TWENTY-FIFTH ANNUAL MEETING — American Aging Association
10th ANNUAL MEETING — American College of Clinical Gerontology
Friday through Tuesday
October 6-10, 1995
The Menger Hotel
San Antonio, Texas

MINISYMPOSIUM:
"Mitochondrial Metabolism"

Organized by Richard Hansford
Chairperson: Richard Hansford

INVITED PAPERS

1. LaNoue, K.F.: **New techniques for evaluating mitochondrial function *in situ***
2. Laderman*, K.A., Penny, J., Attardi, G.: **Cellular models for the study of the role of mitochondrial DNA mutations in aging**
3. Lee, D., Clayton*, D.A.: **Regulation of mitochondrial DNA replication and transcription**
4. Hansford*, R., Pepe, S.: **Mitochondrial function in the aging heart**

SUBMITTED PAPERS

5. Harman, D.: **Free radical theory of aging: Alzheimer's disease**
6. Paradies*, G., Ruggiero, F.M. , Petrosillo, G.: **Impairment of mitochondrial function in heart tissue of aged rats**
7. de la Fuente, M., Fleming*, J.E., Miquel, J.: **Support of the mitochondrial oxidation theory of cell aging in mouse lymphocytes**
8. Fleming*, J.E., Ruchert, P., Thurston, M., Taylor, C.: **Effect of phenolic antioxidants on lifespan and mitochondrial function in *Drosophila melanogaster***
9. Cosmas*, A.C., Manfredi, T.G.: **The effects of aging and physical exercise on mitochondrial distributions in hearts of male rats**

MINISYMPOSIUM:
"Analytical Challenges In Mitochondrial Gerontologic Research"

Organized by Simon Melov
Chairperson: Simon Melov

INVITED PAPERS

10. Shoubridge, E.A.: **Characterization of mitochondrial DNA (mtDNA) mutations in aging skeletal muscle**
11. Melov, S.: ***In situ* approaches to mitochondria and aging**
12. Crapo, J.D.: **Assessment of oxygen radicals, radical scavengers and modulation of mitochondrial injury**
13. Trounce, I.: **Somatic cell genetic approaches to age-related mtDNA alterations in brain**
14. Pfeifer, J.: **Introduction to *in situ* PCR**

SUBMITTED PAPERS

15. Bertoni-Freddari*, C., Fattoretti, P., Caselli, U., Paoloni, R.: **Acetyl-L-carnitine administration modulates the ultrastructural plasticity of synaptic mitochondria in old rats**
16. Koltover, V.K.: **Age changes of EPR signals and free radical production in mitochondria**
17. Gabbita*, S.P., Subramaniam, R., Butterfield, D.A., Carney, J.M.: **Effects of mitochondrial respiration on membrane lipid and protein oxidation: an electron paramagnetic resonance (EPR) investigation using rat brain synaptosomes**
18. Gould*, T.J., Bickford, P.C.: **Localization of age-related ß-adrenergic signal transduction deficits in cerebellar Purkinje cells from F344 rats**

MINISYMPOSIUM:
"Mitochondrial Free Radical Production and Removal"

Organized by Raj Sohal
Chairperson: Raj Sohal

INVITED PAPERS

19. Turrens, J.F.: **Mitochondrial production of reactive oxygen species**
20. McCord, J.M.: **Paradoxes of oxidant/antioxidant balance**
21. Sohal*, R.S., Orr, W.C.: **Oxidative stress may be a causal factor in senescence**
22. Beal, M.F.: **Oxidative damage in human aging**

POST-DOCTORAL COMPETITION

23. Kang*, C.M., Kristal, B.S., Yu, B.P.: **Mitochondrial DNA deletions: effects of dietary restriction**
24. Kristal*, B.S., Chung, H.Y., Yu, B.P.: **Oxidant-induced mitochondrial dysfunction in diabetes**
25. Ikeno*, Y., Yu, B.P.: **Regulation of oxidative stress by dietary restriction in brain: microglia and brain nitric oxide synthase**

26. Pahlavani*, M.A., Harris, M.D., Richardson, A.: **Effect of age on T cell-specific and non-specific transcription factors in lymphocytes from F344 rats**
27. Heydari*, A.R., You, S., Richardson, A.: **The decrease in HSP70 transcription with age occurs because of a defect in heat shock transcription factor**
28. Yin*, D., Yuan, X., Brunk, U.T.: **Peroxidation of mitochondria resulting in lipofuscin formation**

MINISYMPOSIUM: "Mitochondrial DNA Abnormalities"

Organized by Judd Aiken
Chairperson: Judd Aiken

INVITED PAPERS

29. Müller-Höcker, J.: **Defects of the respiratory chain in various human tissues during aging: Enzyme histochemical, immunohistochemical and in situ hybridization studies**
30. Bohr, V.A.: **Mitochondrial DNA repair**
31. Wallace, D.: **Somatic mitochondrial DNA mutations in aging**
32. Aiken, J.: **The effect of dietary restriction on the frequency and abundance of mtDNA deletions**

WALTER R. NICOLAI PRIZE

33. Kim*, M.-J., Aiken, J.M., Ershler, W.B., Weindruch, R.: **Oxidative stress-induced expression of cytokines by peripheral mononuclear cells in rhesus monkeys: influences of age and dietary restriction**
34. Schwarze*, S. R., Laughon, A.S., Aiken, J.M.: **Identification and characterization of age-associated mitochondrial DNA deletions in *Drosophila melanogaster***
35. Lee*, C.M., Weindruch, R., Aiken, J.M.: **Identification of ragged red and cytochrome oxidase negative fibers in old rhesus monkeys and rats**

Submitted Papers - Poster Session

36. Allen*, R.G., Tresini, M., Keogh, B.P., Cristofalo, V.J.: **Effects of cellular aging on the induction of *c-fos* by antioxidant treatments**
37. Almeida*, H., Magalhães, M.D., Magalhães, M.M.: **Adrenal zonation and age-related changes in macrophage number**
38. Bains, J.S.: **Changes in hydrogen peroxide level, thiobarbituric acid reactive substances and antioxygenic enzyme activities in aging fruit fly fed on antioxidants**
39. Chagnon*, P., Robitaille, Y., Gauvreau, D., Bétard, C.: **Brain cytochrome oxidase activity and survival in Alzheimer disease**
40. Chung*, H.Y., Paik, K.J., Kim, J.S., Yokozawa, T.: **The antioxidative mechanisms and active sites of magnesium lithospermate B from salviae miltiorrhizae radix**
41. Chung*, H.Y., Oh, M.H., Kim, J.S., Kim, K.W.: **The antioxidative mechanism of ursolic acid and ginseng saponin**
42. Desai*, V.G., Freeman, J.E., Collins, J.B., Weindruch*, R., Hart, R.W., Feuers, R.J.: **The effect of dietary restriction (DR) on electron transport in aging**
43. Eimon*, P.M., Chung, S.S., Aspnes, L.E., Weindruch, R., Aiken, J.M.: **Age-associated mitochondrial DNA deletions: a study of genome regions and tissue specificity**
44. Fattoretti*, P., Bertoni-Freddari, C., Caselli, U., Paoloni, R.: **Quantitative histochemistry of succinic dehydrogenase (SDH) activity of Purkinje cell mitochondria in aging and vitamin E deficiency**
45. Gakhar*, S.K., Vandana: **Expression of various dehydrogenases in aging malaria vector *Anopheles stephensi***
46. Gudikote*, J.P., Van Tuyle, G.C.: **Mitochondrial DNA rearrangements in the minor arc: deletions involving the light and heavy strand promoter sequences**
47. Hagen*, T.M., Bartholomew, J.C., Do, K.L., Song, M.H., Wehr, C.W., Ames, B.N.: **Age-associated heterogeneity of rat hepatocyte subpopulations based on altered mitochondrial function is reversed by L-acetylcarnitine**
48. Khokhlov*, A.N., Prokhorov, L.Y., Akimov, S.S.: **Comparison of influence of two geroprotectors-antioxidants on cell proliferation**
49. Matsuyama*, S.S., Jarvik, L.F., Bondareff, W., Cummings, J., Leuchter, A., Small, G.: **Apolipoprotein E type 4 allele and early onset Alzheimer disease**
50. Seaton, K.: ***Mitochondria, albumin & protein synthesis***
51. Shen-Tu*, S.-J., Shui-miao, L., *et al.*: **A preliminary investigation on the remedial effect of kidney-nourished and heart-recuperated herbal medicaments on function of cholinergic nerve system of experimental dementic rats**
52. Suzuki*, K., Oberley, T., Pugh, T.D., Weindruch, R.: **Influences of caloric restriction and age on antioxidant enzymes in the prostates of rats**
53. Weiss*, A., Geva, S., Miller-Lotan, R.: **Effects of gonadotropin releasing hormone analogue on uterine growth in adult mice**
54. Vatassery*, G.T., Lai, J.C.K., Smith, W.E., Quach, H.T.: **Alteration in the synaptosomal glutamate uptake with age**
55. Gadaleta*, M.N., Lezza, A.M.S., Petruzzella, V., Cantatore, P.: **Mitochondrial involvement in aging**
56. Barber*, B.J., Babbitt, R.A., Parameswaran, S.: **Age-related changes in glycosaminoglycan content and perivascular distribution**
57. Pipkin*, J.L., Hinson, W.G., Lyn-Cook, L.E., Duffy, P.H., Feuers, R.J., Leakey, J.E.A., Aly, K.B., Hart, R.W., Casciano, D.A.: **P48: a cell cycle regulated nuclear protein in old *ad libitum* rats following a terminal dose of isoproterenol**
58. Manfredi*, T., Wright, D., Dane, J., Cosmas, A., Stravato, J.: **Effect of physical activity, aerobic capacity and age on glycated hemoglobin**
59. Moore, W.A.L., Ivy*, G.O.: **The increased variability of intracellular lipofuscin content with age is decreased by caloric restriction**

60. Valdivia*, E., Apkarian, R.P., Sims, P.: **Ultrastructural studies of mitochondrial membranes**
61. Lotz, M., Rosen, F., McCabe, G., Dudler, J., Seegmiller*, J.E., Terkeltaub, R.: **Mechanisms of interleukin-1β (IL-1β) suppression of transforming growth factor-β (TFGβ)-induced inorganic pyrophosphate (PPi) production by cultured human chondrocytes**
62. Vilenchik*, M.M., Balin, A.K.: **Common deletions in mitochondrial DNA increase in parallel with the death rate in humans**
63. Zhao*, X., Poyton, R.O., Burke, P.V.: **Role of yeast hemoglobin in oxidative stress**
64. Yakes*, F.M., Van Houten, B.: **Analysis of DNA damage to mitochondrial and nuclear DNA following hydrogen peroxide exposure**

MINISYMPOSIUM:
"The Importance of *In Vitro* Aging to *In Vivo* Aging"

Organized by Calvin Harley
Chairperson: Calvin Harley

INVITED PAPERS

65. Cristofalo, V.J.: **Senescence-associated loss of EPC-l expression: a marker gene in G_0 for fibroblasts**
66. Peacocke, M.: **A biomarker for cellular aging *in vitro* and *in vivo***
67. Campisi, J.: **Molecular control of cell senescence - lessons on aging from an old fibroblast**
68. West*, M.D., Tonkin, L.A., Shay, J.W., Wright, W.E., Linskens, M.H.K.: **Alterations in the plasminogen activation system during replicative senescence**
69. Obeid, L.M.: **Lipid signaling in cellular senescence**
70. Linskens, M.H.K.: **Replicative senescence and skin aging**

SUBMITTED PAPERS

71. Guo*, Z.M., Heydari, A.R., Yang, H., Richardson, A.: **Effects of aging on the repair of specific genes in rat hepatocytes**
72. Lehtinen*, S.K., Rahkila, P., Helenius, M., Salminen, A.: **Cellular senescence induced changes in nuclear transcription factors regulating mitochondrial protein expression**
73. Williams*, M.D., Richardson, A., Van Remmen, H.: **Characterization of the catalase promoter**
74. Murakami*, S., Johnson, T.E.: **A single pathway conferring both life extension and stress resistance**

MINISYMPOSIUM:
"Recent Advances in Dietary Restriction Research"

Organized by Byung P. Yu
Chairperson: Byung P. Yu

INVITED PAPERS

75. Smith*, T.C., Salih, M.A., Chen, C., Katz, M.S., Kalu, D.N.: **Intracellular calcium signaling the the rat parotid acinar cells: effects of age and food restriction**
76. McCarter, R.J.M.: **Aging and fuel use: insights from studies of diet restricted rats**
77. Herlihy*, J.T., Klebanov, S., Kim, I.S.: **Modulation of age-related changes in heart by diet and exercise**
78. Kristal*, B.S., Yu, B.P.: **Dietary restriction protects and modulates the permeability transition in liver mitochondria**
79. Ward*, W.F., Shibatani, T.: **Alteration of proteasome function by aging and dietary restriction**
80. Nelson, J.F.: **Do glucocorticoids mediate anti-aging actions of dietary restriction?**

Business meeting of AGE

MINISYMPOSIUM:
"Dietary Alteration of Mitochondrial Aging"

Organized by Richard Weindruch
Chairperson: Richard Weindruch

INVITED PAPERS

81. Weindruch, R.: **Caloric intake, free radicals and mitochondrial aging**
82. Yu, B.P.: **Modulation of mitochondrial damage by dietary restriction**
83. Feuers, R.: **The effect of dietary restriction on electron transport in aging**
84. Pepe, S.: **Dietary fatty acid modulation of cardiac membrane composition: cardiac performance and mitochondrial function in aging**

SUBMITTED PAPERS

85. Moore*, S.A., Lopez, A., Richardson, A.: **Effects of age, dietary restriction and adherence on HSP70 expression in Fisher 344 rat alveolar macrophages**
86. Moore, W.A.L., Davey, V.A., Weindruch, R., Ivy*, G.O.: **Lipofuscin accumulation in mouse brain with age: effects of caloric restriction**

87. Chung*, H.Y., Yu, B.P.: **Regulation of the rat xanthine dehyrogenase/oxidase and uric acid formation by aging and dietary restriction (DR)**

88. Lane*, M.A., Ingram, D.K., Roth, G.S.: **Long-term calorie restriction alters the age-related decline in DHEA and DHEA-sulfate in rhesus monkeys**

Annual Luncheon and Awards:

Excellence in Journalism Award —
Jean Carper

"In recognition of her outstanding contribution to the general public's knowledge and understanding of current biomedical aging research and its potential applications and benefits to all people, Jean Carper, author of *Stop Aging Now!,* is awarded the American Aging Association's Excellence in Journalism Award."

Research Award —
Rajinder S. Sohal, Ph.D.

"This award is presented to Dr. Sohal in recognition of his comprehensive analyses of the effects of oxidative stress and antioxidant defense on the aging process. His studies reveal that the effects of aging at multiple levels of cellular organization stem from oxidative mechanisms and demonstrate a decisive role for oxidant generation and removal in the determination of lifespan."

Distinguished Achievement Award —
James A. Michener

"Established to call attention to the fact that chronological age is not a barrier to a full and productive life, the Distinguished Achievement Award of the American Aging Association for 1995 is presented to James A. Michener. At 88, Mr. Michener is the author of 46 books, all written since the age of 40. His novels have contributed to a greater understanding of the world and its inhabitants; his constant theme is the common bonds that unite people of every race, religion and culture."

Submitted Papers - Oral Presentations

Chairperson: Ritchie Feuers

89. Weiss*, A., Kalemen, A., Shofty, R., Reznick, A.: **Effects of growth hormone on bone loss and knee joint damage caused by denervation in aged mice**

90. Cosmas*, A., Manfredi, T.: **The effects of age and physical activity on myocardial capillary receptor ultrastructure**

91. Porta*, E.A., Llesuy, S., Monserrat, A.J., Benavides, S., Travacio, M.: **Lipofuscin accumulation and cathepsin B activity in rat brain and heart during development and aging**

92. Reddy*, B.N., Sharma, S.P., James, T.J., Vohra, B.P.S.: **Effect of estrogen and progesterone on lipid peroxidation and antioxidant enzymes in cerebella and cerebra of female Wistar rats**

93. Bertoni-Freddari*, C., Fattoretti, P., Caselli, U., Paolini, R.: **Age-related modulation of synaptic plasticity in lesioned rats**

94. Kitani*, K., Ivy, G.O., Carrillo, M.C.: **Long term treatment with deprenyl reduces the optimal dose as well as the effective dose range for increasing antioxidant enzyme activities in old mouse brain**

95. Choi*, J.H., Yoon, H.S.: **Effect of docosahexaenoic acid (DHA) on learning and memory impairments of brain in the senescence-accelerated mouse (SAM-P8) strain**

96. Choi*, J.H., Choi, J.S.: **Effect of reed root extract on learning and memory impairments in the senescence-accelerated mouse (SAM-P8) strain**

97. Khokhlov, A.N.: **Some considerations about the general mechanism of aging of living organisms**

98. Gavrilov*, L.A., Gavrilova, N.S., Semyonova, V.G., Gavrilova, A.L., Evdokushkina, N.N., Lapshin, E.V., Evdokushkina, G.N.: **A summary of human longevity studies**

1

NEW TECHNIQUES FOR EVALUATING MITOCHONDRIAL FUNCTION *IN SITU*. K.F. LaNoue, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Assessing mitochondrial and free radical involvement in aging is a difficult task. Experimental challenges include the facts that: 1) short-lived laboratory animals may not provide good models for bioenergetic problems facing longer lived animals such as humans; 2) human mitochondria obtained by biopsy may not provide a representative sampling of the biopsied tissues; and 3) assessment of mitochondrial DNA structural damage and abnormalities in old compared to young individuals, though important, may not answer critical questions of cause and effect. In combination with data obtained from biopsy material, new methods for measuring mitochondrial function *in situ* may provide useful information. The few mitochondrial proteins coded by mitochondrial DNA are all essential elements in the oxidative synthesis of ATP. Current ways to measure ATP and creatine phosphate and even rates of ATP synthesis noninvasively involve mainly techniques of ^{31}P nuclear magnetic resonance spectroscopy. Rates of recovery of *in situ* ATP after bouts of active ATP utilization, or anoxia, have been assessed by NMR spectroscopy. These published phosphorus NMR studies have shown that muscle creatine phosphate and ATP levels decline as a function of age and the rate of recovery of creatine phosphate and ATP after exercise or ischemia is also an inverse function of age.

To understand the cause of this phenomenon it may be helpful to try to define which steps in oxidative phosphorylation are likely to be rate limiting under normal circumstances in laboratory animals. We have approached this problem in recent years using *in situ* techniques other than NMR spectroscopy. One of the more useful techniques involves measurement of electrical potential gradients across the mitochondrial membrane in perfused rat hearts. Studies of this parameter as a function of work, in conjunction with other data, have convinced us that the ATP synthase is likely to provide the greatest control strength for ATP synthesis in the rat heart. However, it is clear that any of the electron transport chain components, any citric acid cycle dehydrogenase, ATP translocase or the phosphate transporter could become rate limiting with aging if their activities were sufficiently impaired. Use of *in situ* techniques for identifying specifically impaired activities will be discussed.

2

CELLULAR MODELS FOR THE STUDY OF THE ROLE OF MITOCHONDRIAL DNA MUTATIONS IN AGING. K.A. Laderman*, J. Penny & G. Attardi, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

A considerable amount of evidence in recent years has given support to the idea that, during the life of an organism, mitochondrial macromolecules undergo progressive damage by oxygen radicals, with cumulative deleterious consequences (1-3). In particular, it has been hypothesized that accumulation of mitochondrial DNA (mtDNA) mutations, mostly derived from oxidative damage, is a major contributor to aging and degenerative diseases (4,5). Support for this suggestion has recently come from biochemical, histochemical and immunohistochemical evidence of a progressive deterioration with aging of the respiratory capacity of different human tissues, as well as from the demonstration of aging-related mitochondrial DNA damage in the form of large deletions, small deletions and insertions, and oxidative adducts of DNA. However, it has been impossible so far to relate directly the aging-related respiratory decline to mitochondrial DNA damage.

Recent advances in mammalian mitochondrial genetics have provided novel techniques with the potential to directly correlate mtDNA lesions with diseases and aging. In particular, a powerful experimental approach has been developed in this laboratory (6,7), that utilizes the fusion with human cell lines completely devoid of mtDNA of enucleated cells (cytoplasts) or naturally occurring anucleated cell derivatives, platelets, from appropriate human donors. This approach allows the phenotypic effects of mitochondrial gene expression to be observed in a constant nuclear background.

In the past few years, the approach of repopulation with exogenous mitochondria of mtDNA-less (ρ°) cells has been extensively applied in this laboratory to the study of the role of mtDNA in aging, using as mitochondria donors fibroblasts from individuals spanning a broad range of ages. Transmitochondrial cell lines were derived from 31 individuals ranging in age from 20 weeks fetal to 103 years. To obtain a representative sampling, 7 to 31 clones were isolated from each cytoplast x ρ° cell fusion, i.e., a total of 519 transformants. To screen rapidly for possible mtDNA lesions, the growth rate, the cellular respiration rate and the mtDNA content of each transformant were determined 8 to 18 weeks after fusion. A large variation in oxygen consumption rate was observed among the clones derived from the same individual. Since, among the 158 transformants derived from individuals 20 week fetal to 37 years of age, no clone exhibited a rate of oxygen consumption lower than

1 fmol/min/cell, this level was chosen as a cut-off to identify clearly respiratory-deficient transformants. Among clones derived from individuals 39 to 103 years of age, 15 of 361 transformants appeared to be respiration deficient. Furthermore, conventional and nonparametric statistical analysis showed a highly significant decrease with cell donor age in the respiration rates of the 519 clones. In other analyses, a significant age-dependent decline of the mtDNA content of the clones was observed, without, however, a significant correlation between decrease in oxygen consumption rate and decrease in mtDNA content of the defective transformants. These observations suggested the occurrence in the fibroblast-derived transformants of two independent age-related functional alterations of mtDNA.

Currently, experiments of transfer of mitochondria from defective transformants into a secondary ρ° cell line different from the primary ρ° acceptor are being carried out, to confirm the mtDNA origin of the observed aging-dependent respiratory deficient phenotype. A new rapid screening method is also being developed, that will allow the analysis of the respiratory capacity of the transformants sooner after the mitochondria transfer and using a lower number of cells. Furthermore, a technique is being optimized for the fusion of mtDNA-less cells with human cortex-derived synaptic endings (synaptosomes). Synaptosomes are known to contain mitochondria, making them a convenient source of material for the study of the role of mtDNA mutations in brain cells in degenerative diseases and aging.

