TWENTY-FOURTH ANNUAL MEETING - American Aging Association
NINTH ANNUAL MEETING - American College of Clinical Gerontology
Friday through Tuesday, October 14-18, 1994
The Capital Hilton
Washington, DC

MINISYMPOSIUM: "Industrial Initiatives in Antioxidant Interventions"

INVITED PAPERS

1. Papas AM: **Vitamin E research: An industry perspective**
2. Thomas CE: **Rational design and therapeutic applications of antioxidants**
3. Hall* ED, Andrus PK, Zhang J-R: **Age-related changes in hydroxyl radical stress, antioxidants and lipid peroxidation in brain**
4. Clemens* JA, Panetta JA: **Antioxidants are neuroprotective in models of global and focal cerebral ischemia**

SUBMITTED PAPERS

5. Weindruch* R, Kemnitz J, Roecker E: **Dietary restriction and aging in rhesus monkeys**
6. Lane* MA, Cutler RG, Tilmont EM, Ingram DK, Roth GS: **Antioxidant protective systems and dietary restriction: short-term response in young rhesus monkeys**

MINISYMPOSIUM: "Antioxidant Genes"

INVITED PAPERS

7. Siddique* T, Deng H-X, Juneja T, Hentati A, Hung W-Y, Rimmler J, Yaghmour A, Deng G, Kaplan J, Pericak-Vance MA: **Relationship of *SOD1* mutations and disease expression in familial amyotrophic lateral sclerosis**
8. Epstein* CJ, Huang T-T, Carlson E, Chan PH, Phillips JP, Cadet JL: **The effects of increased expression of CuZn-superoxide dismutase in transgenic mice**
9. Orr* WC, Sohal RS: **Life span extension in transgenic *Drosophila* overexpressing antioxidative genes**
10. Van Remmen* H, Williams M, Richardson A: **The transcriptional regulation of catalase**

SUBMITTED PAPERS

11. Busbee* D, Miller S, Merriam E, Srivastava V: **Activity of a DNA polymerase α accessory protein, an ATP-dependent helicase, declines as a function of increased age**
12. Wei* H, Cai O: **Age-dependent increase of indigenous DNA adducts in rat brain is associated with a lipid peroxidation product**

MINISYMPOSIUM: "Free Radicals and Mitochondria"

INVITED PAPERS

13. Hansford* RG, Filburn CR: **Mitochondrial metabolism and calcium transport with aging**
14. Sohal RS: **Relationship between mitochondrial generation of reactive oxygen species and aging**
15. Feuers R: **Mitochondrial enzyme activities as influenced by age and caloric intake**
16. Wei J: **Cardiac mitochondrial changes with aging**
17. Aiken JM: **Age-associated mitochondrial DNA abnormalities in monkeys and mice**

SUBMITTED PAPER

18. Lee* CM, Eimon P, Kaczkowski JM, Weindruch R, Aiken JM: **Characterization of age-associated mitochondrial DNA deletions in rhesus monkeys**

Submitted Papers - Oral Presentations

19. García-de-la-Asunción J, Plá R, Pallardó F, Millán A, Sastre J, Viña* J: **Direct relationship between age associated glutathione oxidation and mitochondrial DNA damage protection by antioxidants**
20. Kumari* KS, Bulliyya G, Reddy K: **Relationship between serum antioxidants and lipids among rural and urban populations**
21. Reddy* KK, Rao AP, Kumari S, Reddanna P: **Free radical mediated lipid peroxidation and DNA damage in industrial population and aging**
22. Salminen* A, Hänninen M, Helenius M: **Aging and replicative senescence associated changes in oxidative stress responsive NF-kB binding activities**
23. Johnson N, Cosmas A, Bronson R, Lipman R, Manfredi* T: **The effect of caloric restriction on capillary density and skeletal muscle fiber area in B6C3F1 mice**
24. Cosmas* A, Edington DW, Manfredi T: **Mitochondrial distributions in hearts of male rats as a function of aging**
25. Khoory* W, Podolsky S: **Vascular complications following treatment of diabetic coma**
26. Nadazdin* AG, Sarma RJ: **Decreased aortic distensibility associated with atherosclerotic plaques in thoracic aorta as assessed by transesophageal echocardiography**

Submitted Papers - Poster Session

27. Koltover VK: **Reliability aspects of free radical theory of aging**
28. Khokhlov AN: **Evolutionary cytogerontology as a new branch of experimental gerontology**
29. Tatariunas AB: **How to explain results of R. Jolly's lab (New Zealand)?**
30. Toth P, Fiddes R, Hammond* P, Levine B, Codispoti J, McNally C: **A double blind, placebo controlled study of the safety and efficacy of indapamide 1.25 mg in elderly patients with mild to moderate hypertension**
31. Jie* M, Qin-Shun D, En-Zong L, Feng-Ping H: **Mechanism of free radical pathology of the chronic cerebral vasospasm in rabbits**
32. Rondó Jr* W, de Felippe Jr J: **Free radicals: qualitative evaluation at bedside**
33. Wei* H, Tian L: **Effect of aging and caloric restriction on lymphocyte function and lipid peroxidation**
34. Khokhlov* AN, Prokhorov LY: **Effects of some geroprotectors-antioxidants on cell proliferation**
35. Prokhorov LY, Petushkova NA, Khokhlov* AN: **Cytochrome P-450 and "stationary phase aging" of cultured cells**
36. Chen* LH, Hu N, Snyder DL: **Effects of age and dietary restriction on liver glutathione transferase activities in male Lobund-Wistar rats**
37. Bains* JS, Kakkar RK, Sharma SP: **Propyl gallate induced modifications in respiratory enzymes in aging fruit fly**
38. Bains* JS, Kakkar RK, Sharma SP: **Effect of** α-**tocopherol on glutathione content in aging** Zaprionus paravittiger
39. Fields* JZ, Robinson CE, Keshavarzian A, Rawal PA, Hagen JP, Wallace RK, Tomlinson PF, Schneider RH: **Anti-oxidant effects of an anti-carcinogenic, natural product - Maharishi Amrit Kalash**
40. Mura* CV, Taylor A: **Calorie restriction modulates age-dependent changes in the expression of antioxidant enzymes in liver of Emory mice**
41. Barber* BJ, Parameswaran S, Dutta S: **Compartmental analysis of extracellular matrix dehydration due to age-related changes**
42. Mune M, Meydani* M, Jahngen-Hodge J, Martin A, Blumberg JB, Taylor A: **Effect of dietary restriction on liver and kidney glutathione (GSH) in aging Emory mice**
43. Schwarze* SR, Chung SS, Weindruch R, Aiken JM: **Fiber bundle analysis of age-associated MtDNA deletions in C56BL/6 mouse skeletal muscle**
44. Seaton K: **Is albumin the life factor?**
45. Santiago* LA, Osato JA, Mori A: **Free radical mechanism and protection of bio-normalizer on brain disorders and autoimmune functions**
46. Cooney C: **Methylation metabolism has a central role in mammalian longevity**

MINISYMPOSIUM: "Antioxidants and Nutrition"

INVITED PAPERS

47. Harman D: **Overview: Role of antioxidant nutrients in aging**
48. Cutler RG: **Testing the oxidative stress hypothesis of aging: a possible role for dietary antioxidants**
49. Meydani* SN, Hayek M, Wu D, Leka L: **Dietary antioxidants and immune function**
50. Gaziano JM: **Dietary antioxidants in cardiovascular disease: epidemiologic studies and randomized trials**
51. Viña* J, Sastre J, Plá R, O'Connor E, Juan G, Pallardó F: **Free radicals induced mitochondrial damage in intact aging cells: protection by antioxidants**

SUBMITTED PAPER

52. Fernandes* G, Chandrasekar B, Venkatraman JT, Kim JD: **Prolongation of life span by omega-3 lipids is linked to higher hepatic and renal antioxidant enzyme activity and mRNA expression in (NZBxNZW)F1 mice**

SPECIAL INVITED GUEST

Hathcock J (US Food and Drug Administration): **Evaluation of scientific evidence related to health claims for antioxidant vitamins**

Annual Luncheon and Awards Presentation

Appointment of Trustee: *Walter R. Baron*
Excellence in Journalism Award: *Robert Whitaker*
Research Award: *Caleb E. Finch*
Distinguished Achievement Award: *Betty Friedan*
Presidential Address: *Sheldon S. Ball*
Finch CE, Research Award: **Invited lecture**

Submitted Papers - Oral Presentations

53. Vilenchik MM: **Better dietary habits for prevention of cancer could be established using a combination of the selected antioxidants protecting both nuclear and mitochondrial parts of the genome against oxidative damage**
54. Busbee* D, Flood L, Jaeger L, Bielec P: **Lipid peroxide-related reduction in microsomal P450 in the livers of animals fed a lipogenic diet**
55. Richie Jr.* JP, Skowronski LA, Leutzinger Y, Zimmerman J, Orentreich N: **The role of glutathione in the enhancement of longevity by methionine restriction in F344 rats**

56. Ianov V, Niedzwiecki* A: **Direct and extracellular matrix mediated effect of ascorbate on vascular smooth muscle cells proliferation**
57. Sharma* SP, Kakkar R, Bains JS: **Effect of ethoxyquin on H_2O_2 levels during development and ageing of Zaprionus paravittiger**
58. Rifkind* JM, Abugo OO, Balagopalakrishna C, Spangler E, Ingram D: **Erythropoietin treatment of rats used to understand the changes in erythrocytes which occur during aging**
59. Carrillo M-C, Ivy*, GO, Milgram NW, Head E, Wu P, Kitani K: **(-)deprenyl increases activities of superoxide dismutase (SOD) in striatum of dog brain**
60. Wei* H, Du H: **Protection of aging-dependent and chemical-induced lymphocyte apoptosis by caloric restriction**

MINISYMPOSIUM: "Neuropathology of Oxidative Stress"

INVITED PAPERS

61. Ratan* R, O'Donovan K, Lee P, Baraban JM: **Apoptotic death in an *in vitro* model of neuronal oxidative stress**
62. Dawson* VL, Dawson TM: **Molecular mechanisms of glutamate neurotoxicity in primary neuronal cultures**
63. Chiueh* CC, Wu R-M, Mohanakumar KP, Miyake H, Obata T, Murphy DL: **MPTP dopaminergic toxicity: free radical mechanism and protection**
64. Joseph* JA, Villalobos-Molina R, Erat S, Strain J: **Experimental manipulation of oxidative stress and the alterations of neuronal signal transduction in aging**

SUBMITTED PAPERS

65. Gould* TJ, Bickford PC: **Effects of chronic treatment with N-tert-butyl-α-phenylnitrone on cerebellar noradrenergic receptor function in aged F344 rats**
66. Matsuyama* SS, Stoddard M, Makhijani N, O'Hara R, Jarvik LF: **Microtubules and Alzheimer disease**
67. Harman D: **Free radical theory of aging: a hypothesis on pathogenesis of Alzheimer's disease**
68. Carrillo MC, Kanai S, Kitani* K: **A protein free diet uncovers the potential age difference in the hepatic detoxifying system, glutathione S-transferase in mice**
69. Troncoso* JC, Martinie D, Singer HS: **Adaptation of neuroblastoma cells to oxidative stress**

1

VITAMIN E RESEARCH: AN INDUSTRY PERSPECTIVE. Andreas M. Papas, Eastman Chemical Company, Kingsport, Tennessee 37662.

Vitamin E research in the past was overshadowed by mystique and faddish lore resulting in major swings in consumer interest and product demand. In contrast, current interest is the result of high quality academic, government and industry research. Industry has concluded that high-quality research is essential for long-term sustainable growth. For this reason, it collaborates or supports vitamin E research, primarily at universities and research institutes, focusing on: (a) its biochemical role as antioxidant; (b) relative bioavailability, dose-response and tissue uptake of various forms using novel techniques such as deuterated tocopherols; (c) its role on the immune system in relation to heavy exercise, aging, etc.; (d) its role in disease prevention with emphasis on heart disease, cancer, cataracts and other chronic diseases; and (e) the effective dose needed for disease prevention, which may be higher than the RDA, and associated safety issues. In addition, industry participates in the dissemination of new findings by sponsoring scientific meetings, providing research information services and by other means. New products or new formulations were developed to meet needs of malabsorbers, to increase its bioavailability, for use in new dosage forms such as drinks and for use in skin creams and cosmetics. Vitamin E is key component of antioxidant nutrient mixtures which have become popular as nutritional supplements.

2

RATIONAL DESIGN AND THERAPEUTIC APPLICATIONS OF ANTIOXIDANTS. Craig E. Thomas, Marion Merrell Dow Research Institute, Cincinnati, Ohio 45215.

Oxidative modification of cellular biomolecules including lipids, proteins and DNA has been proposed to be a causative event in a number of disease states, thus, antioxidants represent a viable approach to the treatment of such disorders. However, each situation may be unique with regards to the site and type of radical generated and to the cellular constituent(s) susceptible to oxidative insult. As answers to these questions become available, then it becomes feasible to design antioxidants which target loci of radical production and/or damage. For example, a series of hydrophilic *ortho*-dimethyl phenols with substituents which lead to rapid distribution and/or accumulation in target tissues have been synthesized and examined for antioxidant activity. MDL 73,404 is a potent anti-lipoperoxidant which contains a quaternary amine functionality leading to its accumulation in heart tissue. Accordingly, MDL 73,404 exhibited significant protection against myocardial ischemia/reperfusion damage in animal models. As another approach, a series of cyclic nitrone radical traps encompassing a wide range of solubilities have been prepared and evaluated for efficacy in treatment of both atherosclerosis and stroke. In vitro experiments demonstrated that hydrophobicity and a corresponding ability to incorporate into low density lipoproteins (LDL) was the primary determinant for prevention of LDL oxidation which may represent a potential anti-atherogenic activity. Selected nitrones were found to trap protein-derived radicals and chelate metals suggesting they may have utility in a number of pro-oxidant states. In a gerbil model of reversible global ischemia several nitrones provided significant protection against hippocampal CA1 damage. A relationship between hydrophobicity and efficacy was not evident and current work is focused on identifying the structural features of the molecules which confer activity. These results provide evidence that antioxidants can be designed in a rational fashion to function as effective therapeutic agents in a variety of abnormal pathophysiologic situations.

3

AGE-RELATED CHANGES IN HYDROXYL RADICAL STRESS, ANTIOXIDANTS AND LIPID PEROXIDATION IN BRAIN. Edward D. Hall*, Paula K. Andrus and Jue-Rong Zhang, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The levels of hydroxyl radicals (•OH), antioxidants (glutathione, vitamin E, ascorbate) and phosphatidylcholine hydroperoxide (PCOOH) were examined in young (3 mo.), middle-aged (15 mo.) and old (20-24 mo.) gerbil hippocampus, cortex and striatum. The •OH levels were quantified via the salicylate trapping products 2,3- and 2,5-dihydroxy-benzoic acid. The levels were significantly increased in middle-aged and old brains compared to young animals in all brain regions. Regional comparison showed higher amounts in cortex than in hippocampus or striatum. The ratio of oxidized to reduced glutathione also rose with age consistent with the increase in oxidative stress. The levels of vitamin E and reduced ascorbate increased in parallel with the increase in oxidative stress. The highest antioxidant concentrations were observed in the hippocampus.

