronic heart failure, *J Appl Physiol*, 94 (2003) 2225.