References:

1. Miguel, J., Economos, A.C., Fleming, J. and Johnson, J.E., Jr. (1980) Exp. Geront. **15**, 575-591.
2. Harman, D. (1981) Proc. Natl. Acad. Sci. **78**, 7124-7128.
3. Shigenaga, M.K., Hagen, T.M. and Ames, B.N. (1994) Proc. Natl. Acad. Sci. **91**, 10771-10778.
4. Linnane, A.W., Marzuki, S., Ozawa, T. and Tanaka, M. (1989) Lancet March **25**, 642-645.
5. Miguel, J. (1992) Mutation Res. **275**, 209-216.
6. King, M.P. and Attardi, G. (1989) Science **246**, 500-503.
7. Chomyn, A., Lai, S., Shakeley, R., Bresolin, N., Scarlato, G. and Attardi, G. (1994) Am. J. Hum. Genet. **54**, 966-974.

3

REGULATION OF MITOCHONDRIAL DNA REPLICATION AND TRANSCRIPTION. D. Lee & D.A. Clayton*, Beckman Center for Molecular and Genetic Medicine, Stanford University of Medicine, Stanford, CA 94305-5427.

Recent advances in detailing both *cis*- and *trans*-acting elements involved in mtDNA replication point to important features held in common in evolution. These include the facts that origin sequences are small and reasonably well defined, although primary template sequence is not highly conserved. Known and putative origins are located adjacent to active promoters, and it is known (or likely) that leading-strand synthesis is primed by RNA species generated from primary transcripts. In contrast, lagging-strand synthesis requires the action of a DNA primase.

We have purified to homogeneity and have available in constructs amenable to genetic analysis the essential components of the yeast mitochondrial basal transcription system. In the case of human mitochondria the same is true of the major, and perhaps only, transcriptional accessory protein. A mutational analysis of this protein has revealed the importance of the carboxyl terminus in activating transcription.

Transcripts initiated by mtRNA polymerase at putative yeast mtDNA origins remain stably associated with the DNA template from which they have been transcribed. In turn, we have not tested for stable RNA-DNA hybrid formation at the documented leading-strand (heavy-strand) origin of human mtDNA replication. Advantage has been taken of the availability of recombinant h-mtTFA (whose major functional regions have now been identified) and a new procedure for isolating Rnase-free human mtRNA polymerase. The data indicate that RNA-DNA hybrids are formed at the human mtDNA origin, at a frequency predicted from the prior data from the yeast system. This finding permits a more refined model for the initiation of leading-strand mtDNA replication.

Rnase MRP is a ribonucleoprotein complex previously shown to have specific catalytic activity on single-strand RNA substrates containing sequences of the leading-strand origin (O_H) of vertebrate mitochondrial genomes. Additional information has come from the above observation that stable hybrids between the nascent RNA and the DNA template are formed by a transcription-mediated mechanism at the yeast and human control regions. This finding suggested a defined model substrate for the processing activity postulated to be responsible for generating primers. We have assembled *in vitro* a stable complex of RNA on closed circular plasmid DNA encoding the mouse mitochondrial leading-strand control region. The RNA strand of the hybrid is site-specifically cleaved by mammalian Rnase MRP.

The reaction products contain 3'-hydroxyl termini and the positions of the cleavage sites correlated with the previously mapped RNA to DNA transitions detected at the mouse control region. Thus, this *in vitro* model system appears to reproduce the essential features of *in vivo* RNA processing events, supporting a major role for Rnase MRP in controlling primer RNA metabolism for mtDNA synthesis at the vertebrate leading-strand origin of replication.

4

MITOCHONDRIAL FUNCTION IN THE AGING HEART. R.G. Hansford* & S. Pepe, National Institute of Health, National Institute of Aging, Baltimore, MD 21224.

We have examined the interaction between aging and dietary lipid on mitochondrial (mito) Ca^{2+} content and the activation of Ca^{2+}-dependent mito dehydrogenases in isolated, perfused rat hearts. The reasons for interest were two-fold. First, one of us (S.P.) had previously shown that feeding saturated fat (SAT) gave rise to less efficient work performance by the heart (measured in terms of external work performed per O_2 consumed) compared to feeding fish oils (FO) rich in Ω-3 polyunsaturated fatty acid: the difference was obliterated by ruthenium red, implicating excess Ca^{2+} cycling at the level of the mito membrane in the SAT. Secondly, we have previously shown in studies with isolated heart mito that rates of the separate uptake and release pathways were both diminished in senescence and these decrements were not associated with any generalized decrease in substrate oxidation or energy conservation. However, it was not clear from this study what the effect of aging would be on the net accumulation of Ca^{2+} by the mito *in situ* in the heart and hence on the activation of the Ca^{2+}-dependent intramitochondrial dehydrogenases. In the present work, male Wistar rats were fed diets rich in FO or SAT for 6 weeks prior to sacrifice at the age of six months or 24 months. Hearts were perfused using the Langendorf protocol with buffer containing 1.5 mM Ca^{2+} and 11 mM D-glucose plus 0.2 mM octanoate as oxidizable substrates. The active, dephospho form of pyruvate dehydrogenase (PDH_A) was measured in freeze-clamped ventricles extracted in a buffer which inhibits kinase/phosphatase activity: PDH_A levels increase with mito matrix free Ca^{2+} (R. Moreno-Sanchez and R. G. Hansford, [1988] Biochem J. 256:403-412). Mitochondria were also isolated from a portion of the ventricles in a rapid protocol which minimizes net uptake or loss of Ca^{2+}. In some experiments, hearts were subjected to 15 minutes of low-flow ischemia, followed by 5 minutes of reflow. It was found that PDH_A content was higher in SAT than in FO, when hearts were stimulated with 1μM norepinephrine to give positive inotropy. After ischemia/reperfusion, PDH_A was much higher in SAT than in FO, and the effect of diet became even larger at 24 months. Perfusion with ruthenium red, which inhibits mito Ca^{2+} uptake, lowered PDH_A and abolished dietary and age-linked differences. Measurement of total Ca^{2+} in rapidly-isolated mito by atomic absorption spectrophotometry, showed a pattern of results similar to those of PDH_A content: viz, mito. Ca^{2+} was raised by norepinephrine to a greater extent in SAT than in FO, with the dietary effect amplified at 24 months. In ischemia/reperfusion, the largest gain in mito Ca^{2+} was in the 24 month SAT group, with the FO 24 month group showing a very marked attenuation. Ruthenium red enforced the same, low level of mito Ca^{2+} throughout. It is concluded that aging potentiates net uptake of Ca^{2+} by mito in the heart in response to these relatively mild positive-inotropic and ischemia/reperfusion paradigms and that this tendency is exaggerated by the SAT and attenuated by the FO diet. In the range of mito Ca^{2+} contents achieved in this study, the physiological range, an increase in Ca^{2+} gives increased dehydrogenase activity and hence potential for energy transduction. However, raised rates of Ca^{2+} cycling across the mito membrane and increased proton leaks due to elevated protonmotive forces may well lower thermodynamic efficiency under conditions giving raised mito Ca^{2+}. Further, the tendency towards increased net Ca^{2+} uptake seen in SAT, and particularly 24 month SAT hearts, likely will predispose them to more damage during more extended ischemia/reperfusion paradigms than that used here.

5

FREE RADICAL THEORY OF AGING: ALZHEIMER'S DISEASE PATHOGENESIS. D. Harman, Dept. of Medicine, University of Nebraska College of Medicine, Omaha, NE 68198-4635.

Senile dementia of the Alzheimer's type (SDAT) is the major cause of dementia. SDAT cases can be divided into two groups: 1) late onset, after about age 60, 90-95 percent of cases; largely non-familial, i.e., sporadic, 2) early onset, before about age 60; 5-10 percent of cases, most, if not all, are familial. It is a systemic disorder whose major manifestations reflect loss of neurons involved in memory. These neurons seem to be aging at a faster than normal rate, for the same patterns of loss are seen in normal individuals at later ages.

It is hypothesized that SDAT is caused by increased free radical reactions levels in the brain neurons associated with SDAT that advance in time patterns of neuronal dysfunctions and cell loss. Measures to this end include: 1) mutations in

mitochondrial (mt) DNA and/or nuclear (nuc) DNA in a somatic cell early in development that adversely affects mitochondrial function, 2) mutations in maternal mtDNA and/or nucDNA that impair mitochondria in offspring, 3) mutations in the amyloid precursor protein (APP), and 4) increased formation of both normal APP and superoxide dismutase levels in the involved neurons.

6

IMPAIRMENT OF MITOCHONDRIAL FUNCTION IN HEART TISSUE OF AGED RATS. G. Paradies*, F.M. Ruggiero & G. Petrosillo, Dept. of Biochemistry and Molecular Biology and CNR Unit for the Study of Mitochondria and Bioenergetics, University of Bari, Bari, Italy.

Aging is a complex biological process associated with a progressive decline in physiological and biochemical performance of individual tissue and organs. Aging has a profound effect on cardiac performance. The well known age-dependent decrement in heart performance may be related to changes in the activity and in the properties of several mitochondrial protein and enzymatic systems involved in energy metabolism. Mitochondrial anion transport proteins are responsible for the flux of metabolites that occur across the inner mitochondrial membrane. Cytochrome oxidase is the terminal enzyme complex of the mitochondrial electron transport chain responsible for virtually all oxygen consumption in mammals. The normal functioning of these membrane-associated proteins is essential for the bioenergetics of eucariotic cell. The effect of aging on the transport activity for the pyruvate, phosphate and ADP carriers, as well as on the cytochrome c oxidase activity in rat heart mitochondria was investigated. The activity of all these protein systems is reduced with aging. This reduced activity is not due to a lower protein content in the membrane. Cardiolipin is known to be essential for the optimal functioning of the pyruvate, phosphate and ADP carriers, as well as the cytochrome oxidase activity. The phospholipid composition of mitochondrial membranes from young and aged rats was analyzed. Among the phospholipid species analyzed, the greatest alteration was found in the cardiolipin, the level of which was markedly reduced with aging. Thus, the age-dependent decrement in the activity of these protein systems may be attributed to a lower cardiolipin content in the membrane. Treatment of aged rats with acetyl-1-carnitine, a natural biomolecule which acts by stimulating cellular energy metabolism, was able to restore the content of cardiolipin, thereby restoring the activity of the anion carrier proteins and that of cytochrome oxidase, to the level of young rats. The observed age-dependent decline in these membrane associated proteins activities may play an important role in the etiopathology of the declining cardiac competence with aging.

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7

SUPPORT OF THE MITOCHONDRIAL OXIDATION THEORY OF CELL AGING IN MOUSE LYMPHOCYTES. M. De La Fuente, Facultad de Ciencas Biologicas, Universidad Complutense, 28040 Madrid, Spain, J.E. Fleming, Eastern Washington University, Cheney, WA 99004, and J. Miquel, Facultad de Medicina, Universidad, 03080 Alicante, Spain.

Our finding of age-related mitochondrial injury (with resulting age pigment accumulation) in the fixed postmitotic cells of *Drosophila* and mammalian testis led us to propose that aging may derive from oxyradical damage to the mitochondria of differentiated cells, including mutation of mitochondrial DNA (Miquel, J. et al., Exp Geront. 15:575, 1980; Fleming, J.E., Gerontology 28:44, 1992). More recently, this concept of intrinsic mitochondrial mutagenesis and concomitant bioenergetic decline has been integrated with the classic views of Minot and Pearl on the role of cell differentiation and metabolic rate. This unified theory, which shares concepts of both programmed and wear-and-tear hypotheses of aging, provides a logical explanation of senescent changes from the molecular to the systemic levels (Miquel, J. Mut. Res. 275:209, 1992; Miquel, J and Blasco, M., Facts. Res. Geront. 8:28, 1994; Miquel, J. and De Le Fuente, M. Age 15:136, 1992).

The above views are supported by our present work which shows age-related degeneration of mitochondria, with accumulation of lipofuscin, in mouse lymphocytes cultured *in vitro*. Moreover, our data show that these changes are less striking in lymphocytes cultured in medium supplemented with glutathione. Also, administration of thioproline to the mice counteracts the senescent decline of their lymphocytes, as shown by preservation of mobility and lymphoproliferative activity. Finally, preliminary clinical studies suggest that the immune competence of aged women is improved by the daily intake of

vitamin C (1 gram) and vitamin E (200 I.U.). These findings justify further work on free radical and antioxidant mechanisms in relation to mitochondrial aging and immunosenescence. (This work has been supported, in part, by grants FISS 94/1348 and 95/1623 from Fondo de Investigaciones Sanitarias, Spain, the Glenn Foundation for Medical Research, and the Northwest Institute for Advanced Studies).

8

EFFECT OF PHENOLIC ANTIOXIDANTS ON LIFESPAN AND MITOCHONDRIAL FUNCTION IN *DROSOPHILA MELANOGASTER*. J.E. Fleming*[1,2], P. Ruchert[2], M. Thurston[2] & C. Taylor[2], 1) Institute of Molecular Medical Sciences, Palo Alto, CA, 94306; 2) Dept. of Biology, Eastern Washington University, Cheney, WA 99004.

The effect of ellagic acid (EA), propyl gallate (PG), catechin (CT), and esculin (EC) on lifespan, respiration, negative geotaxis, and mitochondrial respiratory activity were examined in male imagos of *Drosophila melanogaster*. The phenolic antioxidants EA, PG, CT, and EC were provided in the diet at 0.3% beginning 5-7 days post emergence of the adult flies. No significant difference was observed for respiration among the various antioxidant-fed groups throughout their lifespan. The physical activity of adult flies as measured by a negative geotactic response was slightly reduced in the first third of life for flies fed esculin, ellagic acid, and catechin but not for propyl gallate. In the case of catechin and esculin, flies were more active in the latter half of life. The physical activity of insects fed ellagic acid and propyl gallate were not significantly different from controls in the latter half of life. Mitochondrial ADP/O ratios and respiratory quotients were not significantly different at the beginning of life for the various groups. Both mean and maximum lifespans were significantly increased for flies fed ellagic acid and propyl gallate. Catechin had no effect on the adult lifespan and esculin significantly reduced the lifespan. The mean (SEM) and maximum lifespans in days of the various groups were: Controls, 72.5 (0.8), 86; Esculin, 54.3 (1.3), 77; Catechin, 74.6 (0.9), 94; Ellagic acid, 87.3 (0.8), 96; Propyl Gallate, 82.8 (0.8), 100.

These data suggest that the phenolic antioxidants propyl gallate and ellagic acid delay senescence in *Drosophila* through their protective effects against oxidative damage. Flies fed ellagic acid and propyl gallate have reduced levels of oxidized proteins as measured by carbonyl formation. Ellagic acid is a potent antimutagen and anticarcinogen and has been shown to significantly reduce the level of lipid peroxides induced by carbon tetrachloride. (This work was supported, in part, by the Glenn Foundation for Medical Research and The Northwest Institute for Advanced Studies).

9

THE EFFECTS OF AGING AND PHYSICAL EXERCISE ON MITOCHONDRIAL DISTRIBUTIONS IN HEARTS OF MALE RATS. A.C. Cosmas* & T.G. Manfredi, University of Connecticut, School of Allied Health Professions, Storrs, CT 06269; University of Rhode Island, Exercise Science Program, Independence Square Building, Kingston, RI 02881-0810.

Physical activity has been reported in enhance mitochondrial activity in heart muscle and little is known about the interactive effects of exercise and age on mitochondrial size distributions. The purpose of this study was to examine the effects of physical exercise and age on mitochondrial profiles from specific regions within the left ventricular apex. Twenty male rats of the Charles River strain were subjected to 712 days of physical training on a motor-driven treadmill. Detailed electron microscopic examination of the left ventricular apex was performed in order to obtain mitochondrial size distributions within perinuclear, myofibrillar and cell border regions in trained and control rats of 50, 195, 285, 356, 450, and 1050 days of age. The data indicate that as a function of maturation there seems to be a shift in mitochondrial 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, particularly within the perinuclear and myofibrillar regions of the myocardium. In addition, there appears to be no startling differences between trained and non-trained rats with respect to numbers of mitochondria, but that an intensification of the shift observed initially with maturation occurs in the myocardium of trained rats. This intensification has been attributed to physical training which apparently acts as an induction mechanism in which a subcellular response is manifested by the generation of an increased number of smaller mitochondria. It seems plausible to suggest that the decrease in mitochondrial size seen as a function of maturation and enhanced by physical training, would tend to increase the surface-to-volume ratio which might be an indication of increased physiological function. If a relationship does exist between the surface-to-volume ratio and oxidative capacity, the appearance of a greater

number of smaller mitochondria would be expected in the myocardium of trained rats.

The mitochondrial alterations concomitant with physical exercise may represent a fundamental adaptive mechanism that is associated with long periods of increased cardiac output and may act as a compensatory measure for a tendency toward ischemia under such conditions as stress. Chronic exercise apparently produces an increase in the cardiac workload which is met by subcellular adaptations -- a shift in the size distributions of the mitochondria to the sizes which can most efficiently compensate for the functional overload.

10

CHARACTERIZATION OF MITOCHONDRIAL DNA (mtDNA) MUTATIONS IN AGING SKELETAL MUSCLE. E.A. Shoubridge, Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4.

We characterized mtDNA mutations in skeletal muscle of a group of older patients with late onset skeletal myopathy, as well as in age-matched and disease controls. Multiple mtDNA deletions were demonstrated by PCR analysis in all older individuals but not in younger controls. Although the so-called "common" deletion was found in most patients, the majority of deletion breakpoints, analyzed by DNA cycle sequencing, were not flanked by direct repeat sequences (Class II deletions). *In situ* hybridization analysis of the muscles of the patients demonstrated accumulations of mRNAs transcribed from deleted mtDNAs in a relatively large number of fibers. The activity of cytochrome c oxidase, the terminal enzyme in the electron transport chain, was reduced or absent in these fibers. These data suggest an age-related accumulation of mtDNA deletions in skeletal muscle that reflects the clonal expansion of different deletions in individual muscle fiber segments. This leads to a biochemical phenotype at the cellular level, and we suggest, to a clinical myopathy once the number of affected muscle fibers exceeds a particular threshold.

11

***IN SITU* APPROACHES TO MITOCHONDRIA AND AGING.** S. Melov*, D.A. Hinerfeld & D.C. Wallace, Dept. of Genetics and Molecular Medicine, Emory University School of Medicine, Atlanta, GA 30322.

Determination of changes in mitochondrial function in human disease have traditionally been carried out through enzymology, histochemistry, and molecular characterization. Mitochondrial changes in such diseases as Kearns/Sayre syndrome (KSS) and chronic progressive external ophthalmoplegia are well characterized and dramatic in nature. We have used muscle tissue from a patient with KSS to develop new methods for visualization of mitochondrial changes within muscle fiber. Changes in mitochondrial function as a result of age are typically less obvious and require an extension of the methodologies used in human disease diagnosis. Volumetric three dimensional reconstruction techniques as well as surface/contour based methods were used to visualize mitochondrial changes within mouse brain and muscle with age. Further, we have utilized a mouse knockout of mitochondrial superoxide dismutase to show effects in brain tissue of high levels of free radicals within the mitochondrial with contour based techniques.

12

ASSESSMENT OF OXYGEN RADICALS, RADICAL SCAVENGERS AND MODULATION OF MITOCHONDRIAL INJURY. J. D. Crapo, Duke University Medical Center, Durham, NC 27710.

During the formation of hyperoxic lung injury, the generation of oxygen radicals by mitochondria in lung cells plays a critical role. Dramatic ultrastructure changes occur in both the shape and volume of mitochondria in lung cells, particularly the alveolar type II epithelial cell, in response to hyperoxia. Transgenic animals which overexpress the manganese superoxide dismutase in the mitochondria of alveolar type II epithelial cells demonstrate marked protection against hyperoxic-induced stress. This cell is critical for regeneration of the alveolar epithelium, secretion of surfactant and for the secretion of extracellular superoxide dismutase which plays a critical role in reducing overall inflammation in the lung. Administration of an extracellular superoxide dismutase to the lungs of animals exposed to hyperoxia also dramatically reduces hyperoxic lung injury. The role of both extracellular antioxidants and mitochondrial antioxidants in reducing oxidant stress on lung cells will be explored. Each of the superoxide dismutases found in mammalian tissues are localized to specific compartments. Each plays a critical role in balancing the overall response to injury. By selectively perturbing the antioxidant balance in various compartments within the lung, the role and importance of both mitochondrial and extramitochondrial antioxidants can be determined. These data will help design strategies for the treatment and prevention of oxidant-induced tissue injury.

13

SOMATIC CELL GENETIC APPROACHES TO AGE-RELATED mtDNA ALTERATIONS IN BRAIN. I. Trounce*, S. Melov & D.C. Wallace, Dept. of Genetics and Molecular Medicine, Emory University School of Medicine, Atlanta, GA 30322.

We have developed a method to place into culture mtDNAs from young and old human and mouse brains. Synaptosomes are isolated from fresh post-nuclear brain homogenate with Percoll step gradients and fused by electrofusion to mtDNA-less (p^o) cells. Electron microscopy of synaptosome fractions showed an abundance of synaptosomes with intact membranes and usually containing one to several mitochondrial profiles. Synaptosomes isolated from fresh human brain (0.1-0.5 g) obtained from neurosurgical procedures were fused with human osteosarcoma p^o cells (143 BTK-), yielding approximately one stable cybrid clone per $5x10^4$ p^o cells used. In similar experiments with freshly isolated mouse synaptosomes fused with a mouse LMTK- p^o cell line, cybrids are obtained at a frequency of around one per 10^4 p^o cells used. Attempts to use post-mortem human brain samples have been less successful, and experiments with the mouse cell system suggest that delay in chilling and isolating the synaptosome fraction drastically reduces viability as mitochondrial carriers. Using this system we expect that cybrid clones should re-populate with mtDNA from just one or a few mitochondria from single neurons. We can then test the prediction that age-related mtDNA mutations may accumulate differently in different post-mitotic cells, and investigate functional consequences on oxidative phosphorylation of any mutations found.

14

INTRODUCTION TO *IN SITU* PCR. J. Pfeifer, Dept. of Genetics and Molecular Medicine, Emory University School of Medicine, Atlanta, GA 30322.

Polymerase Chain Reaction (PCR) is a method used to amplify specific genetic sequences. Most PCR is performed on nucleic acids extracted from biological sources. However, the technique of *in situ* PCR allows researchers to amplify nucleic acids in intact cells and tissue. Perkin Elmer has recently introduced a system for more efficient containment of samples on microscope slides and subsequent thermal cycling for *in situ* PCR. Sample results from laboratories using this system will be shown.

