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OXIDATIVE DAMAGE AND CEREBRAL AGING

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CONTENTS

1. INTRODUCTION

The free radical theory of aging is a widely accepted explanation for the progressive accumulation of age-related cell constituent damage. It is also accepted that the central nervous system, the 'control center' for overall body function, is particularly vulnerable to free radical attack due to several of its inate characteristics. Therefore, the main focus of this work is to examine the role of oxidative stress and damage in the senescent brain. However, the large body of literature already reported on age-related changes in the vascular, hepatic, and renal systems associated with alterations in the oxidative status of these systems should not be discounted. This review was undertaken to provide an overview of research on the role of reactive oxygen species, hereafter referred to as ROS, in the central nervous system associated with the aging process. As an ample body of literature exists on each of the separate topics ROS and aging, it was not the intent of the present work to present a complete review of these individual fields of research, but to provide readers with references to each of the pertinent sections. Other topics discussed as background material include a review of the properties of ROS, analytical methods of ROS measurement, factors underlying ROS formation in the nervous system, and mitigation of increased cerebral ROS formation. On the aging front we present data on age-related alterations in cerebral ROS formation, alternative theories of aging, and our own proposal of a consolidated theory of cerebral senescence.

2. THE FREE RADICAL THEORY OF CEREBRAL AGING

When the free radical theory of aging was initially proposed by Harman (1956), the general concept was that free radical reactions cause damage to biological molecules and are either responsible for, or are major contributors to, the normal aging process. Over the next three decades, this theory was further refined to include lifespan studies (Harman, 1957a, 1968), the disease states cancer (Harman, 1961, 1962a), atherosclerosis (Harman 1957b, 1962b), and dietary manipulation experiments (Harman, 1971, 1978, 1979, 1980). These numerous refinements have presented the biogerontology community with Harman's latest version (Harman, 1987) of the free radical aging theory. More recent overviews of the theory have also been published (Porta, 1988; Pacifici and Davies, 1991).

It is widely accepted that the mammalian central nervous system (CNS) contains large amounts of substrates that are susceptible to free radical attack, such as unsaturated lipids and catecholamines. Halliwell and Gutteridge (1985) were probably the first to review the potential role of oxygen radicals in the nervous system. The involvement of these reactive species in hyperoxia, ischemia, trauma, stoke and transition metal-dependent reactions in the brain has since been a topic of considerable interest (Braughler and Hall, 1989; Floyd, 1990). In fact, roles have been proposed for ROS in several human disease states of the CNS, such as, Alzheimer's (Volicer and Crino, 1990), Parkinson's (Youdim *et al.*, 1989; Dexter *et al.*,

1989), tardive dyskinesia (Lohr *et al.*, 1990), Down's syndrome (Brooksbank and Balazs, 1984), and in several mechanisms of neurotoxicity (LeBel and Bondy, 1991a).

The exquisite sensitivity of the brain to oxidative damage suggests that this organ may be a critical target in the aging process. One might expect that brain levels of non-enzymatic antioxidants and antioxidant protective enzymes would decrease with age. However, this has proven not to be the case, leaving unresolved the theory that free radicals are causal to the aging process. The numerous conflictive findings will be discussed in Section 7.

3. GENERAL PROPERTIES OF REACTIVE OXYGEN SPECIES AND THEIR MEASUREMENT

Free radicals are defined as any species with one or more unpaired electrons (Freeman and Crapo, 1982; Halliwell and Gutteridge, 1986). The fact that oxygen is ubiquitous in aerobic organisms has lead to the concept of the oxygen paradox; namely its requirement for aerobic life, but also as the precursor to ROS (Pacifici and Davies, 1991). Superoxide anion and hydroxyl radical qualify as oxygen-centered radicals, while hydrogen peroxide is a potent cellular oxidant that lacks unpaired electrons. These and other ROS have been implicated in a wide range of physiological, toxicological and pathological phenomena

Given the critical nature of the role that ROS are believed to play in biological systems, there is a great need for accurate and reproducible analytical techniques for measurement of these species. However, due to the short half-lives and high reactivity potentials of ROS, the pursuit of sensitive analytical methodologies has been arduous. Furthermore, while many of these assays have been validated in aqueous systems, extrapolation of the measurements to biological systems has been difficult. Many methods of quantitating intracellular oxidative activity are indirect in that they measure breakdown products of oxidative degradation of macromolecules. Thus ROS activity is secondarily implied by measuring catabolic products from lipids and proteins, or by looking for genetic changes in DNA. More sensitive and direct procedures for the evaluation of ROS, include the use of electron probe resonance, and fluorescent probe technology. A thorough and comprehensive review of ROS detection systems, and the caveats associated has been conducted (Weber, 1990).

