IS THERE A BALANCE BETWEEN OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE SYSTEM DURING DEVELOPMENT?

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SUMMARY : For the reason of the biological importance of antioxidant enzymes and reactive oxygen species, it was deemed important to survey the literature for reports on the relation between them. The main aim of this overview not only uncovered the baseline works on aspects of prooxidant and antioxidant processes in different species, but also illuminated the mechanism of the free radical theory of aging. Taken together, this review suggest that the decline in levels of free radicals revealed a coupled with increased antioxidant enzyme activities and the reverse is true, but also critical balance between the generation of oxygen free radicals and antioxidant defense enzymes during development. However, these results are not still entirely accepted because of the difficulties of the direct observation of the active oxygen species in biological systems due to their short lifetime.

Key Words: Antioxidant enzymes, reactive oxygen species.

INTRODUCTION

Mammalian life depends upon oxygen as the final acceptor of electrons in mitochondrial electron transport, but the process also generates toxic metabolites (68); reactive oxygen species (ROS) leak from mitochondria into the cytoplasm where they cause cellular damage by oxidizing a variety of biologically important molecules, including DNA, proteins, lipids, and carbohydrates (70). Lipid and protein peroxidation reactions play an important role in the pathogenesis of a variety diseases (70). Also, Oxidative stress (OS) is caused by an imbalance of oxidants and antioxidants in favor of the former, and is capable of inflicting injury on membrane lipids, proteins and nucleic acids (94,103).

Moreover, oxidative stress is apparent in pathology associated with aging and many age-related chronic diseases, including atherosclerosis, diabetes mellitus, rheumatoid arthritis, and neurodegenerative diseases (47). For the prevention of diseases and control of aging, evaluation and control of oxidative stress *in vivo* may become essential. A wide variety of functional assays are used in the field of research related with oxidative stress. However, direct detection of reactive oxygen species and other free radicals is difficult, because these molecules are short-lived and highly reactive in a nonspecific manner (47). Although ongoing oxidative damage is, thus, generally analyzed by measurement of secondary products, including derivatives of amino acids, nucleic acids, and lipid peroxidation (47), biomarkers to reflect minor changes in the pro-oxidant/antioxidant status under normal, nonpathological conditions in humans might be of special interest.

In mammalian cells, the enzymatic defense system consists mainly of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GSSGR) (3, 79). Hydrogen peroxide formed by the catalytic reaction of superoxide dismutase was further detoxified by catalase and/or glutathione peroxidase (8,69).

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The following is a brief concerted attempt to describe the backgrounds and recent findings which provide specific suggestions in order to explore the balance between the oxidative stress and antioxidant defense system with aging;

1. Formation of antioxidant defense enzymes with age:

Antioxidant enzymes are an important protective mechanism against ROS and, like many other biochemical systems, their effectiveness may vary with the stage of development and other physiological aspects of the organism (38,50). Also, both the development of (molecular) biomarkers (51) and understanding of basic pro-oxidant/ antioxidant processes (50) require knowledge of how the biochemical systems are influenced by key exogenous/ endogenous factors such as developmental stage, age and sex.

Also, L'vova and Abaeva (53) elucidated that, a high antioxidant activity was detected in the brain, liver, heart muscle, skeletal muscle, kidneys and blood serum of 1, 4, 6, 30, and 90 - 120 days old rat newborns. They added that the total antioxidant activity and catalase activity peaked at days 14 - 30 of development. Hussain *et al.* (43) recorded a significant increasing pattern of glutathione content in the cerebellum and brain stem with age. They also demonstrated that the level of antioxidant enzymes varied in different regions of the brain and the overall enzyme activities tend to increase with age progress.

Furthermore, Shivakumar *et al.* (82) recorded that, the total superoxide dismutase activity decreased on day 6, increased again on 10th day, and remained constant thereafter. While the developmental pattern of manganese superoxide dismutase was similar to that of the total superoxide dismutase, the copper-zinc superoxide dismutase levels were low at birth and reached adult levels on the 10th day after birth (82). However, there was no variation in the peroxidase levels during development. The scavenging enzymes, such as SOD, CAT and GPx, have been shown that both systems are immature during early organogenesis (26), also, the activity of the mitochondrial oxidative glucose metabolism is low during early organogenesis (26). Also, Anguiano *et al.* (4) noticed that, the presence of GSH-S-transferase activity at very early stages of the toad embryonic development.

2. What is the Oxidative stress (OS)?

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS), or free radicals and antioxidant defenses, which may lead to tissue injury (37). Also, free radical can be defined as any chemical species that contains unpaired electrons in their outer orbit and thus can react virtually with all cell components (11,70,84). Although, reactive oxygen species are crucial to normal biological processes, they are potentially dangerous (94,103) and are commonly referred to as prooxidants (56). The reactive oxygen intermediates (including superoxide and hydroxyl radicals as well as hydrogen peroxide) can cause direct cellular injury by including lipid and protein peroxidation and damage to nucleic acid (72,89). Common examples of the free radicals include the hydroxyl radical (OH), superoxide anion (O_2), transition metals such as iron and copper, nitric oxide (NO) and peroxynitrite ($ONOO^-$) (22). Moreover, Betteridge (8) reported that, the free radicals can be produced by several different biochemical processes within the body including: (1) The reduction of the molecular oxygen during aerobic respiration yielding superoxide and hydroxyl radicals; (2) By products of chemical reactions such as oxidation of catecholamine and activation of the arachidonic acid cascade product electrons, which can reduce molecular oxygen to superoxide; (3) Production of superoxide and hypochlorous acid (HOC1), a powerful oxidant, by activated phagocytes and (4) Nitric oxide production by vascular endothelium and other cells.