PCOOH levels were measured by HPLC-chemiluminescence and found to be the highest in the striatum in all age groups. Moreover, PCOOH increased in middle-aged and old striatum, but not in cortex or hippocampus. However, analysis of the relationship between oxidative damage and oxidative stress (PCOOH/•OH) revealed a relatively high ratio in young animals that decreased at middle age followed by a subsequent increase between middle and old age in all three brain regions. The ratio was higher in the hippocampus and striatum than in the cortex at all ages consistent with the fact that the latter two regions are more vulnerable to ischemic insults. These data reveal that while there is an increase in brain •OH stress and susceptibility to damage (membrane lipid peroxidation), this is largely compensated for by increases in antioxidant levels or efficiency except in the striatum.

4

ANTIOXIDANTS ARE NEUROPROTECTIVE IN MODELS OF GLOBAL AND FOCAL CEREBRAL ISCHEMIA. James A. Clemens* and Jill A. Panetta, The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

Reactive oxygen species (ROS) are believed to contribute to neuronal cell death that occurs after global ischemia and stroke. During the reperfusion phase, after ischemia, the presence of ROS has been demonstrated. Administration of antioxidants or depletion of substrates capable of generating ROS have been found to attenuate ischemia-induced. Studies on the effects of antioxidants on ischemia-induced brain damage were performed using the 4-vessel occlusion (4-VO) model of global ischemia and the middle cerebral artery occlusion (MCAO) model of focal ischemia. Compounds possessing antioxidant activity were found to be beneficial in attenuating damage in both of these models. While the antioxidant LY231617 was effective in reducing damage in both models, it was found to be more effective in reducing damage from global ischemia that from focal ischemia. LY231617 was found to be neuroprotective when given after 30 minutes of 4-VO. In the MCAO model, LY231617 was more effective when retroperfused into a cerebral vein. Administration of LY231617 to rats subjected to 4-VO prevented the reduction of the hippocampal theta rhythm indicating that it preserved a functionally intact neuronal network. In conclusion, antioxidants may be useful in therapy of stroke and global cerebral ischemia.

5

DIETARY RESTRICTION AND AGING IN RHESUS MONKEYS. Richard Weindruch*, Joseph Kemnitz and Ellen Roecker, Wisconsin Regional Primate Research Center and Departments of Medicine and Biostatistics, University of Wisconsin, and VA GRECC, Madison, WI 53715.

Dietary restriction (DR) without malnutrition is the only intervention that has been consistently shown to slow aging and to extend lifespan of warm-blooded animals. Until recently, this phenomenon has not been examined in a primate species. We are evaluating the effects of 30% DR initiated during early adulthood on aging of male rhesus monkeys. Twenty-eight monkeys have been assessed semi-annually along several dimensions of functional aging. After four years of DR, the main findings are: 1) 30% DR can be safely imposed on animals of this species, gender and age; 2) Restricted (R) monkeys weigh less and have less body fat than Controls (C); 3) overall levels of physical activity do not differ consistently between groups, but R monkeys perform better than C in food-motivated tasks; 4) metabolic rate of R subjects is lower than C during most of the day, even when the contribution of differences in body size and composition are statistically removed; 5) R have increased insulin sensitivity (quantified by the Modified Minimal Model, MMM) and lower plasma insulin levels than C; 6) R have lower fasting plasma glucose levels than C; 7) R have enhanced glucose effectiveness (by MMM) and faster glucose disappearance rates relative to C; and 8) diastolic and mean arterial blood pressures are lower in R than C. These results are generally consistent with and extend observations of beneficial effects of DR in rodents. Additional study is necessary to establish whether or not DR actually slows the basic processes of aging and extends lifespan in primates as it does in rodents and other nonprimate species in which it has been studied.

6

ANTIOXIDANT PROTECTIVE SYSTEMS AND DIETARY RESTRICTION: SHORT-TERM RESPONSE IN YOUNG RHESUS MONKEYS. MA Lane*, RG Cutler, EM Tilmont, DK Ingram, and GS Roth, Gerontology Research Center, National Institute on Aging, NIH, Baltimore, MD 21224.

One proposed mechanism for the biological effects of dietary restriction (DR) increase is an in antioxidant protective systems such that life span is increased and the rate of aging reduced. Several

laboratories have investigated antioxidant defenses during DR in rodent species. However, the majority of these examined indices of oxidative stress state in tissue biopsies after several months of DR. Therefore, little is known concerning the immediate effects of DR on blood levels of the various antioxidant protective systems and to our knowledge no studies of this sort have been reported in nonhuman primates. In the present study we monitored changes in several antioxidants in rhesus monkey sera, during and immediately after, the institution of DR. Six juvenile (2 y. old) rhesus monkeys were fed *ad libitum* prior to the initiation of DR which was phased in over 3 months (10%/month) to a final restriction of 30%. Blood samples were obtained at baseline, 10%, 20%, and 30% restriction and every three months thereafter. A variety of assays were performed to assess lipid- and water-soluble antioxidants, lipoproteins, and iron balance. None of the major lipid soluble antioxidants were altered during or immediately after the initiation of DR. Vitamin C, showed a progressive decline, while uric acid increased during the gradual phase in of DR. LDL cholesterol decreased significantly and total cholesterol and apolipoprotein B exhibited marginal declines. No changes were observed in HDL or triglyceride levels. Concerning iron balance, serum ferritin and available binding capacity increased slightly, while serum iron and percent saturation decreased. These findings suggest that initial responses to DR include generally favorable changes in antioxidant protective systems which if maintained throughout the life span could contribute to the well known effects of DR.

7

RELATIONSHIP OF *SOD1* MUTATIONS AND DISEASE EXPRESSION IN FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS. Teepu Siddique[1]*, Han-Xiang Deng[1], Tony Juneja[1], Afif Hentati[1], Wu-Yen Hung[1], Jackie Rimmler[2], Aysha Yaghmouf[1], Gang Deng[1], Jocelyn Kaplan[1], Margaret A. Pericak-Vance[2]. [1]Northwestern University Medical School, Chicago, IL 60611, and [2]Duke University Medical Center, Durham, NC 27710.

Mutations in four of the five exons of the gene for Cu,Zn SOD (*SOD1*) have been identified in twenty percent of familial ALS families. The mutations correlate with the duration of disease. In families with the A4V mutation, the mean duration from onset of disease to death was 1-2 years, as opposed to the E100G mutation where the mean duration was 4-7 years. This difference is statistically significant. There was no significant difference in the age of onset in individuals with these mutations. The G37R and the V148G mutations also appear to confer a shorter duration of disease. In contrast, the H43A and H46A confer a phenotype with prolonged duration; with mean durations of 18 years and 17 years respectively. However, wide inter-individual variations in onset and duration were seen for most mutations.

8

THE EFFECTS OF INCREASED EXPRESSION OF CuZn-SUPEROXIDE DISMUTASE IN TRANSGENIC MICE. Charles J. Epstein*, Ting-Ting Huang, Elaine Carlson, Pak H. Chan, John P. Phillips, and Jean L. Cadet, University of California, San Francisco, CA 94143; University of Guelph, Guelph, Ontario N1G 2W1; and NIH/NIDA Addiction Research Center, Baltimore, MD 21224.

Heterozygous transgenic mice carrying between 2 and 8 copies of the human genomic sequence for CuZnSOD under control of the native promoter express between 1.8 and 3.1 times the nontransgenic level of CuZnSOD in erythrocytes, fibroblasts, and neurons. High expressing homozygous transgenic animals have 5 times control levels. Transgenic mice with 3 to 5 times increased CuZnSOD are protected to various degrees in vivo against a variety of acute and chronic insults to the central nervous system in which oxygen free radicals (O_2-) and/or nitric oxide (NO) are believed to play a pathogenic role. These insults, both physical and chemical in nature, include cold injury, blunt trauma, ischemia and reperfusion (stroke), and the toxic effects on dopaminergic neurons of MPTP, methamphetamine, and methamphetamine derivatives (which produce models of Parkinson disease). The transgenic mice are also more resistant to the induction of type I diabetes mellitus by the pancreatic islet β-cell toxins, alloxan and streptozotocin. These findings indicate that relatively small changes in CuZnSOD activity can have profound effects on the cellular response to a variety of stimuli that are believed to cause damage through the generation of oxygen free radicals. As a result, CuZnSOD transgenic mice serve as useful probes for investigating the potential roles of such radicals in pathological processes.

9

LIFE SPAN EXTENSION IN TRANSGENIC *DROSOPHILA* OVEREXPRESSING ANTIOXIDATIVE GENES. William C. Orr* and Rajindar S. Sohal, Department of Biological Sciences, Southern Methodist University, Dallas, TX 75275.

The purpose of this study was to directly test the oxidative stress theory of aging by examining the effect of over-expression of antioxidative genes on life span and various aging parameters in transgenic *Drosophila.*

All initial studies in the introduction of single, extra copies of either Cu-Zn SOD or database, driven by their native promoters was found to have a relatively minor effect on mean life span and no significant impact on maximum life span of the transgenic lines. Since SOD and catalase act in tandem in the elimination of O_2^- and its stoichiometric product, H_2O_2, we proceeded to determine the impact of simultaneous overexpression of both catalase and Cu-Zn SOD in the same transgenic lines. As compared to diploid controls, transgenic flies carrying three copies of each of these genes exhibited an extension of average and maximum life spans and mortality rate doubling time by up to one-third, a reduced level of protein and DNA oxidative damage and a delayed loss of motor ability. These results provide the first direct support for the oxidative stress hypothesis of aging.

This research was supported by Grant RO1AG8459 from the National Institutes of Health-National Institute on Aging.

10

THE TRANSCRIPTIONAL REGULATION OF CATALASE. H. Van Remmen*, M. Williams, and A. Richardson, GRECC at Audie Murphy Memorial VA Hospital, and Departments of Physiology and Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

The cellular defense system against free radical damage involves free radical scavenging enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. Catalase converts hydrogen peroxide, which is produced by the action of superoxide dismutase and other enzymes, to water and oxygen. It is located primarily in the cytoplasm within peroxisomes, cellular organelles responsible for degradation of fatty acids and other molecules. The activity of catalase, which is highest in liver and kidney, has been shown to decrease with age in several tissues and this decrease has been reversed/retarded by dietary restriction. The changes in catalase activity with age and diet have been shown to arise at the level of transcription. Therefore, we are interested in elucidating the transcriptional regulation of catalase expression. At the present time, very little is known about the regulation of catalase transcription or the catalase promoter region. Therefore, we have begun to determine the length of the catalase 5'-flanking region necessary for expression. Various lengths of the catalase promoter have been attached to the CAT (chloramphenicol acetyltransferase) reporter gene, and the ability of these constructs to drive the expression of CAT are being tested. We have transfected either a porcine kidney cell line ($LLPCK_1$) or a human hepatoma cell line (HepG2) with a transgene construct containing 3.3kb of the 5'-flanking region of the catalase gene. These transfected cells exhibited significant CAT activity. However, our preliminary studies show that when the 5'-flanking region was reduced to 1.7kb, a significant decrease in CAT activity was observed in transfected cells. We are also studying the transcriptional activity of the various transgene constructs in transgenic mice. These experiments will allow us to determine the elements in the catalase gene that regulate the tissue specific expression of catalase.

11

ACTIVITY OF A DNA POLYMERASE α ACCESSORY PROTEIN, AN ATP-DEPENDENT HELICASE, DECLINES AS A FUNCTION OF INCREASED AGE. David Busbee*, Susan Miller, Elizabeth Merriam and Vinod Srivastava. Department of Anatomy and Public Health, College of Veterinary Medicine, College Station, Texas 77843.

An accessory protein for DNA polymerase α (pol α), which was isolated from L1210 cells, was found to bind both dsDNA origin sequences and DNA pol α, and to exhibit ATP-dependent helicase activity. Murine hepatic DNA pol α showed age-related decreases in enzyme activity and in the amount of enzyme isolated per gram of tissue, while pol α isolated from human fibroblasts (HDF) showed age-related declines in the amount of enzyme isolated per cell, the activity of pol α isozymes isolated, and in the response of pol α to addition of the exogenously isolated accessory protein, αAP. Treatment of pol α from old HDF with αAP resulted in increased pol α binding to dsDNA DNA and pol α activity, while high activity pol α isolated from fetal-derived or transformed HDF showed increased dsDNA binding but little or no activity enhancement in the presence of αAP. These data indicate that there is a decrease in total recoverable

pol α activity and pol α specific activity in HDF as a function of increased age of the cell donor, as well as a decrease in the specific activity of pol α from essentially amitotic murine hepatic tissues. The data further indicate that transformation of HDF is associated with increased expression of pol α, but suggest that increased expression alone is not sufficient to explain the difference in pol α activity levels between parental and transformed HDF. Lastly, the data suggest that interaction of pol α with an accessory protein which has ATP-dependent helicase activity may be altered as a function of age, an alteration that may be correlated with the decline in pol α DNA binding and specific activity. Changes in expression of the accessory protein as a function of increased age have not been determined.

12

AGE-DEPENDENT INCREASE OF INDIGENOUS DNA ADDUCTS IN RAT BRAIN IS ASSOCIATED WITH A LIPID PEROXIDATION PRODUCT. Huachen Wei* and Qiuyin Cai, Department of Environmental Health Science, University of Alabama at Birmingham, Birmingham, AL 35294.

Indigenous DNA adducts (I-Adducts) are considered to be a biomarker of the aging tissues. So far, few studies have been conducted to investigate the accumulation of I-adducts in the brain during aging, or the identification of age-dependent I-adducts. We determined the amount of I-adducts in the brains of male Fisher 344 rats at ages of 1, 6, 12, 18, and 24 months using the ^{32}p-postlabelling. We found that I-adducts increased in the brain age-dependently from 6 to 24 months of age. The maximum adduct formation occurred at age of 18 months. The brain of 1-month old rats contained high levels of I-adducts, which might be due to the hypermetabolic status. Co-incubation of Malondialdehyde (MDA) with deoxynucleosides shows the formation of adduct spots in the reactions containing dGMP and dTMP. However, only dG-MDA adducts overlapped with the I-adducts of the brain DNA on the TLC sheet. *Via* co-chromatography, five MDA-dG adducts have been identified to be responsible for the spots in the brain DNA adduct map. The brain contains high levels of lipids, and MDA is a key product of lipid peroxidation. The accumulation of I-adducts with aging might be an index of indirect oxidative damage to DNA as evidenced by the presence of MDA-DNA adducts.

13

MITOCHONDRIAL METABOLISM AND CALCIUM TRANSPORT WITH AGING. R.G. Hansford* and C.R. Filburn, National Institute on Aging, Gerontology Research Center, Baltimore, Maryland.

In a series of studies of substrate oxidation by cardiac mitochondria we have found no general pattern of loss of activity of oxidative phosphorylation with aging (24 mo vs 6 mo male Wistar rat). Instead, there are substrate-specific decrements such that, for instance, the rate of oxidation of both short-chain and long-chain acylcarnitine species is diminished by approximately 30%. This appears to relate to the lower carnitine content of the aging rat heart and consequently lowered rates of acylcarnitine translocation across the mitochondrial membrane. There is also a significant decrease in the activity of mitochondrial Ca^{2+} -ion transport, involving both the "uniporter" which catalyses Ca^{2+} uptake and the "antiporter", or 2 Na^{+}/Ca^{2+} exchanger which catalyses release of the ion. Since intra-mitochondrial free Ca^{2+} is a critical regulator of oxidative phosphorylation, through its role in activating pyruvate dehydrogenase phosphatase and *a*-ketoglutarate dehydrogenase, these decreases in velocity of transport of the Ca^{2+} ion would be expected to translate into a damped response of the activity of oxidative phosphorylation to changes in work-load of the heart, as signaled mainly through the *B*-adrenergic system.