15

ACETYL-L-CARNITINE ADMINISTRATION MODULATES THE ULTRASTRUCTURAL PLASTICITY OF SYNAPTIC MITOCHONDRIA IN OLD RATS. Carlo Bertoni-Freddari*, Patrizia Fattoretti, Ugo Caselli and Roberta Paoloni, Neurobiology of Aging Laboratory, "N. Masera" Research Dept., 60121 Ancona, Italy.

Our previous studies have documented that the morphological deterioration of synaptic contact areas in the dentate gyrus supragranular layer of old rats can be consistently recovered by long term administration of acetyl-L-carnitine (ALCAR) (Brain Res. 656: 359-366, 1994). We supported that the positive action of ALCAR may be due to an improvement of neuronal metabolism. To check this assumption a computer-assisted morphometric investigation has been carried out on the ultrastructural features of dentate gyrus synaptic mitochondria in rats of 6, 13, 25 months of age and in 25 month-old animals treated with ALCAR at a daily dose of 50 mg/100g body weight from the age of 1 month up to the day of sacrifice. The volume fraction of the mitochondria/μm^3 of tissue (Volume density: Vv), the number of mitochondria/μm^3 of tissue (Numerical density: Nv), the average mitochondrial volume (V) and length (Skeleton: Sk), the surface density of cristae+inner membrane/μm^3 of mitochondrion (Svc) and the average length (L) of the cristae were the parameters measured by means of a semiautomatic procedure applying currently used morphometric formulas. In old animals we found a significant reduction of Vv and Nv. V was unchanged between 13 and 25 month-old animals, but it was significantly smaller vs 6 month-old group. Sk and Svc did not change with time. L was the same at 13 and 25 months of age, but was significantly longer vs the 6 month-old rats. The treated group showed an increase of Vv, Nv and L, while V, Sk and Svc were unchanged vs 13 and 25 months of age. L was significantly longer comparing treated and 6 month-old animals. The mitochondrial population taken as a model for the present investigation subserves the energy needs at the cholinergic synaptic regions of the dentate gyrus supragranular layer, an area of the hippocampal formation currently reported to be a preferred target for age-related deteriorations. We interpret the changes found in old rats to represent a morphofunctional decline in the plastic response of the synaptic metabolic hardware to the alterations due to time. Chronic administration of ALCAR has a modulating effect on the morphological plasticity of the organelles present at the hippocampal cholinergic terminals and results in a significant recovery of the mitochondrial ultrastructural parameters altered with aging.

16

AGE-CHANGES OF EPR SIGNALS AND FREE-RADICAL PRODUCTION IN MITOCHONDRIA. V.K. Koltover, N. Semenov Institute of Chemical Physics of RAN, Moscow Region, 142432, Russia.

Up to date, low-temperature EPR spectroscopy has shown its great power as the most direct instrument in the definition, often discovery, of free radicals and paramagnetic metal ion components in highly integrated whole-tissue preparations of all origins. We examined the effects of the life-prolongation substance, SKN-carbon enterosorbent, on the EPR signals in tissues of adult (4-6 months) and old (24-26 months) male Wistar rats. The mitochondrial signals of iron-sulfur proteins (g=1.94), semiquinone free radicals (g=2.003), and Mo-sulfite oxidase (g=1.97), as well as the endoplasmic signals of cytochrome P-450 (g=2.25), Mn-proteins, and nitrosyl complexes of iron (g=2.035) in liver of old rats are lower in their intensities compared to the similar signals of adult animals. SKN remained the sulfite oxidase signal of adult rats without change while diminished the other signals. The changes in intensity of the EPR signals reflect the changes in activity of the relevant enzymes. Furthermore, the effects of a single administration of another life-prolongation substance, antioxidant butylated hydroxytoluene (BHT), on the EPR signals in heart and blood of the rats were studied. The decrease of the signal of iron-sulfur proteins of heart mitochondrial electron-transport chains occurs during the first 6 h after BHT injections while the free-radical semiquinone signal tends to increase. Since the intensity of the signal of iron-sulfur proteins is directly proportional to the amount of these proteins in the reduced form, it means that BHT increases oxygenation of the heart tissue. In the spectra of the animals' blood, the ratio of the intensity of the signal of transferrin (g=4.3) to that of ceruloplasmin (g=2.05) decreased after the injections of BHT. Moreover, the BHT-induced EPR signal of NO-hemoglobin complex arose in the animals' blood. The intensity of this signal was lower in the case of old rats. The data of our recent papers clearly demonstrated that hypoxia/ischemia impairs mitochondrial membranes and makes a drastic increase in production of the superoxide by-products of respiration. The hypoxia/ischemia conditions make increase in reactivity of the mitochondrial ubisemiquinone radicals (SQ·) to oxygen as it was proved by following intensity of the EPR signals of the redox-cycling SQ· (Nohl et al, 1992). The loss of control of the electron flow through SQ· correlates with the increase of membrane lipid fluidity measured by the spin-label method (Koltover, 1995). As hypoxia/ischemia triggers superoxide release, BHT appears to perform indirect antioxidative protection by means of fostering the extent of oxygenation of heart cells via NO-cGMP-mediated mechanisms.

17

EFFECTS OF MITOCHONDRIAL RESPIRATION ON MEMBRANE LIPID AND PROTEIN OXIDATION: AN ELECTRON PARAMAGNETIC RESONANCE (EPR) INVESTIGATION USING RAT BRAIN SYNAPTOSOMES. S.P. Gabbita,* R. Subramaniam, D.A. Butterfield, J.M. Carney, Graduate Center of Toxicology, Center of Membrane Sciences and the Dept. of Chemistry, University of Kentucky, Lexington, KY 40506.

Previous studies have implicated mitochondria in both the aging process and age-related diseases. It has been established that the generation rate of reactive oxygen species (ROS) like O_2 *-, OH* and H_2O_2 increases with age. These ROS also have been implicated in the pathogenesis of neurodegenerative diseases like Alzheimer's, Parkinson's, etc. The increased mitochondrial respiration driven by succinate (through complex II of the electron transport chain) is found to result in the generation of ROS which can combine with spin trapping agents and is detected by EPR. The current study was designed to determine if mitochondrial respiratory stimulation by succinate caused extensive oxidative damage to membrane cytoskeletal proteins and lipids in the brain. Young (6 months) Brown Norway female rat brain cortex or cerebellum homogenate containing a mixture of synaptosomes and mitochondria were prepared by centrifugation techniques. Stimulation of succinate dehydrogenase (complex II) of the mitochondria was accomplished using 20 mM succinate at 25°C for 3 hours. EPR spin labeling was performed on resulting membranes from the preparation using the lipid-specific spin label, 5-nitroxyl stearate (5-NS). This spin label acts as the reporter molecule and changes in the EPR signal amplitude is indicative of the oxy-free radical damage to the membrane lipid microenvironment. Changes in the physical state of cytoskeletal proteins due to succinate respiration-induced oxidation were measured by using MAL-6 (2,2,6,6,-tetramethyl-4 maleimidopiperdin-l-oxyl), a thiol-specific nitroxyl spin label. MAL-6 binds to thiol groups of cytoskeletal proteins in weakly (W) or strongly (S)- immobilized sites. The ratio of the amplitudes of the weak to strongly immobilized spin label reaction sites (W/S ratio) is used to determine any alteration in protein conformation. Previous studies in our laboratory have established that a decreased W/S ratio is associated with increased protein oxidation. Our results indicated significant lowering of the W/S ratio in both cerebellum (31% decrease, $p<0.0001$) and cortex

(30% decrease, $p<0.0001$) upon stimulation of the mitochondria with 20 mM succinate. Significant decreases were also found in the amplitudes of 5-NS spin labeled cerebellum (83% decrease, $p<0.002$) and cortex (80% decrease, $p<0.0001$). In related studies, we also found that there was a significant lowering ($p<0.05$) in the order parameter (an EPR measure of membrane fluidity) of the 5-NS spin labeled synaptosomes with increasing succinate stimulation of the mitochondrial electron transport chain. Such a decrease in membrane fluidity may possibly be attributed to the formation of polar lipid hydroperoxides in the membrane bilayer due to reaction with ROS. Thus, we conclude that respiratory stimulation of mitochondria with succinate generates oxy-free radicals which result in significant membrane lipid and protein oxidation. Supported in part by NIH (AG-10836).

18

LOCALIZATION OF AGE-RELATED β-ADRENERGIC SIGNAL TRANSDUCTION DEFICITS IN CEREBELLAR PURKINJE CELLS FROM F344 RATS. T.J. Gould*[2] & P.C. Bickford[1,2], [1]VAMC; [2]Dept. of Pharm. Univ. Colorado, Health Sciences Ctr., Denver, CO 80262.

In aged F344 rats the ability of β-adrenergic receptors to modulate GABAergic inhibition of Purkinje cells is impaired. The age-related deficit in β-adrenergic function has been correlated with an age-related deficit in motor learning. Treatment of rats with either caloric restriction or N-tert-α-butyl-phenylnitrone (PBN) has shown ameliorative effects on age-related deficits in β-adrenergic function and/or motor learning. These results suggest free radical damage may contribute to the age-related functional and behavioral declines. The β-adrenergic receptor is a G_s-linked receptor in which the signal transduction mechanism involves adenylate cyclase activation of cAMP which activates PKA. The specific site (or sites) of the age-related deficit in the β-adrenergic receptor signal transduction cascade, however, is unknown. In order to identify the mechanisms underlying the age-related decline in β-adrenergic receptor function, this study examined the signal transduction cascade of Purkinje cell β-adrenergic receptors in 18-21 month old F344 rats. In anesthetized rats, cerebellar β-adrenergic receptor function was assessed by iontophoresis of GABA and the β-adrenergic agonist isoproterenol (ISO). Forskolin was used to directly activate adenylate cyclase and adenosine 3',5'-cyclic monophosphothioate (Sp-cAMPs) was used to activate PKA using pressure ejection. The ability of ISO and forskolin or Sp-cAMPs to modulate GABAergic inhibition was compared within rats and differences between forskolin and Sp-cAMPs were compared across rats. Initial results with forskolin show that ISO and Forskolin reveal similar age-related impairments in the ability to augment GABAergic inhibition. This suggests that the problem is located at or beyond the level of adenylate cyclase. Initial tests with Sp-cAMPs show that in Purkinje cells where ISO augments GABAergic inhibition, Sp-cAMPs has similar modulatory effects. Tests of Sp-cAMPs on cells with age-related deficits are underway. This approach allows for identification of specific points in the β-adrenergic transduction cascade affected by aging. This work was supported by USPHS grant AG04418, grant AG05686-01 and the VAMRS.

19

MITOCHONDRIAL PRODUCTION OF REACTIVE OXYGEN SPECIES. J.F. Turrens, Dept. of Biomedical Sciences, College of Allied Health Professions, University of South Alabama, Mobile, AL 36688.

The chemistry of oxygen is a direct consequence of its unusual electron distribution, having two unpaired electrons with the same spin in the outer layer. Thus, the complete reduction of oxygen to water (four electrons) must occur in four one-electron steps. This generates a variety of intermediates that are usually called reactive oxygen species (ROS). These partially reduced oxygen derivatives include superoxide anion ($O^{\cdot}_2$, the product of one electron reduction), hydrogen peroxide (H_2O_2, after two electrons are transferred to oxygen) and hydroxyl radical ($\cdot OH + H_2O$, three electrons reduction). The latter species is extremely reactive and will oxidize any biological molecule, starting a chain reaction in which molecular oxygen acts as a propagator. This process is generally known as oxidative stress. Cells are protected against these species by several antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxide), as well as by low molecular weight antioxidants (i.e., vitamin E, β-carotenes and glutathione).

At least two of the electron carriers in the mitochondrial respiratory chain may directly transfer electrons to oxygen generating O_2, the enzyme NADH-dehydrogenase and ubisemiquinone. The rate of O_2 formation varies with the reduction level in the respiratory chain. For example, when mitochondria are actively synthesizing ATP, the chain becomes more oxidized and the formation of ROS decreases. If oxygen concentration increases, given the non-enzymatic nature of these reactions, the formation of ROS will increase linearly with oxygen. The basal rate of H_2O_2 formation also increases with aging.

In addition to oxygen derived oxidants; a naturally occurring oxidant derived from nitric oxide (NO, endothelial cell-derived relaxing factor) has recently been identified. NO reacts with intracellular O_2^- generating the ionized form of peroxynitric acid (peroxynitrite), a potent oxidant similar in reactivity to ·OH. Recent studies using isolated perfused rat lungs suggest that this species is responsible for most of the steady state oxidative stress in normal cells. We postulate that peroxynitrite may be formed in the mitochondrial matrix as a product of the reaction between mitochondrial O^-_2 and cytosolic NO diffusing into the mitochondrial compartment.

20

PARADOXES OF OXIDANT/ANTIOXIDANT BALANCE. J.M. McCord, Webb-Waring Institute, University of Colorado Health Sciences Center, Denver, CO 80262.

The study of free radical biology has created a great deal of controversy and apparently conflicting observations. This is especially true with regard to the use of the antioxidant enzyme superoxide dismutase as a protective or therapeutic agent.

21

OXIDATIVE STRESS MAY BE A CAUSAL FACTOR IN SENESCENCE. R.S. Sohal* and William C. Orr, Dept. of Biological Sciences, Southern Methodist University, Dallas, TX 75275.

There are several lines of evidence implicating oxidative stress as a significant contributor to the causation of senescence in animals. Direct evidence is based on studies in transgenic *Drosophila melanogaster*. Overexpression of Cu, Zn superoxide dismutase and catalase was found to: (i) extend life span of the flies by up to 34%, (ii) slow down the age-associated increase in the rate of generation of reactive oxygen species (ROS), (iii) decrease the accrual of molecular oxidative damage to DNA and proteins, (iv) decrease the susceptibility of tissues to acute oxidative stress, (v) increase the physical vigor of the flies, and, importantly, (vi) increase the metabolic potential, i.e., total amount of oxygen consumed during life, by about 30%. Indirect evidence supporting the predictions of oxidative stress hypothesis derives from comparative studies, which indicate that: (i) maximum life span potential (MLSP) in different mammalian and insect species is inversely correlated to the rate of mitochondrial ROS generation, the steady-state level of molecular oxidative damage, and the susceptibility to such damage, (ii) caloric restriction regimen in mice, and lowering the metabolic rate in flies reduce the level of oxidative stress and increase the life span of animals.

22

OXIDATIVE DAMAGE IN HUMAN AGING. M.Flint Beal, Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

A role for free radicals and oxidative damage is becoming increasingly compelling. Limited evidence however exists in man. We examined concentrations of 8-hydroxy-2-deoxyguanosine, a marker of oxidative damage to DNA, in both nuclear and mitochondrial DNA in postmortem cerebral cortex and cerebellum of individuals aged 42 to 97. An age-dependent increase was found in both nuclear and mitochondrial DNA. The amount of oxidative damage to mitochondrial DNA was 10 fold greater than that in nuclear DNA, and 15 fold greater in individuals older than age 70. This is consistent with other observations that mitochondrial DNA may be particularly vulnerable to oxidative damage. We also found age-dependent increases in the common 5 Kb mitochondrial DNA deletion which were greatest in the putamen. This is consistent with the work of several other groups. In human postmortem cardiac tissue mitochondrial deletions and 8-hydroxy-2-deoxyguanosine show age-dependent increases which correlate. A critical issue is whether age-dependent oxidative damage to mitochondrial DNA has functional significance. In aged rhesus monkey cerebral cortex we found age-dependent decreases in complex I and complex IV activities of the electron transport chain. Similar observations have been made in human muscle biopsies. In human neurodegenerative diseases oxidative damage may be exacerbated beyond that seen with normal aging. Oxidative damage to mitochondrial DNA was 3 fold increased in cerebral cortex of Alzheimer's disease patients as compared to age-matched controls. These observations support a role of oxidative damage to mitochondrial DNA in both normal human and in human neurodegenerative diseases.

23

MITOCHONDRIAL DNA DELETIONS: EFFECTS OF DIETARY RESTRICTION. C.M. Kang*, B.S. Kristal, & B.P. Yu, Dept. of Physiology, University of Texas Health Science Center, San Antonio, TX.

Mitochondria have been proposed as "biologic clock of aging." According to this view, the maintenance of mitochondrial integrity is a prime importance to organisms. It has been hypothesized that age-related degenerative processes may be related to

defects in mitochondrial DNA (mtDNA) and damaged mtDNA molecules, which accumulate with age. In the present study, we explored the extent of mtDNA deletion with age, and its attenuation by dietary restriction (DR). We determined the relative quantities of deleted mtDNA at different ages and measured the effects of DR by using the polymerase chain reaction (PCR) in rat liver. The livers were obtained from 6, 12, 18, and 24-month old male Fischer 344 rats. Dietary restriction was implemented by reducing the average food intake of ad libitum feeding by 40%. L-7689 and H-13753 primers were used to detect the deleted mtDNAs and L-15758 and H-117 were used to detect the constant region (D-loop) of mtDNA; the cycle times were 1 min denaturation at 94°C, 1 min annealing at 61°C, and 1 min extension at 72°C for 30 cycles.

PCR analysis of mtDNA revealed the presence of mutated molecules containing a deletion of 4834 bp, which encompasses the genes from ATPase 6 to ND5. The results show clearly that with advancing age, the rates of mtDNA deletions were progressively increased; mtDNA deletions were remarkably suppressed by dietary restriction.

Our data show the effective attenuation of mtDNA deletion by DR. In conjunction with other data from our laboratory, this work shows the potential ability of DR to maintain mitochondrial integrity as the animal ages.

24

OXIDANT-INDUCED MITOCHONDRIAL DYSFUNCTION IN DIABETES

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An important role for mitochondrial dysfunction in aging and age-related disease has been hypothesized, but it is difficult to directly address this role, especially since the pathways underlying progressive mitochondrial dysfunction remain unclear. Diabetes presents a useful system in which to address this question. Diabetes-associated mitochondrial deterioration has been well-documented, but the mechanisms underlying it remain poorly understood. In the current work, we conducted experiments on diabetes-induced mitochondrial alterations to address the etiology of mitochondrial abnormalities in diabetes and to provide a model with which to begin to examine the ways in which mitochondrial dysfunction might progress during the aging process. Rats were rendered diabetic with streptozotocin, then sacrificed approximately 3 months later; the following mitochondrial functions were assessed: 1) Transcription, 2) Oxidant-resistance, 3) Permeability transition, and 4) Respiratory parameters (State 3 and 4 respiration, RCR, ADP/O ratio). The results show that diabetes-associated loss of mitochondrial transcription is progressive, and can approach 95% in the most severely affected animals. Studies of the susceptibility of mitochondrial transcription to oxidant-inhibition and susceptibility of mitochondrial lipids to lipid peroxidation indicate that loss of gene expression is tightly associated with markers for exposure to oxidative stress. It was determined that the source of this oxidative stress was not a result of diabetes-associated abnormalities in mitochondrial membrane fluidity or in the calcium-regulated permeability transition (although changes in the transition do exist, they do not appear responsible for the increased oxidant damage). Mitochondria from the animals in this study show a 3-to-10-fold increase in uncoupled utilization of oxygen from mitochondria isolated from diabetic animals that occurs in the absence of overt loss of respiratory function in these mitochondria; this defect localizes at Center P of Q-cycle portion of Complex III in the electron transport system. These results provide a mechanistic explanation for the onset and progression of mitochondrial defects in diabetes; the data support the hypothesis that diabetes-associated radicals generated at Center P in Q-cycle portion of the respiratory chain. These results further provide a model to use in designing more critical approaches to the study of the effects of aging and age-related disease on mitochondrial function. The utility of this model is strengthened by the observation that some of the same changes observed in the diabetic are also observed in the aging rat.

25

REGULATION OF OXIDATIVE STRESS BY DIETARY RESTRICTION IN BRAIN: MICROGLIA AND BRAIN NITRIC OXIDE SYNTHASE.

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Free radicals are known to be involved in the aging process. It is also known that the anti-aging action of dietary restriction (DR) attenuates free radical damage and maintains anti-oxidant defense systems. We have proposed that the modulation of oxidative stress is the underlying mechanism responsible for the anti-aging action of DR. Our earlier data showed that age-related increases in serum total iron and ferritin concentration are attenuated by DR, a finding consistent with DR's antioxidative action. This report presents further

insights regarding the mode of modulation of oxidative stress and its beneficial action on neuronal function. Iron mediated reactive oxygen species (ROS) production plays an important role in pathogenesis of several age-related brain diseases. We focused on iron and ferritin containing microglia as the source of oxidative stress through ROS production in brain. We also examined nitric oxide (NO), a free radical which plays an important physiological role on neuronal function as a neurotransmitter. The brains removed from 6, 12, 18 and 24-month old male F344 rats from both ad libitum (AL) fed and 40% dietary restricted groups were utilized for this study. For quantitation of microglial cells, the frozen sections, including hippocampus, were used for ferritin immunohistochemistry and iron histochemistry. The lipid peroxidation level was assessed by measuring the malondialdehydes (MDA) production using hippocampus homogenate. To quantitate the amount of brain nitric oxide synthase (bNOS), the cytosol fraction from hippocampus was used for Western blot analysis. Our results show that the number of iron and ferritin containing microglial cells correlated with increased lipid peroxidation level with age. Dietary restriction suppressed these age-related changes. AL rats showed slight decreases in bNOS with age. Although the amount of bNOS was lower in DR group than AL group at 6 months, DR up-regulated bNOS and maintained a slightly higher level than the AL group in aged brain. Our findings suggest the possibility that iron mediated ROS production in microglia may play a major role in age-related deterioration in brain; DR has antioxidative action in maintaining iron homeostasis; and bNOS may be important in preserving the neuronal function in brains of aged DR rats. The significance of our work shows that the beneficial effects of dietary restriction on neuronal function in aged brain may relate to the tight regulation of the free radicals' dual function, to suppress microglial iron mediated ROS production and maintain the NO level as a neurotransmitter.

26

EFFECT OF AGE ON T CELL-SPECIFIC AND NON-SPECIFIC TRANSCRIPTION FACTORS IN LYMPHOCYTES FROM F344 RATS. M.A. Pahlavani, M.D. Harris & A. Richardson, GRECC, Audie L. Murphy V.A. Hospital & Dept. of Medicine, University of Texas Health Science Center, San Antonio, TX 78284.