In the following sections we provide an overview of the existing literature regarding mechanisms of oxidative damage in the nervous system.

4. FACTORS UNDERLYING CEREBRAL REACTIVE OXYGEN SPECIES FORMATION

Approximately two percent of oxygen consumed by mitochondria, is incompletely reduced and appears as oxygen radicals (Boveris and Chance, 1973). This proportion may be increased when the efficient functioning of mitochondrial electron transport systems is compromised.

In addition, lowered pH resulting from excess glycolytic activity may not only accelerate the process of liberating protein-bound iron in organisms, but it may also lead to an impairment of oxidative ATP generation, and to the appearance of the pro-oxidant protonated superoxide (Siesjo, 1988).

Liberation of protein-bound iron may enhance the degradation of important iron-binding proteins such as ferritin and transferrin. A small increase in levels of free iron within cells can dramatically accelerate rates of ROS production (Minotti and Aust, 1989). A key feature in establishing the rate of production of ROS by tissue, is the cytosolic concentration of free metal ions possessing the capacity to readily change their valence state. Iron is considered the most important of these but levels of free manganese and copper may also be significant factors (Aust *et al.*, 1985).

Enhanced phospholipase activity can lead to the release of arachidonic acid. This polyunsaturated fatty acid contains four ethylenic bonds and is readily auto-oxidizable. In fact, impure preparations of this chemical may explode spontaneously on exposure to air (Halliwell and Gutteridge, 1989). The enzymic conversion of this compound to prostaglandins, leukotrienes and thromboxanes by cyclooxygenases and lipoxygenases leads to considerable ROS generation (Freeman and Crapo, 1982; Saunders and Horrocks, 1987). Increased levels of cytosolic free calcium, may result from either breakdown of the steep concentration gradient of calcium across the plasma membrane, or by liberation of the large amounts of calcium bound intracellularly within mitochondria or endoplasmic reticulum. Elevation of calcium can activate phospholipases and thus stimulate ROS production. The activation of phospholipase D has been functionally linked to superoxide anion production (Bonser *et al.*, 1989). A reciprocal relation exists, since free radicals can enhance phospholipase A_2 activity within cerebral capillaries (Au *et al.*, 1985).

Phospholipase A_2 may selectively induce oxidative changes to the GABA-regulated chloride channel, and thus increase cell excitability (Schwartz *et al.*, 1988).

Chemical induction of cytochrome P450-containing mixed function mono-oxidases can increase the rate of Phase I detoxification reactions. The oxidative metabolism of many lipophilic compounds, while necessary for their conjugation and excretion, often involves the transient formation of highly reactive oxidative intermediates such as epoxides (Sevanian *et al.*, 1990). While mixed function oxidases predominate in the liver, they are also present in the nervous system, largely within neurons (Ravindranath *et al.*, 1989). At the intracellular level, most of these cerebral oxidases are mitochondrial rather than microsomal, and like the corresponding hepatic enzymes, they are inducible (Perrin *et al.*, 1990). Xanthine oxidase is a prime generator of superoxide anion and may be a significant exacerbating factor in several pathological states.

The key reducing power of intracellular glutathione stores can be compromised by agents forming tight complexes with sulfhydryl groups, or by inhibition of the related glutathione reductase or peroxidase.

Glutathione depletion can result in neurological deficit (Orlowski and Karkowsky, 1976).

Extracellular formation of superoxide anion by phagocytes has long been recognized as a bacteriocidal mechanism. Similar oxidative activity has been observed in cerebral microglia (Halliwell and Gutteridge, 1989). Astroglial activation is a common event following neural trauma, and reactive astrocytes are active in clearance of cell debris and ultimately in the formation of glial scar tissue. Although the phenomenon of reactive oxygen species generation has not been documented in the injured brain during neuronophagia, the ROS-enhancing potential of such events is worthy of further study.