In a study of mt-DNA and respiratory chain complex activity in specific brain regions as a function of aging of the rat, we have found manyfold increases in the fractional incidence of a large (near 5 kb) deletion, which resembles the "common" deletion of human mt-DNA. When cerebral cortex, cerebellum, hippocampus and striatum were examined, the largest increase in incidence of deleted mt-DNA was found in striatum and the smallest in cerebellum. This pattern is closely analogous to that found in aging humans. Despite 10-30 fold increases with aging (23 month versus 6 month) in abundance of mt-DNA carrying the deletion, absolute amounts of deleted DNA remained very small (less than 1% of total). When Complex (NADH-dichlorophenol indophenol oxidoreductase), Complex IV (cytochrome c oxidase) and Complex V (F1Fo ATP-ase) activities were measured, no age-linked changes were found in any of the four brain regions. These components of the machinery of oxidative phosphorylation were chosen as having subunits coded for on mt-DNA, as well as others coded on the nuclear genome. Any physiological

importance of the age-linked increases in deleted mt-DNA seems to be minimized by these findings on enzyme activity. However, this is not necessarily so, as the mt-DNA carrying the deletion may be distributed among neurons in a mosaic fashion, with a minor fraction of the neurons having a preponderance of the deletions, in analogy to what seems to occur in heart. If so, their energy metabolism may be compromised to the point that they may face an "energy-crisis", perhaps in response to excitatory amino acid stimulation, and die. Single-cell PCR and histochemical techniques will be required to resolve this question.

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RELATIONSHIP BETWEEN MITOCHONDRIAL GENERATION OF REACTIVE OXYGEN SPECIES AND AGING. R. S. Sohal, Department of Biological Sciences, Southern Methodist University, Dallas, TX 75275.

There is considerable evidence to support the concept that mitochondria play a major role in the aging process as being the primary generators of reactive oxygen species (ROS) as well as the victims of oxidative damage. Supportive evidence is provided by the following set of observations:

i) The rates of mitochondrial superoxide anion radical (O_2^-) and H_2O_2 generation increase during aging in mammals and insects.

ii) The rate of mitochondrial O_2^- and H_2O_2 generation is inversely related to experimentally induced intraspecies variations in life spans. For example, caloric restriction lowers the rate of mitochondrial O_2^- and H_2O_2 generation and extends life span.

iii) The life span potential of a selected group of mammalian species was found to be inversely related to the rate of mitochondrial O_2^- and H_2O_2 generation.

iv) Mitochondria undergo considerable age-related accrual of protein and DNA oxidative damage, which is inversely related to the life expectancy of the organisms.

v) Experimental oxidative damage to mitochondria leads to a further enhancement of mitochondrial ROS generation.

Overall, the existing evidence suggests that the age-related increase in mitochondrial ROS generation elevates the in vivo level of oxidative stress and accelerates the aging process.

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AGE-ASSOCIATED MITOCHONDRIAL DNA ABNORMALITIES IN MONKEYS AND MICE. Judd M. Aiken, Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI 53706.

We are studying the rhesus monkey and the mouse for the presence of age-associated mitochondrial DNA (mtDNA) deletions. In both species, we have identified and characterized multiple mtDNA deletions, the number of which increases with age of the animal. DNA sequence analysis of the mtDNA deletion breakpoints suggests that two different mechanisms of deletion formation exist; one requiring and one independent of direct repeat sequences. Similar to other studies we have found that the abundance of specific deletions, when determined from tissue homogenates, to be too low to exert a significant physiologic impact. Accordingly, a critically important issue concerns the cellular distribution of the deletions. MtDNA deletions may be either: i) present at low levels in almost every cell or ii) present in cells at variable abundance with some cells having comparatively high levels of mtDNA deletions while other cells contain low levels. Analysis of mtDNA from defined mouse skeletal muscle fiber bundles (100 fibers/bundle) indicates that mtDNA deletions are not present in every cell at identical levels. We can, therefore, conclude that mtDNA deletion are not evenly distributed throughout all cells but rather there is considerable cell to cell variability in mtDNA deletion abundance. The significance of this conclusion is that to assess the potential impact mtDNA deletions may have on aging, mtDNA deletions need to be characterized at the cellular level.

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CHARACTERIZATION OF AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS IN RHESUS MONKEYS. Connie M. Lee[1]*, Peter Eimon[1], Jean M. Kaczkowski[1], Richard Weindruch[2], and Judd M. Aiken[1]. [1]Department of Animal Health and Biomedical Sciences and [2]Department of Medicine and VA-GRECC, University of Wisconsin-Madison, Madison, WI 53706.

We have previously shown the existence of multiple age-associated mitochondrial DNA (mtDNA) deletions in the skeletal muscle of rhesus monkeys. By cloning and sequencing several deletion breakpoints, we have found that deletions unique to a particular animal generally did not have direct repeats at the breakpoints. In

contrast, deletions common to several animals had direct repeats flanking the breakpoints, indicating that at least two different mechanisms exist by which age-associated mtDNA deletions are formed. Of the common deletions analyzed in rhesus monkeys, a 5.7 kb deletion was found in all animals over the age of 9 years. This deletion is flanked by a 17 and 18 bp imperfect direct repeat and its frequency increases with age. Although initially detected in skeletal muscle, this deletion was also detected at lower levels in cardiac and brain tissue, as well as in platelet mtDNA. Platelets are easily isolated from blood specimens and may provide an excellent, noninvasive source by which to measure an individual's mtDNA deletion load. (Supported by P01 AG11915).

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DIRECT RELATIONSHIP BETWEEN AGE ASSOCIATED GLUTATHIONE OXIDATION AND MITOCHONDRIAL DNA DAMAGE PROTECTION BY ANTIOXIDANTS. José García-de-la-Asunción, Rosa Plá, Federico Pallardó, Arantxa Millán, Juan Sastre, José Viña*, Departamento de Fisiología, Facultad de Medicina, Universidad de Valencia.

The aim of this work was to test the effect of aging on mitochondrial DNA damage and on glutathione oxidation and the possible protective effect of thiolic antioxidants on this process. We used the following methodological approach: 1. C57BLJ mice of 6 or 18 months of age were used. They were fed ordinary chow diet or the same diet supplemented with thiolic antioxidants, which were given between mo 6 and 18 of age. 2. Mitochondria from liver and brain were used. 3. 8-hydroxy-2'deoxyguanosine (8-OHdG) was measured by hplc with electrochemical detection. 4. GSH was measured enzymatically and GSSG by an hplc method that we recently developed to minimize GSH oxidation. The results that we found are: 1. Mitochondrial glutathione is oxidized with age. 2. The level of 8-OHdG in mitochondrial DNA increases with age. 3. Oral administration of glutathione or of a derivative of thiazolidine carboxylate results in a decrease in GSSG and in 8-OHdG in mitochondria. 4. There is a direct relationship between mitochondrial glutathione oxidation and DNA damage evidenced by formation of 8-OHdG associated with aging. We conclude that there is a relationship (r=0.958) between mitochondrial glutathione oxidation and oxidative damage to mitochondrial DNA associated with age. Both glutathione oxidation and mitochondrial DNA damage can be prevented by oral administration of antioxidants.

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RELATIONSHIP BETWEEN SERUM ANTI-OXIDANTS AND LIPIDS AMONG RURAL AND URBAN POPULATIONS. K. Soorya Kumari*, G. Bulliyya, K.K. Reddy, Department of Biochemistry and Physical Anthropology, S.V. University, Tirupati-517 502, India.

The association of serum antioxidants and lipids was studied in 350 urban individuals with an age range of 40-76 years, in comparison with a control rural sample of equal size. Glutathione Peroxidase (GPx) activity was found to be significantly higher in urban population when compared to rural population. Urban population were characterized by elevated levels of serum cholesterol (SC), low density lipoprotein cholesterol (LDLC) and triglycerides (TG) in comparison with rural population. In urban females both tocopherol (T) and GPx were negatively related with age. The serum lipid levels were found to be increased with age in urban population when compared to rural population. Further the correlation co-efficients revealed that LDLC positively and TG inversely related to ascorbic acid (AA), and an inverse association of SC, high density lipoprotein cholesterol (HDLC), and LDLC with T in urban population. In rural population GPx shown a good positive correlation with lipid levels. The results of this study revealed that accumulation of lipids with unaltered antioxidants may be the consequence of urbanization.

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FREE RADICAL MEDIATED LIPID PEROXIDATION AND DNA DAMAGE IN INDUSTRIAL POPULATION AND AGING. K.K. Reddy*, A. Papa Rao, Soorya Kumari, P. Reddana, Department of Physical Anthropology and Biochemistry, Sri Venkateswara University, Tirupati-517 502, India.

Oxidative stress and free radical mediated lipid peroxidation cause damage to the biological membranes and DNA which may lead to disease like atherosclerosis and malignancies. The objective of this study was aimed to know the DNA damage, free radical production and antioxidant status among Industrial Population with reference to aging. After separation of lymphocytes from whole

blood DNA damage and free radical production were studied besides estimation of antioxidants and lipids. Significant increase in the production of superoxide anion and H_2O_2 were observed in the Industrial Population. We found increased DNA strand breakage in lymphocytes of Industrial Population. The main mechanism of cellular defense against free radical mediated stress effectively functioned until the concentration of lipid peroxides reached a level of 4.0 nmol/ml, in both Industrial and Rural Population: further increase in lipid peroxides resulted in a depletion of antioxidants. The percentage of individuals possessing > 4.0 nmol/ml of lipid peroxides with age was increased in the Industrial Population. Linear relationship between lipid peroxides and lipids were observed among Industrial Population, whereas body mass index were not correlated. In summary the Industrial Population who were exposed to various environmental contaminants and toxicants and toxicants may be vulnerable to stress and disease status.

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AGING AND REPLICATIVE SENESCENCE ASSOCIATED CHANGES IN OXIDATIVE STRESS RESPONSIVE NF-kB BINDING ACTIVITIES. A. Salminen*, M. Hänninen, M. Helenius, University of Jyväskylä, Department of Cell Biology, SF-40100 Jyväskylä, Finland.

Oxidative stress is one contributor to cellular aging. In eukaryotic cells oxidative stress activates the cytoplasmic transcription factor NF-kB which after nuclear translocation activates or represses the transcription of a great variety of genes. Our purpose was to study whether aging of mice and replicative senescence of fibroblasts affect NF-kB binding activities. EMSA (electrophoretic mobility shift assay) method was used to characterize the binding activities of NF-kB, AP-1, and Sp-1 in nuclear extracts. Inactive cytoplasmic NF-kB was activated with deoxycholate. We observed considerably higher NF-kB binding activities in nuclear extracts from the tissues of old (2 years) than young (3-4 months) male and female mice. The difference in NF-kB binding was observed in all tissues studied (brain, liver, kidney, and heart) but not in AP-1 or Sp-1 bindings. Instead, the total cytoplasmic NF-kB binding activity was higher in young mice suggesting the greater activation of cytoplasmic NF-kB complex in old mice. Preliminary results from replicative senescence of human WI-38 and IMR-90 fibroblasts showed reduced binding activity of AP-1 in senescent fibroblasts but nearly similar bindings of NF-kB. It is not known whether increased NF-kB binding activity reflects pathological changes, increased oxidative stress, or endogenous aging process in the tissues of old animals.

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THE EFFECT OF CALORIC RESTRICTION ON CAPILLARY DENSITY AND SKELETAL MUSCLE FIBER AREA IN B6C3F1 MICE. Nicole Johnson[1], Arthur Cosmas[2], Roderick Bronson[3], Ruth Lipman[3], and Thomas Manfredi[1]*, [1]University of Rhode Island, Kingston, RI. 02881 [2]University of Connecticut, Storrs, CT 06269; [3]USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Caloric restriction by 40% of ad libitum intake increases longevity and has beneficial effects on a variety of physiologic parameters that change with age including the manifestations of free radical damage. These alterations may be related to tissue oxidative capacity which may be influenced by cell size. The purpose of this study was to investigate the effects of 40% caloric restriction in B6C3F1 hybrid male mice at 12, 24 and 30 months of age on skeletal muscle fiber area, capillary density (CD) and capillary fiber ratio (C:F). Six mice were studied in each age-diet cohort. At the time of sacrifice, the animals were perfused with Bouin's fixative. Samples of skeletal muscle were doubled fixed with glutaraldehyde and osmium tetroxide and prepared for light/electron microscopic examination. Cross sections were cut at 1 micron and stained with methylene blue. National Institute of Aging software was used to measure fiber areas from sections captured with a color video camera attached to a light microscope. Approximately 100 fibers per animal were measured. CD and C:F were measured within a defined area and expressed per square mm. A 2-way analysis of variance demonstrated that age had a significant effect on muscle fiber area ($p<.05$) and that diet had a significant effect on CD ($p<.05$) and C:F ($p<.01$). By 30 months, fiber area had declined approximately 30% in both dietary groups and CD was 54% lower in the restricted mice. The C:F ratios were 1.126 and .905 in the ad libitum and restricted mice, respectively at 30 months of age. We conclude that muscle fiber area in mice decreases with age, regardless of diet and that caloric restriction appears to increase diffusion distance in muscle.

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MITOCHONDRIAL DISTRIBUTIONS IN HEARTS OF MALE RATS AS A FUNCTION OF AGING. Arthur Cosmas[1]*, D.W. Edington[2], and Thomas Manfredi[3], [1]University of Connecticut, Storrs, CT 06269; [2]University of Michigan, Ann Arbor, Michigan 48109; [3]University of Rhode Island, Kingston, RI 02881.

Aging has been reported to have profound effects on tissue energy production. The purpose of this study was to examine the size distributions of mitochondria in rat myocardium as a function of age. Mitochondrial profiles from specific regions within the hearts of twenty male rats of the Charles River strain were examined following fixation for routine electron microscopy. Detailed electron microscopic examination of the left ventricular apex was performed in order to obtain size distributions of more than 100,000 mitochondria within perinuclear, myofibrillar and cell border regions in rats of 50, 195, 285, 356, 450, and 1050 days of age. The electron microscopic data indicates that apparently as a function of maturation there seems to be a shift in size distributions in the direction toward an increased percentage of smaller organelles in all subcellular regions examined. However, as senescence is approached, the distribution shifts toward a greater percentage of larger mitochondria, particular within the perinuclear and myofibrillar regions of the myocardium. It seems plausible to suggest that the decrease in mitochondrial size which occurs during maturation, would tend to increase the surface-to-volume ratio which might be an indication of increased physiological function. Further, as aging continues, the increased percentage of larger mitochondria observed is consistent with the decreased physiological capacity of the myocardium during senescence.

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VASCULAR COMPLICATIONS FOLLOWING TREATMENT OF DIABETIC COMA. Wissam Khoory* and Stephen Podolsky, VA Outpatient Clinic and Boston University School of Medicine, Boston MA.