In addition, Shivakumar *et al.* (82) recorded that, the levels of thiobarbituric acid reactive products, indicative of lipid peroxidation, were very low at birth and increased to adult levels by the 16th day after birth. Moreover, the free radicals and lipid peroxidation have been reported to be increased in the aged brain of rats (40). Also, the lipid peroxidation showed an elevated increase with the aging; this fact is more evident in neuronal than in glial cells of rats (32). The increased levels of thiobarbituric acid-reactive substances (TBARS) suggest a net increase in the levels of oxygen free radicals which could be due to their increased production and/or decreased destruction (33).

3. The free radical theory of aging:

The free radical theory of aging is based upon the adverse effects of oxidative stress (OS), and studies in humans and other organisms have been shown that indices of OS generally increase with advancing age (7).

However, the OS may also be a normal physiological response in youth, when ROS act as signal transducers during normal growth and development (68). Also, univalent reduction of O_2 yields superoxide anion radical, hydrogen peroxide and hydroxyl radical; these metabolites are capable of reacting with a variety of cellular components, including nucleic acids, lipids, proteins, free amino acids and carbohydrates. The resulting oxidative free radicals are obligate intermediates of many metabolic reactions but may also cause pathological damage (15,28). Thus, control of redox balance is critical to cellular development, differentiation and homeostasis (2). Also, the body has evolved a number of interrelated antioxidant mechanisms to maintain redox homeostasis (94,96). Moreover, Heme oxygenase (HO) is one of the major antioxidant enzymes (55); catalyzes the rate limiting step in heme degradation and the products of this reaction are biliverdin, carbon monoxide and iron. Biliverdin is rapidly reduced by biliverdin reductase to bilirubin, and thus the product of HO is thought to be primarily bilirubin. Furthermore, physiological concentration of bilirubin functions as a potent antioxidant by reacting with ROS and, subsequently, becomes oxidized (55, 86). Hence, BOM rather than bilirubin per se could be a good marker for evaluating the antioxidant activity of bilirubin under oxidative stress (62).

Thus, the balance between production and elimination of ROS is maintained by antioxidants and enzymes (94,103). Also, antioxidant enzymes counteract excessive formation and deleterious effects of reactive oxygen metabolites (Cotgreave et al., 1988). For example;

(1) Superoxide dismutase (SOD) catalyzes the conversion of superoxide anion radical to H_2O_2.

(2) Catalase (CAT) reduces H_2O_2 to water;

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

(3) Glutathione peroxidase (GSH-Px) acts in conjunction with other enzymes to $2H_2O_2$ and to terminate lipid peroxidation. Changes in antioxidant activities may occur under conditions that alter the rates of formation of reactive oxygen radicals.

(4) The (GSSGR) enzyme catalyses the reduction of glutathione in the presence of NADPH, which was oxidized to $NADP^+$;

$$NADPH + H^+ + GSSG \longrightarrow NADP^+ + 2GSH$$

(GSH is reduced Glutathione form, while GSSG is the oxidized one).

4. Effect of co-factors on the antioxidant enzymes:

Expression of antioxidant metalloenzyme activities in developing tissues may be limited by the availability of their trace metal co-factors. For example, catalase activity in perinatal rat lung is highly influenced by the pregnant dam's intake of the enzyme co-factor, Fe (90). On the other hand, a decrease in Mn concentration is associated with a fall in MnSOD activity in chick liver during the first week after hatching (20). Also, Cu-ZnSOD activity of chick liver more than doubles during this postnatal week in spite of decreasing hepatic Cu and Zn concentrations (20). Antioxidant metalloenzyme co-factors; Fe, Cu and Zn are essential for maturational processes in the chick embryo, since deficiencies in their supply lead to severe structural and functional abnormalities (10). As for the normal pattern of trace metal distribution, Dewar *et al.* (17) reported that concentrations of Fe, Cu and Zn in whole chick embryo decrease between days 5 and 18 in ovo, with the most rapid declines occurring from day 5 to day 10. Upon examining individual organs of the chick, the concentrations of Fe, Cu and Zn are markedly greater in liver than in brain and are similar to those previously reported for the liver and brain of neonatal mice (46).

Moreover, as for prenatal development, antioxidant enzyme activities have been measured during late stages of gestation in mammals (19,74) but have not been related to trace metal concentrations. Studies of trace metal concentrations in avian embryos (73) did not make comparisons with antioxidant defenses. Thus, it is not known if the availability of metal co-factors limits antioxidant enzyme activity during the prenatal period. Wilson *et al.* (100) reported that, prenatal development of antioxidant defenses is related to spontaneous changes in the supply of O_2, the maturation state of tissues and the concentrations of metalloenzyme cofactors.