The expression of interleukin-2 (IL-2) gene has been found to decrease with age. Recently, it has been shown that the transcription of IL-2 is under control of T cell-specific transcription factor, the nuclear factor of activated T cells (NFAT) and T cell non-specific transcription factors (AP-1, NF-kB, and OCT-1). Therefore, in this study we have examined the induction of NFAT, AP-1, NF-kB, and OCT-1 binding activity in spleen lymphocytes isolated from young (6 months) and old (24 months) rats. Cells were stimulated with concanavalin A (con A) for various times and the binding activity of these factors in the cell extract was measured by gel shift assay using a murine-specific oligonucleotide as a probe. The levels of NFAT, AP-1, NF-kB, and OCT-1 binding activities were induced markedly by con A in spleen lymphocytes. The maximum level of NFAT binding activity was reached 6 h after con A induction. The AP-1 and OCT-1 binding activities were maximum at 3 h and the NF-kB binding activity was maximum at 2 h after con A stimulation in lymphocytes from both young and old rats. The NFAT, AP-1, OCT-1, and NF-kB binding activities were approximately 40 to 55% lower for lymphocytes isolated from the old rats. Because c-Fos and c-Jun proteins have been implicated as constituents of AP-1 and of the nuclear component of NFAT complex, we examined the induction of c-Fos and c-Jun expression by con A in lymphocytes. The c-Fos protein and mRNA level did not change with age; however, the expression of c-Jun decreased 44% in lymphocytes from old rats. Thus, the age-related decline in AP-1 and in NFAT binding activity appears to be correlated with a decline in c-Jun gene expression. (Supported by NIH grants AG-00165 and AG01548).

27

THE DECREASE IN HSP70 TRANSCRIPTION WITH AGE OCCURS BECAUSE OF A DEFECT IN HEAT SHOCK TRANSCRIPTION FACTOR. A.R. Heydari*, S. You & A. Richardson, GRECC, Audie Murphy Memorial VA Hospital and the Dept. of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, TX 72824.

A characteristic feature of senescence is the progressive decline in the ability of an organism to respond to stress. Because heat shock proteins protect cells from a variety of stresses, we studied the expression of hsp70 by hepatocytes and spleen lymphocytes isolated from young (4 to 6 months of age) and old (24 to 28 months of age) male Fischer F344 rats. The induction of hsp70 synthesis and hsp70 mRNA levels by heat shock (42.5°C for 30 to 60 min) was 40-50% lower for cells isolated from old rats compared to cells isolated from young rats. Using *in situ* hybridization, it was found that the decline in hsp70 mRNA levels was not due to an age-related decrease in the number of cells that responded to heat shock; essentially all cells isolated from young or old rats responded to the heat shock and expressed hsp70. The

age related decline in the induction of hsp70 synthesis and mRNA levels was paralleled by a decline in the nuclear transcription of hsp70. Therefore, the age-related decline in hsp70 expression occurs at the level of transcription. The effect of aging on the binding activity of the heat shock transcription factor 1 (HSF1) to the heat shock element (HSE) was studied using a gel shift assay. Cell extracts from hepatocytes isolated from old rats showed significantly reduced (approximately 50%) HSF1 binding activity. Using a polyclonal antibody to HSF1, we have measured the levels of HSF1 protein in hepatocytes isolated from young and old rats by Western blots. The reduced HSE binding activity of HSF observed in old cells was not due to a decrease in the level of HSF1 protein; in fact, the HSF1 protein level was significantly higher in hepatocytes from old rats. Interestingly, the age-related decrease in the induction of HSF1 binding activity in rat hepatocytes was reversed by caloric restriction (CR), the only experimental manipulation known to prolong the mean and maximum life span of laboratory rats, and did not appear to be due to an accumulation of inhibitory molecules with age. Interestingly, the level of HSF1 protein was significantly higher in hepatocytes isolated from the old rats fed *ad libitum* compared to hepatocytes obtained from rats fed the CR-diet even though the levels of HSF1 binding activity were lower for hepatocytes isolated from the old rats fed *ad libitum*. The levels of the mRNA transcript for HSF1 was not significantly altered by age or CR. Thus, the changes in HSF1 binding activity with age and CR do not arise from changes in the level of HSF1 protein available for activation. Rather, it appears that the age-related decrease in the induction of HSF1 binding activity by heat shock and its impediment by CR is due to a defect in the ability of the HSF1 in old *ad libitum* fed animals to be converted from its inactive, monomeric form to its active (HSE binding) oligomeric form.(supported by NIA grant AG 01548 and a grant from AFAR)

28

PEROXIDATION OF MITOCHONDRIA RESULTING IN LIPOFUSCIN FORMATION. D. Yin*, X. Yuan, U.T. Brunk, Dept. of Pathology II, Linköping University, S-581 85, Linköping, Sweden.

Cysteine-stimulated oxidation of a rat liver lysosomal-mitochondrial fraction (LMF) was studied. The process would simulate oxidative stress-related events during the degradation of autophagocystosed material within secondary lysosomes, which may contribute to the formation of lipofuscin or age pigments. Millimolar concentration of cysteine was needed to stimulate LMF lipid peroxidation, measured as thiobarbituric acid reactive substances (TBARS). The amount of endogenous LMF iron was 545 µg/l and was enough to initiate peroxidation, probably through the reduction of ferric to ferrous iron by cysteine with induction of Fenton chemistry. Peroxidation could be completely inhibited by the addition of the iron chelator desferal or the antioxidant butylated hydroxytoluene (BHT). A substantial amount of the formed TBARS was associated with trichloroacetic acid (TCA) precipitable proteins. Elevated protein carbonyls were observed 1-2 hours after the increase of TBARS. The tryptophan-tyrosine related protein autofluorescence (280/335 nm) decreased sharply during the first few hours of incubation. In contrast, a lipofuscin-type autofluorescence (345/430 nm) appeared only after a few days, suggesting that the latter fluorophore is not an immediate product of protein oxidation.

The sequential formation of TBARS, protein carbonyls and lipofuscin-type autofluorescence as well as their dependence on iron and a reducing agent add further support to the concept that lipofuscin forms in secondary lysosomes as a result of iron-catalyzed oxidative reactions involving autophagocystosed materials.

29

DEFECTS OF THE RESPIRATORY CHAIN IN VARIOUS HUMAN TISSUES DURING AGING: ENZYME HISTOCHEMICAL, IMMUNOHISTOCHEMICAL AND *IN SITU* HYBRIDIZATION STUDIES. J. Müller-Höcker, Institute of Pathology, Ludwig-Maximilians-Universität, Thalkirchnerstraße 36, 80337 München.

Among the many aspects of aging, loss of organ reserve capacity is one of the outstanding features. Loss of mitochondrial function appears to be involved in this process. In fact, enzyme histochemical studies of cytochrome-c-oxidase have revealed an age-related loss of cytochrome-c-oxidase activity (complex IV) especially in permanent tissues such as the diaphragm, limb, extraocular and heart muscle and also in the substantia nigra of the brain. Similar defects occur also in the mitochondria-rich oxiphilic cells of the parathyroid gland and in the liver of humans.

Characteristically the defects are randomly distributed and partly or completely expressed, indicating cellular heterogeneity of the aging process. In the parathyroids, it has been shown that defects of complex III and IV occur selectively and in combination with over 80% of the defects involving complex IV.

The defects density varies in different tissues. In extraocular muscles, a five to six times higher rate has been observed than in other striated muscles (ca. 370/cm^2) in advanced age.

Immunohistochemistry has shown that the loss of enzyme activity is paralleled by a loss both of nuclearly and mitochondrially coded subunits of complex IV.

Molecular genetic studies have revealed that in the external eye muscles an accumulation of the common deletion (4977 bp) and of various point mutations (np 3243, 5692, 8344, 10006, 12246) occur. *In situ* hybridization of mtDNA has shown an accumulation of the common deletion in about one third of extraocular muscle fibers and of depletion of mtDNA in a minor portion. In contrast, in the respiratory chain defects of the parathyroids, neither the common deletion nor the above mentioned point mutations could be detected.

The results indicate that interorgan and intercellular heterogeneity are major aspects of the aging process. Most likely different pathogenetic mechanisms including probably also nuclear effects on the mitochondrial genome and on the decline of the respiratory chain function are involved in aging. It is, however, unclear whether deficiency of the respiratory chain in aging is a primary event leading to cellular aging or merely a consequence of it. Irrespective of the pathogenetic mechanisms, loss of respiratory chain activity during aging may be an important factor for the decline of functional organ reserve capacities in senescence.

30

MITOCHONDRIAL DNA REPAIR. V.A. Bohr*, D. Croteau, R. Hansford & R.M. Anson, Laboratory of Molecular Genetics and Laboratory of Cardiovascular Sciences, National Institutes on Aging, NIH, 4940 Eastern Avenue, Baltimore, MD 21224.

It has been the general notion for the past decades that mitochondria have no DNA repair capacity. This would then explain the observed accumulation of DNA lesions in mtDNA with aging or under other conditions. However, it has now become evident that there are DNA repair mechanisms in mitochondria, but they differ from those in the cellular nucleus.

We have taken several approaches to study DNA repair in mitochondria. In one approach we use a bacterial DNA repair enzyme to detect 8-oxo-deoxyguanosine lesions in the mitochondrial DNA, and these studies have revealed an efficient repair of this important lesion in mtDNA. After oxidative stress, the number of lesions introduced in mtDNA and in a nuclear gene are similar, but the repair is faster in mtDNA than in the endogenous nuclear gene, dihydrofolate reductase (DHFR). We are currently comparing the induction and removal of 8-OH guanosine after different types of oxidative stress to the cells.

In another approach which is still at the preliminary stage, we are examining the repair process in mitochondrial extracts from rat liver. We can detect a nicking activity after oxidative cellular damage, but not after UV irradiation. We are currently improving this assay and using it for the detection of DNA repair capacity after various types of DNA damage to enable us to characterize the nature of the repair process in mitochondria.

31

SOMATIC MITOCHONDRIAL DNA MUTATIONS AND OXYGEN RADICALS IN AGING AND DEGENERATIVE DISEASE. D.C. Wallace*, S. Melov, J. Shoffner, L. Esposito, T. Horton, M. Corral-Debrinski & C. Epstein†, Emory University School of Medicine, Atlanta, GA 30322, and †University of California Medical School, San Francisco, CA.

Mitochondrial oxidative phosphorylation (OXPHOS) enzyme activity declines with age in post mitotic mammalian tissues in parallel with the accumulation of somatic mitochondrial DNA (mtDNA) mutations. Quantitation of the common 5 kilobase (kb) human mtDNA deletion by the deletion-PCR method revealed that this deletion accumulates in normal heart after age 40 and in the cerebral cortex and basal ganglia of normal brains after age 75 with cortical deletion levels reaching 2-4% of the mtDNAs and the basal ganglia levels reaching 10 to 20%. The levels of the 7.4 and 10.6 kb deletions also accumulate.

One possible cause of these mutations is damage to the mtDNA by OXPHOS generated reactive oxygen species (ROS). Hearts with coronary artery disease experience bursts of ROS generated during the periodic ischemia and reperfusion. In such hearts, the somatic mtDNA deletion levels can be increased between 8 and 2200 fold.

While correlative with age, the level of any one mtDNA mutation is too low to cause pathology. Consequently, the pathology must result from the effects of a variety of different mutations. To assess these mutations, we have developed two new methods to assess the breadth of mtDNA mutations in aging tissue: long-extension-PCR (LX-PCR) and Southern blots of undigested mtDNA. LX-PCR permits amplification of the complete mtDNA using two adjacent primers in opposite orientation. When combined with field inversion gel electrophoresis (FIGE), this procedure reveals a wide range of mtDNA species in muscle biopsies from old subjects but not young. The presence of altered mtDNAs in older skeletal muscle is also shown by Southern blots of undigested DNAs. Older subjects have an extra mtDNA band in the same region as that seen in patients harboring known deletions, but this band is consistently absent in young individuals.

Evidence of the toxicity of ROS to mitochondrial structure and function have come from studies on mice in which the mitochondrial manganese superoxide dismutase (MnSOD) is inactivated. Homozygous mutant animals die by one week of age with a dilated cardiomyopathy. This is associated with a dramatic

reduction in the activities of mitochondrial enzymes containing iron-sulfuric centers, particularly succinate dehydrogenase and aconitase. Thus ROS toxicity occurs at two levels, acute inhibition of the iron-sulfur center enzymes of OXPHOS and progressive mtDNA mutation leading to decline in OXPHOS biogenesis.

32

THE EFFECT OF DIETARY RESTRICTION ON THE FREQUENCY AND ABUNDANCE OF mtDNA DELETIONS. J. Aiken, Dept. of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, Madison, WI 53706.

The association of mitochondrial DNA (mtDNA) deletions and age has been observed in a wide range of species. In mammals, these age-associated deletions appear to be most pronounced in nerve and muscle and hold great promise for explaining the major diseases and disorders old age brings to these tissues. My laboratory has been study the influence of age and dietary restriction (DR) on the accumulation of these age-associated mtDNA deletions. We have focused upon specific skeletal muscle groups of C57BL/6 mice (gastrocnemius) and Lobund-Wistar rats (adductor longus) as well as two dietary restriction regimens, early onset (mice) and late onset (rats). We found that the age-associated accumulation of mtDNA deletions is significantly affected by DR. Although early onset DR had a more dramatic effect, late onset DR was also effective in decreasing the accumulation of mtDNA deletions.

33

OXIDATIVE STRESS-INDUCED EXPRESSION OF CYTOKINES BY PERIPHERAL MONONUCLEAR CELLS IN RHESUS MONKEYS: INFLUENCES OF AGE AND DIETARY RESTRICTION. M.-J. Kim*, J.M. Aiken, W.B. Ershler & R. Weindruch, Depts. of Nutritional Sciences, Animal Health & Biomedical Sciences & Medicine University of Wisconsin-Madison & VA-GRECC, Madison, WI 53706.

Cytokines play an important role in the regulation of immune function and levels of several cytokines are known to change with age and age-associated diseases. Recent data suggest that free radicals may be involved in the physiological control of the expression of cytokines and may contribute to the age-associated dysregulation of these cytokines. We are, therefore, investigating influences of oxidative stress, as induced by the xanthine and xanthine oxidase (X/XOD) system on the expression of interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6) and interleukin-8 (IL-8) by peripheral mononuclear cells (PBMC). To investigate age effect, PBMC from normally fed young (7-10 yr.) and old (>20 yr.) rhesus monkeys were studied. Since DR attenuates these age-associated changes and decreases free radical damage, we investigated the DR effect by studying PBMC from middle aged monkeys (14-17 yr.) that were either fed normally or subjected to five years of DR (70% of ad lib).

X/XOD treatment induces significant IL-6 expression in PBMC as compared to controls and PBMC from old monkeys are more sensitive to X/XOD treatment than those from young monkeys. Further, DR attenuates IL-6 expression in PBMC treated with X/XOD. These results suggest that free radicals may play a role in the dysregulation of cytokines seen with aging. Further, this indicates that DR may attenuate the age-associated dysregulation of cytokines in a nonhuman primate model, the rhesus monkey (Supported by PO1 AG 11915).

34

IDENTIFICATION AND CHARACTERIZATION OF AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS IN *DROSOPHILA MELANOGASTER*. S.R. Schwarze[1], A.S. Laughon[2] & J.M. Aiken[1], [1]Dept. of Animal Health & Biomedical Sciences & [2]Dept of Genetics, University of Wisconsin, Madison, WI 53706.

Age-associated mitochondrial DNA (mtDNA) deletions have been extensively studied in humans and other animals, however, we sought to develop *D. melanogaster* as a model for studying mtDNA deletions because of its increasing usefulness as an experimental organism. This system would allow us to use mutagenesis, transgenics and injections as a means to answer many questions in the field. Various stages of juveniles (embryos, first, second and third larval instar) and ages of adults (2, 30, and 60 days) were selected from an Oregon-R strain of *D. melanogaster*. Total DNA was isolated and polymerase chain reaction (PCR) based technology was used to identify mtDNA deletions. Steady increases in the number of deletions were observed from the embryo through the instar stages to adulthood. Once adulthood was reached, however, no further increases in the number of deletions were detected. Our results suggest that the majority of mtDNA deletions occur during development where rapid growth and high metabolic activity occurs.

35

IDENTIFICATION OF RAGGED RED AND CYTOCHROME OXIDASE NEGATIVE FIBERS IN OLD RHESUS MONKEYS AND RATS. C.M. Lee[1]*, R. Weindruch[2] & J.M. Aiken[1], [1]Dept. of Animal Health & Biomedical Sciences & [2]Dept. of Medicine & VA-GRECC, University of Wisconsin, Madison, WI 53706.

Mitochondrial enzymatic abnormalities have been identified histologically in aged human heart and muscle tissue, but have been studied less extensively in animal tissue. We chose to examine heart and skeletal muscle from aged rhesus monkeys and Lobund Wistar rats for localized mitochondrial enzymatic defects. Adjacent frozen sections of rat vastis lateralis were stained for succinate dehydrogenase (SDH) and cytochrome oxidase (COX) activities. Hyperactivity with SDH stain inferred the presence of a ragged red fiber (RRF). Several fibers from 30-month-old rats were identified as RRF, COX negative, or both. RRF or COX deficient fibers were not found in tissue from three-month-old rats. Similarly, analysis of quadriceps tissue from a 39-year-old rhesus monkey identified several RRF and COX negative fibers, none of which were found in tissue from younger rhesus monkeys (6-18 years of age). Areas of COX deficiency were also detected in aged rhesus monkey heart tissue, however, such changes in hearts from aged rats were more subtle. We are currently using *in situ* hybridization to determine whether these abnormal enzymatic activities are associated with mitochondrial DNA (mtDNA) deletions. Such an analysis will help elucidate the overall contribution of mtDNA deletions to the physiological decline of aged muscle tissue.

36

EFFECTS OF CELLULAR AGING ON THE INDUCTION OF *C-FOS* BY ANTIOXIDANT TREATMENTS. R.G. Allen, M. Tresini, B.P. Keogh & V.J. Cristofalo, Center for Gerontological Research Medical College of Pennsylvania & Hahnemann University, Philadelphia, PA 19129.

Oxidants are known to induce the expression of some transcriptional factors such as *c-fos*, but the effects of antioxidants on these factors are largely unknown. Using northern and western blot analysis, we have examined the effects of three antioxidants on the induction of *c-fos* and one pathway of signal transduction believed to be involved in *c-fos* induction. All three antioxidants induced *c-fos* in proliferatively young human fetal lung fibroblasts (WI-38); however, each antioxidant stimulated induction over a unique time course. The induction of *c-fos* by nordihydroguaiaretic acid was to some extent mediated by activation of protein kinase C, because down regulation of PKC by pretreatment with phorbol ester blocked NGA induction of *c-fos*. Both *N-acetyl* cysteine (NAC) and Trolox C stimulate *c-fos* transcription by PKC-independent mechanisms. It has previously been reported by others that the induction of *c-fos* transcription was irreversibly blocked in senescent cells. We have also determined the effects of NAC and NGA in proliferatively young and senescent WI-38 cells. NAC induced *c-fos* transcription in both proliferatively young and senescent cells, while NGA induced *c-fos* transcription in young cells but failed to stimulate it in senescent cells. Since we had previously observed an age-related decline in PKC translocation and because PKC activation appears to be involved in NGA induction of *c-fos* we examined the relative protein abundances of several PKC isoforms in young and senescent cells. Additionally, we examined the protein abundance of several members of the MAP kinase pathway (including: RAF-1, MEK-1, ERK-1, ERK-2 and ERK-3) which could play a role in *c-fos* induction by the PKC-dependent pathway. We were unable to detect PKC-ß or θ in WI-38 cells. Senescent cells contained a slightly greater abundance of PKC α, γ and ε than young cells. No other differences in PKC isoforms or in members of the MAP kinase family were observed in young and senescent cells. These results clearly demonstrate that at least some pathways leading to *c-fos* induction remain intact in senescent cells. While we were unable to detect any decreases in PKC isoforms or MAP Kinase proteins we cannot exclude the possibility that functional decrements accumulate in these proteins during senescence.

37

ADRENAL ZONATION AND AGE-RELATED CHANGES IN MACROPHAGE NUMBER. H. Almeida*, M.C. Magalhães & M.M. Magalhães, Institute of Histology & Embryology of the Faculty of Medicine, & Institute of Molecular & Cellular Biology of the University of Porto (IBMC). Faculdade de Medicina do Porto, 4200 Porto, Portugal.

In the adrenal cortex, macrophages are often located in subendothelial space, in close relation to the parenchymal cells. Variation in their amount has been observed in some experimental conditions. However, data on aging is lacking and thereafter a morphologic quantitative study was performed.

Wistar male rats with free access to water and laboratory diet were randomly selected and divided in groups of 6 animals each. Animals with 2, 6, 12, 18 and 24 months of age were studied. After anesthesia with sodium pentobarbital, the adrenals were removed and processed for electron microscopy by

conventional methods. Semi-thin sections stained with Azur II, were used to identify the 3 cortical zones (glomerulosa, ZG, fasciculata, ZF, and reticularis, ZR). Stereological methods were employed for the determination of the volume density (Vv, % of total volume of the zone) and numerical density of the macrophages (Nv, number of cells × 10^3 per mm^3 of zone) in each zone.

Macrophages, displaying elongated or notched nuclei and coarse, heterogeneous cytoplasm were present in all zones, being remarkably more common in ZR. The quantitative study of 6 rats/group revealed the following average results:

	Vv (%)			Nv (× $10^3/mm^3$)		
age	ZG	ZF	ZR	ZG	ZF	ZR
2 m	0,15	0.24	1.26	5.64	6,49	13.94
6 m	0,21	0.21	2.01*	4.02	4.27	27.76*
12 m	0,24	0.33	2.78*	5.23	9.44	30.55*
18 m	0,31	0.28	3.76*	3.43	5.46	30.06*
24 m	0,24	0.44	4.89*	1.89‡	7.26	30.51*

* $p<0.01$; ‡ $p<0.05$

Comparing 2 months old animals with 12, 18 and 24 months, a statistically significant increase in macrophage Vv of ZR was observed ($p<0.01$). In respect of Nv, statistically significant increase ($p<0.01$) was obtained in ZR when 2 months old animals were compared to all the other groups; ZG revealed a statistically significant decrease ($p<0.01$) in 24 months rats when compared to 2 months ones.

The data suggest that macrophages have a functional role in the adrenal cortex. The significant increase of the number of macrophages in ZR during aging must be related to an enhanced macrophagic activity in this zone.