Diabetics Mellitus is a major age-related disease. Over 90% of the 14,000,000 persons with diabetes in the U.S. have Type II (noninsulin dependent) diabetes, often erroneously referred to as "mild diabetes". Type I (insulin dependent) diabetes is much less common, and typically presents in children and young adults. Both forms of the disease can develop acute, potentially fatal metabolic decompensation: Diabetic Ketoacidosis (DKA) in Type I Diabetes and Hyperosmolar Nonketotic Diabetic Coma (HNC) in Type II diabetes. Blends of HNC and DKA can occur. Prior to the discovery of insulin in 1922, DKA was invariably fatal. The National Commission on Diabetes reported to Congress in 1976 that almost 10% of hospitalizations for DKA still ended in death of the patient. During the 1970's and early 1980's, HNC was widely reported to have a 40-70% mortality rate, despite hospital treatment. Following increased awareness by physicians, plus more aggressive treatment of HNC, its mortality rate has fallen even more rapidly than that of DKA in recent decades. Still, the mortality rate from HNC continues to run 2-3 times higher than that of DKA.

Improvements in management of potentially fatal acute hyperglycemic complications of diabetes are clearly saving many lives. Nevertheless, it is now recognized that some survivors are at risk to develop acute vascular complications. These included arterial thrombosis, thromboembolic complications and disseminated intravascular coagulation. Although mesenteric and iliac thrombosis have been reported, lower extremity ischemia is most common. Some survivors have developed gangrene in the hospital, requiring lower extremity amputation. These acute vascular complications of diabetic coma are far more likely to occur in HNC than in DKA. Like DKA and HNC themselves, such vascular complications in recovering patients may be largely preventable.

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DECREASED AORTIC DISTENSIBILITY ASSOCIATED WITH ATHEROSCLEROTIC PLAQUES IN THORACIC AORTA AS ASSESSED BY TRANSESOPHAGEAL ECHO-CARDIOGRAPHY. Aleksandar G. Nadazdin* and Radha J. Sarma, USC School of Medicine, Rancho Los Amigos Medical Center, Downey-Los Angeles, CA 90033.

To investigate whether presence of atherosclerotic plaques (AP) in thoracic aorta is associated with change in aortic distensibility 28 patients underwent Transesophageal echocardiography (TEE) examination. Seventeen patients (Group I) were found to have AP in thoracic aorta whereas eleven (Group II) had no evidence of aortic pathology. No significant difference between the groups was found in age, gender, aortic diameter and LV function. In group I ten patients (subgroup IA) and AP in either ascending or descending thoracic aorta whereas other seven (subgroup IB) had AP in both. Aortic strain (STR=diameter

change/minimal diameter x 100%) and Aortic stiffness (STF=pulse pressure/%STR) were estimated following simultaneous brachial artery blood pressure measurements. STR was greater in group II than in group I (8.7±2.9% vs. 6.1±2.0%; $p<0.05$) and subgroup IA (8.7±2.9% vs. 6.7±2.2%; $p<0.05$) whereas no significant difference in STF was found between group I, IA and II (11.4±6.0 vs. 10.5±6.3 vs. 6.2±2.3; NS). No difference was found between IA and IB in STR and STF. However, subgroup IB vs. group II showed significant difference in both STR (5.1±1.2% vs. 8.7±2.9%; $p<0.05$) and STF (12.7±5.6 vs. 6.2±2.3; $p<0.05$). Conclusions: 1. Decreased aortic distensibility is associated with the presence of AP in thoracic aorta as clinically expected, 2. The severity of distensibility impairment appear to be related to the extent of AP within the aortic wall, 3. TEE is feasible method for rapid assessment of AP and related distensibility changes in thoracic aorta.

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RELIABILITY ASPECTS OF FREE RADICAL THEORY OF AGING. Vitali K. Koltover, Institute of Chemical Physics of Russian Academy of Science, Moscow Region, 142432, Russia.

Aging of an organism is determined by stochastic changes which pace in a finite number of critical structures of limited reliability. Reliability-theory approach enables to link the free radical concept with such quantitative features of aging as the mortality data and the interspecies correlations between maximum lifespan and oxidative metabolism intensity for animals (Koltover, 1992). Transient hypoxia/anoxia conditions can transform mitochondria into active generators of superoxide radicals. Decrease in reliability of mitochondria is caused by increase in the membrane lipid fluidity and relevant increase in mobility of the ubiquinone-binding proteins (Nohl, Koltover, and Stolze, 1993). Any factors, capable of turning conditions aside from the physiological optimum, also increase super-oxide production. No more than 4 superoxide radicals from every million may penetrate the SOD-defense. However, the slipped radicals may stimulate NO-synthase producing NO-radical. Moreover, NO can be produced in reactions of peroxides with ammonium and other by-products of nitrogen metabolism. Abnormal quantities of NO can activate guanylate cyclase and, thus, disorder transcription machinery of cells (Koltover, 1987). We have estimated that longevity of human brain could reach 250 years, were it not for cell injuries due to the oxygen free radicals.

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EVOLUTIONARY CYTOGERONTOLOGY AS A NEW BRANCH OF EXPERIMENTAL GERONTOLOGY. A.N. Khokhlov, Evolutionary Cytogerontology. Sector, Biology Faculty, Moscow State University, 119899 Russia.

Evolutionary cytogerontology deals with comparative studies of aging mechanisms on different cultured cells (from bacteria and algae to higher organism cells). Aging of multicellular organism is the increase of death probability with age but in the case of unicellular organism population (or cell culture) aging is revealed as decrease of relative number of living cells with time. The cells die because of "age" changes induced, as we think, by cell proliferation restriction. Thus in the first place such changes accumulate in the cells of stationary cultures. Detailed study of the "stationary phase aging" phenomenon (degradation of resting cultured cells similar to that of aging multicellular organism cells) enables to compare "age" changes in cells of nearly any origin. We plan to: 1) analyze the "age" destruction of different cultured cells in the stationary phase of growth; 2) try to develop universal methodological approach to studies of various objects which could enable further data comparison even if they are obtained on very different (from the evolutionary point of view) cells; 3) try to reveal the mechanisms of cellular aging universal for all species (or, at least, for most of them).

The second topic interesting for evolutionary cytogerontology is analysis of kinetics of growth and stationary phase death of different cultured cells. It is known that, based on growth kinetics and saturation density of cell culture, it is possible to determine "age" of the cell population studied. With the help of our cell kinetics model we can conclude, basing on the growth curve changes under effect of the factor studied, if this factor is geroprotector or geropromoter. Since this regularity is peculiar to very different cells we suppose it is advisable to develop the model further using cultures of bacteria, algae, plant and animal cells in order to find out how much their responses to different geroprotectors and geropromoters are similar. It is rather probable that for testing the different classes of new (from the gerontological point of view) compounds and physical factors the different cell types will be the most suitable.

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HOW TO EXPLAIN RESULTS OF R. JOLLY'S LAB (NEW ZEALAND)? Antanas B. Tatariunas, Kaunas Medical Academy, Kaunas 3000, Lithuania.

Major protein of Ceroid-Lipofuscin Granules (CLG) from ovine affected by Neuronal Ceroid Lipofuscinosis disease (NCL) has been isolated in R. Jolly's Lab. It was shown that the protein sequence is alike subunit c of mitochondrial ATPsynthase (cMt-ATPe). But as it has been found Mt from affected sheep contains normal amounts of this polypeptide. There were also no mutations in nuclear genes P1, P2 coding for cMt-ATPe. It was accepted in these studies that cMt-ATPe synthesis exists on cytoskeleton bound ribosomes. According to G. Ivy's idea about the protease inhibitor model of aging storage material in hereditary NCL has been examined by M. Katz, et al. They have found that the disease-related storage body proteins were rich in S-methylmethionine and ε-N-trimethyllysine. These modificated amino acids could block intracellular degradative pathway of cMt-ATPe. It should be mentioned that in plasma membrane (PM) proton translocation also exists and so called cMt-ATPe could be found as the domain of this channel. From the view point that CLG can be derived from endoplasmic reticulum (ER) one possible explanation of results of R. Jolly's Lab is that the products of cell synthesis are accumulating in the CLG and they can't reach the PM. For elucidation of this idea one would be purposeful to show the presence of the so called cMt-ATPe in PM (for instance by antibodies technique). In this case the synthesis of such kind of hydrophobic protein must exist on ribosomes of rough ER.

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A DOUBLE BLIND, PLACEBO CONTROLLED STUDY OF THE SAFETY AND EFFICACY OF INDAPAMIDE 1.25 MG IN ELDERLY PATIENTS WITH MILD TO MODERATE HYPERTENSION. P. Toth, R. Fiddes, P. Hammond*, B. Levine, J. Codispoti, and C. McNally, Midwest Institute for Clinical Research, Indianapolis, IN 46208; Southern California Research Institute, Whittier, CA 90606; Loma Linda VA Medical Center, Loma Linda, CA 92357; VA Medical Center, Los Angeles, CA 90073; Rhône-Poulenc Rorer Pharmaceuticals, Inc., Collegeville, PA 19426.

The safety and efficacy of once daily 1.25 mg indapamide (Lozol) was evaluated in elderly patients (≥65 years) with mild to moderate hypertension. Two hundred four (204) patients were randomized (after 4 weeks of placebo [P] washout) to either low dose indapamide 1.25 mg or P. After 8 weeks and at study endpoint, indapamide 1.25 mg produced significantly greater decreases in supine diastolic BP, supine systolic BP, standing diastolic BP, and standing systolic BP than placebo ($p \leq 0.0037$). In addition, after 8 weeks, significantly more patients in the indapamide group were considered treatment responders (55% vs. 37%; $p=0.020$). The incidence of drug-related adverse events was similar between the indapamide and placebo treated groups. There were no clinically meaningful differences in laboratory values, including serum potassium, between patients in the indapamide and placebo groups.

In summary, indapamide 1.25 mg given once-daily for 8 weeks was safe and effective as monotherapy with respect to BP reduction in an elderly population with mild to moderate hypertension.

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MECHANISM OF FREE RADICAL PATHOLOGY OF THE CHRONIC CEREBRAL VASOSPASM IN RABBITS. Ma Jie, Dai Qin-Shun, Liu En-Zong, Han Feng-Ping, Neurosurgical Institute of Harbin Medical University Harbin, PRC 150001.

In an effort to study the possible pathological mechanism of free radicals in the development of chronic cerebral vasospasm, different contents of the blood were injected into cisterna magna. The animals were randomly assigned to one of five groups: whole arterial blood, RBC-free blood, arterial RBC, venous RBC and serum (control). MDA was detected on the 1st, 3rd and 5th day after the injections of above blood contents in CSF. A direct method, electron spin resonance (ESR) technique, was used to detect the free radical of active oxygen (OFR) of CSF in the three groups of arterial RBC, venous RBC and control, and angiographic, pathological investigations were also performed. The results showed that MDA level was gradually increased in 3 and 5 days in the three groups of the whole arterial blood, arterial RBC and venous RBC, all of which contained RBC. There was no significant change in other groups ($P>0.05$). Correspondingly, the content of OFR increased significantly in arterial RBC group, compared with venous RBC and control group ($P<0.01$). The reduction of the caliber of vertebrobasilar arteries were correlated with the level of MDA and OFR. There were morphological changes in the arterial wall which underwent chronic spasm, and its severity was correlated with the degree of the

spasm. These results suggest that the pathological reaction of free radicals initiated by RBC lysis plays an important role in the genesis of chronic cerebral vasospasm and platelet may not involve in the development of chronic CVS in rabbits.

Key words: Cerebral Vasospasm (CVS); Myocardial Malondialdehyde (MDA); Electron Spin Resonance (ESR); Oxygen Free Radical (OFR).

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FREE RADICALS: QUALITATIVE EVALUATION AT BED-SIDE. Dr. Wilson Rondó Jr.* and Dr. José de Felippe Jr., Hospital Sao Leopoldo, Sao Paulo, SP-04532-000, Brazil.

The increase in the free-radical generation is one of the intermediary mechanisms of several diseases which affect the critically ill patients: sara, low flow states, infection, sepsis, trauma, myocardial infarct etc. The increase in the generation of free radicals affects vital structures of cells and disturbs several metabolic pathways. It is interesting to note their reaction with the components of the whole blood forming sub-products which provoke morphologic alterations in the peripheral blood, which can be seen under a common microscope. There alterations depend on the kind and quantity of free radicals and on the strength of the immune system. The qualitative evaluation of the free radicals is made by the H.L.B. blood test, created by researchers Heitan La Garde-Bradford.

Used were 54 dry glass plates of 12 critically ill patients. (3 polytrauma, 3 post-op, 1 unstable angina, 1 myocardial infarct, 1 asthma, 1 vascular hemorrhagic cerebral accident, 1 hypertensive crisis, 1 hepatic insufficiency.) The number of blades varied from 3 to 8 per patient, having been obtained on admission, on important events and on discharge/obit. The reading was done by 2 independent researchers not aware of the clinical state of the patients. A series of blood drops is collected on a dry glass plate, by one slight puncture of the fingertip until we get the desired decrease of drop thickness. Dry for 10 minutes and read.

There was a significant co-relation between the degree of the oxidative lesion and the degree of the patient's lesion, clinically and laboratory evaluated. In only 10 minutes the H.L.B. blood test gives the diagnosis of oxidative pathology and it mainly permits the monitoring of the evolution and the changes determined by the therapeutics.

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EFFECT OF AGING AND CALORIC RESTRICTION ON LYMPHOCYTE FUNCTION AND LIPID PEROXIDATION. Huachen Wei* and Ligun Tian, Department of Environmental Health Science, University of Alabama at Birmingham, Birmingham, AL 35294.

Effects of aging and caloric restriction on T cell function have been studied in two experiments consisting of 5 male Fisher BN rats per group at ages of 5, 18 and 31 months. In experiment one, lymphocytes were isolated from the spleens and treated with phytohemagglutinin (PHA, 1.25 - 10 μg/ml) and Concanavalin A (Con A, 0.625 - 5 μg/ml). T-cell proliferation was evaluated by measuring the incorporation of ^{3}H-thymidine. The results showed that both PHA- and Con A-stimulated T-cell proliferation in *ad libitum* groups significantly decreased with aging. However, the aging-dependent decline in T-cell proliferation is more pronounced in PHA than in Con A stimulated lymphocytes. Caloric restriction significantly prevents the decline of the mitogen-mediated T cell proliferation in all three ago groups. Experiment two showed that T cell proliferation exhibited a similar pattern as Experiment one in response to PHA (10 μg/ml) or Con A (2.5 μg/ml) stimulation in both *ad libitum* and calorically restricted animals. Analyses of plasma lipid peroxides indicate an inverse correlation between lipid peroxidation and the decline in T-cell immunity. We speculate that increased oxidative damage to macromolecules during aging might be responsible for the decline in the cellular immunity, and the aging phenotype.

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EFFECTS OF SOME GEROPROTECTORS-ANTIOXIDANTS ON CELL PROLIFERATION. A.N. Khokhlov* and L.Yu. Prokhorov, Evolutionary Cytogerontology. Sector, Biology Faculty, Moscow State University, 119899 Russia.