5. The variation of OS and antioxidant in different species:

a. In birds and mammals:

Comparisons between birds and mammals may reveal developmental patterns that have been conserved during evolution. For example, hepatic GPx and CAT specific activities increase in both birds (100) and mammals (74,85) during the final week before birth.

Also, Wilson *et al.* (100) found that, aside from hepatic GPx and CAT, however, the expression of antioxidant enzymes differs between chick and mammalian embryos in a number of ways. They have suggested that, the changes in SOD and GPx which occur in chick brain during the final 2 weeks of embryonic development may result from the particular timing of neuronal and glial proliferation and differentiation in this species. Although this explanation remains speculative, it is clearly evident that the developmental patterns of chick cerebral GPx and CAT differ from those of mammalian species. In the brain of the guinea pig embryo, for example, the activities of GPx and CAT increase during days 45-60 of gestation (59). In the rat brain, on the other hand, the neonatal period from 19 days gestational age through 2 days after birth is marked by decreases in the specific activities of both enzymes (18). Contrastingly, in embryonic chick brain during the final 2 weeks in ovo the specific activity of GPx doubles and that of CAT falls 4-folds (100). The ontogeny of SOD also varies between vertebrate species. Cu-ZnSOD is the predominant isozyme in late gestational and neonatal rat brain (18) but MnSOD predominates in embryonic chick brain (100). Additionally, the specific activities of SOD enzymes in brain vary markedly during the development of embryonic chick (100), whereas they are maintained at constant level in guinea pig during days 45-60 of gestation (59) and in rat from day 19 of gestation through 2 days after birth (18).

b. In aquatic organisms:

Despite the relative scarcity of information on the relationship between age and oxidative stress in aquatic organisms, the results for *G. locusts* appear to be consistent with the general definition of ageing as "the progressive accumulation of changes that are responsible for the decreased ability of organisms to maintain physiological homeostasis, which may eventually lead to functional impairment and even death" (27,93,102).

Furthermore, Dandapat *et al.* (16) found that the antioxidant defenses play an important role in providing protection to the developing larvae from oxidative assault. Production of ROS during larval development is likely to depend upon metabolic status of the cell and the ambient oxygen tension. Although a definite role of the ROS and antioxidants is established in various cellular processes such as development, differentiation, regeneration and regression (1), knowledge on the role of ROS and antioxidants during the embryonic and larval development of aquatic animals in general and crustaceans, in particular, is scanty (6). A number of studies have demonstrated potential for ROS generation; antioxidant enzyme and free radical scavenger responses; and oxidative damage in species of invertebrate, mainly in molluscs (51,52).

On the other hand, during the course of egg and embryonic development, a gradual increase of oxygen uptake was seen in the prawn *M. malcolmsonii* that appeared to be counteracted by an increase in CAT, SOD and GPx activities (6). Similarly, SOD activity was highest in embryos of turbot *Scophthalmus maximus* and decreased with growth to 11-day old larvae, concomitant with known decreases in respiration rate over this period (52,66). In contrast, whole body CAT and GPx activities in *S. maximus* increased during the same period (66). Considering a much later stage of development and growth, CAT activities and levels of other antioxidants in the *mussle M. edulis* were lower in older than younger animals, consistent with lower oxygen consumption rates in the former (99).

As a consequence of the reactivity of ROS and their potential to damage cells and tissues, marine and other organisms balance the production of these radicals with a wide variety of cellular antioxidant defenses. Prominent among these antioxidants are the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (21,38). Antioxidant enzymes can be induced by various environmental pro-oxidant conditions (i.e. increased ROS generation), e.g. exposure to various types of pollution, as well as being affected by other endogenous/exogenous factors, such as age (6, 41, 66, 99), diet (67) and seasonably/reproductive cycle (75, 98). In addition, there have been many studies on the endogenous antioxidant enzyme systems in fish (60,76) particularly in relation to specific oxidative stresses (65,71) but also including studies in relation to age and development (63).

6. Effect of antioxidant enzymes on the cell proliferation:

Temporal changes in antioxidants within a tissue may reflect proliferation and differentiation of particular cell types. In embryonic chick brain, for example, both GPx

specific activity (100) and glial cell number (49,95) increase markedly during the second half of the in ovo incubation period. This is consistent with previous findings that GPx specific activity is higher in glial cells than in neurons (32,80). Furthermore, Wilson *et al.* (100) found that, the late induction of GPx in brain may result from glial proliferation and differentiation.

Similarly, SOD activities may vary because of changes in the relative abundance of particular cell types. For example, among adult rat brain cells, the specific activity of total SOD is approximately 10-fold higher in glial cells than in neurons (80). In addition, immunolocalization methods indicate that Cu-ZnSOD occurs in neurons and oligodendroglia, but not in astroglia (91). Cultures of chick embryonic neurons also have been shown to express Cu-ZnSOD (92). Analysis of the chick embryonic telencephalon revealed that essentially all postmitotic neurons are generated between days 4 and 9 but most astroglial cells originate after day 10 (95). Similarly, glial cells are not detectable in chick embryonic optic tectum before day 9 (49). These observations suggest that, on the one hand, the brain's Cu-ZnSOD peak on day 8 is associated-with ablative abundance of neurons and, on the other hand, the subsequent increase in MnSOD coincides with augmented numbers of astroglia (100). Also, the balance between antioxidant enzymes, SOD and CAT, is relevant for cell functions (31). An imbalance between Cu-ZnSOD and GPx was said to be crucial in the prevention of toxicity of free radicals (42).