5. FACTORS ASSOCIATED WITH EXCESS FORMATION OF CEREBRAL REACTIVE OXYGEN SPECIES

One of the consequences of physical damage to brain tissue, is vascular hemorrhage. The entry of blood into tissue leads to the breakdown of erythrocytes and consequent escape of hemoglobin into the extracellular compartment. Protein degradative events can lead to the liberation of iron from hemoglobin, and this is a primary initiator of ROS in the nervous system (Sadrzadeh *et al.*, 1987; Halliwell, 1989a). Stroke leads to cerebral ischemia that can be of varying duration as the capillary supply may be restored. In addition to hemorrhage caused by the extravasating type of stroke, all transient interruptions of vascular supply have the potential to lead to severe post-ischemic damage.

The pathological changes consequent to restoration of the normal blood supply may be initially related to excessively high levels of cytosolic calcium (Siesjo, 1986), and this may lead to excess levels of ROS (Dykens *et al.*, 1987; Oleson, 1986; Murphy *et al.*, 1989). It has been reported that the high metabolic rates associated with reperfusion injury can lead to excess oxygen radical production (Vlessis *et al.*, 1990). At the onset of ischemia there is an accumulation of hypoxanthine due to breakdown of adenine nucleotides. Upon reperfusion, a combination of oxidative and proteolytic events converts xanthine dehydrogenase to the direct oxygen acceptor, xanthine oxidase. The final combination of elevated enzyme and substrate leads to superoxide production and consequent oxidative stress.

The complex relation between oxygen-induced events and anoxia is illustrated by the finding that damage to the penumbra region, around an ischemic region, can be more severe than at the core (Choi, 1990). The presence of ROS in post-ischemic tissue has been directly demonstrated (Cao *et al.*, 1988). Even a brief period of ischemia can lead to ROS generation and thence to delayed neuronal death (Kitagawa *et al.*, 1990).

A deficiency of neuronal energy generating systems can result in intracellular accumulation of excess sodium and accompanying water. The subsequent expulsion of sodium must then be effected by the Na/H exchanger, since the sodium pump (Na,K ATPase) cannot function without ATP. By these means, cell swelling can be attenuated but the cost involves a reduction of pH (Asano *et al.*, 1989). A lower cytosolic pH can accelerate the formation of the intensely pro-oxidant protonated superoxide anion (Essman and Wollman, 1989) and can increase the reactivity of the hydroxyl radical (Tadolini and Cabrini, 1990). In the absence of optimal rates of oxidative phosphorylation, this drop in pH can be exacerbated by the increasing dependence on glycolysis and consequent accumulation of lactate. Finally, the sodium pump may be initially activated but ultimately specifically inhibited by ROS (Asano *et al.*, 1989; Malis and Bonventre, 1986).

Seizure activity can elevate the content of ROS in the brain (Armstead *et al.*, 1989). Excessive neuronal activity may lead to an influx of calcium that is sufficient to overwhelm the sequestering and pumping processes by which calcium homeostasis is maintained. There is an increasingly clear relation between excitotoxic events and oxidative damage (Kontos, 1989). Cerebral superoxide generation is elevated during seizures (Armstead *et al.*, 1989; Essman and Wollman, 1989). In addition to calcium mediating this interrelation, the NMDA receptor may be in part regulated by ROS (Tauck and Ashbeck, 1990), but there are conflicting reports concerning the directionality of this regulation (Levy *et al.*, 1990; Pellegrini-Giampietro *et al.*, 1990). For example, a mechanism by which ROS generation can induce seizure activity is by the direct oxidative inactivation of glutamine synthetase thereby permitting an abnormal buildup of the excitatory transmitter, glutamic acid (Oliver *et al.*, 1990). In this manner neuronal hyperactivity and ROS induction may co-operate in the genesis of ischemia-induced neuronal damage (Pellegrini-Giampietro *et al.*, 1990).

6. MITIGATION OF CEREBRAL OXIDATIVE STRESS

Protection against excess levels of reactive oxygen species is effected by several antioxidant mechanisms intrinsic to the cell. ROS-induced damage is thus normally maintained at low levels. In addition, many forms of dietary additions and pharmacological interventions may enhance the protection of tissues from ROS. Such treatments may involve elevating the content of naturally occurring chemicals, or the introduction of novel xenobiotic agents.