Continuing our investigations of geroprotectors and geropromoters in cell culture experiments (Age, 1991, 14, 139; 1992, 15, 128) we studied effects of antioxidants butylated hydroxytoluene (BHT), epigid (2-ethyl-6-methyl-3-oxypyridine hydrochloride), glutathione and anphene (new compound kindly supplied by Dr. L.K. Obukhova from Institute of Chemical Physics of Russian Academy of Sciences) on colony-forming ability of transformed Chinese hamster cells of different culture growth phases ("young" - logarithmic phase of growth, "old" - stationary phase of growth). We found that BHT at 10-30 mg ml

concentrations decreases the number of large colonies and increases the number of small colonies formed by "young" cells but has no effect on general colony-forming ability (GCFA) of the cells. After raising the BHT concentration to 300 mg/ml GCFA decreased to 5%. At the same time BHT at 10-30 mg/ml concentrations significantly increased GCFA of "old" cells. It means, we think, that the antioxidant is more effective for the cells which "feel bad". We showed also that epigid at 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M does not influence on GCFA of "young" cells, at 10^{-3} M decreases it to 45%, and at 10^{-2} M decreases it to zero. The number of small colonies significantly increased at 10^{-5} and 10^{-4} M epigid concentrations. Glutathione at 10^{-5} M did not influence on GCFA of "young" cells, at 10^{-4} M decreased it to 11%, and at 10^{-3} and 10^{-2} M - to zero. Anphene at all the concentrations tested (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M) had no effect on GCFA of "young" cells. This fact allowed us to consider it the least cytotoxic compound from all the substances investigated.

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CYTOCHROME P-450 AND "STATIONARY PHASE AGING" OF CULTURED CELLS. L. Yu. Prokhorov, N.A. Petushkova, and A.N. Khokhlov*, Evolutionary Cytogerontology. Sector, Biology Faculty, Moscow State University, 119899 Russia.

Cytochrome P-450 is involved in detoxification in liver of exogenous and endogenous compounds that are dangerous for organism. Besides, it plays the important role in metabolism of cholesterol, steroids, fatty acids, etc. Unfortunately, this enzyme system can produce also some harmful metabolites: free radicals, epoxides, N- and S-oxides, hydroperoxides. They, in their turn, can damage DNA, proteins and membrane phospholipids inducing the processes of aging, mutagenesis and carcinogenesis. Numerous studies of cytochrome P-450 changes during aging, performed as a rule on rodent liver, revealed nearly zero enzyme activity in embryos, its maximum value in adult animals and small shift down in senescence. At the same time we are not informed about some investigations of "age" cytochrome P-450 changes in cell culture experiments. We studied cytochrome P-450 activity in cultured transformed Chinese hamster cells and human embryo diploid fibroblasts during their "stationary phase aging" (model description - Age, 1992, 15, 135). The enzyme activity was determined by fluorescent method in whole cell homogenates (cells were grown in the present of 1 mM 3-methylcholanthrene or without it). The following substrates were used: 7-benzyloxyresorufin, 7-ethoxyresorufin, 7-pentoxyresorufin, 7-methoxycoumarin, 7-ethoxycoumarin. It was found that the pattern of cytochrome P-450 activity changes during "stationary phase aging" of the cells depends strongly on the enzyme substrate used. We suppose that this fact is caused by the differences of "stationary phase aging" changes of various enzymes forming the cytochrome P-450 complex system. In particular, cytochrome P-450 activity revealed with 7-benzyloxyresorufin was absent in actively proliferation cells (logarithmic phase of growth) but became evidently detectable in "old" cultures. In our future experiments we plan to define more precisely the pattern of these "age" changes of cytochrome P-450 activity including the changes in the late stationary phase of growth ("terminally senescent cells") that was not investigated in this work.

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EFFECTS OF AGE AND DIETARY RESTRICTION ON LIVER GLUTATHIONE TRANSFERASE ACTIVITIES IN MALE LOBUND-WISTAR RATS. Linda H. Chen*, N. Hu and David L. Snyder, Department of Nutrition and Food Science, University of Kentucky, Lexington, KY 40506 and Lobund Laboratory, University of Notre Dame, Notre Dame, IN 46556.

Glutathione-S-transferases (GST) are a group of enzymes which detoxify electrophilic xenobiotics (including drugs, carcinogens and their metabolites), and thus may be involved in the age-related pathologic process. Effects of a 30% dietary restriction on liver GST activities toward 7 substrates were studied in male Lobund-Wistar rats at 6, 12, 18,24 and 30 months of age. The enzyme activities in the ad libitum (AL) group toward 1-chloro-2,4-dinitrobenzene (CDNB), bromosulfophthalein (BSP), 4-nitropyridine-N-oxide (NPNO), p-nitrobenzyl chloride (NBC), trans-4-phenyl-3-buten-2-one (PBO) and styrene oxide (STOX) did not change with age, while those toward 1,2-dichloro-4-nitrobenzene (DCNB) decreased after middle age. The enzyme activities in the dietary restricted (DR) group toward CDNB and STOX did not change with age, while those toward DCNB, BSP, NPNO, NBC and PBO decreased after middle age. The DR group had significantly higher GST activities that the AL group, especially at 18 months, when BSP, NPNO, NBC and PBO were used as the substrates. Dietary restriction did not affect GST activities toward all 7 substrates at old age. These results are substrate-specific, indicating

that isozyme-specific changes in GST activities occur with dietary restriction and aging. The results suggest that dietary restriction enhances liver detoxification capability associated with GSH conjugation in middle age, which may contribute to the delaying of the age-related pathologic process until later point in life in this animal model.

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PROPYL GALLATE INDUCED MODIFICATIONS IN RESPIRATORY ENZYMES IN AGING FRUIT FLY. J.S. Bains[1]*, R.K. Kakkar[2] and S.P. Sharma[3], [1]Department of Pathology, University of Calgary, Calgary, Canada T2N 4N1; [2]Department of Pathology, University of Saskatchewan, Saskatoon, Canada S7N OWO; [3]Department of Zoology, GND University Amritsar, India 143 005.

Antioxidants enhance the mean life span of insects and rodents. Propyl gallate (PG) acts as an antioxidant by scavenging the superoxide (O_2) radical. The present investigation was designed to observe the effect of PG on mitochondrial enzyme activities. Various concentrations (1, 10, 25, 50 µg/ml) of PG were mixed in the standard corn meal agar (CMA) medium. The insects were reared and maintained on standard and PG mixed diets at 26±2°C. The most suitable concentration (25 µg/ml) was calculated from the life span table. The flies were maintained on this concentration throughout the experimentation. The activities of succinate dehydrogenase (SDH) and NADH oxidase were measured at 1, 8, 15, 22, 29, 36 and 43 days of age following standard methods. The SDH and NADH oxidase activities increased in the reproductive period while they declined during senescence. PG feeding showed a significant reduction in the activities of both the enzymes in male and female insects. These results suggest that enhanced longevity in insects on antioxidant feeding may partly be due to the decreased metabolic rate and reduced free radical formation.

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EFFECT OF α-TOCOPHEROL ON GLUTATHIONE CONTENT IN AGING ZAPRIONUS PARAVITTIGER. J.S. Bains[1]*, R.K. Kakkar[2], and S.P. Sharma[3], [1]Department of Pathology, University of Calgary, Calgary, Canada T2N 4N1; [2]Department of Pathology, University of Saskatchewan, Saskatoon, Canada S7N 0W0; [3]Department of Zoology, GND University, Amritsar, India 143 005.

α-tocopherol is a chain breaking antioxidant which protects the organism from free radicals. Glutathione is a ubiquitous cellular constituent and functions in the maintenance of thiol groups of proteins. We have previously reported that the antioxidants increase the glutathione content and enhance the longevity of insects (Bains et al., 1992). The cultures of Zaprionus paravittiger (Diptera: Drosophilidae) were reared on standard corn meal agar (CMA) medium at 26±2°C. α-tocopherol (5 µg/ml-calculated from the dose response curve) was fed to the insects throughout their life. The glutathione (GSH) content was measured at 1, 8, 15, 22, 29, 36 and 43 days of survival in whole body homogenate. Maximum level of glutathione content (GSH) was observed during reproductive period. During reproduction there is a high demand of metabolic rate that could result in the increased production of oxygen free radicals. Female flies showed slightly higher levels of GSH at all age intervals. α-tocopherol increased the GSH content significantly in both the sexes. Maximum increase was observed at 15th day of age in males and 22nd day of survival in females. These results indicate that the antioxidants may enhance the longevity of insects by strengthening he defense mechanism of the organism.

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ANTI-OXIDANT EFFECTS OF AN ANTI-CARCINOGENIC, NATURAL PRODUCT-MAHARISHI AMRIT KALASH. JZ Fields*, CE Robinson, A Keshavarzian, PA Rawal, JF Hagen, RK Wallace, PF Tomlinson, and RH Schneider, Research and Medical Services, VA Hospital, Hines IL 60141; Departments of Pharmacy and Medicine, Loyola University Medical School, Maywood, IL; Department of Physiology[c], Maharishi International University, Fairfield, IA.

Maharishi Amrit Kalash (MAK), a natural product available as a pair of food supplements (M4 & M5), and claimed to have many health benefits, was reported to be anti-carcinogenic (HM Sharma et al, Pharm Bioch Behav 35 (1990) 767-773). The mechanism is unknown. Since aging & many major diseases including cancer have been linked to damage due to reactive oxygen species (ROS). we hypothesized that the anti-carcinogenic properties of MAK are due to the scavenging of ROS. We found that, in vitro, an aqueous extract of M4 or M5 completely scavenged superoxide anions (generated by xanthine/xanthine oxidase & measured by reduction of ferricytochrome C) & did so as completely as vitamins C or E, without inhibition of xanthine oxidase, as indicated by uric acid

production. In an ex vivo preparation-isolated human neutrophils (PMN's) - M4 or M5 was as effective as SOD, i.e., it scavenged 100% of superoxide anions. There was no alteration in PMN function as indicated by PMN viability (trypan blue exclusion [95%], oxygen burst). Again using PMN's, M4 or M5 lowered levels of the oxidant HOCL (iodometric assay) as effectively as catalase or as known MPO inhibitors. In an in vivo model of ROS-induced tissue damage, in which mitomycin-C (3.25 mg/kg, IP) induces inflammation in the colonic mucosa of rats, dietary pretreatment using an M4 & M5 supplement to the diet significantly attenuated the extent of inflammation as indicated by pathology scores (mean±sem): con=4.6±0.2, n=13; mito-C=17.6 ±1.0, n=5; mito-C ± MAK=10.0±3.1, n=6; $p<0.05$. Our data suggest that scavenging of ROS by MAK may constitute a major molecular mechanism for the reported anticarcinogenic effects of MAK and for its putative health benefits.

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CALORIE RESTRICTION MODULATES AGE-DEPENDENT CHANGES IN THE EXPRESSION OF ANTIOXIDANT ENZYMES IN LIVER OF EMORY MOUSE. Casilda V. Mura* and Allen Taylor, HNRCA at Tufts University, Boston MA 02221.

Calorie restriction (R) is the only means known to extend life span and retard the onset of many age-associated degenerative diseases. Oxygen-derived free radicals have been implicated in the aging process. Thus the expression of the genes for the antioxidant enzymes could impact the general well being and the longevity of the organism. The aim of this work was to study the effect of calorie restriction on the expression of enzymes that are involved in scavenging oxygen-derived free radicals: superoxide dismutases, Cu/ZnSOD (cytosolic), MnSOD (mitochondrial), catalase (CAT), and glutathione peroxidase (Glpx). Total RNA was isolated from livers of 7 young (4.5 months) and 10 old (22 months) Emory mice fed a C (control) or R diet (40% restricted). Northern analysis was carried out using cDNA probes labeled with ^{32}P. Levels of Glpx and MnSOD (4.4kb transcript) mRNA decreased to 32% ($p<0.0001$) and 40% ($p<0.002$), respectively, in the old compared to the young, C animals. There was no age effect of Glpx and MnSOD mRNAs in the R-fed animals. At 4.5 months R animals had 45% ($p<0.0001$), 63% ($p<0.009$), 85% ($p<0.02$), 53% ($p<0.07$) lower levels of Glpx, CAT, CuSOD and MnSOD. No significant differences in CuSOD mRNA levels and in CAT mRNA levels were observed between young and old animals. There seems to be greater age-related decrease in mRNA for some enzymes in C, but absolute levels of mRNA in C and R animals were indistinguishable at older ages.

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COMPARTMENTAL ANALYSIS OF EXTRA-CELLULAR MATRIX DEHYDRATION DUE TO AGE-RELATED CHANGES. B.J. Barber*, S. Parameswaran and S. Dutta, Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40546.

It is generally agreed that there is an age related decline in hydration, however, confusion abounds due to differences in how water was measured and expressed, and the body water compartment studied; in particular, distribution of dehydration between intra and extracellular spaces remains unclear. A mathematical model that integrates microvascular exchange with whole body parameters is lacking. To aid in resolving discrepancies a compartmental model of body water was constructed consisting of vascular and extravascular extracellular and intracellular compartments; the effect on whole body parameters of microvascular exchanges of water and protein can be predicted. Potential consequences of free-radical production due to glycation include enhanced globulins due to autoimmune reactions and decreased extracellular matrix (ECM) glycosaminoglycan (GAG) due to enhanced protease activity. The mathematical model is used to test the hypothesis that age-related increases in γ-globulins lead to ECM dehydration due to the following cause and effect chain: increased serum globulins tend to cause an increase in serum colloid osmotic pressure (COP); the liver reduces albumin synthesis to restore serum COP; this causes a decrease in serum and tissue ECM albumin; the rise in serum globulins has little effect on tissue globulins because of low capillary leakage for these large macromolecules; therefore tissue COP decreases; this shifts Starling equilibrium toward ECM water reabsorption; ECM dehydrates until an increasingly negative swelling pressure restores balance. The model predicts our data from rats showing a hydration change of - 0.012 wk^{-1} in ECM with tissue albumin decreasing 0.016 (g/dl)/wk and serum globulin increasing 0.028 (g/dl)/wk. Similar dehydration is predicted by changes in GAG concentration in the model. Further data is needed to differentiate between globulin and GAG hypotheses. We conclude that

the hypothesis that an age-related increase in serum globulins causes ECM dehydration is consistent with normal rat physiological parameters.

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EFFECT OF DIETARY RESTRICTION ON LIVER AND KIDNEY GLUTATHIONE (GSH) IN AGING EMORY MICE. M. Mune, M. Meydani*, J. Jahngen-Hodge, A. Martin, J.B. Blumberg and A. Taylor, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111.

Increases in antioxidant defense capacity are associated with the increased health and life span of calorie restricted animals. Emory mice develop late life cataract, a lesion associated with oxidative damage and loss of lens glutathione (GSH). The effect of calorie restriction on GSH in other tissues in this model has not been explored. GSH and oxidized GSH (GSSG) were measured by HPLC in liver and kidney of Emory mice fed a control diet (C; 85% calories of ad-lib fed mice) or 60% calorie intake of C (R; 40% calorie restriction) for 22 mo. Liver GSH concentration increased significantly in C and R mice from 4.5 to 12 mo with no difference observed between the two groups. At 22 mo, liver GSH was lower than at 12 mo in both groups; this decrease was markedly · greater in C ($p=0.0001$) than R ($p=0.02$) with C=22.1±8.3 and R=32.8±5.1 µmol/g protein ($p<0.01$). Liver GSSG was similar in C and R at 12 mo but increased in R at 22 mo (R=5.43±1.48, C=3.22±1.02 µmol/g protein, $p<0.01$). At 22 mo, liver GSH+GSSG in R was higher than in C (43-6±5.9 vs 28.6±9.0 µmol/g protein, $p<0.001$). At 12 mo, liver GSH/GSSG in both groups was higher than in 4.5 and 22 mo mice and C had higher GSH/GSSG than R (17.5±3.7 vs. 11.2±2.0, $p<0.02$); this difference diminished at 22 mo. There was no significant difference in GSH, GSSG, total GSH or GSH/GSSG in kidney from C and R at these ages. Thus, calorie restriction minimizes decreases in liver GSH antioxidant capacity with age. Supported by NIH grant #08566 to A.T.