On the other hand, ROS play an essential role in the regulation of cell proliferation, e.g. within the central and peripheral nervous system, ROS initiate and promote the establishment of neuronal patterns and subsequent neurogenesis (97). Furthermore, the ROS not only act as external triggers of apoptosis, but also play a crucial role as mediators of apoptosis (29,54).

7. The role of glutathione (GSH) on the biological processes:

Glutathione, a tripeptide thiol consisting of glycine, cystein and glutamic acid moieties, presents in high concentration virtually in all types of living cells (12,61). McLennan *et al.* (57) demonstrated that glutathione plays an important role in the protection of the cells from oxidative damage by (1) reducing disulphide groups of proteins and other cellular molecules, or by (2) scavenging free radicals and active oxygen species. The latter action occurs mainly by detoxifying hydrogen peroxides and lipid hydroperoxides through reactions catalyzed by glutathione peroxidase as reported by the same authors.

GSH is an abundant intracellular thiol in toad early embryos and it is known to participate in the control of cellular redox status in different organisms (48). GSH participates in various critical cellular processes including detoxification and the regulation of cellular proliferation and development (2,77). Also, GSH has been reported as a co-factor in thiol-disulfide exchange reactions in sea urchin eggs (78) and in the protection of protein-SH groups. These groups are involved in cell division, and their oxidation results in damage to this important function (35). In fact, the progress of embryonic development is delayed by oxidative stress and protein-thiol group oxidation (36).

On the other hand, GSH in the lung fluid plays a critical role in protecting the lung from oxidative stress by detoxifying exogenous toxicants and quenching ROS (39). The inverse relation between peroxidative decomposition of membrane polyunsaturated fatty acid and GSH is well established (81). Also, when the capacity of the cell to regenerate GSH is exceeded- primarily due to an insufficient supply of NADPH- oxidized glutathione (GSSG) is released from the cell and protein synthesis turns off (25).

Although the reduction in the GSH pool produced by the enhanced activity of the enzyme could not be minimized, because it may result in: DNA damage (87); cell cycle (58); and development arrest (36) and increased susceptibility to oxidative damage. Thus, the depletion in GSH content can be explained by: (1) The higher levels of free radicals that convert more reduced glutathione (GSH) to its oxidized form (GSSG) (64); and (2) A decreased activity of glutathione reductase (GSSGR) (14), the enzyme that regenerates reduced glutathione in a NADPH-dependent reaction. Moreover, Dringen and Hirrlinger (23) showed that the antioxidant glutathione is essential for the cellular detoxification of reactive oxygen species in brain cells. In addition, the glutathione plays a vital role in the regulation of the redox state and prevention of the cell damage induced by oxidative stress (protective mechanism of the cells) (9, 44).

Furthermore, experimental studies (45,88) have reported that cellular GGT has a central role in glutathione homeostasis by initiating the breakdown of extracellular glutathione, a critical antioxidant defense for the cell; thereby providing a supply of constituent amino acids for uptake and reutilization in intracellular GSH synthesis. Paradoxically, there is evidence that, under physiological conditions, GGT is directly involved in the generation of ROS, especially in the presence of iron or other transition metals (24,34).

In normal metabolism (i.e. without the influence of stress conditions), a balance exists between the generation of ROS and other pro-oxidants, and their detoxication and removal by antioxidant defense mechanisms (101). However, either an increase in ROS production above the level that can be removed by antioxidant defenses, could result in oxidative damage to key molecules, including DNA, protein and lipids (lipid peroxidation) (38). Also, the activity of the mitochondrial oxidative glucose metabolism is low during early organogenesis (26). ROS not only produce peroxidation of polyunsaturated fatty acids, injurying plasma membranes, leading to abnormal function and loss of membrane integrity, but also attack on proteins resulting in their aggregation or fragmentation, inactivating membrane enzymes, receptors, and transport proteins, and modifying cellular antigenic properties (70).

On the other hand, Correia *et al.* (13) reported that, the comparison of antioxidant enzyme activities between species is limited, because of the relatively small database, the use of different units in some studies, the unknown influence of seasonality and other factors. Considering that antioxidant enzyme activities represent only part of the antioxidant defense capability, and the limitations of applying the assays due to small animal biomass, it is suggested that additional and/or alternative methods would be useful in future studies, including measurement of total oxyradical scavenging capacity and the application of immunochemical analyses.

Although one may get the impression that the risk of damage by reactive oxygen species is limited in developing embryos, they are equipped with enzymatic antioxidants such as CAT, SOD and GPx (5) that develop at different rates during the intrauterine period (30,59). Are these developments of enzymatic antioxidant activities subjected to a developmental inherited program alone or can changes in environmental oxygen stress modulate them?