38

CHANGES IN HYDROGEN PEROXIDE LEVEL, THIOBARBITURIC ACID REACTIVE SUBSTANCES AND ANTIOXYGENIC ENZYME ACTIVITIES IN AGING FRUIT FLY FED ON ANTIOXIDANTS. J.S. Bains, Dept. of Pathology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Oxygen free radicals (OFRs) have been postulated to be involved in the aging process. Antioxidants can delay aging by scavenging the OFRs. Many studies have shown that dietary antioxidants extend the average life span of various organisms. However, the mechanism of action of these exogenously given antioxidants is not clear. In the present investigation, two synthetic (butylated hydroxy anisole, BHA and propyl gallate, PG) and one natural (alpha-tocopherol, TP) antioxidants were used as dietary supplementation. Suitable concentrations (calculated from life span table) of antioxidants were added to standard corn meal agar (CMA) medium. The flies were reared and maintained on control and antioxidant mixed diets throughout their life. Mean life span of insects is 33±4 days for males and 40±3 days for females. Biochemical estimations were made starting from freshly emerged (1 day old) flies to well beyond the average life span with weekly interval between different age groups. Level of hydrogen peroxide (H_2O_2) and thiobarbituric acid (TBA) reactive substances was increased gradually with age but it declined significantly on feeding antioxidants. The maximum decline in H_2O_2 was observed with TP followed by PG and BHA respectively. Similar trend was revealed for TBA reactive substances in both the sexes. Activities of catalase and glutathione reductase (GR) were measured using standard protocols. Both catalase and GR enzyme activities were decreased with aging. Antioxidants enhanced the catalase activity significantly during all age intervals in male and female insects. However, BHA feeding increased the GR activity at 22 days in male and at 22 and 29 days of survival in female flies. PG and TP showed increase in GR activity at all ages. These results indicate that antioxidants reduce free radical damage by strengthening the antioxidative system thereby leading to prolonged life span.

39

BRAIN CYTOCHROME OXIDASE ACTIVITY AND SURVIVAL IN ALZHEIMER DISEASE. P. Chagnon*, Y. Robitaille, D. Gauvreau & C. Bétard, Projet IMAGE, Centre hospitalier Côte-des-neiges, Montréal.

Cytochrome oxidase (CO) is the terminal complex of the mitochondrial respiratory chain which generates ATP by oxidative phosphorylation. Recent investigations have suggested that a defect in CO activity may be involved in the pathogenesis of Alzheimer disease (AD). We have measured CO activity in two different brain regions of a cohort of 35 sporadic patients with senile dementia of the Alzheimer type (SDAT), and performed mitochondrial DNA (mtDNA) analysis.

CO activity was significantly reduced for SDAT patients who had a shorter survival (≤ 5 years) as compared with patients who had a longer survival (> 10 years). The 6 to 10 year survival group had intermediate CO activity levels as compared with the two other groups.

The cause of the reduction of CO activity was investigated at the molecular level in order to reveal a mtDNA mutation which encodes three subunits of CO. We report on the results of the analysis of the genotypic variants.

These results indicate that reduced CO activity might play an important role in the physiopathology of

SDAT by accelerating the processes of degeneration leading to death.

40

THE ANTIOXIDATIVE MECHANISMS AND ACTIVE SITES OF MAGNESIUM LITHOSPERMATE B FROM SALVIAE MILTIORRHIZAE RADIX. H.Y. Chung*, K.J. Paik, J.S. Kim & T. Yokozawa‡, Dept. of Pharmacy, Pusan National University, Pusan, Korea, Research Institute for Wakan-Yaku, Toyama Medical & Pharmaceutical University, Toyama, Japan†.

Natural compounds, phenolic derivatives and flavonoids have antioxidant actions, and can function as free radical terminators and often, as metal chelators. In this study, we attempt to examine an antioxidant isolated from naturally occurring herb medicine. A phenolic compound, magnesium lithospermate B (MLS), was isolated and identified as a major biologically active component of an herbal material called Salviae Miltiorrhizae Radix (SMR). In the present study, antioxidative activities of MLS were investigated in CCL_4-intoxicated mice to elucidate the antioxidant mechanism of MLS. MLS was injected intraperitoneally into mice at a dose of 10 mg/kg/day for two days, while control mice were treated with an equal volume of saline. The mice received 0.4 ml/kg CCL_4 dissolved in olive oil 1 hour after administration of MLS. All mice were fasted 24 hours after CCL_4 administration, then were killed by decapitation and their liver taken. MLS significantly increased Cu, Zn-SOD, catalase and nonprotein-SH levels by 19% and 34% compared to 34% observed in control CCL_4-treated mice while marked decreases were seen by 18% malondialdehyde in the CCL_4-treated mice. These results suggest that the antioxidative action of MLS is partly attributable to increases in endogenous antioxidants.

In a separate study, antioxidative effects of the caffeic acid isolated from Salviae miltiorrhizae Radix, its dimer (trans-rosmarinic acid), trimer (lithospermate A), and tetramer (lithospermate B) were studied in mouse liver homogenate. Caffeic acid inhibited *in vitro* lipid peroxidation induced by H_2O_2 and $FeSO_4$ to 70% of the control values. To elucidate the structural requirement, essential functional groups of MLS were investigated by modifying the structure. The derivatives, which changed structure at dihydroxyphenyl moiety of caffeic acid, ferulic acid, 3,4-demithoxycinnamic acid, and cinnamic acid, showed a decreased antioxidative effect compared to that of caffeic acid. In addition, caffeic acid derivatives which have changed structure at side chain part, p-hydroxyphenylpropionic acid, p-hydroxyphenylacctic acid, p-hydroxybenzoic acid, and hydrocinnamic acid also have less antioxidative effects than that of caffeic acid. These results suggest that dihydroxyl group of caffeic acid and the double bond of its side chain play an essential role in its antioxidative action.

41

THE OXIDATIVE MECHANISM OF URSOLIC ACID AND GINSENG SAPONIN. H.Y. Chung*, M.H. Oh, J.S. Kim, & K.W. Kim‡ , Dept. of Pharmacy, Dept. of Molecular Biology†, Pusan National University, Pusan, Korea.

Naturally occurring substances such as phenolic compounds and flavonoids are known to have various efficacies, including antioxidant action. Natural products, ursolic acid, a triterpenoic derivative, and ginseng saponin have steroid-like anti-inflammatory abilities. However, their antioxidative activity is currently unknown.

In the present study, the antioxidative activity and mechanism of ursolic acid and ginseng saponin were examined in *in vivo* systems. Ursolic acid and ginseng saponin (Rb_2) were intraperitoneally treated with doses of 10 μmol/ kg/day for 5 days. The treatment with ursolic acid and ginseng saponin also significantly enhanced the activities of catalase by 24% and 26% respectively. Protein and lipid oxidation were assessed by measuring the carbonyl content and malondialdehyde (MDA) respectively. Treatment with ursolic acid or ginseng saponin significantly decreased the content of carbonyl group and MDA. Furthermore, by binding ursolic acid and ginseng saponin to glucocorticoid receptor, we generated a competition assay through replacement. Ursolic acid, ginseng saponin, and dexamethasone markedly decreased dexamethasone binding indicating that these compounds competitively bind the gluco-corticoid receptor.

To examine the effect of ursolic acid and ginseng on the antioxidant scavenging enzyme, protein synthesis inhibitor, and cycloheximide, catalase activity was studied in a cultured liver cell to prove whether or not ursolic acid and ginseng saponin induced catalase through protein synthesis. Results showed that through treatments of ursolic acid and ginseng saponin, cycloheximide abolished the induction of catalase. Ursolic acid and ginseng saponin markedly increased the expression of catalase mRNA compared to the control group. In conclusion, ursolic acid and ginseng saponin showed an antioxidative effect by inducing endogenous catalase.

42

THE EFFECT OF DIETARY RESTRICTION (DR) ON ELECTRON TRANSPORT IN AGING. V.G.Desai*, J.E.Freeman, J.B.Collins, R. Weindruch†, R.W. Hart & R.J. Feuers, Natl. Cen. Tox. Res. (NCTR), Jefferson, AR 72079, †Dept. of Medicine, University of Wisconsin, Madison, WI 53706.

Age-associated alterations in mitochondrial electron transport system ((ETS) may lead to free radical generation and contribute to aging. The complexes of ETS were screened spectrophotometrically in gastrocnemius of young (10 month) as well as older (20 month) B6C3F1 female mice fed *ad libitum* (AL) or DR diets (40% less food than AL mice). The activities of complexes I, III, and IV decreased significantly by 62%, 54%, and 74% respectively, in old AL mice (OAL) compared to young AL mice (YAL). Complexes I, III, and IV from YDR mice had activities which were significantly lower than those seen in YAL mice (suggesting lower total respiratory rate or improved efficiency). In contrast, complex II activity did not decrease with age (actually increased, but not significantly) in OAL mice. Complex II was decreased across age in DR mice. Mitochondrial DNA (mtDNA) encodes for specific subunits for complexes I, III, and IV and decreased activities observed for these complexes in OAL mice could be the result of mtDNA damage. K_m for ubiquinol-2 of complex III was significantly increased in YAL animals (0.33 mM vs 0.26 mM in YDR mice) and was further increased with aging (0.44 mM in OAL vs 0.17 mM in ODR mice). This suggests insufficient binding and potential inhibition of electron transport in aging which could yield free radical generation. Complex IV possesses a high affinity and a low affinity binding site and is constructed of 13 subunits. Total complex as measured by V_{max} was highest in YAL mice, but the proportion of complex as high affinity sites was lower (69%) than in either YDR (80%) or ODR (80%). Thus, additional amount of complex IV present in YAL as measured by V_{max} may be the result of substrate induction since binding is compromised. Further, the percentage of high affinity sites decreased to only 45% in OAL mice and V_{max} was reduced by 75%. At physiologic concentration of reduced cytochrome c, significant dysfunction of complex IV would be expected and overall electron transport would be obstructed. Therefore, the age associated loss of activity and function of complexes I, III, and IV may contribute to increased free radical production. Lack of sufficient DNA repair in mitochondria and juxtaposition to ETS adds to susceptibility and accumulation of mtDNA damage. DR seems to retard this deterioration of mitochondrial respiratory function by preserving enzymatic activities and function. Supported in part by the NCTR and the NIA.

43

AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS: A STUDY OF GENOME REGIONS AND TISSUE SPECIFICITY. P.M. Eimon[1], S.S. Chung[1], L.E. Aspnes[2], R. Weindruch[3] & J.M. Aiken[1], [1]Dept. of Animal Health & Biomedical Sciences, [2]Dept. of Nutritional Sciences, [3]Dept. of Medicine & VA-GRECC, University of Wisconsin, Madison, WI 53706.

Multiple age-associated mitochondrial DNA (mtDNA) deletions have been characterized in humans, rhesus monkeys and mice, and may contribute to the aging process. The major arc region has been the main focus of studies analyzing age-associated mtDNA deletions because 95% of deletions in myopathy patients occur in this region. Since estimating the total number of mtDNA deletions throughout the genome is critical in determining their potential physiological impact, we have examined different regions of the mtDNA for the distribution of deletions in skeletal muscle of 24-month-old mice. Three similarly sized regions of the genome were examined: one region entirely within the minor arc; one region entirely within the major arc; and one region spanning the light strand origin. Using polymerase chain reaction we have found, on average, two-fold more deletions in the major arc region than the minor arc region while deletions in the region containing the light strand origin were rarely detected. Thus, previous estimates of deletion abundance are most likely an underestimate of the true deletion load.

We sequenced several deletion products from mice and determined the location of their breakpoints. A deletion that was common among mice contained a large 14 bp imperfect direct repeat while other deletions that were unique to animals usually did not. This common deletion, located in the minor arc region, was found in both brain and skeletal muscle tissues. A 7.0 kb deletion was found in brain samples from 24-month-old mice but not in skeletal muscle from the same mice. Similarly, common deletions have been identified that appear to be cardiac-specific. We are interested in whether tissue-specific deletions are a phenomenon in other species, and so are currently expanding our search to include rats.

44

QUANTITATIVE HISTOCHEMISTRY OF SUCCINIC DEHYDROGENASE (SDH) ACTIVITY OF PURKINJE CELL MITOCHONDRIA IN AGING AND VITAMIN E DEFICIENCY. P. Fattoretti*, C. Bertoni-Freddari, U. Caselli & R. Paoloni, Neurobiology of Aging Laboratory, "N. Masera" Research Dept., 60121 Ancona, Italy.

Free radical attacks to biological molecules represent a well-documented critical event in nerve cell aging. The absence of the biological antioxidant vitamin E (α-tocopherol) from the diet of young laboratory animals is reported to reduce the protection from free radical aggressions. To check the efficiency of nerve cell metabolism in aging and in conditions of increased oxidative stress, a computer-assisted morphometric study has been carried out on the ultrastructure of succinic dehydrogenase (SDH)-positive mitochondria histochemically evidenced by the copper-ferrocyanide reaction in Purkinje cells of 3, 12 and 24 month-old rats and in 11 month-old animals fed a vitamin E deficient diet from the age of 1 month up to the day of sacrifice. The mitochondrial volume fraction (Volume density: Vv), the number of organelles/μm^3 of Purkinje cell cytoplasm (Numerical density: Nv) and the average mitochondrial volume (V) were automatically calculated by applying currently used morphometric formulas. Vv was significantly decreased in old animals vs the other age groups. Nv was significantly higher at 12 than at 3 and 24 months of age, while it was significantly decreased in old vs young rats. V was significantly decreased at 12 and 24 months of age, but no difference was envisaged between adult and old rats.Vitamin E deficiency resulted in a significant decrease of Vv and V vs the normally fed animals of any age. Nv was significantly lower in the vitamin E deficient rats vs the age-matched control group, but it was not statistically different from young and old animals. With aging, the percentage of organelles larger than 0.32 μm^3 was above 10%, while in the adult group the enlarged mitochondria account for less than 1%. In the vitamin E deficient animals the reduction of the percentage of elongated organelles is clearly evident with the largest size class (0.24-0.32 μm^3) accounting for about 1%. The present quantitation of SDH histochemistry provides information on the metabolic competence of perykarial mitochondria considered as functional units of neuronal bioenergetics. In old Purkinje cells, mitochondrial structural dynamics appear to be capable of impaired plastic reactive responses unable to restore the metabolic hardware refined and tuned during development and maturation. Vitamin E deficiency resulted in morphological alterations of the SDH positive mitochondria similar to those observed in old rats. Because of the known protective role of α-tocopherol from free radical attacks our present findings lend support to the assumption that the consistent decay of the metabolic competence in brain aging may be due to the marked deterioration of mitochondrial ultrastructure in which an increased oxidative stress seems to play a key role.

45

EXPRESSION OF VARIOUS DEHYDROGENASES IN AGING MALARIA VECTOR ANOPHELES STEPHENSI. S.K. Gakhar* & Vandana, Dept. of Biosciences, Maharshi Dayanand University, Rohtak-124001, Haryana, India.

The studies focusing on the genetic programming of the expression of specific gene enzyme systems such as dehydrogenases show promise for elucidating regulatory events involved in cellular differentiation and differential gene expression, which forms the basis of eukaryotic development. The expression of three dehydrogenases, i.e., Glycerophosphate dehydrogenase (GPDH), Lactate dehydrogenase (LD) and Malate dehydrogenase (MDH) has been studied b electrophoretic and spectrophotometric techniques during the aging of malaria vector, Anopheles stephensi.

A total of three isoenzymes of GPDH (GPDH-1 to GPDH-3; in the order of decreasing mobility) were resolved on the agarose gel in the adult life of the mosquito. A single locus seems to govern the developmental expression of three isoenzymes. GPDH-2 enzyme was adult specific. The GPDH-1 and -2 disappeared during senescence. GPDH-1 has been indicated to be more suitable for energy metabolism and GPDH-3 for lipid biosynthesis. Three codominant alleles control the four electrophoretic variants of GPDH revealed in the laboratory strain.

Five isoenzymes of LDH (LDH-1 to LDH-5; in the order of increasing mobility) expressed during the complete life of A. stephensi, however, LDH-3 did not express in the adults. The newly emerged adults had only one isoenzyme (LDH-2) while three new additional isoenzymes (LDH-1, -4 and -5) appeared in the aging females. Five different electromorphs of LDH, presumed to be governed by five codominant alleles, were recorded for the laboratory strain.

Two separate gene loci seem to govern the developmental expression of two different forms of MDH, probably cytoplasmic (cMDH) and mitochondrial (mMDH) forms. Laboratory survey revealed mMDH locus monomorphic and cMDH polymorphic with five different electromorphs governed by four alleles. cMDH was present throughout the life, while mMDH appeared from pupa

onwards. This locus seems to be more suited to fuel the predominant flight activity of adults.

Quantitatively, the GPDH activity declined by about 2o% in males and 60% in females during senescence as compared to their respective peak levels (early adult life). This period is characterized by the disappearance of GPDH-1 in males and in addition, GPDH-2 in females. In the case of LDH, after a peak on day 4 in both the sexes, no significant fluctuations were noted during the rest of adult life. The MDH activity increased continuously during the complete development and aging.

Regarding the sex-specific differences, the adult males had a significantly higher level of GPDH and MDH than their female counterparts while the reverse was true for LDH.

The disappearance of some isoenzymes and the appearance of the others at the onset of adult life and during aging seem as the evolutionary necessity or an adaptive effort for the change from aquatic to terrestrial life.

LDH is a more suitable enzyme for energy production in a relatively anaerobic aquatic environment. Since larvae are not very active as compared to adults, it is quite sufficient for them to utilize LDH reaction.

High level of GPDH-1 in the thorax indicated that this isoenzyme functions in energy metabolism. The new adult enzyme, GPDH-2 may be more suited to fuel the predominant flight activity. It seems more advantageous for the adult insect to metabolize the pyruvate and NADH via mitochondria rather than utilize LDH reaction to malate. The higher levels of MDH than the other two enzymes in the adult seem to be associated with the overall high energy requirements of adults for flight activity, mating and reproduction, etc.

46

MITOCHONDRIAL DNA ARRANGEMENTS IN THE MINOR ARC: DELETIONS INVOLVING THE LIGHT AND HEAVY STRAND PROMOTER SEQUENCES. J.P. Gudikote* & G.C. Van Tuyle, Dept. of Biochemistry & Molecular Biophysics, Virginia Commonwealth University, Richmond, VA 23298.

The purpose of this study was to analyze mitochondrial DNA (mtDNA) rearrangements in the minor arc between the heavy and light strand origins of replication in rat brain samples. The mitochondrial DNA from five brain samples of rats belonging to three different age groups were analyzed for rearrangements using the polymerase chain reaction (PCR). Products from nested PCR amplification were cloned and sequenced. Four deletions were identified in one-day old pups, seven in a 22-month old rat, and nine in 33-month old rats. The deletions ranged in length between 4423 bp and 5177 bp. Heavy strand promoter sequence was lost in all of the deletions reported here. All but one of the deletions identified in this study contained short direct repeats of $\leq$ 9 bp at the end points. In thirteen of the deletions, precisely one member of the direct repeat was lost with the deleted segment. Three of the deletions exhibited partial loss of one member of the repeat while retaining the other member completely. The other two deletions showed the loss of one complete member of a repeat and part of the second member. One end of all 19 deletions terminated in the D-loop. The opposite ends of 12 of the deletions were in the ND 2 gene, 3 were in the $tRNA^{Ala}$ gene, 3 were in the $tRNA^{Asn}$ gene and one ended in the $tRNA^{Trp}$ gene. Ten of the deletions terminated on one side at a common locus of 9 bp in length, located in the D-loop region. The opposite ends of three of these ten deletions were also located in a common locus of 9 bp in length at the 3' end of the $tRNA^{Asn}$ gene. The light strand promoter sequence, which serves to initiate mtDNA replication, was missing in all four of the deletions identified in the pup brain sample and in two of the deletions identified in the 22-month old rat brain sample. None of the deletions identified in the brain samples obtained from 33-month old rats was missing the light strand promoter sequence, suggesting that the frequency of these deletions may decrease as a function of age. Interestingly, only one of the 19 deletions was found to be common to two different template samples, namely 22-month and 33-month old rats. Thus our study indicates that a complex array of large-scale mtDNA rearrangements in the short arc between the origins of replication can be detected throughout the life span of rats.

47

AGE-ASSOCIATED HETEROGENEITY OF RAT HEPATOCYTE SUBPOPULATIONS BASED ON ALTERED MITOCHONDRIAL FUNCTION IS REVERSED BY L-ACETYLCARNITINE. T.M. Hagen*, J.C. Bartholomew, K.L. Do, M.H. Song, C.W. Wehr, & B.N. Ames, The University of California, Berkeley & the Lawrence Berkeley Laboratory, Berkeley, CA 94720.

Mitochondria decay with aging. Old tissues contain mitochondria which exhibit marked morphological heterogeneity, increased oxidant production, and altered membrane potential compared with young tissues (PNAS 91: 10771-10778). Such age-associated changes in mitochondria render their isolation from old tissues difficult; mitochondria obtained using standard purification techniques may not be entirely representative of a given tissue due to

mitochondrial fragility and lysis during purification. Moreover, mitochondrial isolation may not reflect more distinct age-associated changes of mitochondria occurring in cells or cell linages within an organ. Such changes may be due to epigenetic mitochondrial damage or mitochondrial DNA mutation. Clearly, a means to separate cells based on functional differences in mitochondria could provide new insights into their role in the aging process.

Isolated hepatocytes from 24 month old rats exhibit marked heterogeneity based on their average mitochondrial membrane potential, as measured by rhodamine 123 (R123) fluorescence. Three distinct subpopulations were separated by centrifugal cell elutriation; each exhibits unique R123 fluorescent staining patterns, with the largest subpopulation of old cells having significantly lower fluorescence than that seen in 3 month old rat cells. Two subpopulations had lower respiratory control ratios than young hepatocytes. Correspondingly, these two subpopulations had significantly higher rates of oxidant production, as measured by the 2',7'-dichlorofluorscin diacetate assay. Hepatocyte subpopulations exhibiting high oxidant production also have increased levels of nuclear DNA damage as measured by accumulation of DNA-protein crosslinks. In addition, increased mitochondrial deletions occur in hepatocytes from old animals when compared with hepatocytes obtained from young animals.

Supplementation of old rats with 1.5%(w/v)L-acetylcarnitine (ALCAR) in drinking water increased significantly the mean R123 fluorescence in 2 of the 3 isolated subpopulations. ALCAR also caused a reversal of an age-associated decline in cardiolipin levels in these subpopulations of hepatocytes. Thus, ALCAR supplementation may not only increase the ability of altered mitochondria to transport fatty acids for β-oxidation but may also allow cardiolipin-dependent proteins to function more efficiently.