Enzymes such as superoxide dismutase, catalase and peroxidase are able to destroy the superoxide radical and hydrogen peroxide respectively. While these oxidant species are not in themselves very active, they are able to interact in the presence of trace amounts of iron, and by the Haber–Weiss reaction, give rise to the highly reactive, short-lived hydroxyl radical. Thus, superoxide dismutase, if induced in the absence of similar induction of hydrogen peroxide degrading enzymes, may have a pro-oxidant effect (Seto *et al.*, 1990). In addition, hydrogen peroxide, in the presence of iron can be converted to the hydroxyl radical by the Fenton reaction. Iron sequestering proteins such as ferritin and transferrin are important means of ensuring extremely low levels of cytosolic free iron. Some serum proteins, notably albumin can also act as free radical scavengers

(Halliwell, 1988). The low protein content of cerebrospinal fluid makes both albumin and ferritin largely unavailable to the CNS (Halliwell, 1989b). Lower antioxidant activity in Parkinson's disease has been related to decreased levels of ferritin in the brain (Dexter *et al.*, 1990). Basal levels of protective enzymes are also relatively low in brain (Savolainen, 1978). Much of this antioxidant capacity lies within cerebral capillaries and glial cells (Tayarani *et al.*, 1987), presumably to prevent diffusion of pro-oxidants into neurons, since the cerebral microvasculature is also a major site of lipid peroxidative activity (Hall and Braughler, 1989). Many protective enzymes are relatively concentrated in cerebral microvessels (Tayarini *et al.*, 1987), where mixed function oxidase activity is also high (Ghersi-Egea, *et al.*, 1988). Treatment of neurons with nerve growth factor (NGF) is able to confer resistance to oxidative damage apparently by induction of antioxidant enzymes (Jackson *et al.*, 1990). This factor is known to promote survival of those neurons taking up NGF from target tissues.

The presence of diffusible antioxidant vitamins and provitamins provides protection against oxygen radicals. Such molecules may be predominantly lipophilic (β-carotene, α-tocopherol, retinoic acid), or water soluble (ascorbic acid). β-Carotene is distinguished by its effectiveness at the low partial pressures of oxygen found in tissues under physiological conditions (Burton and Ingold, 1984). In addition to its antioxidant properties, α-tocopherol (vitamin E) may have other membrane-stabilizing properties (reviewed by Clement and Bourre, 1990). There is a close correlation between the cerebral content of α-tocopherol and polyunsaturated fatty acids during development (Clement and Bourre, 1990). While lipid soluble vitamins are essential for retardation of oxidative damage to lipid membrane constituents, their reducing power needs continual replenishment, by water soluble reserves, of reducing power. However, ascorbic acid has powerful pro-oxidant potential when significant concentrations of iron are present. This constitutes yet another mechanism by which iron acts as an ROS inducer.

Cellular glutathione is normally present at high concentrations intracellularly (1–5 mM), and constitutes the major reducing capacity of the cytoplasm (Halliwell and Gutteridge, 1985). It is maintained very largely in the reduced form by glutathione reductase acting in concert with NADPH. Thus the cell's reducing powers ultimately depend largely on the production of NADPH by the glycolytic pentose phosphate shunt. Glutathione reserves can be depleted by oxidative stress (Maellaro *et al.*, 1990) and such depletion in the brain can cause neurological deficits (Calvin *et al.*, 1986). The brainstem has a relatively low glutathione content and a high content of mixed function oxidase. It has been proposed that this combination may render the region especially vulnerable to oxidative damage, and that this is relevant to nigral damage in Parkinson's disease (Perry *et al.*, 1982, Ravindranath *et al.*, 1989).

α-Tocopherol supplementation of patients with impaired capacity for absorption of this vitamin has significantly improved their neurological deficits (Sokol, 1990). Animal studies have shown that pretreatment with α-tocopherol reduces the edema and ischemia that follows compression injury to the brain (Busto *et al.*, 1984; Saunders and Horrocks, 1987). Antioxidative therapy of Parkinsonism is currently under trail (Shoulson, 1989). The beneficial effects of vitamin E may be due to its membrane-stabilizing properties as well as its antioxidant capacity, since fatty acid release following reperfusion of ischemic tissue, is inhibited by this vitamin (Yoshida *et al.*, 1985). Conversely, marginal deficiencies of antioxidant vitamins over extended periods may increase vulnerability to chronic exposure to low levels of free-radical promoting environmental contaminants. The effect of this may be expressed as subclinical events that are very difficult to quantitate but may relate to the overall well-being of an individual.