CONCLUSION

The biological effects of antioxidant defenses and oxidative stress endproducts can be summarized as follows; under the normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems used by organisms to deactivate and protect themselves against free radical toxicity (83). The pro-oxidant/antioxidant balance and detoxication of potentially damaging ROS is crucial for cellular homeostasis (50,101). The increase in the oxidative stress may be a reason for such decrease and exhaustion of antioxidant defense system, as a result of increased endogenous production of the free radicals. Thus, I hypothesize that the formation of antioxidant enzymes during development is related to the changes in the levels of free radicals.

REFERENCES

1. Alien RG : Oxygen-reactive species and antioxidant responses during development: the metabolic paradox of cellular differentiation. Proc Soc Exp Biol Med, 117, 117-129, 1991.

2. Alien RG, Balin AK : Oxidative influence on development and differentiation: an overview of a free radical theory of development. Free Radic Biol Med, 6, 631-661, 1989.

3. Ames BN, Shigenaga MK, Hagen TM : Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci, U.S.A., 90, 7915-7922, 1993.

4. Anguiano OL, de Castro AC, de D'Angelo AMP : The role of glutathion conjugation in the regulation of early toad embryos9 tolerance to pesticides. Comparative Biochemistry and Physiology Part C 128, 35-43, 2001.

5. Ar A, Mover H : Oxygen tensions in developing embryos: System inefficiency or system requirement? Isr J Zool, 40, 307-326, 1994.

6. Arun S, Subramanian P : Antioxidant enzymes in freshwater prawn Macrobrachium malcolmsonii during embryonic and larval development. Comp Biochem Physiol Part B 121, 273-277, 1998.

7. Ashok BT, Ali R : The aging paradox: free radical theory of aging. Exp Gerontol, 34, 293-303, 1999.

8. Betteridge DJ : What is oxidative stress? Metabolism 49 (2), suppl 1, 3-8, 2000.

9. Bounous G, Molson JH : The antioxidant system. Anticancer Res 23 (2B), 1411-1415, 2003.

10. Butler EJ : Role of trace elements in metabolic processes. In: Physiology and Biochemistry of the Domestic Fowl. Ed by BM Freeman, Academic Press, London, pp 175-190, 1983.

11. Collier A, Rumiey A, Rumley AG, John RP, John PL, Gordon DO, Micheal S : Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Dia-

betes 41, 909-913, 1992.

12. Comporti M : Glutathione depleting agents and lipid peroxidation. Chem Physics Lipids, 45, 143-169, 1987.

13. Correia AD, Costa MH, Luis OJ, Livmgstone DR : Age-related changes in antioxidant enzyme activities, fatty acid composition and lipid peroxidation in whole body Gammarus locusta (Crustacea: Amphipoda). Journal of Experimental Marine Biology and Ecology 289, 83-101, 2003.

14. Costagliola C : Oxidative state of glutathione in red blood cells and plasma of diabetic patients : In vivo and in vitro study. Clin Physiol Biochem, 8, 204-210, 1991.

15. Cross CE, Halliwell B, Boush ET, Pryor WA, Ames BN, Saul J, McCord D : Har-mon, Oxygen radicals and human disease. Ann Int Med, 107, 526-545, 1987.

16. Dandapat J, Chainy GBN, Rao KJ : Lipid peroxidation and antioxidant defence status during larval development and metamorphosis of giant prawn, Macrobrachium rosenbergii. Comparative Biochemistry and Physiology Part C 135, 221-233, 2003.

17. Dcwar WA, Teague PW, Downie JN : The transfer of minerals from the egg to the chick embryo from the 5th to the 18th days of incubation. Br Poult Sci, 15, 119-129, 1974.

18. Del Maestro RF, McDonald W : Distribution of superoxide dismutase, glutathione peroxidase and catalase in developing rat brain. Mech Ageing Dev, 41, 29-38, 1987.

19. Del Maestro RF, McDonald W : Subcellular localization of superoxide dismutases, glutathione peroxidase and catalase in developing rat cerebral cortex. Mech Ageing Dev, 48, 15-31, 1989.

20. DeRosa G, Keen CL, Leach RM, Hurley LS : Regulation ofsuperoxide dismutase activity by dietary manganese. J Nutr, 110, 795-804, 1980.

21. Di Giulio RT, Benson WH, Sanders BM, Van Veld PA : Biochemical mechanisms: metabolism, adaptation, and toxicity. In: Fundamentals of Aquatic Toxicology, Effects, Environmental Fate, and Risk Assessment. Ed by G Rand. Taylor and Francis, London, pp 523-561, 1995.

22. Dormandy TL : An approach to free radical. Lancet 29, 1010 -1014, 1983.

23. Dringen R, Hirrlinger J : Glutathione pathways in the brain. J Biol Chem, 384 (4), 505-516, 2003.

24. Drozdz R, Parmentier C, Hachad H, Leroy P, Siest G, Wellman M : γ-Glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transfemn system. Free Radic Biol Med, 25, 786-792, 1998.

25. Eaton JW, Brewer GJ : Pentose phosphate metabolism. In: The Red Blood Cell, 2nd Ed., New York, Academic Press, 436-471, 1974.