48

COMPARISON OF INFLUENCE OF TWO GEROPROTECTORS-ANTIOXIDANTS ON CELL PROLIFERATION. A.N. Khokhlov*, L. Yu. Prokhorov & S.S. Akimov, Evolutionary Cytogerontology Sector, School of Biology, Moscow State University, 119899 Russia.

Effects of two geroprotectors-antioxidants, butylated hydroxytoluene (BHT) and 2-ethyl-6-methyl-3-oxypyridine hydrochloride (epigid), on cell proliferation were investigated in experiments with "young" (logarithmic growth stage) and "old" (stationary growth phase) cultured cells. It was shown that BHT either had no effect (10 and 30 μg/ml) on saturation density of "young" transformed Chinese hamster cells (CHC) or lowered it (500μg/ml). However, the compound could improve viability of "old" cells at 500 μg/ml it decreased mortality rate of CHC and, besides at all the concentrations studied increased their cloning efficiency). In contrast to BHT, epigid (at 10^{-5} M) stimulated growth of "young" cells (increased saturation density of human embryo diploid fibroblasts and CHC) and improved their viability (increased human fibroblast cloning efficiency). However, epigid decreased survival of "old" cells (number of living "stationary phase aged" cells went down faster than in control). The results allowed us to conclude that mechanisms of the antioxidants investigated influence on cell proliferation are rather different.

49

APOLIPOPROTEIN E TYPE 4 ALLELE AND EARLY ONSET ALZHEIMER DISEASE. S.S. Matsuyama*[1,3], L.F. Jarvik[1,3], W. Bondareff[4], J. Cummings[1,2,3], A. Leuchter [1,3], G. Small [1,3], [1]West Los Angeles VA Medical Center, the Depts. of [2]Neurology & [3]Psychiatry & Biobehavioral Sciences, Neuropsychiatric Institute, University of California, Los Angeles & the [4]Dept. of Psychiatry, Division of Geriatric Psychiatry, University of Southern California.

Alzheimer disease (AD) is a devastating neurodegenerative disease affecting an estimated 2.5 million Americans with the number expected to double by the year 2000. There is general consensus that apolipoprotein E type 4 allele (APOE-4) is a major risk factor for late-onset sporadic and familial AD. Whether APOE-4 is associated with early onset AD is controversial and may reflect the presence of amyloid precursor protein or chromosome 14 mutations in some families. The APOE gene, located on chromosome 19 (19q13.2), has three allelic variants (e2, e3, and e4) resulting in six genotypes (2/2, 2/3, 2/4, 3/3, 3/4, and 4/4). We examined APOE genotypes of 44 AD patients, 22 autopsy confirmed and 22 clinically diagnosed cases. APOE genotyping was performed by polymerase chain reaction assay of DNA specimens derived from brain tissues, fibroblast cultures, and/or lymphocytes.

The e4 allele frequency in the AD cases was 0.34, within the range reported in numerous other studies, while none of 14 healthy volunteers (mean age 68.00±10.22) had an e4 allele. There was no significant difference in e4 allele frequencies between autopsy confirmed (0.36) and clinically diagnosed (0.32) cases. Thirty of the 44 AD cases were late onset (>60 years) and the e4 allele frequency was 0.33. The e4 allele frequencies were similar for those with a negative (n=11) and positive (n=17 family history for dementia (0.32 and 0.35 respectively). For

two cases, there was insufficient information to assess family history. We examined mean age at onset as a function of APOE genotype and there was a trend toward earlier onset with increasing e4 dosage (no e4=74.46 years, 1=69.71 years, and 2=63.33 years). However, in early onset cases, there was no difference in mean age at onset as a function of e4 dosage (no e4=55.00 years, 1=55.00 years, none homozygous for e4). In our small sample of 14 early-onset (≤60 years) cases (ten autopsy confirmed) the e4 allele frequency was 0.36, within the range from 0.24 to 0.52 reported for late onset cases. The e4 frequency was lower for those with a negative (n=7) family history than those with a positive (n=7) family history (0.29 and 0.43 respectively) but this difference was not significant. Our results suggest that e4 also represents a risk factor for early onset AD and the association may be modified by family history for dementia.

50

MITOCHONDRIA, ALBUMIN AND PROTEIN SYNTHESIS. Kenneth Seaton, High Technology Hygiene, 1712 8th Avenue, Huntington, WV. 25703.

This continuing longitudinal study, commenced in 1974, uncovers a major link between albumin levels, healthy cells, protein synthesis and Mitochondrial numbers and function.

The Mitochondria "Power Plant", breaks down fatty acids, sugars and amino acids, using the released energy to make Adenosine Triphosphate. ATP is analogous to a "Battery" which stores energy for all the various cellular machinery. Lack of oxygen, damage to cell membranes, drugs, poisoning or low albumin can stop ATP production, and within a minute, the ATP/ADP ratio can fall to 1/10th of the normal value. Liver cells may contain up to 2500 mitochondria, also have the highest levels of albumin in the surrounding environment, and perhaps are the most "Ageless" of all cells. Albumin delivers calcium, magnesium, fatty acids, also it is a "Packet" of the correct amino acids, all of these are essential for proper functioning of the mitochondria. Mitochondria average a half-life in rat liver cells of 10 days, containing many enzymes, DNA and RNA capable of self replication and perhaps 13 proteins; all coming from the Mother. Mitochondria may undergo marked age-associated changes. Myelin-like substances may appear and those from old animals are less stable on storage. There is a fall in numbers under stressful conditions. Albumin levels can mirror these changes in aging and stress. Cells grown in the presence of optimal levels of albumin, grew profoundly longer and stronger (Todaro & Green, 1964).

I observed growth, general health, protein synthesis and wasting in association with albumin levels, in a variety of domesticated animals on an experimental farm in New South Wales, Australia, between 1974 and 1976, fish propulsion and bird flight between 1974 and 1981, and 30 canines in the U.S.A. from 1987 to current. The domesticated animals were fed a variety of high protein diets. The fish were observed in water tanks for maximum speed, and the birds were observed by following them in 4 wheel drives along the beach or by boats in the water.

In all animals regardless of age, low serum albumin levels were associated with poor growth, wasting and lessor performance. Restoration of serum albumin levels, accompanied marked improvement in protein synthesis, growth, and performance. These observations supported those observed in >4000 human volunteers between 1980 to current. Serum albumin levels in humans need to be >47g/L, with an A/G ratio 2.0, particularly in the elderly to prevent wasting, lack of energy, and cellular death that normally accompanies aging, cancer and AIDS. Optimal albumin profiles can only be achieved by reducing antibodies and acute phase proteins, allowing more "Osmotic Room." This can only be achieved by better hygiene. High protein diets have no effect.

In summary, albumin, degraded and recycled, controls protein synthesis. Optimal mitochondria survival and performance appears deeply associated with the levels of serum albumin in the interstitial fluids. Perhaps albumin, because of its high net charge, even supplies electrons. Albumin surrounds cells, collecting wastes, neutralizing drugs and poisons that may damage the cell and mitochondria. Albumin is the dominant antioxidant. Calorie restriction in association with optimal albumin profiles sets the scene for the purest possible conditions; for maximum cell growth and longevity.

51

A PRELIMINARY INVESTIGATION ON THE REMEDIAL EFFECT OF KIDNEY-NOURISHED AND HEART-RECUPERATED HERBAL MEDICAMENTS ON FUNCTION OF CHOLINERGIC NERVE SYSTEM OF EXPERIMENTAL DEMENTIC RATS. S.-J. Shen-Tu, L.Shui-miao, et al., Gerontology Institute, Shanghai University of Traditional Chinese Medicine, Shanghai, P.R. China 200032.

Dysmnesia of Alzheimer's patients has been shown to be associated with the dysfunction of the cholinergic nerve system, and characterized biochemically by reduction of choline acetyltrans-

ferase (ChAT) and acetylcholinesterase (AchE) activities, and by decreased nerve transmitter content in cortical areas and in the number of their corresponding receptors.

Remedial effects of five prescriptions of herbal medicaments (Kidney-nourished, KN; breathe-beneficial; blood-invigorated; heart-recuperated, HR; and expectorants) were invertigated on memory function of dementic rats induced by unilateral electrolytic lesions of the nucleus basalis. Significant enhancement of the acquisition of passive avoidance conditioned responses by KN and HR was observed in the lesion rats by 80% increase of memory accuracy, estimated by the Y maze method, compared to control rats ($p<0.05$). The demented rats treated with KN and HR demonstrated a significantly elevated activity of ChAT (6.77 umol/hr/g and 6.79 umol/hr/g, respectively) in the cerebral cortex in comparison with control rats ($p<0.01$), and a markedly increased maximum binding capacity (Rt) of the muscarinic receptor in the hippocampus (1174.28 fmol/mg protein and 1007.39 fmol/mg protein, respectively). The output of nerve transmitters in the cerebral cortex, including norepinephrine (0.26 ng/mg), dopamine (0.51 ng/mg), and 5-hydroxytryptamine (0.12 ng/mg), were found to increase considerably in the rats (0.21, 0.25 and 0.10 ng/mg, respectively) in response to both of the decoctions ($p<0.05$ and 0.01, respectively). No remarkable effect was observed with the other three decoctions. These findings indicated that KN and HR decoctions have therapeutic effects on demented rats. Although the mechanism of action remains unclear, it could conceivably be ascribed partly to biochemical modulation and recovery of function of the disordered cholinergic nerve system, with resultant memory acquisition.

52

INFLUENCES OF CALORIC RESTRICTION AND AGE ON ANTIOXIDANT ENZYMES IN THE PROSTATES OF RATS. K. Suzuki*, T. Oberley, T.D. Pugh & R. Weindruch, Depts. of Medicine & Pathology & Laboratory Medicine, University of Wisconsin-Madison & VA-GRECC, Madison, WI 53706.

It is well established that caloric restriction (CR) in rodents prevents the development of age-associated diseases such as cancer and renal disease. Lobund-Wistar (L-W) rats are known to develop spontaneous metastatic prostatic cancer after 30 months of age and are susceptible to chemically induced forms of the disease. Recently, we have described microscopic neoplastic changes that occur spontaneously in the lateral lobes of the prostates in L-W rats by 20 months. We herein report the results of immunohistochemical staining and localization for 10 antioxidant enzyme proteins in the prostatic tissues of aging L-W rats subjected to 30 percent CR from 16 months of age and in controls. Antioxidant enzymes (AE) included manganese superoxide dismutase (MnSOD), copper-zinc superoxide dismutase (CuZnSOD), catalase (CAT), glutathione peroxidase (GPX), and 6 glutathione S-transferase (GST) isoenzymes, (Ya, Yb1, Yb2, Yc, Yk, and Yp). At 3 months of age, the lining epithelium of the dorso-lateral lobes of the prostates consisted of tall columnar cells with basal nuclei. Immunostaining for AEs showed light reaction product for all tested antibodies in young rats fed ad lib for 3 months. By 20 months, the staining was strongly positive. CAT showed dense, granular deposits at the poles of epithelial cells in the lateral lobes. MnSOD and GPX stained lightly and diffusely in the cytoplasm with moderate apical concentrations of stain, whereas CuZnSOD was more intensely stained, occasionally localized at the apical pole of the lateral epithelium. GST Ya staining was diffuse throughout the cytoplasms and notably absent from the apical zone stained by CAT. Immunoreactive product of AEs in prostate tissues decreased with advancing age, especially in the lateral lobes. In the CR rats at 20 months of age, staining was nearly the same as in controls for all antioxidant enzymes examined. At 30 months of age, there was minimal or no staining of AE proteins in the prostates of control fed rats. Rats in the CR group showed a higher level of immunoreactivity for MnSOD, CuZnSOD, CAT and GSTs in the lateral lobes compared with control rats. Thus, CR started in middle-age prevented the reduction of immunoreactive protein of AEs in the lateral lobes of prostate observed at 30 months of age in the controls.

53

EFFECTS OF GONADOTROPIN RELEASING HORMONE ANALOGUE ON UTERINE GROWTH IN ADULT MICE. A. Weiss*, S. Geva & R. Miller-Lotan, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology & Rambam Medical Center, Haifa 31096, Israel.

Uterine myomas are the most common form of benign neoplasia in women. Clinical trial studies indicate that sustained treatment with GnRH analogues can suppress further growth and even reduce the volume of fibroids, which in many cases will prevent the need for surgical intervention. However, because of side effects, still more basic and clinical research is required to explore the issue of risks and benefits of GnRH analogues. Mice develop

spontaneously uterine tumors with aging or when treated with excessive doses of estrogen, thus may present a valuable investigative model for studies on fibroids, yet numerous studies reported that the pituitary-gonadal axis cannot be suppressed by GnRH analogues in mouse. In order to explore this issue, 3 month old female mice were treated by daily sc injections of 1 μg of D-trp6-GnRH for a period of two months. The body weight of treated animals decreased by 10% in comparison to control ($p<0.5$). The mean weights of ovaries and uteri decreased by 37% and 27% respectively ($p<0.001$), while that of kidneys was not affected. A marked decrease in the thickness of uterine walls and the length of processi was observed. Also, the height of the epithelial layer and the number and size of uterine glands were markedly diminished. The incorporation of 3H-thymidine into uterus was decreased by 67% ($p<0.001$) in comparison to control. Autoradiography revealed that cell proliferation was decreased in all of the cell layers of the uterus: epithelium, myometrium and endometrium. The ovaries of the D-trp6-GnRH treated animals were devoid of follicles and the number of 3H-thymidine labeled cells was significantly diminished. In addition, immunocytochemical studies with polyclonal antibody against human estrogen receptor revealed a significant decrease in the number of cells containing the receptor.

In summary, the findings of this study reveal that D-trp6-GnRH can suppress the reproductive axis in mice, hence the mouse can be used as a model for studies of the effects of GnRH analogues on uterine cell proliferation and fibroid growth.

54

ALTERATION IN THE SYNAPTOSOMAL GLUTAMATE UPTAKE WITH AGE. G.T. Vatassery*, J.C.K. Lai, W.E. Smith, & H.T. Quach, VA Medical Center, Minneapolis, MN 55417 & Idaho State University, Pocatello, ID 83209.

Glutamate and aspartate are two of the major excitotoxic amino acid neurotransmitters in the central nervous system. Abnormal activity of these transmitters can lead to excitotoxicity and this process has been implicated in the pathogenesis of age-associated diseases such as Alzheimer's disease. Excitotoxicity can result from alterations in the release or uptake of these amino acids into the synapse and this can be explored using synaptosomes (i.e., nerve ending particles). In this study we have examined the kinetics of uptake of radiolabeled glutamate by rat brain synaptosomes isolated from the cerebra of Fischer 344 rats aged 4 and 24 months. Samples of cerebra were dissected out of the brain and synaptosomes isolated by a centrifugation procedure involving Ficoll density gradients (7.5 and 10%). The washed synaptosomes were suspended in buffer (pH 7.4) that simulated extracellular fluids and had the following composition: 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM sodium phosphate, 4 mM HEPES and 10 mM glucose. The samples were incubated in tubes at 37° C and C-14-labeled glutamate was added. The samples were filtered after different periods of incubation and the filter was washed twice quickly with ice-cold buffer. The amount of radioactivity in the synaptosome that was retained by the filter was counted using standard liquid scintillation techniques. The nonspecific binding of glutamate was measured by cooling the sample immediately after the addition of glutamate and filtering the mixture immediately. This nonspecific binding was always subtracted from the total uptake of radioactivity to get the specific uptake of glutamate by synaptosomes. The Km for the high affinity uptake of glutamate was ~9 mM which is similar to the value of 15 mM reported for synaptosomes from mice. The glutamate uptake at 12 and 16 minutes was lower in synaptosomes from older animals than those from younger animals. The data suggest that the glutamate re-uptake is less efficient in the older animals. This may lead to higher concentration of glutamate in the synaptic cleft and may enhance the susceptibility of the older animal to excitotoxicity.

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55

MITOCHONDRIAL INVOLVEMENT IN AGING. M.N. Gadaleta*, A.M.S. Lezza, V. Petruzzella & P. Cantatore, Dept. Biochem. Mol. Biol., Via Orabona 4, 70126 Bari, Italy.

Aging is a complex phenomenon mainly associated with loss of mitochondrial bioenergetic capacity. We planned to ascertain the role of mitochondrial DNA (mtDNA) in the degenerative processes of senescence.

Mitochondrial DNA (mtDNA) carries the information for 13 of the 60 polypeptides of mitochondrial respiratory complexes: therefore, it is responsible together with nuclear DNA (nDNA) of their biogenesis.

MtDNA is localized near the cell's most active compartment for the production of oxygen free radicals, it is unprotected by hystone-like proteins, it lacks efficient repair mechanisms and it has a highly compact informational content. Oxygen radicals might, therefore, easily attack mtDNA causing various kinds of somatic mutations. Recently, sporadic and inherited mutations of mtDNA have been found in different human mitochondrial pathologies supporting

the idea that aging might be the most widespread mitochondrial pathology[1]

Rat is our experimental model to study the role of the mitochondrial genetic system in aging. To pursue this goal we used: Northern hybridization, *in vitro* RNA synthesis, Southern hybridization, gel electrophoresis adapted to reveal triple strands DNA molecules, qualitative and quantitative PCR. We found in aged rat brain and heart: a reduced steady-state level of mt transcripts[2] due to reduced RNA synthesis, an unchanged mitochondrial DNA copy number per cell[3], a reduced number of mtDNA molecules harbouring the triplex strand structure in the "D-loop region"[4], a low but increasing age-related content of mtDNA molecules harbouring a 4.8 Kb deletion[3]. We found, furthermore, that the reduced steady-state level of mt transcripts is reversible: 1 h acetyl-L-carnitine (AC) pre-treatment of senescent rats is able to bring back the level of mt transcripts to the value of adult rat[2]. Recently we reported that mitochondrial protein synthesis is also reduced in aging rat[5]. Reduced mitochondrial protein synthesis might not be only a consequence of reduced organelle's transcription. We propose, in fact, that reduced transcription and translation in aged rat mitochondria are mainly a consequence of the age-dependent decline of the bioenergetic state of mitochondria[5], of the altered composition of mitochondrial membranes and of the transport of some metabolites[6]. Data recently obtained by us on human tissues seem to support this possibility[7].

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56

AGE-RELATED CHANGES IN GLYCOSAMINOGLYCAN CONTENT AND PERIVASCULAR DISTRIBUTION. B.J. Barber*, R.A. Babbitt & S. Parameswaran, Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40546-0236.

Extracellular matrix structural and transport properties are largely determined by collagen and gel (glycosaminoglycan or GAG) content. An age-related decrease in gel would reduce tissue hydration and alter protein transport. The purpose of the present study was to visualize the perimicrovascular spatial distribution of glycosaminoglycans by staining rat mesenteric tissue *in situ* with Alcian Blue (AB) dye solution, thereby determining how perivascular AB stain distribution differs between young and old rats.

An intestinal loop was exteriorized from an anesthetized rat, and the exposed mesenteric tissue bathed with pH 2.8 saline, HCl solution for 5 min followed by AB dye solution; after 30 min the tissue was washed with destain solution. Photographs were taken of the same region of mesenteric microvasculature before and after staining. Staining by AB of tissue plasma proteins other than GAGs was tested for by cellulose acetate electrophoresis of tissue samples and plasma protein standards. Collagen standards were tested for AB staining at pH 2.8, 5.0, 6.0, and 7.0. Hyaluronan (HA), a prominent GAG in loose connective tissue, was independently measured by radio-assay (RA) for control and suffused mesentery in young and old rats.

Gradients in AB staining were found similar to those previously reported for perimicrovascular plasma protein quantity (Barber and Nearing, Amer. J. Physiol. 258:H556-H564, 1990). In old rats staining was greatly reduced and the pattern was altered; in particular, gradients were essentially absent. To confirm this observation, HA in tissue samples distal to microvasculature was measured by RA. Hyaluronan was significantly ($p<0.005$) reduced in old rats, but no significant ($p=0.55$) difference was found between control and bathed tissue nor was there a significant ($p=0.56$) interaction between age and superfusion effects. When tissue samples were subjected to electrophoresis, AB did not stain tissue plasma proteins. Collagen staining by AB decreased with pH; linear regression gave a zero staining intercept at pH 4.0. AB staining of collagen was undetectable at pH 2.8 after treatment with hyaluronidase.

The results demonstrate that AB staining is increased near the microvasculature in young rat mesentery and suggests that there is increased GAG in the perimicrovascular region. Old rats show much less AB staining and gradients are absent. Hyaluronan concentration is reduced in older rat avascular mesenteric loose connective tissue.

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57

p48: A CELL CYCLE REGULATED NUCLEAR PROTEIN IN OLD *AD LIBITUM* RATS FOLLOWING A TERMINAL DOSE OF ISOPROTERENOL. J.L. Pipkin*, W.G. Hinson, L.E. Lyn-Cook, P.H. Duffy, R.J. Feuers, J.E.A. Leakey, K.B. Aly, R.W. Hart & D.A. Casciano, National Center for Toxicological Research, Jefferson, AR 72079.

The synthesis ([^{25}S]-incorporation) of stress proteins (sps, i.e., 24, 25, 70, 90) and of nuclear protein 48 (p48) was investigated in several tissues with three groups of male Fischer 344 rats following administration of isoproterenol (IPR). Two groups of rats, young *ad libitum* (Y/AL - 3 mo.) and old/AL (O/AL - 28 mo.), had full access to rat chow, and a third group of old diet restricted (O/DR - 28 mo.) rats were maintained on a diet restricted intake of 40% of the Y/AL animals. Sp synthesis in the bone marrow and heart nuclei of O/AL was significantly reduced as compared with Y/AL and O/DR rats following their induction with IPR. A 1 mg/kg dose of IPR was lethal for O/AL but not for Y/AL or O/DR animals. This lethal dose induced synthesis of p48 in bone marrow nuclei of O/AL rats only. p48 existed in isoform states and when a lethal dose of IPR was administered, it was expressed in O/AL rats in a cell-cycle regulated pattern. In an association assay p48 was shown to conjugate with sp 70 and sp 90. p48 in bone marrow and heart nuclei from O/AL rats was antigenically identical with p48 observed in HL60 nuclei following exposure of these cells to lethal temperature. The presence of p48 is correlated with mortality and with an *ad libitum* diet in old rats since it is absent in old diet restricted animals.

58

EFFECT OF PHYSICAL ACTIVITY, AEROBIC CAPACITY AND AGE ON GLYCATED HEMOGLOBIN. T. Manfredi*, D. Wright, J. Dane, A.C. Cosmas, J. Stravato, University of Rhode Island, Exercise Science, Independence Square, Kingston, RI. 02881-0810; South County Hospital, Cardiac Rehabilitation, Wakefield, RI 02879; University of Connecticut, School of Allied Health, U101, Storrs, CT 06269.