Deferoxamine, a potent iron chelator can protect against cold-induced cerebral edema (Ikeda *et al.*, 1989). The specificity of this chelator toward iron, is however, not complete (Wahba *et al.*, 1990), and its precise mode of action remains uncertain (Halliwell, 1989a). However deferoxamine pretreatment can block methyl-mercury induced elevation of ROS within the brain (LeBel *et al.*, 1991).

Glucocorticoids are anti-inflammatory and protective against lipid peroxidation (Hall and Braughler, 1989) and dexamethasone can attenuate ischemia-induced hippocampal damage (Dun *et al.*, 1990). The mode of action of dexamethasone may involve stimulation of the polypeptide factor, lipotropin, which inhibits phospholipase-stimulated liberation of arachidonic acid (Blackwell *et al.*, 1980). Some recently developed drugs such as the 21-aminosteroid, U74006F, combine the potential benefits of both a steroidal effect and chelation capacity (Hall *et al.*, 1988). However, some estrogens, by redox cycling, can exacerbate ROS production (Liehr and Roy, 1990).

Several other classes of chemical have been reported to be protective against neural damage associated with excess oxidative activity. These include chloroquine, mepacrine and dibucaine, which can inhibit phospholipases (Au *et al.*, 1985; Malis and Bonventre, 1986), and indomethacin, an inhibitor of cyclo-oxygenases (Kontos and Povlishock, 1986). Ganglioside GM1, a membrane-stabilizing agent effective within the CNS, may also mitigate some components of oxidative damage to neural tissue. This ganglioside has been reported protective against ischemia (Mahadik *et al.*, 1989), edema (Koga *et al.*, 1990, Skaper *et al.*, 1989), and direct oxidative activity (Bondy and McKee, 1990). The therapeutic application of macromolecular proteins as a means of reducing ROS production is obviously somewhat limited. However, superoxide dismutase linked to albumin has been reported to prevent cold induced brain edema (Ando *et al.*, 1989).

7. AGE-RELATED ALTERATIONS IN THE BRAIN ASSOCIATED WITH REACTIVE OXYGEN SPECIES

As mentioned earlier, it has been postulated that age associated decreases in non-enzymatic antioxidant concentrations and decreased protective antioxidant enzymes should be observed in the senescent brain. However data in this area appear inconclusive.

Conflicting reports have been published on age-related changes in rat brain for peroxidative potential (Sawada and Carlson, 1987; Devasagayam, 1989; Cand and Verdetti, 1989; LeBel and Bondy, 1991b), activities of superoxide dismutase, catalase, glutathione peroxidase and cytochrome oxidase (Vitorica *et al.*, 1984; Scarpa *et al.*, 1987; Cand and Verdetti, 1989; Vanella *et al.*, 1989; Barja de Quiroga *et al.*, 1990; Semsei *et al.*, 1991; LeBel and Bondy, 1991b), in the glutathione system (Benzi *et al.*, 1989; Devasagayam, 1989; Barja de Quiroga *et al.*, 1990) and in neuronal calcium turnover (Gibson and Peterson, 1987). Membrane rigidity has also been an area of controversy in aging research. While some investigators have demonstrated no age-related alterations in cerebral membrane order (Bondy *et al.*, 1989; LeBel and Bondy, 1991b), others have reported changes (Nagy *et al.*, 1983; Miliutin *et al.*, 1984; Freund *et al.*, 1986).

There are areas of research that investigators do tend to agree on, those being iron content, lipofuscin accumulation, and protein modification. Increased levels of iron associated with aging are reported in rats (Floyd *et al.*, 1984), and in the human Parkinsonian brain (Dexter *et al.*, 1989). The accumulation of lipofuscin in post mitotic tissue, such as the brain, is widely accepted and has been thoroughly reviewed by Porta (1991). Vitamin E deficiency in both experimental animals and man, also leads to an excessive accumulation of lipofuscin (Sokol, 1989). It should however be mentioned that the mechanism by which lipofuscin accumulation occurs, whether via increased oxidative stress in the brain, or by proteolytic decline remains unresolved. Finally there is a growing body of evidence demonstrating age-dependent changes in brain protein turnover (Ragusa *et al.*, 1989; LeBel and Bondy, 1991b; Carney *et al.*, 1991), findings that are suggestive of altered brain function with senescence.