26. El-Hage S, Singh SM : Temporal expression of genes encoding free radical-metabolizing enzymes is associated with higher mRNA levels during in utero development in mice. Dev Genet, 11, 149-159, 1990.

27. Emerit I, Chance B (Eds.) : Free Radicals and Aging. Birkhauser Verlag, Basel, 1992.

28. Floyd RA : Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J, 4, 2587-2597, 1990.

29. Fleury C, Mignotte B, Vayssiere JL : Mitochondrial reactive oxygen species in cell death signaling, Biochimie 84, 131-141, 2002.

30. Frank L, Sosenko IRS : Prenatal development of lung anti-oxidant enzymes in four species. J Pediatr, 110, 106-110, 1987.

31. Freitas RM, Nascimento VS, Vasconcelos SMM, Sousa FCF, Viana GSB, Fonteles MMF : Catalase activity in cerebellum, hippocampus, frontal cortex and striatum after status epilepticus induced by pilocarpine in Wistar rats. Neuroscience Letters 365, 102-105, 2004.

32. Geremia E, Baratta D, Zafarana S, Giordano R, Pinizzotto MR, La Rosa MG, Garozzo A : Antioxidant enzymatic systems in neuronal and glial cell-enriched fractions of rat brain during aging. Neurochem Res, 15 (7), 719-723, 1990.

33. Giugliano D, Ceriello A, Paolisso G : Oxidative stress and diabetic vascular complications. Diabetes Care 19, 257-267, 1996.

34. Glass GA, Stark AA : Promotion of glutathione-gamma-glutamyi transpeptidase-dependent lipid peroxidation by copper and ceruloplasmin: the requirement for iron and the effects of antioxidants and antioxidant enzymes. Environ Mol Mutagen, 29, 73-80, 1997.

35. Goddard MJ, Pratt HPM : Control of events during early cleavage of the mouse embryo: An analysis of the "2-cell block". J Embryol Exp Morph, 73, 111-133, 1983.

36. Goto Y, Noda Y, Narimoto K, Umaoka Y, Mori T : Oxidative stress on mouse embryo development in vitro. Free Radic Biol Med, 13, 47-53, 1992.

37. Halliwell B : Free radicals, antioxidants and human disease: Curiosity, cause or consequence, Lancet, 344, 721-724, 1994.

38. Halliwell B, Gutteridge JMC, (Eds.) : Free Radicals in Biology and Medicine. Oxford Univ. Press, Oxford, 1999.

39. Heffner JE, Rapine JE : Pulmonary strategies of antioxidant defense. Am Rev Respir Dis, 140, 531-554, 1989.

40. Hiramatsu M, Edamatsu R, Mori A : Free radicals, lipid peroxidation, SOD activity, neurotransmitters and choline acetyltransferase activity in the aged rat brain. EXS, 62, 213-218, 1992.

41. Hole LM, Moore MN, Bellany D : Age-related cellular reactions to copper in the marine mussel Mytilus edulis. Mar Ecol Prog Ser, 94, 175-179, 1993.

42. Huggle S, Hunsaker JC, Coyne CM, Sparks DL : Oxidative stress in sudden infant death syndrome. J Child Neurol, 11, 433-438, 1996.

43. Hussain S, Slikker WJr, Ali SF : Age-related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of mouse brain. Int J Dev Neurosci, 13 (8), 811-817, 1995.

44. Irita K, Okabe H, Koga A, Kurosawa K, Tagawa K, Yamakawa M, Yoshitaka J, Takahashi S : Increased sinusoidal efflux of reduced and oxidized glutathione in rats with endotoxin / D- galaclosamine hepatitis. J Circ Shock, 42, 115-120, 1994.

45. Karp DR, Shimooku K, Lipsky PE : Expression of gamma-glutamyi transpeptidase protects Ramos B cells from oxidation-induced cell death. J Biol Chem, 276, 3798-3804, 2001.

46. Keen CL, Hurley LS : Developmental changes in concentrations of iron, copper and zinc in mouse tissues. Mech Ageing Dev, 13, 161-176, 1980.

47. Kohen R, Nyska A : Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol, 30, 620-650, 2002.

48. Kosower NS, Song KR, Kosower EM : Glutathione IV. Intracellular oxidation and cellular injury. Biochem Biophys Acta, 192, 23-28, 1969.

49. Linser PJ, Perkins M : Gliogencsis in the embryonic avian optic tectum: neuronal-glial interactions influence astroglial phenotype maturation. Dev Brain Res, 31, 277-290, 1987.

50. Livingstone DR : Contaminated-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar Pollut Bull, 42, 656-666, 2001.

51. Livingstone DR, Chipman JK, Lowe DM, Minier C, Mitchelmore CL, Moore MN, Peters LD, Pipe RK : Development of biomarkers to detect the effects of organic pollution on aquatic invertebrates: recent molecular, genotoxic, cellular and immunological studies on the common mussel (Mytilus edulis L.) and other mytilids. Int J Pollut, 13, 56-91, 2000.

52. Livingstone DR, O'Hara SCM, Frettsome F, Rundle J : Contaminant-mediated pro/anti-oxidant processes and oxidative damage in early life-stages offish. In: Environment and Animal Development. Genes, Life Histories and Plasticity. Ed by D Atkinson, M Thomdyke, BIOS Scientific Publishers, Oxford, pp 173-201, 2001.