With age, continual exposure to elevated glucose levels causes glycation of proteins and a decline in glucose sensitivity. Physical activity enhances glucose sensitivity and active adults have been reported to have more positive glucose and lipid profiles compared to their sedentary counterparts. The intent of this study was to compare glycated hemoglobin (GHb) values in very active (V), active (A), and inactive (I) young (Y)(20-39 Ys), middle-aged (M)(40-59 yrs), and older (O)(60+) males and females. One hundred and forty seven subjects were initially screened for body composition using bioelectrical impedance and estimated fitness level using a validated Physical Activity Index (PAI). Glycated hemoglobin (GHb) levels were measured on a subset of 94 subjects. Age and activity levels had significant effects on estimated maximal oxygen consumption (V_{O2max}) in males ($p<0.01$). In females, age had a significant effect on (V_{O2max}) in moderately active subjects ($p<0.05$). Within activity levels, GHb values were significantly lower in AY Vs AM men and AY Vs AO men. Within age groups, GHb values were lower ($p<0.01$) in VM compared to AM males ($p<0.01$). Age and activity levels had no effect on GHb levels in females. We conclude that age and exercise may have greater effects on GHb levels in males and not females. The lack of age and activity effects in females may be due in part to menopausal status.

59

THE INCREASED VARIABILITY OF INTRACELLULAR LIPOFUSCIN CONTENT WITH AGE IS DECREASED BY CALORIC RESTRICTION. W.A.L. Moore, R. Walford, R. Weindruch & G.O. Ivy*, Office of Post Grad Education, Clarke Institute of Psychiatry, Toronto, ON, Canada M5T 1R8; Life Sciences Division, University of Toronto, Scarborough, ON, Canada M1C 1A4.

For most physiologic functions the co-efficient of variation about the mean increases with age. This axiom of aging has traditionally been observed in populations of organisms but has not been tested for populations of cells within an organism.

Lipofuscin (LF), which is a marker for changing metabolic function with aging in post mitotic cells, is usually measured as mean cell content or total (representative) tissue content.

The purpose of this study was to delineate the variability of lipofuscin content within a population of granule cells in the hippocampus of mice, as a function of age and caloric restriction (CR). Three age groups of CR and control mice, including groups of maximum age life span, were studied.

We have found that the variability of lipofuscin content within a population of granule cells of the hippocampus was significantly greater in old animals and was significantly less in mice calorically restricted.

We hypothesize that an increased variability of metabolic function between cells, as measured by increased variability of lipofuscin content, may represent a definitive mechanism underlying the overall decreased efficiency of tissue/organ functioning with age. This idea is supported by the fact that CR, which has been shown to maintain the "youthful" state of many physiologic functions, has

now been shown in this study to maintain the "youthful" state of low variability lipofuscin content within a population of brain cells.

The increasing heterogeneity of function within a cell population with age may result in more disorganized intercellular or tissue function. Consequently, physiologic function may be progressively compromised until it is compatible with organ function, and, ultimately, with the life of the organism. This model may provide evidence for entropy as an ultimate mechanism underlying the aging process.

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60

ULTRASTRUCTURAL STUDIES OF MITOCHONDRIAL MEMBRANES. E. Valdivia*, University of Wisconsin, Madison, WI 53705; R.P. Apkarian, Emory University, Atlanta, GA 30322; P. Sims, University of Wisconsin, Madison, WI 53705.

The purpose of this investigation is to visualize the morphology of mitochondrial membranes. The methodology involves isolation of the organelles from animal hearts and liver, fragmentation of the isolated organelles, incubation to stabilize functional states, cryofixation and processing for ultrastructural examination with the electron microscope. Transmission electron microscopy has used negative staining to demonstrate surface structures. The membrane surfaces have been visualized by scanning electron microscopy with and without specimen coating. The results of these observations demonstrate that the inner surface facing the matrix has patches of subunits which have the appearance of the structures believed to represent mitochondrial ATPase enzymes. Similar cluster morphology is observed by electron microscopy of mitochondrial membranes treated with the Gomori technique used to demonstrate ATPase activity. Examination of the external surface of the isolated mitochondria shows patchy elements which are also visible as regular mosaic of clusters and numerous bridges joining mitochondria. These observations demonstrate that isolated mitochondria are not individual organelles and that the mitochondrial membranes have clusters of structures in a mosaic pattern.

61

MECHANISMS OF INTERLEUKIN-1β (IL-1β) SUPPRESSION OF TRANSFORMING GROWTH FACTOR-β (TGFβ)-INDUCED INORGANIC PYROPHOSPHATE (PPi) PRODUCTION BY CULTURED HUMAN CHONDROCYTES. M. Lotz, F. Rosen, G. McCabe, J. Dudler, J.E. Seegmiller*, & R. Terkeltaub, Sam & Rose Stein Institute for Research on Aging, VA Medical Center & University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0664.

This study centers on the mechanisms by which TGFβ acts to increase substantially the production of PPi by human chondrocytes and examines the suppressive effect of IL-1β on both this stimulation by TGFβ on the basal production of PPi by these cells. PPi was measured by the radiochemical method of Cheung and Suharolnik (Anal Biochem 83:52-56, 1977). Protein was determined using a dye-binding method (Bio Rad DC Protein Assay). DNA concentrations were determined by a modified Burton method (Leyva & Kelly, Anal Biochem 62:173-179, 1974), nucleoside triphosphate pyrophosphohydrolase (NTPPPH) activity of Plasma Cell Membrane glycoprotein-1 (PC-1) and its mRNA were determined as described by Huang et al (J Clin Invest, 1994, 94:560-567). Alkaline phosphatase was assayed as described by Oyajabi et al (J Bone Miner Res, 1994, 9:1259-1266).

A 96 hour incubation of chondrocytes with TGFβ (10 ng/ml of DMEM) produced a 74% increase in intracellular PPi and a 2 to 5-fold increase in extracellular PPi. The effects were most pronounced in chondrocytes during their first or second passage in culture and became minimal or absent by the fifth to the eighth passage concurrent with the de-differentiation of the chondrocytes to a fibroblastoid phenotype. Under the same conditions IL-1β (5 ng/ml) decreased intracellular PPi by 33% and extracellular PPi by 53%. When both agents were added together, IL-1β caused a substantial inhibition of the TGFβ-induced increase in PPi levels. Similar results were obtained with even 1 ng/ml IL-1β with chondrocytes from over 11 different donors with similar results in either the presence or absence of fetal calf serum.

To address mechanisms involved with the regulation of PPi synthesis by IL-1β and TGFβ we analyzed the activity of NTPPPH involved in PPi synthesis and alkaline phosphatase involved with its hydrolysis. The elevation in PPi production induced by TGFβ was accompanied by comparable increases in activity of NTPPPH, PC-1 protein and its mRNA levels in chondrocytes as well as a 90% reduction in activity of alkaline phosphatase. IL-1β increased alkaline phosphatase activity to 44% of control values either with or without TGFβ.

Increased IL-1β has been reported in plasma of patients with rheumatoid arthritis and increased IL-1β production has also been found in their cartilage. In the past the fragile bones of these patients have been largely attributed to their use of steroids. These observations suggest the possibility of these two

cytokines playing an important role in mineralization in chondrocytes with IL-1β inhibiting TGFβ-induced PPi production and PC-1 expression.

62

COMMON DELETIONS IN MITOCHONDRIAL DNA INCREASE IN PARALLEL WITH THE DEATH RATE IN HUMANS. M.M. Vilenchik & A.K. Balin, Longevity Achievement Foundation, Chester, PA 19013, and Medical College of Pennsylvania & Hahnemann University, Philadelphia, PA

We have determined, based on data from the literature, that there is an exponential increase in the occurrence of a specific 5 kb deletion in mtDNA isolated from human skeletal muscle and of a 7.4 kb deletion in mtDNA isolated from human heart muscle, as a function of chronological age. The increase in mtDNA deletions in certain tissues are correlated with an age-dependent increase in the death rate in developed countries. The ratio of deleted mtDNA: wild (normal) mtDNA and the death rates in developed countries doubles each 7-8.8 years after age 30-40. This observation does not establish cause or effect. It is possible that the observed mtDNA deletion is one of a family of deletions that result from continual random oxidative damage on uniquely oxygen sites. However, it is also possible that an intrinsic programmed mechanism exists within the mitochondria that results in a specific mtDNA deletion as a function of age. In either case, it is possible that the mtDNA deletions may have deleterious consequences and contribute to the age-related decline in the functional capacity of tissue.

63

ROLE OF YEAST HEMOGLOBIN IN OXIDATIVE STRESS. X.-J. Zhao*, D. Raitt, P.V. Burke, A. Clewell, S. Pepperl, & R.Q. Poyton, University of Colorado, Dept. Of Molecular, Cellular and Developmental Biology, Boulder, CO 80309-0347.

As a part of a study on the relationship(s) between mitochondrial dysfunction and oxidative stress, we are studying the function and expression of the flavohemoglobin of the yeast *Saccharomyces cerevisiae*. This protein is a member of a growing family of flavohemoproteins, which contain both heme and flavin binding domains and which are capable of transferring electrons from NADPH to heme iron. Yeast cells which carry a deletion in the flavohemoglobin gene, YHB, are sensitive to thiol oxidants and redox recycling compounds like paraquat, indicating that the flavohemoglobin plays a role in oxidative stress. We propose that this protein functions to bind and reduce superoxide by a mechanism that is analogous to the terminal steps in oxygen reduction by cytochrome c oxidase.

Normally, actively respiring yeast cells have very low levels of the flavohemoglobin. However, its intracellular levels are greatly increased in cells in which the mitochondrial electron transport chain has been compromised by either mutation[1] or inhibitors of respiration[2]. The expression of YHB is also increased in strains that lack superoxide dismutase suggesting that the accumulation of superoxide serves as a signal for induction. Together these findings suggest that mitochondria in which the complete respiratory chain has been blocked release superoxide or other reactive oxygen species and that these in turn up-regulate YHB. This suggests a signaling pathway from the mitochondrion to the nucleus[3].

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64

ANALYSIS OF DNA DAMAGE TO MITOCHONDRIAL AND NUCLEAR DNA FOLLOWING HYDROGEN PEROXIDE EXPOSURE. F.M. Yakes* & B. Van Houten, Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555.

Exposure of cells to reactive oxygen species (ROS) is known to produce specific DNA lesions including 8-oxoguanine, pyrimidine hydrates, albasic sites, and strand breaks. Such damage, if left unrepaired, may contribute to the onset of human disease and/or may contribute to the complex process of aging. Classic methods for the measurement of oxidative DNA damage often requires large amounts of DNA. The purpose of this study was to: 1) develop technologies to measure oxidative DNA damage from minute quantities of DNA, and 2) to quantify the formation and repair of oxidative DNA damage in a nuclear gene and the mitochondrial DNA following exposure to hydrogen peroxide. Damage was assayed by the use of quantitative PCR for a 17.7 kb fragment from the β-globin gene and 16.2 kb fragment from the mitochondria. This novel approach is based on the principle that damaged DNA templates will not participate in the PCR. Under quantitative PCR conditions the assay accurately measured the induction of pyrimidine hydrates in nanogram quantities of purified human DNA treated with osmium tetroxide. Human fibroblasts treated with hydrogen peroxide (50-800 μM) for 1 hour resulted in

a dose-dependent decrease in amplification of the β-globin gene fragment and the mitochondrial genome. Damage to the mitochondrial DNA was 4-5 times more extensive than nuclear DNA. Alkaline agarose gel and Southern analysis of mitochondrial DNA indicated that the QPCR-based assay detects both single-strand breaks and base damage. DNA damage in the β-globin gene, but not the mitochondria DNA was not repaired during a 3 hour recovery period. This lack of mitochondrial repair correlates well with the loss of mitochondrial respiratory function as measured by the loss of the ability to reduce the tetrazolium salt, MTT. These data indicate that the QPCR damage detection assay can accurately measure oxidative DNA damage in specific gene sequences from the equivalent of approximately 1000 cells. These observations suggest that the mitochondrial DNA is a critical target for damage by ROS. This work was supported by grants from the NIEHS to BVH (1R01ES07038-02 and 1R01ES07218-01).

65

SENESCENCE-ASSOCIATED LOSS OF EPC-1 EXPRESSION: A MARKER GENE IN G_0 FOR FIBROBLASTS. *V.J. Cristofalo, Center for Gerontological Research, Medical College of Pennsylvania and Hahnemann University, Philadelphia, PA 19129.

In the early 1960's Hayflick and Moorhead proposed that the limited replicative life span of human fibroblast cell cultures and the attendant changes in cell phenotype were a manifestation of aging at the cellular level. This discovery provided a paradigm by which one could study, under controlled environmental conditions, the molecular mechanisms for cell senescence.

Human cells in culture represent the artificial expansion of a population of one of the cell types in the human organism. The cell cultures that have received the most study, viz. fibroblasts have four functions *in vivo*; to migrate, to proliferate, to lay down matrix materials such as collagen, and to elaborate cytokines. Cells that are replicatively senescent *in vitro* have severely reduced ability to migrate, proliferate, make type III collagen and reduced ability to elaborate at least two cytokines, IGF-1 and EPC-1. It seems reasonable to hypothesize that significant aging related changes which occur in this cell type *in vivo* for these functions would have profound effects on the physiology of the organism.

We have been studying the elaboration of a cytokine, EPC-1, by fibroblasts in culture at different *in vitro* ages. EPC-1 is a 50 kD secreted protein which has approximately 30% sequence homology with the family of serine protease inhibitors, although we have no evidence that it functions as a protease inhibitor. EPC-1 is synthesized and secreted by young cells when they are growth arrested either through confluency (density dependent inhibition of proliferation) or by serum/growth factor deprivation. In other experiments we have shown that EPC-1 down-regulates cell proliferation. Cells *in vitro* lose the ability to synthesize the mRNA for EPC-1 gradually throughout their *in vitro* replicative life span so that at the end of the life span virtually no *EPC-1* mRNA can be detected. Similarly, cells taken from 27 donors of different ages ranging from fetal donors to those in the tenth decade of life show a striking decline in EPC-1 with age that is independent of proliferative age *in vitro*. EPC-1 mRNA expression was significantly lower ($r=-0.79$; $p<.000001$) in the older donors. Thus, loss of EPC-1 is clearly a highly reproducible marker for senescence *in vitro* and for changes *in vivo* as they are expressed in cell cultures from different age donors. Current experiments are underway to identify similar changes in fibroblasts in situ.

These studies support the validity of the cell culture model of *in vitro* aging in that they suggest that aging changes *in vivo* are expressed in cultures which are not proliferatively senescent and the loss of EPC-1 suggests that this aging change may contribute to the various hyperplasias that are characteristic of human aging including perhaps, cancer and atherosclerosis. These studies were supported by USPHS grants AG00378, AG00532 and AG00131.

66

A BIOMARKER FOR CELLULAR AGING *IN VITRO* AND *IN VIVO*

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[No Abstract Available]

67

MOLECULAR CONTROL OF CELL SENESCENCE: LESSONS ON AGING FROM AN OLD FIBROBLAST. J. Campisi*, Dept. of Cancer Biology, Lawrence Berkeley National Laboratory, Berkeley, CA 94720.

Organismic aging is a multi-faceted, complex process that affects mitotic and postmitotic tissues, and extracellular components. To understand the prime causes of aging, model systems have been developed. As expected, models have made it possible to explore the bases for aging in much greater detail than is possible in intact organisms. But, also as expected, the relevance of model systems to organismic aging has been legitimately questioned.

One model for the aging of mitotic tissue is cells, principally human fibroblasts, grown in culture. Virtually all somatic cells, with the possible exception of primitive stem cells, divide only a finite number of times before they irreversibly arrest growth and show altered functions by a process termed cellular or replicative senescence. How pertinent is the senescence of cells in culture to aging *in vivo*? And what have we learned from senescent fibroblasts that pertains to organismic aging?

The evidence that senescence in culture reflects processes that occur during aging is: 1) Cells from old donors senesce after fewer divisions than cells from young donors. Thus, renewable tissues may progressively exhaust their replicative potential with age; 2) Cells from short-lived species senesce more rapidly than cells from long-lived species, and cells from donors with hereditary premature aging syndromes senesce more rapidly than age-matched controls supports this idea. Thus, the genes controlling replicative lifespan and organismic lifespan may overlap; 3) Some processes, e.g., stress resistance, the heat shock, are altered similarly by senescence in culture and aging *in vivo*; 4) A senescence biomarker expressed in culture shows that senescent cells accumulate with age in human tissue.

Cell culture studies provided insights into why replicative senescence evolved, and how senescent cells may alter tissue function or integrity. Senescent cells arrest growth due to repression of a few cell cycle activators and over-expression of cell cycle inhibitors. The p53 and Rb tumor suppressors have been shown to be critical for this arrest. These and other data suggest that senescence is a tumor suppressive mechanism. Thus, replicative senescence may have evolved to ensure relative freedom from cancer during the period of reproductive fitness. Post-reproductively, it may fail increasingly, and have the unselected effect of contributing to a decline in tissue function and integrity because senescent cells express altered functions. Skin fibroblasts, for example, over-express collagenase and under-express collagen and collagenase inhibitors when senescent, thus changing from a matrix-producing to a matrix-degrading cell. Senescent cells also over- or under-produce cytokines. These types of changes in cell function can obviously have wide-ranging effects of tissue function and integrity. Finally, senescent cells acquire resistance to apoptotic death. Thus, with age, tissues appear to accumulate senescent cells which cannot divide, have altered functions, and do not die.

68

ALTERATIONS IN THE PLASMINOGEN ACTIVATION SYSTEM DURING REPLICATIVE SENESCENCE. M.D. West*[1], L.A. Tonkin[1], J.W. Shay[2], W.E. Wright[2] & M.H.K. Linskens[1], [1]Geron Corporation, Menlo Park, CA 94025; [2]Dept. Of Cell Biology & Neuroscience, The University of Texas Southwestern Medical Center at Dallas, TX 75235-9039.

The finite replicative capacity of somatic cells *in vitro* is well documented. However, evidence of replicative senescence *in vivo* and information regarding the mechanisms by which senescence plays a role in the etiology of age-related disease is relatively lacking. Since the components of the extracellular matrix frequently play a role in the pathogenesis of age-related disease, we examined the expression of relevant extracellular proteins, in particular, components of the plasminogen activation system in young and senescent cells under controlled conditions of growth. Young and senescent cells were compared in quiescent and activated growth conditions for the secretion of tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2). Young cells showed decreased levels of PAI-1 in both the secreted and extracellular matrix pools upon serum deprivation, whereas senescent cells displayed a more constitutive pattern of gene expression, with markedly higher levels than young cells in a low concentration of serum. RNA analysis revealed that senescent lung and skin cells constitutively express relatively high levels of u-PA and PAI-1 comparable to that of a young activated cell, whereas these are down-regulated in quiescent young cells. Both t-PA and PAI-2 were markedly over-expressed in senescent skin but not lung cells under all growth conditions. Total plasminogen activator activity in conditioned medium was approximately 50-fold higher in senescent-cell medium compared to young when cultured in 0.5% fetal calf serum (FCS) for five days, with the majority of the activity co-migrating on zymograms with u-PA. Similar increases in PAI-1 and u-PA were observed in senescent human umbilical vein endothelial cells. In summary, senescent cells of various types display alterations in plasminogen activator activity with replicative senescence. The inappropriate over-expression of plasminogen activator activity *in vivo* may be expected to lead to a progressive disruption of extracellular matrix maintenance and possibly a loss of proliferative homeostasis. These observations suggest that cellular replicative senescence is associated with an altered expression of genes regulating tissue maintenance which, in turn, could

play a role in tissue disrepair in age-related degenerative disease.

69

LIPID SIGNALING IN CELLULAR SENESCENCE.

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Cellular senescence is the finite life span phenotype whereby cells are no longer able to respond to growth factors and proliferate. Lipid mediated signal transduction pathways have recently been implicated in cell growth regulation. Phospholipids appear to predominantly be involved in proliferation whereas very recently sphingolipids have been shown to play a critical role in growth arrest, and apoptosis. Our laboratory's focus is understanding these lipid mediated signal transduction pathways involved in growth regulation in young cells and their alterations in senescent cells. Using the Wi38 human diploid fibroblast (HDF) model we investigated the response of young cells to serum stimulation. We demonstrated that a critical phospholipid mediated mitogenic pathway is activated such that diacylglycerol is generated, protein kinase C and phospholipase D are activated. In contrast, senescent cells are unable to respond. In addition, senescent cells have significantly increased sphingomyelinase activity and ceramide levels. Exogenous ceramide was able to induce a senescent phenotype as measured by its ability to inhibit DNA synthesis, Rb phosphorylation, and AP1 activation. Interestingly, ceramide is also able to inhibit phospholipase D activation. This indicated that the phospholipase D/diacylglycerol/protein kinase C mitogenic pathway is defective in senescence, and that the sphingomyelinase/ceramide growth inhibitory pathway is activated in senescence. It also indicates that cross-talk between these pathways can result in mitogenesis or senescence.

70

REPLICATIVE SENESCENCE AND SKIN AGING.

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Replicative senescence is the observation that somatic cells grown in culture do not divide indefinitely, but have a limited life span. This finite replicative capacity correlates with telomere length: as mortal cells undergo more doublings, they lose more telomeric DNA. The exit from cell division during replicative senescence correlates with a typical short telomere length. The telomere hypothesis describes the role of the telomere as a cell-division counter in somatic cell strains and postulates that telomere length may play a causal role in the process of replicative senescence.

A second characteristic of replicative senescence is the marked change in phenotype when the cells approach the end of their life span. Changes in gene expression during replicative senescence have been documented for a number of genes. We expanded this catalog by using an adapted version of the differential display technique. We have identified 12 known and 11 novel genes that are expressed differentially during replicative senescence in human lung and foreskin fibroblasts. We are testing whether these genes are expressed differentially in several other human skin fibroblast strains during senescence. This work will identify those genes that are regulated in several different cell strains during senescence.

To determine whether changes in gene expression during replicative senescence correlate with *in vivo* aging, several probes for genes that are differentially expressed were used in *in situ* hybridization experiments on skin samples from young and old donors. Genes that are expressed in young fibroblasts are expressed in skin samples from young donors, but not from old donors. Further work will determine whether this correlation can be made for other genes.