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FIBER BUNDLE ANALYSIS OF AGE-ASSOCIATED MTDNA DELETIONS IN C57BL6 MOUSE SKELETAL MUSCLE. Steven R. Schwarze[1]*, Susan S. Chung[1], Richard Weindruch[2], and Judd M. Aiken[1]. [1]Department of Animal Health and Biomedical Sciences and [2]Department of Medicine and VA GRECC, UW-Madison, WI 53706.

In post-mitotic tissues such as brain and skeletal muscle, the accumulation of mtDNA deletions may represent an important aging process. There has been, however, considerable debate as to whether these deletions have any physiological significance. MtDNA deletions may be either present at low levels in almost every cell or present in few cells at variable abundance. If the first hypothesis is correct, it is probable that deletions would exert minimal physiologic effects. If, however, the second hypothesis is correct, deletions may increase to a sufficiently high frequency in a few cells to cause cellular dysfunction. We examined the cellular distribution of mtDNA deletions in small skeletal muscle fiber bundles from C57BL6 mice. Fiber bundles (100 fibers/bundle) were dissected from the *vastus intermedius* of mice. Total DNA was isolated and polymerase chain reaction (PCR) based technology was used to identify and quantitate mtDNA deletions. The number of deletions per 100 fibers was found to increase with age. Bundles from 5, 19, and 24 month old mice contained 0.54, 1.82, and 2.50 deletions per 100 fibers, respectively. Not every fiber bundle has detectable deletions, but those that do appear to be unique to that bundle. Quantitation of a specific deletion within a select fiber bundle indicates that the deleted genomes comprise 1.2% of the total mtDNA present, while this abundance within a total hind limb homogenate is only 0.00023% of wild type. Our results suggest that mtDNA deletions in skeletal muscle increase with age and that these deletions are not located in every cell but rather are localized to individual cells.

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IS ALBUMIN THE LIFE FACTOR? Kenneth Seaton. Hi-Tech Hygiene.

Longitudinal studies, analysis of hospital and surgical outcome, and nursing home observations, clearly indicate that serum albumin levels are the most important predictor of mortality and morbidity. Calorie restricted rodents maintain higher albumin and A/G ratios. Perhaps, because albumin is so abundant in animals, particularly in humans, it is by far, the most important antioxidant. Studies clearly indicate that albumin levels decline in aging and all pathological conditions. Further, it is probably the best single indicator of overall homeostasis. Albumin is a "Super Protein" with versatile roles in many areas of biochemistry/physiology, such as: Fluid homeostasis, calcium, thyroid, sex hormones, cortisol and aldosterone transport, cell stability, growth and cancer control, nutrient transport, waste

neutralization and removal, electron collection and donation, proper fluid viscosity and resonance, connective tissue stability, brain purification, blood brain barrier maintenance, transportation of fatty acids and control of lipid/cholesterol levels. It also dominates/controls other serum protein levels, stabilizes red blood cells and transports bilirubin, helps buffer pH, and is vital in optimal growth of the fetus. Any attempt for a longer and more productive life will have to maintain optimal levels of serum albumin (48-60g/L) throughout life. Albumin levels do not respond to diet or infusion under normal conditions, this is the "Ultimate in metabolic misunderstanding". Mean albumin is approx. 40g/L. During the last 13 years I have examined the impact of new techniques of scientific hygiene with thousands of human volunteers/clients in attempts to raise/maintain optimal albumin levels (>48g/L). Scientific cleaning of the fingernail area, nasal cavity, eyes, skin and hair can profoundly reduce a whole range of infectious and allergic diseases, reducing the level of antibodies and acute phase proteins, thus, restoring naturally, optimal levels of serum albumin and A/G ratios to a level never before thought possible. Improvements in sanitation have been the most important factor in extending the average life span from approximately 40 years to approximately 75 years of age in the last century. Animals that spend a great deal of their life in water live substantially longer, such as polar bears 45 yrs vs. brown bears 18 yrs, moose 25 yr vs. deer 10 yrs, seals 40 yrs vs. dogs 13 yrs. The simplest solution is usually correct.

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FREE RADICAL MECHANISM AND PROTECTION OF BIO-NORMALIZER ON BRAIN DISORDERS AND AUTOIMMUNE FUNCTIONS. Librado A. Santiago[1,2*], James Akira Osato[2,3] and Akitane Mori[1], [1]Department of Neuroscience, Okayama University Medical School, Okayama 700, Japan; [2]Osato Research Institute, Gifu 500, Japan; [3]The United Graduate School of Agricultural Sciences, Gifu University, Gifu 500, Japan.

Oxidative Stress has been implicated in neurological disorders and age-related autoimmune dysfunctions, hence the development of effective antioxidants that have significant clinical potential utility. Bio-normalizer, a white granular yeast-fermented nutritional health food from *Carica papaya*, other tropical herbal plants and traditional Japanese foodstuffs, has been shown to exhibit anti-oxidant actions by scavenging hydroxyl radicals. Bio-normalizer has been unequivocally demonstrated to inhibit lipid peroxidation in various disease models of post-traumatic epilepsy, aging, and brain ischemia-reperfusion injury in rats and gerbils. By microdialysis, it was shown to inhibit the excitatory release of dopamine, serotonin, and their metabolites in the intrastriatal perfusate of iron-injected rats. It has the ability also to modulate the release of [^{3}H]aspartate and [^{3}H]GABA evoked by high K+ and/or 2.2 azobis (2-amidinopropane), and a water-soluble peroxyl radical generator, from mouse hippocampal slices. The mitochondrial and cytosolic SOD activities were shown to increase further with age in various brain regions of rats administered with Bio-normalizer. Correspondingly, the SOD, NADPH oxidase activity and superoxide radical production were increased in inflamed murine macrophage after Bio-normalizer treatment. The interferon-γ production was enhanced whereas the liver transaminases (GOT & GPT) were suppressed in the human blood serum treated with Bio-normalizer. These findings support the possibility of a nutritional approach using Bio-normalizer in the management and prevention of brain disorders and autoimmune dysfunctions.

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METHYLATION METABOLISM HAS A CENTRAL ROLE IN MAMMALIAN LONGEVITY. Craig A. Cooney, Biology Department, Beckman Research Institute, City of Hope, Duarte, CA 91010.

I have proposed a mechanism of aging for mammals in which somatic cells have inherent deficiencies in methylation metabolism with respect to their DNA methyltransferase activity and DNA cytosine methylation. These proposed deficiencies are present from the time animals are young and, over time, accumulated DNA methylation loss contributes to genetic instability, senescence and cancer. This provides a new and evolutionary consistent explanation for DNA methylation loss observed in mammalian somatic cell aging.

It is known that DNA methylation levels are influenced by factors, such as diet, that affect methylation metabolism. I am currently developing dietary means to manipulate methylation metabolism and intervene in mammalian aging. I am also studying DNA methylation with dietary intervention and with aging.

Measurements of plasma homocysteine and other key factors in methylation metabolism show that deficient methylation metabolism is a risk factor for numerous age related human diseases including heart disease, stroke and at least some

cancers. I propose that mammals are inherently deficient in methylation metabolism with respect to longevity and that normal levels of methylation metabolism and normal homocysteine levels are parts of aging mechanisms in mammals. This has numerous important implications for the role of nutrition in aging and for possible means of intervening in the development of age-related disease and overall aging.

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OVERVIEW: ROLE OF ANTIOXIDANT NUTRIENTS IN AGING. Denham Harman, University of Nebraska, College of Medicine, Department of Medicine, Omaha, Nebraska 68198-4635.

Aging is the accumulation of changes that increase the risk of death. Aging changes are attributed mainly to the action of free radical reactions (FRR). These are largely initiated by superoxide radicals and hydrogen peroxide generated by mitochondria - at an increasing rate with age owing to impairment of the respiratory chain by mutations in mitochondrial DNA. The FRR initiation rate can be decreased by caloric restriction, and deleterious effects produced by them reduced by FRR-inhibitors-antioxidants. Precisely how FRR-induced changes cause disease and death is not known, nor how FRR inhibitors slow aging changes.

Addition of one of a number of inhibitors to the diet increased the lifespan of all species studied. In man, the main source of antioxidants in the diet, fruits and vegetables, is associated with lowered risks for chronic diseases. This is probably due in part to the presence of micronutrients that induce detoxication enzymes - a property shared by some antioxidants, e.g., ethoxyquin and butylated hydroxytoluene - which protects cells from oxidative stress resulting from the one-electron metabolism of quinones and other substances.

The growing knowledge of antioxidant action in biological systems, coupled with efforts to slow FRR initiation rates by mitochondria, should result in significant increases in the functional lifespan of man.

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TESTING THE OXIDATIVE STRESS HYPOTHESIS OF AGING: A POSSIBLE ROLE FOR DIETARY ANTIOXIDANTS. Richard G. Cutler, GRC, NIA, Baltimore, Maryland 21224.

The steady state level of oxidative damage at any level of organization in an animal is defined as its oxidative stress state (OSS) and is dependent on 1) level of production of reactive oxygen species (ROS) 2) the efficiency of removal of ROS by antioxidants (AO) and 3) the rate of removal or repair of the resultant damage. Although ROS in specific cases do have important functional value, OSS itself, as defined here, is considered largely to be decremental and a trade off of essential normal energy metabolism pathways. Consequently based on this argument OSS has been proposed as a primary cause of aging. Many different strategies are possible to control the OSS of an organism where tissue levels of AO's represent only one example. There is also evidence suggesting that OSS is under tight homeostasis control where longer lived species would be expected to have a lower "set point" of OSS maintenance. So it has been predicted that over a wide range of exercise, and dietary intake of AO's that OSS would remain stable and unchanged. Moreover, dietary supplement of AO would be expected to be most effective only when deficiencies in diet, or absorbance of AO's is present. This argument could explain the lack of significant lifespan extension in rodent species on AO supplement. Yet large doses of carotenoids and Vitamin E and C in humans do appear to reduce the frequency of specific disease of aging and enhance the function of the immune system. Thus it appears that extra Healthspan beyond what might be considered evolutionary normal for a species can be achieved by extra AO's in the diet.

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DIETARY ANTIOXIDANTS AND IMMUNE FUNCTION. S.N. Meydani*, M. Hayek, D. Wu and L. Leka, USDA-HNRCA at Tufts University Boston, MA 02111.

Age-related decline of the immune response and its contribution to several age-associated disease is well documented. The biochemical and molecular bases for these changes, however, are not well defined. The aged exhibit low status of several antioxidant nutrients known to play an important role in maintenance of the immune response. In the last several years our laboratory has conducted a series of experiments in older mice and humans using dietary antioxidants to: 1) develop dietary strategies to improve the immune response in the aged, and 2) determine the contribution of eicosanoids and other products of lipid peroxidation to the age-associated changes of the immune response. Vitamin E supplementation of both old mice and older adults increased interleukin (IL)-2 production, mitogenic response to Con A and

the delayed-type hypersensitivity skin response (DTH). This was recently confirmed in 2 double blind placebo-controlled long term studies using healthy elderly. Peripheral blood mononuclear cells (PBMC) from older subjects synthesized more prostaglandin (PG)E_2 than young subjects. Vitamin E supplementation significantly decreased PGE_2 production. In another study we showed that PBMC from older subjects had significantly lower glutathione (GSH) levels than young subjects. In vitro GSH supplementation significantly increased PBMC's GSH level, mitogenic response to Con A and PHA, and IL-2 production while decreasing PGE_2 production in both young and old subjects. Moreover, a significantly higher percent increase was observed in older subjects than young subjects. Experiments were then conducted to further delineate the role of PGE_2 and other eicosanoids in the age-associated decline of T cell function. Splenocytes from old mice had significantly higher production of PGE_2, leukotriene (LT) B_4 and LTC_4 than young mice. No difference was observed in production of 12-hydroxyeicosatetraenoic acid (HETE), 15-HETE or H_2O_2 between the two age groups. In vitro inhibition of PGE_2 but not LTB_4 and LTC_4 resulted in increased mitogenic response. Furthermore, in vitro addition of PGE_2 significantly inhibited T cell proliferation. Preliminary experiments indicate that the age-associated increase in PGE_2 production is not due to increased substrate level, i.e. membrane arachidonic acid level, but rather it represents increased activity and/or level of the enzyme cyclooxygenase. In conclusion, increased PGE_2 production contributes to the age-associated decrease in T cell-mediated function. Supplementation with dietary antioxidants decreases PGE_2 production and increases immune response in the aged.

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DIETARY ANTIOXIDANTS IN CARDIOVASCULAR DISEASE: EPIDEMIOLOGIC STUDIES AND RANDOMIZED TRIALS. J. Michael Gaziano*, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02215-1204.

Over the past several decades, the atherogenic potential of LDL cholesterol has been clearly identified. Recent evidence suggests that oxidation of LDL may enhance its atherogenicity, raising the possibility that antioxidant vitamins, which can inhibit the oxidation of LDL, may reduce the risks of CHD. The hypothesis that consumption of antioxidant vitamins may reduce the risk of CHD has been explored in a number of epidemiologic studies using various methodologies. The findings from observational epidemiologic studies, while not entirely consistent, generally suggest an association between intake of antioxidant vitamins and reduced risk of CHD. Limited data from randomized trials are conflicting. Data from a recent large-scale randomized trial suggest no benefit of supplemental vitamin E or beta carotene in the prevention of CHD death.

Several additional ongoing large-scale randomized trials will provide reliable data upon which to base clinical decision making and public health policy. At present, antioxidant vitamins represent a promising, but as yet unproven, means to decrease risks of CHD.

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FREE RADICALS INDUCED MITOCHONDRIAL DAMAGE IN INTACT AGING CELLS: PROTECTION BY ANTIOXIDANTS. José Viña*, Juan Sastre, Rosa Plá, Enrique O'Connor, Gloria Juan, Federico V. Pallardó, Departamentos de Fisiología y Bioquímica, Facultad de Medicina, Universidad de Valencia, 46010 Valencia, Spain.

The aim of this study was to test the function of mitochondria in whole cells and not in mitochondria isolated from old cells. Free radicals are important in the pathophysiology of aging. Mitochondria are primary targets of such damage. However all evidence in support of this "mitochondrial theory of aging" was based in experiments using isolated mitochondrial from old animals. In this way, mitochondrial cytosolic relationships are ignored. Also the impaired performance of old mitochondria might be due to increased susceptibility during preparation. We used the following methodological approaches: 1. Study mitochondrial function and oxidative stress within cells using flow cytometry; 2. Study the rate of metabolic pathways that involve both cytosol and mitochondria; and 3. Study the molecular expression of hnRNA of specific proteins that we had found damaged in previous phases of the study. The results that we have found can be summarized as follows: 1. There is a decrease in the rate of gluconeogenesis from lactate plus pyruvate (mitochondrial dependent) but not from glycerol or fuctose (independent from mitochondria); 2. This is due to an impaired function of the mitochondrial malate carrier; 3. Molecular translation of hnRNA coding for their protein in oocytes from Xenopus did not change with aging; 4. Mitochondrial membrane potential was decreased in intact old cells; 5. Peroxide generation was increased in

mitochondria from old animals; 6. Lipid peroxide content was increased in cells from old rats. We conclude that there is an age-associated damage that can be found in mitochondria studied in intact cells from old animals. Acknowledgments: This work was supported by grants from FISS (92/0238) and CICYT (DEP 497/91) to JV.