Figure 1 summarizes the types of macromolecular lesions effected by random oxidative events, that could impinge on the process of aging. Such gradual impairment of macromolecular function could compromise the immediate capacity of the cell to maintain its structure. In addition, reducing the efficiency of transcription and translation processes, carries grave long-term implications for the cell.

8. PROPOSED ALTERNATIVES TO THE FREE RADICAL THEORY OF AGING

As pointed out, the role of oxidative processes in neural aging is still an unsettled question with considerable conflicting data (Halliwell, 1989c). As we have stated before (LeBel and Bondy, 1991b), these numerous disparate reports have clouded the understanding of the aging process. Sohal and Allen (1990) have postulated that the level of oxidative stress increases during cellular differentiation and aging. Given that all organisms generate a basal level of ROS, the pro-oxidant status is counterbalanced by non-enzymatic antioxidants and antioxidant enzymes, but with maturation, increasingly tends to a pro-oxidant direction. All organisms exhibit a basal level of undirected oxidative events. In situations that place the brain under heightened oxidative conditions, such as genetic Vitamin E deficiencies (Sokol, 1989) and neurotoxicity (LeBel and Bondy, 1991a) the redox status of the brain shifts to a higher pro-oxidant

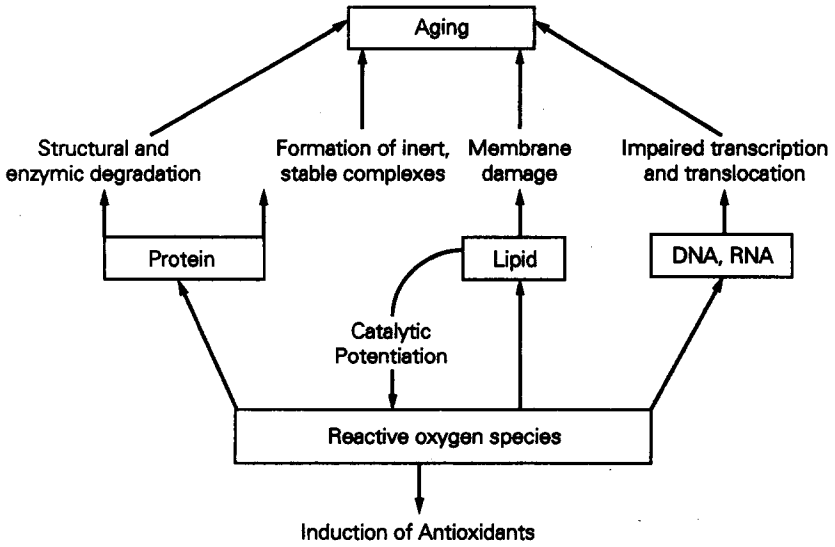

FIG. 1. Enhancement of age-related entropic processes by reactive oxygen species. The pathways derived from reactive oxygen species (ROS) are bidirectional in that the homeostatic control of ROS steady-state levels is a function of the antioxidant and oxidative damage components. Macromolecules like protein, lipid, and DNA/RNA are routinely damaged by ROS-induced mechanisms. Peroxidation of lipids can potentiate ROS activity through a chain propagation mechanism. Under oxidative stress conditions which may occur numerous times during the lifespan of an organism, the intensity of damage is increased. The sum total of these degradative and denaturing events may contribute to the aging phenomenon by way of altered functionalities or through accumulation of inert complexes.

status. Sohal and Allen (1990) pose this question to the traditional free radical theory of aging: How do randomly occurring oxidative damage impose a predictable and sequential pattern of age-associated events?