53. L'vova SP, Abaeva EM : The tissue antioxidant system in the early postnatal development of rats. J Ontogenez, 27 (3), 204-207, 1996.

54. Madeo F, Frohlich E, Ligr M, Grey M, Sigrist SJ, Wolf DH, Frohlich KU : Oxygen stress: a regulator of apoptosis in yeast. J Cell Biol, 145, 757-767, 1999.

55. Maines MD : The heme oxygenase system: A regulator of second messenger gases. Ann Rev Pharmacol Toxicol, 37, 517-554, 1997.

56. Mates JM, Perez-Gomez C, Nunez de Castro I : Antioxidant enzymes and human diseases. Clin Biochem, 32, 595-603, 1999.

57. McLennan SV, Heffernan S, Wright L, Rae C, Fisher E, Yue DK, Turtle JR : Changes in hepatic glutathione metabolism in diabetes. Diabetes 40, 344-340, 1991.

58. Messina JP, Lawrence DA : Cell cycle progression of glutathione depleted human peripheral blood mononuciear celt is inhibited at S phase. J Immunol, 143, 1961-1974, 1989.

59. Mishra OP, Delivoria-Papadropoulos M : Anti-oxidant enzymes in fetal guinea pig brain tissue during development and the effect of maternal hypoxia. Dev Brain Res, 42, 173-179, 1988.

60. Murata W, Sakai T, Yamauchi K, Ito T, Tsuda T, Yoshida T, Fukudome M : In vivo lipid peroxidation levels and antioxidant activities of cultured and wild yellowtail. Fisheries Science 62, 64-68, 1996.

61. Ogino T, Kawabata T, Awadi M : Stimulation of glutathione synthesis in iron-loaded mice. Biochim Biophys Acta, 1006, 131-135, 1989.

62. Otani K, Shimizu S, Chijiiwa K, Yamaguchi K, Kuroki S, Tanaka M : Increased urinary excretion of bilirubin oxidative metabolites in septic patients: A new marker for oxidative stress in vivo. J Surg Res, 96, 44-49, 2001.

63. Otto DME, Moon TW : Endogenous antioxidant systems of two te'leost fish,'the rainbow trout and the black bullhead, and the effect of age. Fish Physiol Biochem, 15, 349-358, 1996.

64. Ou P, Nourooz-Zadeh J, Tritschler HJ, Wolff S : Activation of aldose reductase in rat lens and metal-ion chelation by aldose reductase inhibitors and lipoic acid. Free Radic Res, 25: 337-346, 1996.

65. Pedrajas JR, Peinado J, Lopez-Barea J : Oxidative stress in fish exposed to model xenobiotics. Oxidatively modified forms of Cu, Zn-superoxide dismutase as potential biomarkers. Chem Biol Interact, 98, 267-282, 1995.

66. Peters LD, Livingstone DR : Antioxidant enzyme activities in embryologic and early larval stages of turbot. J Fish Biol, 49, 986-997, 1996.

67. Peters LD, Porte C, Albaiges J, Livingstone DR : 7-Ethoxyresorufm O-deethylase (EROD) activity and antioxidant enzyme activities in larvae of sardine (Sardina pilchardus) from the north coast of Spain. Mar V Pollut Bull, 28, 299-304, 1994.

68. Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Rahbari-Oskouia F : Increased oxidative stress in younger as well as in older humans. Clinica Chimica Acta, 328, 83-86, 2003.

69. Piper GM, Jordan M, Dondlinger LA, Adans MB, Roza AM : Peroxidative stress in diabetic blood vessels. Reversal by pancreatic islet transplantation. Diabetes 44, 884-889, 1995.

70. Przekwas M, Matgorzewicz S, Zdrojewski Z, Debska-Slizien A, Lysiak-Szydtowska W, Rutkowski B : Influence of Predialysis Oxidative Stress on Peroxidation Processes After Renal Transplantation. Transplantation Proceedings 35, 2170-2173, 2003.

71. Rana SVS, Singh R : Species differences in glutathione-dependent enzymes in the liver and kidney of two fresh water fishes and their implications for cadmium toxicity. Ichthyol Res, 43, 223-229, 1996.

72. Richard C, Lemonnier F, Thibault M, Ccuturier M, Auzepy P : Vitamin E deficiency and lipoperoxidation during adult respiratory distress syndrome. J Crit Care Med, 18, 4-9, 1990.

73. Richards MP, Stcele NC : Trace element metabolism in the developing avian embryo: a review. J Exp Zool Suppl 1, 39-51, 1987.

74. Rickett GM, Kelly FJ : Developmental expression of antioxidant enzymes in guinea pig lung and liver. Development 108, 331-336, 1990.

75. Ringwood AH, Conners DE : The effects of glutathione depletion on reproductive success in oysters, Crassostrea virginica. Mar Environ Pollut, 50, 207-211, 2000.

76. Roche H, Boge G : Effects of Cu, Zn and Cr salts on antioxidant enzyme activities in vitro of red blood cells of a marine fish Dicentrarchus labrax. Toxicol. In Vitro 7, 623-629, 1993.