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PROLONGATION OF LIFE SPAN BY OMEGA-3 LIPIDS IS LINKED TO HIGHER HEPATIC AND RENAL ANTIOXIDANT ENZYME ACTIVITY AND mRNA EXPRESSION IN (NZBxNZW)F1 MICE. Gabriel Fernandes*, Bysani Chandrasekar, Jaya T. Venkatraman and Jong D. Kim, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284-7874.

Calorie restriction (CR) is known to extend life span by inhibiting the formation of reactive oxygen intermediates. Since CR is not practical clinically, an alternate approach with supplementing regular food with ω-3 lipid capsules appears to be gaining attention. The present study, therefore, compares the effects of corn oil (CO, ω-6 lipids) and fish oil (FO, ω-3) lipids) containing diets with equal levels of antioxidant supplements, on antioxidant enzymes (CAT, GSH-Px, and SOD) activity and mRNA expression in liver and mRNA expression in kidneys. Weanling B/W female mice were fed *ad libitum* (AL), a semi-purified diet containing CO or FO at 10% (wt/wt) level (isocaloric). A cross sectional study was done at 6.5 months (m). The results indicate that mice fed FO as compared to CO had 1) equal body weight with less proteinuria, at 6.5m; 2) had higher hepatic eicosapentaenoic and docosahexaenoic acids, and lower arachidonic and linoleic acids; 3) significantly lower estimated peroxidation index and TBARS generation; 4) higher CAT, GSH-Px and SOD enzyme activity and mRNA expression; 5) higher renal antioxidant enzymes mRNA levels; and 6) significantly extended life span [FO, 402±26.1 days; CO, 266.7±12.5 days, $p<0.0001$).

In summary, these results indicate that diet supplemented with ω-3 lipids when fed AL (without CR) delays autoimmune disease and prolongs life span of B/W mice possibly through maintenance of higher hepatic and renal antioxidant enzyme levels.

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BETTER DIETARY HABITS FOR PREVENTION OF CANCER COULD BE ESTABLISHED USING A COMBINATION OF THE SELECTED ANTIOXIDANTS PROTECTING BOTH NUCLEAR AND MITOCHONDRIAL PARTS OF THE GENOME AGAINST OXIDATIVE DAMAGE. Michael M. Vilenchik, College of Veterinary Medicine, Cornell University, Ithaca, NY 14851.

Antioxidants such as beta-carotene, usually used for prevention of cancer, are lipophilic agents, accumulating in membranes, rather than in nucleus. But for cancer prevention, it is necessary to also protect nuclear genes such as antioncogenes against the oxidative DNA damage, 8-hydroxyguanine (OHG). Previously, we developed a mixture of carotenoids, including beta-carotene, which are very stable to oxidation and able to prevent precarcinogenic damage in overall DNA of liver of rats exposed to human hepatocarcinogens (V.M. Michailenko, M.M. Vilenchik, and M.S. Furman, Bullet. Experim. Biol, 10, 481-483, 1988). Recent studies of this mixture confirm that it also significantly decreases the frequency of mammary cancer in control rats and in rats exposed to tritium (HTO) or gamma rays and increase the life span of the animals (Beljaev et al., 1992). There is also evidence, the age-dependent accumulation of OHG in mtDNA is involved in pathogenesis of the common chronic disease. Thus antioxidant mixture for the healthy longevity could be developed, based on: (a) stabilization of the mixture against oxidation; (b) minimization of the antioxidative stress which at certain conditions results in activation of some of oncogenes; and (c) protection of both parts of the genome against serious damage such as OHG and/or promotion of repair of the damage (M. Vilenchik, A strategy for protection of human DNA against the damage involved in pathogenesis of common chronic disease, manuscript).

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LIPID PEROXIDE-RELATED REDUCTION IN MICROSOMAL P450 IN THE LIVERS OF ANIMALS FED A LIPOGENIC DIET. David Busbee*, Larry Flood, Laurie Jaeger and Paul Bielec, Department of Anatomy and Public Health, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Parameters of hepatic microsomal metabolism were investigated in animals fed a lipogenic vs a non-lipogenic diet. Constitutive and induced levels of hepatic microsomal cytochromes P450 and P420.

7-ethoxyresorufin-O-deethylase (EROD), NADPH cytochrome C reductase (NCCR) catalytic activities and lipid peroxides were compared in tissues from sexually-immature hatchery-raised and wild-caught red drum. Data indicate a diet-and/or exercise-related reduction in active P450 and increase in P420, the denatured form of cytochrome P450, occurred in hatchery-raised redfish. EROD catalytic activity, generally considered to be a measure of cytochrome P450-1A1 (CYP1A1) enzymatic activity, was decreased in hatchery-raised fish whereas wild-caught fish exhibited significantly higher levels of both cytochrome P450 and EROD activity. No significant alterations in NCCR activity were noted in hatchery-raised fish. Histologic examination showed hepatopancreii from hatchery-raised redfish to have very significantly increased levels of intracellular lipids, with lipid peroxide levels averaging about 8-fold higher in animals fed lipogenic diets than in wild caught animals. These data indicate that fish fed diets that promoted hepatic lipid storage exhibited elevated levels of hepatic lipid peroxides and reduced drug metabolizing capacity. The data further suggest that hepatic lipid storage levels were coincident with elevated lipid peroxide levels, and with a dramatic decline in cytochrome P450 content and increase in the P450 breakdown product, P420. These conditions present potentially dangerous therapeutic and/or toxicologic implications for vertebrate animals with significant hepatic lipid deposition.

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THE ROLE OF GLUTATHIONE IN THE ENHANCEMENT OF LONGEVITY BY METHIONINE RESTRICTION IN F344 RATS. J.P. Richie, Jr.*, L.A. Skowronski, Y. Leutzinger, J. Zimmerman, and N. Orentreich, American Health Foundation, Valhalla, NY 10595; St. John's University, Jamaica, NY 11439; and Orentreich Foundation for the Advancement of Science, Cold Spring, NY 10516.

Glutathione (GSH) deficiency appears to be a general phenomenon of senescent tissues and may play a key role in the aging process. Recently we observed that dietary restriction of the GSH precursor methionine (Met) resulted in a 40% increase in longevity and a doubling of GSH levels in blood throughout the life span of the rat. The objective of this study was to examine the mechanisms by which Met restriction (MR) alters GSH metabolism and inhibits the aging process. Male F344 rats, beginning at 6 weeks of age were fed a defined amino acid diet containing either 0.86% (control) or 0.17% (MR) Met as the sole source of sulfur amino acid. At various times thereafter, rats were sacrificed and blood and tissues were removed, extracted with 5% metaphosphoric acid and analyzed for thiols and disulfides. MR resulted in 42-44% increases in mean and maximum life span and a 44% decrease in body weight throughout adulthood ($P<0.01$). Blood GSH levels in 30 mo. old MR animals were increased 164% over controls ($P<0.002$). Liver was apparently the source for this increase as hepatic GSH levels decreased to 40% of controls ($P<0.002$). Except for a 25% decrease in kidney, GSH was unchanged in other tissues. All changes in GSH occurred within 2 months of the diet. Altogether, these results suggest that dramatic adaptations in sulfur amino acid metabolism occur as a result of chronic MR leading to conversation of tissue GSH levels and prevention of the GSH deficiency of aging.

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DIRECT AND EXTRACELLULAR MATRIX MEDIATED EFFECT OF ASCORBATE ON VASCULAR SMOOTH MUSCLE CELLS PROLIFERATION. Vadim Ivanov and Aleksandra Niedzwiecki*, Linus Pauling Institute of Science and Medicine, Palo Alto, CA 94306.

Proliferation of vascular smooth muscle cells (VSMC), and deposition of extracellular matrix proteins are important events in forming atherosclerotic plaques. We have investigated effect of ascorbate and other antioxidants on proliferation of VSMC isolated from guinea pig aortas. In the presence of various concentrations of ascorbate, cells showed bi-phasic growth curve. In 125 μM ascorbate, cells growth was stimulated by 25%, however, its higher concentrations gradually decreased cell proliferation up to 50% in 2 mM ascorbate. Vitamin E, glutathione and N-acetylcysteine, used individually and in combination with 1 mM ascorbate, also decreased VSMC growth. Ascorbate inhibited cell proliferation also through matrix-mediated effect. Cells grown in ascorbate-free media on endogenously synthesized matrices by VSMC had up to 50% lower proliferation on matrices derived from 2 mM ascorbate-supplemented than ascorbate-deficient cells. Various matrix components differently affected VSMC growth. This effect was specific for VSMC and human fibroblasts growing on VSMC-originated matrices, but not for human endothelial cells. Possible role of ascorbate in regulation of cell proliferation may be considered through its antioxidant/prooxidant effect as well as its role in extracellular matrix composition.

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EFFECT OF ETHOXYQUIN ON H_2O_2 LEVELS DURING DEVELOPMENT AND AGING OF ZAPRIONUS PARAVITTIGER. S.P. Sharma*, R. Kakkar and J.S. Bains, Department of Zoology, Guru Nanak Dev University, Amritsar, India; Department of Pathology, College of Medicine, University of Saskatchewan, Saskatoon, Canada.

Hydrogen peroxide (H_2O_2) is the precursor for highly reactive hydroxyl radical (OH) and is associated with life span of an organism. In the present study, the effect of ethoxyquin (EQ) at optimal concentration (75 ug/ml) was investigated on H_2O_2 levels in whole body and mitochondrial fractions during development and aging of Z. paravittiger. The optimal concentration of EQ (75 ug/ml) was selected from life table analysis. EQ feeding resulted in increase in median (37.14) and 34.37%) and maximum (19.90 and 19.20%) life span of males and females respectively. The H_2O_2 levels were measured in the control and EQ fed insects at various developmental stages and in adults at 1, 9, 17, 25 and 33 days of age. The H_2O_2 level increased during development (2nd instar larvae to late pupae) and with age in males and females in whole body as well as mitochondrial fractions. Females exhibited lower H_2O_2 levels as compared to males. EQ feeding decreased H_2O_2 levels at various developmental stages and in adults of both the sexes. The reduction was more pronounced in females than males. The findings suggest that the increased life span of Z. paravittiger with EQ feeding could be due to decreased levels of H_2O_2.

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ERYTHROPOIETIN TREATMENT OF RATS USED TO UNDERSTAND THE CHANGES IN ERYTHROCYTES WHICH OCCUR DURING AGING. Joseph M. Rifkind*, Omoefe O. Abugo, Chavali Balagopalakrishna, Edward Spangler and Donald Ingram, Laboratory of Cellular and Molecular Biology, NIH/NIA Gerontology Research Center, Baltimore, MD 21224.

Previous studies by this group and other groups indicate that older subjects have a younger distribution of erythrocytes. These cells have additional altered properties including increased sphericity and decreased deformability which are indicative of some form of stress. In an attempt to mimic and thereby further understand the changes in blood properties during aging, 6 month old Fisher rats have been chronically treated with recombinant human erythropoietin (Epogen, Epo-5 units injected s.c. every other day). During the first three months of treatment there is an increase in hematocrit which coincides with decreased sphericity and deformability. After 6 months of treatment there is a marked decrease in the life span of the cell as indicated by a drop in the hematocrit and the cells are also larger and more spherical. These subsequent changes are thought to reflect oxidative damage to the erythrocyte, which results from circulation of the less deformable cells. The changes found in old subjects are actually similar to the long term chronic treatment and suggest both stressed erythropoiesis and oxidative stress.

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(-)DEPRENYL INCREASES ACTIVITIES OF SUPEROXIDE DISMUTASE (SOD) IN STRIATUM OF DOG BRAIN. M-C. Carrillo[1], G.O. Ivy[2]*, N.W. Milgram[2], E. Head[2], P. Wu[2], and K. Kitani[3], [1]Tokyo Metropolitan Institute of Gerontology, Tokyo; [2]University of Toronto, Scarborough; [3]Radioisotope Research Institute, Faculty of Medicine, University of Tokyo, Tokyo 113.

Deprenyl prolongs the life span of rats. Although the mechanism(s) underlying this effect of the drug remains unresolved, it was shown that the drug can increase SOD as well as catalase (CAT) activities in striatum and s. nigra but not in hippocampus of rat (and mouse) brains. The present study attempted to elucidate whether a similar observation can be made in animals other than rodent species. Seven beagle dogs were administered sucrose (control animals) or different doses (0.1-1.0 mg/kg) of (-)deprenyl orally by means of capsules for 3 weeks. Activities of Cu Zn-SOD and Mn-SOD were determined in striatum and hippocampus in these animals. There was a significant dose-dependent increase in activities of total as well as of both types of SOD enzymes in striatum, demonstrating a significant positive correlation between enzyme activities (u/mg; Y axis) and dose (mg/kg, X axis) in striatum (e.g. for Cu, Zn-SOD, Y-7.5 + 8.9 X, $r=0.928$, $P<0.005$) but not in hippocampus ($P>0.05$). The results suggest that the drug can increase SOD enzyme activities in striatum but not in hippocampus in the dog, thus showing a brain region selectivity like that in rats. Furthermore, since the deprenyl doses used equally inhibited MAO B activities in both striatum and hippocampus, it is unlikely that the effect of deprenyl on increasing SOD activities in striatum is caused by MAO B inhibition.

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PROTECTION OF AGING-DEPENDENT AND CHEMICAL-INDUCED LYMPHOCYTE APOPTOSIS BY CALORIC RESTRICTION. Huachen Wei* and Hong Du, Department of Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, AL 35294.

Caloric restriction is known to be an effective intervention in the aging process and aging-dependent disorders. However, the precise mechanisms(s) of caloric restriction-delayed-aging remains unclear. We have investigated the age-dependent and chemical-induced lymphocyte apoptosis of *ad libitum* and caloric restricted male Fisher 344 BN rats at ages of 5, 18, and 31 months. Lymphocytes were isolated from rat spleens and incubated for different times with or without H_2O_2 and dexamethasone (DXM). Apoptosis was identified by a DNA ladder on 0.8% agarose gel and terminal DNA transferase (TDT) staining. The results showed that untreated lymphocytes undergo apoptosis in a time-dependent manner at all age groups. The lymphocyte apoptosis in older rats is more pronounced than in young rats. However, no significant difference between 18 and 31 month old rats was observed. Both H_2O_2 and DXM could induce apoptosis as compared to controls. The lymphocytes from caloricallly-restricted rats appeared more resistant to chemical-induced apoptosis than *ad libitum*-fed rats and the protective effects was more obvious in 5 month old rats. Our results appeared to support the hypothesis that caloric restriction reduces metabolism and oxidative damage to macro-molecules and delay cellular apoptosis.