Another theory proposes that the blood–brain barrier (BBB) experiences age-related declines in its ability to maintain its multifunctional role in nutrient, hormone, and neurotransmitter delivery to the brain, and in the exclusion of environmental or endogenous neurotoxicants (Mooradian, 1988). If altered BBB function occurs, this could be deleterious to normal central nervous system function. This theory is based on histopathologic effects seen in Alzheimer's disease and various measures of BBB function (Mooradian, 1990; Oztas *et al.*, 1990; Saija *et al.*, 1990). Many of the effects reported appear to be subtle changes, and as pointed out by Pardridge (1988), the role that the BBB plays in the aging process still lacks a concrete hypothesis. Since senescence is common to most organs, its major basis must be sought in a more general phenomenon.

Nevertheless, these alternate theories should provide biogerontology research with other venues to investigate, although their infancy will require considerable investigation in order to determine whether age related deficits do exist.

9. PHYSIOLOGICAL EXPLOITATION OF FREE RADICALS

Considerable attention has been focused on the destructive damage producing effects of ROS on cell constituents. However, it should be mentioned that not all ROS are considered deleterious to biological systems, evidenced by the recent proposal that nitric oxide may be a novel neuronal messenger (Palmer *et al.*, 1987; Bin, 1991; Bredt *et al.*, 1991). The physiologic target of nitric oxide is the heme of soluble guanylate cyclase, an event that acts in concert with the second messenger cGMP in a variety of cellular processes. In fact, superoxide anions have also been proposed to act in a similar mechanism (Saran and Bors, 1989, 1990). In view of the large range of chemical species encompassed by the term "free radical," the evolution of a range of physiologic functions utilizing these species is unsurprising.

10. A CONSOLIDATED THEORY OF CEREBRAL OXIDATIVE STRESS AND AGING

The authors wish to acknowledge that the heuristic value contributed by Harman's free radical theory of aging has been the driving force behind new research ideas in biogerontology for four decades. We would like to take this opportunity to consolidate the present theory along with current data available in studies of neural origin.

It is becoming increasingly clear that ROS have multiple functionalities which include deleterious cell damage, phagocytic debris removal, and potential second messenger mechanisms. In addition, an overview of the disparate data on the levels of non-enzymatic antioxidants and antioxidant enzyme activities would indicate that aging is associated with dynamic changes in redox status of the brain. Investigations are generally limited in that single experimental time windows into the aging process are used. In our own experiments in which we studied 1, 6 and 20 month-old rats (LeBel and Bondy, 1991b), we determined that an inverse relationship existed between increasing age and decreased ROS generation rate. The ROS levels assayed in this study, represented the situation at a single instant of time. In the same work, we also found evidence that aged brains contained increasing amounts of degraded oxidized proteins that presumably represented oxidative events integrated over a long period of time. It is known that an accumulation of inert macromolecular breakdown products with the neuron can, by altering its form, lead to neuropathological changes (Purpura and Suzuki, 1976). Such "geometric toxicity" has been described for several hereditary gangliosidoses. The possibility exists that such an intracellular build-up of materials may contribute to the aging process, especially in very long-lived, non-dividing cells such as neurons. The development of a continuous non-invasive marker for ROS formation throughout the lifespan of an animal, would be very useful.

It is likely that the sum total of oxidative stress is a contributor to senescence, but by no means the sole cause. We can analogize our organismal boundaries to land coastlines, in that what occurs over our lifespan is a biological erosion, gradual, yet continual. Occasionally, we experience a natural disaster, such as what the Eastern Coastal United States received in Hurricane Bob in the summer of 1991. This environmental stress (analogous to an oxidative stress in our model) caused immense destruction on our coast. Eventually we returned to what appeared to be normal daily function, however much of our landscape has been irreversibly altered. In the brain, we propose that occasionally a transient oxidative stress occurs via disease states, or via environmental and dietary causes. Compensatory mechanisms in the brain, such as ROS repair processes, attempt to return the nervous system to what appears to be normal function. This may result in an acceleration of oxidative aging processes that current methodologies lack the sensitivity to detect. We posit that the accumulation of these events over a lifespan can modulate the velocity of the rate process. As pointed out by Sohal and Allen (1990), maintenance of high dietary antioxidant therapy does not result in a complete removal of oxidant. Most organisms appear to respond to a flux in the redox potential by re-establishing a homeostatic redox status. ROS must be present in normal organisms, and although dietary manipulations may slow the rate of aging, there will be a limitation to the protection afforded.

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