77. Rudneva II : Antioxidant system of Black Sea animals in early development. Comp Biochem Physiol, Part C 122, 265-271, 1999.

78. Sakia II : A ribonucleoprotein which catalyzes thioldisulfide exchange in the sea urchin egg. J Biol Chem, 242, 1458-1461, 1967.

79. Sardesai VM : Role of antioxidants in health maintenance. Nutr Clin Pract, 10, 19-25, 1995.

80. Savolainen H : Superoxide dismutase and glutathionc peroxidase activities in rat brain. Res Cornmw Chem Pathol Pharmacol, 21, 173-175, 1978.

81. Shen X, Aw TY, Jones DP : Glutathione dependent protection against oxidative injury. Pharmacol Ther, 47, 61-71, 1990.

82. Shivakumar BR, Anandatheerthavarada HK, Ravindranath V : Free radical scavenging systems in developing rat brain. Int J Dev Neurosci, 9 (2), 181-185, 1991.

83. Sies H : Oxidative stress: Oxidants and Antioxidants. New York, Academic Press, 1991.

84. Slater TF : Free radical mechanisms in tissue injury. J Biochem, 222, 1-15, 1984.

85. Stefanini S, Farracc MG, Ceru Argcnto MP : Differentiation of liver perwisomes in the foetal and newborn rat. Cytochemistry of catalase and D-aminoacid oxidase. J Embryol Exp Morphol, 88, 151-163, 1985.

86. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN : Bilirubin is an antioxidant of possible physiological importance. Science 235, 1043-1046, 1987.

87. Stohs SJ, Lawson TA, Anderson L, Beuding E : Effect of ottipraz, BHA, ADT and cabbage on glutathion metabolism, DNA damage and lipid per- oxidation in old mice. Mech Ageing Dev, 37, 137-145, 1986.

88. Takahashi Y, Oakes SM, Williams MC, Takahashi S, Miura T, Joyce-Brady M : Nitrogen dioxide exposure activates gamma-glutarnyl transferase gene expression in rat lung. Toxicol Appl Pharmacol, 143, 388-396, 1997.

89. Takeda K, Shimada Y, Amano M, Sakal T, Okada T, Yoshiya I : Plasma lipid peroxides and alpha-tocopherol in critically ill patients. J Crit Care Med, 12, 957-959,1984.

90. Tanswell AK, Freeman BA : Pulmonary antioxidant enzyme maturation in the fetal and neonatal rat. II. The influence of maternal iron supplements upon fetal lung catalase activity. Pediatr Res, 18, 871-874, 1984.

91. Thaete LG, Crouch RK, Spicer SS : Inununolocalization of copper-zinc superoxide dismatase. J Histochem Cytochem, 33, 803-808, 1985.

92. Tholey G, Ledig M, Kopp P, Sargentini-Maier L, Leroy M, Grippo AA, Wedler FC : Levels and sub-cellular distribution of physiologically important metal ions in neuronal cells cultured from chick embryo cerebral cortex. Neurochem Res, 13, 1163-1167, 1988.

93. Tollefsbol TO, Cohen HJ : Expression of intracellular biochemical defects of lymphocytes in aging: proposal of a general aging mechanism which is not cell-specific. Exp Gerontol, 21, 129-148, 1986.

94. Toyokuni S : Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int, 49, 91-102, 1999.

95. Tsai HM, Garber BB, Larramendi LMH : [^{3}H]Thymidine autoradiographic analysis of telencephalic histogenesis in the chick embryo; I. Neuronal birthdates of telenccphalic compartments in situ. J Camp Neural, 198, 275-292, 1981.

96. Tsukahara H : Pathophysiological roles of nitric oxide in inflammatory diseases. In: Pediatric Asthma and Other Allergic Diseases. Ed by A Morikawa. Gunma: JOMO NEWSPAPER pp 145-152, 2002.

97. Verity MA : Oxidative damage and repair in the developing nervous system. Neurotoxicology 15, 81-91, 1994.

98. Viarengo A, Canesi L, Pertica M, Livingstone DR : Seasonal variations in the antioxidant defence systems and lipid peroxidation of the digestive gland of mussels. Comp Biochem Physiol, 100C, 187-190, 1991a.

99. Viarengo A, Canesi L, Pertica M, Livingstone DR, Orunesu M : Age-related lipid peroxidation in the digestive gland of mussels: the role of the antioxidant defence systems. Experientia 47, 454-457, 1991b.

100. Wilson JX, Lui EMK, DEL Maestro RF : Developmental profiles of antioxidant enzymes and trace metals in chick embryo. Mechanisms of ageing and development 65, 51-64, 1992.

101. Winston GW, Di Giulio RT : Prooxidant and antioxidant mechanisms in aquatic organisms Aquat Toxicol. 19, 137-161, 1991.

102. Yu BP : Cellular defenses against damage from reactive oxygen species. Physiol Rev, 74, 139-162, 1994.

103. Zimmerman JJ : Redox/radical repertoire rapport: Pathophysiology and therapeutics. Acta Anaesthesiol Scand 42, 1-3, 1998.

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