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APOPTOTIC DEATH IN AN *IN VITRO* MODEL OF NEURONAL OXIDATIVE STRESS. R. Rajiv*, Kevin O'Donovan, Paul Lee and Jay M. Baraban, Departments of Neurology, Neuroscience, and Physical Medicine and Rehabilitation, Johns Hopkins University School of Medicine

Immature embryonic rat cortical neurons are susceptible to glutamate neurotoxicity through a non-receptor mediated mechanism involving cystine transport inhibition, glutathione depletion and oxidative stress. We utilized this *in vitro* model of neuronal oxidative stress to assess mechanisms by which free radicals induce cell death. We found that glutathione depletion leads to hypercondensation and fragmentation of chromatin, a morphologic signature of apoptosis. These morphologic changes are accompanied by laddering of DNA into multiple oligonucleosomal fragments and can be prevented by a spectrum of antioxidants. Cell death induced by glutathione depletion can also be prevented by inhibitors of macromolecular synthesis. In this paradigm, protection by these agents derives from shunting of the amino acid cystine from global protein synthesis into the formation of the antioxidant glutathione. These results suggest that oxidative stress can induce apoptosis in neurons and that protein synthesis inhibitors may protect by augmenting antioxidant defenses.

To test the hypothesis that transition metals are necessary for the formation of damaging free radicals under conditions of glutathione depletion, we treated glutamate exposed neurons with iron chelators. Several structurally distinct iron chelators were protective. Chelators could be applied 7-9 hours after glutathione depletion to 30% of control and completely suppress death. These observations define a "commitment point" to oxidative stress induced apoptosis and suggest that cystine deprivation and glutathione depletion will be a valuable model system in which to define the primary molecular targets of oxidative stress-induced neuronal death.

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MOLECULAR MECHANISMS OF GLUTAMATE NEUROTOXICITY IN PRIMARY NEURONAL CULTURES. Valina L. Dawson* and Ted M. Dawson, Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Derangements of glutamate neurotransmission have been implicated in several neurodegenerative diseases, including ALS, Huntington's disease and Alzheimer's disease and stroke. Activation of the N-methyl-D-aspartate (NMDA) receptor subtype of glutamate receptors results in formation of nitric oxide (NO) from NO synthase (NOS). Formation of excess NO is responsible at least in part for glutamate neurotoxicity in primary neuronal cultures and in models of focal ischemia.

Alzheimer's disease is characterized by a progressive neurodegeneration of unknown etiology which may be associated with the abnormal metabolism or deposition of the beta-amyloid protein. In primary neuronal cultures, beta-amyloid and fragments of the beta-amyloid protein enhance glutamate neurotoxicity. Preincubation with fragments of the beta-amyloid protein (beta-amyloid 1-40 and 25-30) in primary neuronal cultures potentiates non-toxic concentrations of NMDA to produce neurotoxicity. Inhibitors of NOS provide marked protection against beta-

amyloid induced potentiation of NMDA neurotoxicity. The protection is reversed by competition with excess NOS substrate, L-arginine. These observations implicated the formation of NO in the mediation of beta-amyloid enhanced NMDA neurotoxicity.

The mechanism by which NO or other free radicals can elicit neuronal cell death is unknown. One potential mechanism may be through the activation of the "suicide" response to (non-apoptotic) DNA damage mediated by poly(ADP-ribose) synthetase (PARS) resulting in cellular depletion of NAD and ATP. NO can induce DNA strand breaks leading to stimulation of poly (ADP-ribose) synthase (PARS). NO stimulates ADP-ribosylation of PARS in brain tissue. PARS inhibitors such as benzamide, are neuroprotective against glutamate neurotoxicity with relative potencies paralleling their ability to inhibit PARS activity. Therefore, NO appears to elicit neurotoxicity by damaging DNA, which activates PARS leading to the depletion of NAD and subsequently ATP. Loss of these intracellular energy sources leads to loss of cellular homeostasis, membrane integrity, and cell death.

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MPTP DOPAMINERGIC TOXICITY: FREE RADICAL MECHANISM AND PROTECTION. C.C. Chiueh*, R.-M. Wu, K.P. Mohanakumar, H. Miyake, T. Obata, and D.L. Murphy, Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health Clinical Center, Bethesda MD 20892.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetra-hydro-pyridine) is known to cause a selective destruction of pigmented substantia nigra neurons and Parkinsonism in humans and primates. Its toxic metabolites 1-methyl-2,3-dihydro-pyridine (MPDP) and 1-methyl-4-phenyl-pyridinium ion (MPP^+) induced a sustained biphasic increase in dopamine efflux, more from the A9 nigrostriatal system than the A10 mesolimbic system. There is a positive linear correlation between MPP^+-induced dopamine efflux and hydroxyl free radical (·OH) generation reflected by ·OH adduct products of salicylate.

MPP^+-induced severe nigral injury and striatal dopamine depletion were suppressed by dimethylsulfoxide (DMSO, a ·OH scavenger) and U-78517F (a novel vitamin E analogue) that interrupted lipid peroxidation. Furthermore, MPP^+ stimulated free radical generation and related nigral injury were suppressed by the classical type B monoamine oxidase inhibitor deprenyl (selegine). This neuroprotective action of U-78517F, DMSO, and deprenyl may be the consequence of their antioxidant properties.

These *in vivo* results support a hypothesis that oxidation of released dopamine in the iron-rich basal ganglia could lead to formation of cytotoxic ·OH free radical, lipid peroxidation, and nigral injury. The dopaminergic neurotoxin MPTP could hasten this oxidative neurodegenerative process especially in the iron-rich and pigmented substantia nigra compacta A9 neurons. Antioxidants including DMSO, U-7851F, and deprenyl may preserve nigral neurons against dopaminergic toxicity by scavenging free radicals and/or interrupting oxidant stress. The present *in vivo* findings that ·OH scavengers protect brain neurons against oxidant stress have evident clinical implications for formulating neurorescue and/or neuroprotective strategies for the treatment of progressive neurodegenerative disorders, such as Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's dementia.

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EXPERIMENTAL MANIPULATION OF OXIDATIVE STRESS AND THE ALTERATIONS OF NEURONAL SIGNAL TRANSDUCTION IN AGING. J.A. Joseph*, R. Villalobos-Molina, S. Erat, and J. Strain, USDA-ARS Human Nutrition Research Center, Boston, MA 02111.

While the "free radical hypothesis of aging" has been proposed for more than 40 years, the data regarding the effects of these very reactive molecules in aging is controversial. Evidence suggests that free radicals may contribute to the pathogenesis of age-related degenerative disorders (e.g., Alzheimer's disease and Parkinson's disease). However, the relationship between other indicators such as antioxidant levels and oxidative stress in normal aging is much more tenuous. Reports on the levels of antioxidants (e.g. superoxide dismutase and catalase) have been inconsistent. There is more agreement regarding the increase in iron content and the deleterious effects of dopamine as a function of age. However, the case for involvement of oxidative stress on neuronal deficits in aging could be strengthened if models could be found which would show relationships between functional age-related deficits and oxidative stressors. Over the past several years, we have attempted to establish such models by focusing upon the rather ubiquitous loss of sensitivity that occurs in several receptor systems as a function of age. This loss is expressed as age-related alterations in motor and cognitive behaviors. Two of the most important are decreases in receptor concentrations and altered

signal transduction deficits. By utilizing the decrease of oxotremorine enhancement of K^+-evoked dopamine release (K^+-ERDA) from superfused striatal slices as an indicator of reduced signal transduction in aging, we have shown that: a) These reductions can be restored with *in vivo* and *in vitro* administration of the nitrone trapping agent, N-tert-butyl-alpha-phenylnitrone (PBN). b) Oxo-enhancement of K^+-ERDA and motor behavioral deficits similar to those seen in aging could be induced in young animals irradiated with ^{56}Fe irradiation. c) *In vitro* application of the nitric oxide generator, sodium nitroprusside or the OH·-generator H_2O_2, reduced dopamine release or oxo-enhancement of K^+-ERDA, respectively, from striatal slices, and either alpha-tocopherol or PBN prevented the striatal dopamine decrease in both age groups. Additional data has suggested that within this paradigm (oxo enhancement of K^+-ERDA), membrane cholesterol (which increases during aging) can function as a pro- or antioxidant. These results suggest that at least one important marker of receptor sensitivity in senescence, signal transduction, can be altered through increases or decreases of the levels of oxidative stress. We feel that these findings may have important implications for the delineation of more effective treatment procedures to restore cognitive and motor function in senescence.

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EFFECTS OF CHRONIC TREATMENT WITH N-TERT-BUTYL-α-PHENYLNITRONE ON CEREBELLAR NORADRENERGIC RECEPTOR FUNCTION IN AGED F344 RATS. Thomas J. Gould[2]* and Paula C. Bickford[1,2] VAMC[1] & Department of Pharm., [2]University of Colorado Health Science Center, Denver, CO 80262.

One theory of aging, the free radical theory, postulates that accumulation of free radicals during a life span leads to deterioration of cell function. A wide variety of neural age-related deficits exist. For example, aged rats have a deficit in cerebellar noradrenergic (NE) function. It is possible that free radical production and accumulation may be related to deficits in cerebellar NE function. We examined if a 2 week treatment with the spin trapping agent N-tert-butyl-α-phenylnitrone (PBN) would alleviate age-related deficits in cerebellar NE function of aged male rats compared to age-matched controls. The compound PBN reduces oxidized protein levels and thus may reduce free radical damage.

Six litter mate male 21-22 month old F344 rats were tested. Two rats served as controls and the 4 remaining rats received 2 daily injections of PBN (10 mg/kg, i.p.) for 2 weeks. After treatment, PBN and control rats were anesthetized with urethane and Purkinje cell and extracellular recordings were made in lobules VI and VII of cerebellar vermis. During recording with multibarrel micropipettes, baseline responses to iontophoresis of GABA were established and then the β-adrenergic agonist isoproterenol (ISO) was applied. Modulation of the GABA response during ISO application was recorded.

The ability of ISO to modulate GABAergic inhibition of Purkinje cells in PBN-treated rats was significantly greater than in age-matched control rats ($p < 0.02$, χ^2 test). Noradrenergic receptor function of aged PBN-treated rats was similar to young rats whereas for non-treated aged rats it was typical of that previously recorded in aged rats. Thus, treatment with PBN may reverse age-related deficits in cerebellar NE receptor function. These deficits could be related to production of free radicals.

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MICROTUBULES AND ALZHEIMER DISEASE. Steven S. Matsuyama*, Mitchell Stoddard, Nalini Makhijani, Ruth O'Hara and Lissy F. Jarvik, West Los Angeles VA Medical Center, Brentwood Division, and UCLA, Department of Psychiatry & Biobehavioral Sciences, Los Angeles, CA 90073.

Impairment of the microtubule (MT) system may represent a basic underlying defect in Alzheimer disease (AD)(PNAS 86: 8152-56, 1989; Exptl Neurol 125:163-71, 1994). We previously reported that following treatment of skin fibroblasts with the MT-disrupting agent colchicine, AD cells exhibited a significant delay in the reappearance of the MT network upon release from treatment with colchicine (AGE 16:152-58, 1993). That study utilized cell cultures commercially available from the Human Genetic Cell Repository (Camden, NJ). Whether cultures derived from fresh skin biopsies would exhibit a similar MT response was investigated in the present study. We established cell cultures from skin biopsies obtained from 15 clinically diagnosed AD patients and 15 normal controls ranging in age from 50 to 83 years. There was no significant difference in age or sex composition between the two groups. Stepwise discriminant analyses indicated that the MT response could discriminate between AD and controls. Specifically, the discriminant function was able to accurately classify 80% of the AD cases and 73% of the normal controls ($p<0.01$). These results continue to support our earlier findings and are consistent with the MT hypothesis of AD.

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FREE RADICAL THEORY OF AGING: A HYPOTHESIS ON PATHOGENESIS OF ALZHEIMER'S DISEASE. Denham Harman, University of Nebraska, College of Medicine, Department of Medicine, Omaha, Nebraska 68198-4635.

Senile dementia of the Alzheimer's type (SDAT) is the major cause of dementia. It is a systemic disorder whose major manifestations reflect loss of neurons involved in memory. Apparently the neurons are aging at a faster than normal rate for the same losses are normally seen at later ages. Aging is caused by free radical reactions: largely initiated by mitochondria(mt), and at an increasing rate with age lowing to mutations in mtDNA. A mtDNA mutation early in development results is the distribution in the growing organism of cells that differ in the number of mutated mtDNA's.

It is hypothesized that SDAT is caused by a mtDNA mutation early in life that increases production of superoxide radical and hydrogen peroxide: significantly impaired neuronal descendants are distributed to the areas associated with SDAT where they are the first to become energy deficient and to die.

The incidence of SDAT may be decreased by maternal antioxidant supplementation and/or increased consumption of fruits and vegetables, while the function of SDAT patients should be temporarily improved by measures, such as with ubiquinone, employed with other mitochondrial disorders.

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A PROTEIN FREE DIET UNCOVERS THE POTENTIAL AGE DIFFERENCE IN THE HEPATIC DETOXIFYING SYSTEM, GLUTATHIONE S-TRANSFERASE, IN MICE. M-C. Carrillo[1], S. Kanai[1], and K. Kitani[2]*. [1]Tokyo Metropolitan Institute of Gerontology, Tokyo; [2]Radioisotope Research Institute, Faculty of Medicine, University of Tokyo, Tokyo 113.

Although many past studies have reported an age-dependent decline in various enzyme activities in the liver, our own group has emphasized that hepatic enzyme activities often stay unchanged with age. In order to elucidate the actual role of aging in regulations of age-stable enzyme activities, female C57BL mice of six different ages (from 6 to 26 months) were given a protein free-diet (PFD) for one week and then given a normal diet (ND) thereafter. These PFD mice as well as control mice fed a ND for 2 weeks were examined for enzyme activities of hepatic glutathione S-transferase (GST) with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, at different times during these dietary manipulations. Enzyme activities were very close among the six different age groups in control animals. After one week of PFD, enzyme activities significantly decreased in all age groups; again activities were mostly comparable for six different age groups, showing no significant correlation ($P>0.05$) between enzyme activities (Y) and animal age (X) for these groups. However, when animals were examined 2 days after the start of ND refeeding following one week of PFD, a highly significant negative correlation ($P<0.005$, $r=-0.89$) was demonstrated. After 5 day ND refeeding, this negative correlation again disappeared. We conclude from the present study, that basal GST activity is stable with age; however, GST activities are a clear function of animal age after a dietary manipulation such as a PFD and ND refeeding.

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ADAPTATION OF NEUROBLASTOMA CELLS TO OXIDATIVE STRESS. Juan C. Troncoso*, Daniel Martinie, and Harvey S. Singer, Departments of Pathology and Neurology, Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2196.

Recent studies have underscored the importance of oxidative injury in age-associated neurodegenerations, such as amyotrophic lateral sclerosis and Alzheimer's disease. However, our understanding of the responses of neuronal cells to oxidative stress is limited. We have begun to study the regulation of oxidative stress in cultured neuroblastoma cells (N18). In cultures exposed to 8 mM H_2O_2 for one hour at 37°C, we observed 30-50% cellular death as detected by nuclear shrinkage and incorporation of propidium iodide. However, cultures pretreated with a low concentration of H_2O_2 (i.e., 50 μM or 100 μM) for 30 minutes and allowed to rest for one hour in medium without oxidants showed only a 5-10% cell mortality when challenged with 8 mM H_2O_2. This protective effect was blocked when protein synthesis was inhibited by cycloheximide (50 mg/ml). These preliminary results indicate that neuronal cells have mechanisms that allow them to adapt to oxidative (peroxide) stress in a way similar to that described in bacteria. The survey and identification of proteins induced by peroxide stress in N18 cells are underway.