Known genes that are expressed differentially during replicative senescence indicate that changes in the extra cellular matrix contribute significantly to the senescent phenotype. The cloning of the novel genes which are expressed differentially *in vitro* and *in vivo* will facilitate the study of their normal function and their role in the senescent phenotype. The progress in the understanding of the molecular biology of replicative senescence allows a more detailed investigation of the role that replicative senescence may play in skin aging.

The data so far suggests that some alterations found *in vitro* during replicative senescence correlate with changes observed during skin aging *in vivo* and may contribute to age-related alterations in the skin. Therefore, reversal of senescent gene expression may be a novel strategy in the treatment of age-related skin diseases.

71

EFFECTS OF AGING ON THE REPAIR OF SPECIFIC GENES IN RAT HEPATOCYTES. Z.M. Guo, A.R. Heydari, H. Yang & A. Richardson, GRECC, Audie L. Murphy Memorial VA Hospital & Dept. of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

DNA repair is heterogeneous, i.e., different genes are repaired at different rates. At the present time, essentially all the information we have on measuring DNA repair in specific genes comes from studies with mammalian cell lines. In this study, we show that repair of specific DNA regions can be measured in primary cultures of hepatocytes isolated from rats. Primary cultures of hepatocytes also gives one a system to assess the effect of aging on the DNA repair capacity of a cell. Hepatocytes were isolated from 4- and 24-month-old Fischer 344 rats by *in situ* collagenase perfusion, and the hepatocytes were cultured for 24 hours in William's E media. DNA damage was induced in the primary cultures of hepatocytes by exposing the cells to different doses of UV-irradiation. The UV-induced pyrimidine dimers (PD) in a 14-kb *BamH I* fragment containing the H-ras gene and a 23 kb fragment containing the albumin gene were measured using T4 endonuclease V. Hepatocytes were exposed to doses of UV irradiation ranging from 5 to 30 j/m^2. The PD frequency in the fragment of H-ras and albumin gene ranged from 0.5 to 1.2 PD/10kb. When UV dose increased to 60 j/m^2, the PD frequency in the fragment of albumin gene (2.17 PD/10kb) was higher than the H-ras gene (1.6 PD/10kb). The removal of PDs from the *BamH 1* fragments of the H-ras and albumin genes by primary cultures of hepatocytes from young and old animals was measured over a 24 hour period. The PDs were removed preferentially from the albumin gene (35%) compared to the H-ras gene (15%) after 10 j/m^2 of UV-irradiation. Cultured hepatocytes from young rats removed 70% of the PDs in the albumin gene 24 hours after 5 j/m^2 of UV irradiation. In contrast, hepatocytes from old animals removed only 10% of PDs. (Supported by NIH grants AG01188)

72

CELLULAR SENESCENCE INDUCED CHANGES IN NUCLEAR TRANSCRIPTION FACTORS REGULATING MITOCHONDRIAL PROTEIN EXPRESSION. S.K. Lehtinen*, P. Rahkila, M. Helenius, A. Salminen, University of Jyvaskyla, Dept. of Cell Biology, Box 35, FIN-40351 Jyvaskyla, Finland.

Aging and cellular senescence are associated with several mitochondrial dysfunctions. Most of the mitochondrial proteins are encoded by nuclear genome and imported to mitochondria. Transcription of these nuclear genes are coordinated by specific transcription factors which regulate the expression of mitochondrial proteins. Our purpose was to study whether cellular senescence of human WI-38 and IMR-90 fibroblasts and SV-40 immortalization of WI-38 cells affect the proteins binding to Oxbox-Rebox and NRF-1 promoter regions. Electrophoretic mobility shift assays (EMSA) were used to show the protein-DNA binding activities. Mitochondrial distribution in cells was studied by immunocytochemical staining of mtMDH. Replicative senescence of both WI-38 and IMR-90 fibroblasts strongly reduced the binding activity of Oxbox-Rebox -proteins. Instead, the SV-40 immortalization prominently increased the binding activity of proteins. Cellular senescence and SV-40 immortalization did not affect significantly the binding activities of NRF-1 factors. A brief UVB-treatment (photoaging) of WI-38 fibroblasts arrested the proliferation of cells and reduced the binding activity of Oxbox-Rebox -proteins. Immunocytochemical staining of mtMDH showed that mitochondria were distributed around nuclei in young proliferating and especially in SV-40 immortalized WI-38 fibroblasts, whereas in flat senescent fibroblasts mitochondria were dispersed evenly in cytoplasm. Our results suggest that selective alterations during aging in transcriptional regulation of mitochondrial proteins encoded by nuclei could induce disturbances in mitochondrial function and lead to cellular senescence.

73

CHARACTERIZATION OF THE CATALASE PROMOTER. M.D. Williams*, A. Richardson & H. Van Remmen, Dept. of Physiology, The University of Texas Health Science Center at San Antonio, & GRECC at Audie Murphy Memorial VA Hospital, San Antonio, TX 78284.

Damage from free radicals has long been implicated in the aging process. Oxygen-derived free radicals cause damage to lipids, proteins and DNA and are believed to be involved in a variety of chronic diseases, e.g. atherosclerosis, emphysema, heart disease and cancer. To protect against free radical damage, cells have evolved a complex antioxidant system to detoxify free radicals. Cellular defense against free radical damage involves free radical scavenging enzymes such as catalase which converts hydrogen peroxide to oxygen and water. Because little is known about the regulation of catalase, we are currently characterizing the catalase promoter by measuring the transcriptional activity of various lengths of the catalase promoter region attached to

the CAT (chloramphenicol acetyltransferase) reporter gene in several cell lines. In mammalian tissues, catalase activity is highest in the kidney and liver and lowest in brain. A construct containing 3.4kb of the 5'-flanking region of the catalase gene (pCat/CAT) exhibits significant activity in a porcine kidney epithelial cell line ($LLCPK_1$), a human hepatoma cell line (HepG2) and a human glial cell line (HTB16). To further study the catalase promoter region, fragments (2.6, 2.1, 1.9, 1.7, 0.2 and 0.05kb) of the 5'-flanking region were produced by restriction enzyme digestion and attached to the CAT reporter gene. There is a consistent decrease in transcriptional activity in all 3 cell lines as the promoter region is shortened from 2.6kb to 1.7kb. In the glial cell line, the 1.7kb fragment has essentially no transcriptional activity. Further reduction to 200bp does not alter the transcriptional activity in the kidney and hepatoma cells, while activity increases in the glial cells. Reduction to 50bp resulted in essentially no transcriptional activity in all three cell lines. These data suggest that there are important tissue specific regulatory elements within the catalase promoter, especially in the region between 2.6kb to 1.7kb. The pCat/CAT transgenes have also been used to produce transgenic mice which are currently being characterized as to the tissue specific expression of the CAT reporter gene. (Supported by NIA grant AG01548 and by the Office of Research and Development, Dept. of Veterans Affairs.)

74

A SINGLE PATHWAY CONFERRING BOTH LIFE EXTENSION AND STRESS RESISTANCE. S. Murakami* & T.E. Johnson, Institute for Behavioral Genetics, University of Colorado, CO 80309

Several single-gene mutations that lengthen life up to 100% have been found in *Caenorhabditis elegans*. Diverse explanations have been put forward to explain life-extension of the mutants (age-1: an aging specific mechanism, daf-2 and daf-23: activation of a dauer-specific program and spe-26: reduced fertility). To understand the basic mechanism of longevity extension in *C. elegans*, we tested whether longer-life mutants show resistance to UV light as an index of environmental stress. Ultraviolet (UV) light is a well-characterized DNA-damaging agent which can also cause alterations in other cellular components, such as lipids and proteins, through the formation of free radicals. We have found that all the longer-life mutants show increased resistance to ultraviolet irradiation in adult hermaphrodites. In all cases, the ultraviolet resistance was suppressed by mutations in daf-16, which also suppressed longevity of all the mutants. The daf-16 mutations did not suppress the reduced-fertility defect of spe-26.

These results suggest: (1) There is a single, daf-16 dependent pathway, conferring life extension in four quite seemingly disparate mutations; (2) the daf-16 dependent pathway also confers resistance to UV radiation; (3) the reduced-fertility defect of spe-26 does not appear to be responsible for life extension. This pathway appears to confer increased resistance to a wide variety of stresses, since at least three of these mutants show increased thermotolerance and age-1 confers increased resistance to oxidative stress. We propose that a normal function of these gerontogenes is to negatively regulate both life extension and stress resistance in adults.

75

INTRACELLULAR CALCIUM SIGNALING IN RAT PAROTID ACINAR CELLS: EFFECTS OF AGE AND FOOD RESTRICTION. T.C. Smith*, M.A. Salih, C. Chen, M.S. Katz, & D.N. Kalu, Depts. of Physiology & Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

In parotid acinar cells, both ∝-adrenergic (∝AR) and muscarinic agonists stimulate intracellular calcium ($[Ca^{2+}]_i$) mobilization and Ca^{2+}-linked K^+ washout. Previous studies indicate that the response to (-)epinephrine (Epi), but not to carbachol, decreases with age. In addition, food restriction increases lifespan in laboratory animals and prevents many age-related physiological changes. In this study, we have characterized ∝AR and muscarinic induced $[Ca^{2+}]_i$ changes and ∝AR induced washout in dispersed acinar cells from male Fischer 344 rats (6-24 mo) fed *ad libitum* (AL) or 60% *ad libitum* intake (FR). Cells were prepared by collagenase/hyaluronidase digestion. $[Ca^{2+}]_i$ was measured by video image, fluorescent microscopy in single acinar cells loaded with FURA2. K^+ washout was estimated from ^{86}Rb release in cell suspensions pre-loaded with the isotope. Neither estimated age nor food restriction altered the peak $[Ca^{2+}]_i$ achieved in response to carbachol (100 μM). Similar results were obtained for Epi (100 μM) stimulation in 6 and 12 mo animals. However, the peak $[Ca^{2+}]_i$ response to Epi declined between 12 and 18 mo in both dietary groups (e.g., AL: 12 mo = 387±21 nM, 18 mo = 253±10 nM; FR: 12 mo = 430±22 nM, 18 m0 = 325±14 nM). The decline in response to Epi seen with age was less in FR than in AL animals at 18 mo, but not at 24 mo. Stimulation of K^+ washout (% total ^{86}Rb lost in 5 min; mean ± SE) from parotid cell suspensions by EPI shows a similar dependence on age (e.g., AL: 6 mo=43.5±1.8%, 18 mo=29.2±1.4%, 24 mo=34.1±2.8%). Food restriction

did not alter the response of K^+ washout to Epi stimulation. We examined whether the reduced response to epinephrine with age is related to alterations in parotid alpha-adrenergic receptor number and binding characteristics. The B_{max} for [^{3}H]prazosin increases by 41 and 18% in AL and FR rats, respectively, between 3 and 24 months, and is lower in FR than AL rats at all ages. The K_d for [^{3}H]prazosin binding increases by 70% in AL ($p<0.001$) rats between 3 and 24 months, but decreases in FR rats between the same ages. K_d is reduced by approximately 45% in FR versus AL rats at ages from 6 to 24 months. The results support the view that calcium mobilization in parotid acinar cells from male Fischer 344 rats in response to αAR, but not to muscarinic, stimulation is impaired with age. Food restriction may slow, but does not prevent, the functional decline. This effect may be partly mediated by enhanced epinephrine binding in FR animals.

76

FUEL USE AND AGING: INSIGHTS FROM STUDIES OF DIET RESTRICTED RATS. R.J. McCarter, Dept. of Physiology, University of Texas Health Science Center, San Antonio, TX 78284.

Metabolic fuels may occupy a central role in aging processes since they are inherently active and can react in ways which diminish rather than sustain cellular function. In particular the rate of fuel use, or metabolic rate, has long been viewed as a primary determinant of aging. In contrast, dietary restriction (DR) has been demonstrated to retard aging in laboratory rodents. The mechanism of action is unknown but the effect is a consequence of reduced input of calories rather than decreased consumption of any single dietary component. Since decreased caloric input is known to reduce metabolic rate, it has been widely speculated that decreased rate of fuel use underlies the anti-aging action of DR. Our previous research shows that decrease in metabolic rate is not an essential component of this mechanism. Our current research is on the use of glucose as a fuel, focusing on whole animal and skeletal muscle metabolism. Data from these studies suggest that DR re-sets conditions of fuel use such that glucose is metabolized under conditions less damaging to the maintenance of cellular homeostasis. For example, levels of plasma glucose and insulin are significantly lower in DR versus *ad libitum* fed rats, but the number of grams of glucose metabolized per day is the same per unit of metabolic mass in both dietary groups. Skeletal muscle is the primary site of disposal of glucose following a meal. In DR animals skeletal muscle exhibits enhanced ability to transport glucose under conditions of low extracellular glucose and insulin, mainly as a consequence of increased glucose effectiveness. The data are consistent with the view that DR enables fuel use under conditions which are less damaging to the maintenance of cellular function. Thus aging may be an inevitable consequence of life processes, in that fuel use is essential to life but the presence of reactive fuels is damaging in the long term.

77

MODULATION OF AGE-RELATED CHANGES IN HEART BY DIET AND EXERCISE. J.T. Herlihy*, S. Klebanov, J.S. Kim, Dept. of Physiology, University of Texas Health Science Center, San Antonio, TX 78216.

The cardiovascular system exhibits a variety of age-related functional and biochemical alterations. These alterations range from a loss in neural regulation, as observed with the decline in baroreflex control of heart rate, to changes in the expression of intracellular proteins, as seen with the cardiac myosin isozyme profiles. The mechanisms underlying these aging changes are not fully understood. Calorie restriction (CR) and exercise (EX) have profound anti-aging actions and are, therefore, useful probes of the aging processes. Unfortunately, these interventions have not been used widely to probe the aging cardiovascular system. However, the data that are available suggest multiple mechanisms of action. On the one hand, CR retards the age-related deterioration of baroreflex responsiveness and prevents the loss in cardiac norepinephrine content. Part of the mechanism for this retardation may be due to a decrease in free radical damage, since both CR and EX decrease mitochondrial and microsomal malondialdehyde content and enhance the cardiac anti-oxidant defense systems. On the other hand, CR accelerates, rather than retards, certain age-related changes. Aging is associated with a loss in β-receptor mediated relaxation of aortic smooth muscle, and CR accelerates that loss. Similarly, the cardiac alpha myosin isozyme profile changes with age from predominantly fast alpha myosin isozyme seen in young rat hearts to predominantly slow beta isozyme seen in old rat hearts, and CR augments the age-related changes. Since arterial β-adrenergic responsiveness and cardiac myosin isozyme profiles depend upon thyroid hormone status, these results suggest that thyroid hormone may mediate these age- and diet-induced alterations in the cardiovascular system. The ramifications of the diverse actions of CR and EX on the aging cardiovascular system will be discussed.

78

DIETARY RESTRICTION PROTECTS TRANSCRIPTION AND MODULATES THE PERMEABILITY TRANSITION IN LIVER MITOCHONDRIA. B.S. Kristal* & B.P. Yu, Dept. of Physiology, University of Texas Health Science Center, San Antonio TX.

Mitochondrial function is dependent on defense mechanisms that protect critical systems from oxidant- and calcium-mediated damage. We examined the effects of age and dietary restriction (DR) on the ability of the transcription system to resist oxidant attack, and on the ability to resist induction of a permeability transition.

Our previous observation that mitochondrial transcription is extremely sensitive to oxidant-mediated inhibition, coupled with the evidence of the existence of age-associated, oxidant-mediated damage led to the hypothesis that aging might lead to overt loss of resistance to oxidant-mediated damage, and that DR might prevent this decline. Our primary findings were that age increased variability of resistance to both hydrophilic and hydrophobic oxidants, but did not necessarily decrease it. DR consistently increased resistance to hydrophobic oxidants by middle age, and reduced the age-associated variability in resistance to this stress, but had less effect on resistance to hydrophilic stress until later in life.

Mitochondrial transport and storage of calcium is critical both for buffering cytosolic calcium and for maintaining proper energy production. One measure of the mitochondrial capacity to both effect and affect calcium homeostasis is the ability to resist induction of an event termed the mitochondrial permeability transition (PT). A PT is most commonly associated with calcium- and oxidant-mediated activation of a specific inner mitochondrial membrane channel. We examined PT induction in animals of different ages maintained on *ad libitum* feeding or dietary restriction regimens. Little effect of age is observed in *ad libitum* fed animals after 12 months of age. In contrast, we will present data demonstrating that DR, which increases longevity and enhances antioxidant defense systems, greatly enhances resistance to PT induction. Resistance is enhanced to several distinct inducers -- calcium alone, phosphate alone, calcium and phosphate, calcium and t-Butyl hydroperoxide, as well as to calcium and the lipid peroxidation byproduct 4-hydroxyhexenal, one of the most potent inducers discovered to date. In some cases (e.g., PT induction by calcium and phosphate), resistance can be increased as much as 12-fold. It is also interesting to note that DR animals further enhance their resistance to PT induction during aging, with a notable increase occurring between 12 and 24 months of age. Although a portion of the resistance is clearly mediated by the increased resistance of animals to oxidative stress, it appears that the primary modulator of the increased resistance to PT induction in DR animals is an improved ability to regulate intramitrochondrial calcium homeostasis. These studies highlight the susceptibility of the PT to the physiological status of the organisms and provide mechanistic information about the interaction of calcium and oxidants in PT induction. In addition, these studies also help to further explore how DR helps prevent free radical and lipid peroxidation-mediated toxicity that may occur during the aging process.

79

ALTERATION OF PROTEASOME FUNCTION BY AGING AND DIETARY RESTRICTION. W.F. Ward* & T. Shibitani, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7756.

Protein degradation declines with age and this decline is ameliorated by food restriction. It has been suggested that age-related decline in protein degradation is due to the alteration of cytosolic protein degradation pathway. Proteasome has been described as a major alkaline protease in the cytosol. Therefore, it is of importance to investigate the effect of age and food restriction on the proteasome function. We have investigated 1) quantitation of the cellular proteasome level, 2) three major peptidase activities: chymoatrypsin-like (ChT-L), trypsin-like (T-L), and peptidylglutamyl peptide hydrolyzing activities (PGPH), and 3) proteolytic activity. The studies have been carried out using liver tissue from 7,16, and 26 month-old Fischer 344 rats. The proteasome level was measured by Western blot analysis. The level of the proteasome was not affected by either age or food restriction ChT-L and T-L peptidase activities of the proteasome increased ~15% and ~30% with age, respectively, and these increases were prevented by food restriction. In contract, PGPH activity declined ~40% with age, and food restriction maintained a higher rate of the activity throughout life. The proteolytic activity of the proteasome was assessed using casein as a substrate and polylysine as a proteasomal activator. The proteolytic activity was not changed by either age or food restriction. In conclusion, the proteasome peptidase activities appear to be differentially regulated by age and food restriction without altering its proteolytic activity. We hypothesize that the effect of age and food restriction are mediated by alteration the subunit composition of the proteasome, analogous to the effects of y-interferon on expression of LMP2 and LMP7.

80

DO GLUCOCORTICOIDS MEDIATE ANTI-AGING ACTIONS OF FOOD RESTRICTION? J.F. Nelson, Dept. of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78216-7756.

Food restriction (FR) induces changes in gene expression which prolong life, presumably by enhancing cytoprotective mechanisms. These changes occur throughout the organism and are therefore probably orchestrated in part systemically, by hormonal and neural mediators. Our working hypothesis--that glucocorticoids are among the hormonal mediators--is based on several lines of evidence. First, levels are elevated in FR animals, and glucocorticoids are key components in the protection against stress. Second, the FR rat employs multiple strategies to sustain a hyperadrenocortical state, suggesting that the maintenance of that state is physiologically important. In the early phase of FR, plasma corticosterone is elevated. When that elevation disappears, plasma corticosterone binding globulin declines and free corticosterone remains elevated. Corticosterone levels also remain elevated despite decreased ACTH, due to enhanced adrenal sensitivity to ACTH. Third, many corticosterone-regulated genes and proteins exhibit changes consistent with a hyperadrenocortical state: pituitary ACTH, PRL, TSH and GH; hypothalamic CRH, proopiomelanocortin and neuropeptide Y; liver lipocortin I, phospholipase A2 and insulin receptor. Many of these changes are in directions consistent with greater life span or cytoprotection. Finally, inflammatory processes are more rapidly resolved in FR animals. Suppression of inflammation a well known effect of glucocorticoids, is implicated in the attenuation of several age-related pathologies., including cancer, cardiovascular disease and dementia. These arguments support the possibility that glucocorticoids contribute to the altered metabolic state of FR and argue for further testing of their role in the anti-aging actions of FR.

81

CALORIC INTAKE, FREE RADICALS AND MITOCHONDRIAL AGING. R. Weindruch, University of Wisconsin & VA GRECC, Madison, WI 53705.

A major issue in caloric restriction (CR) research concerns the mechanisms by which CR retards aging in rodents. There is increasing evidence that CR may act by reducing mitochondrial production of free radicals and modulating free radical metabolism in tissue-specific ways culminating in decreases in oxidative stress. A tissue which appears important to investigate further in the context of CR is skeletal muscle. It accounts for a large part of the body's total oxygen consumption at rest (due to its large mass) and the majority of oxygen consumption during vigorous physical activity. Also, skeletal muscle and nervous tissues therein do not posses the very high repair capacities found in more mitotically active tissues. Further, muscle's main cellular replacement system (satellite cells) is less robust in old animals. Accordingly, oxidative stress may accumulate with age in muscle and in the neurons therein and contribute to the development of sarcopenia (muscle mass loss). This possibility has not been thoroughly investigated.

Recent findings using the CR paradigm support this concept. Studying hindlimb skeletal muscle samples from male (Brown-Norway x F344) F1 rats of various ages fed normally or subjected to CR at 14 weeks of age, we found that the total muscle mass recoverable in CR rats was ~30% less than that of ad lib-fed controls at 11 months of age. However, CR countered the 50% loss of muscle mass observed in very old (34 mo) ad lib-fed rats. Antioxidant enzyme activities were measured. Activities of glutathione peroxidase and catalase from the 1000 x g supernatant and a 12,000 x g fraction increased with aging in ad lib-fed rats, and these increases were opposed by CR. These data can be explained by substrate-induced increases in the activities glutathione peroxidase and catalase in muscle from *ad lib* fed-rats. This induction is a probable consequence of increased free radical production in ad lib-fed animals which may be responsible (at least in part) for the development of mitochondrial damage and sarcopenia. New findings from collaborating investigators (e.g., Aiken, Feuers, Sohal) provide additional support for this explanation. Supported by the NIA (P01 AG11915, R01 AG10536) and the American Cancer Society (#CN57).

82

MODULATION OF MITOCHONDRIAL DAMAGE BY DIETARY RESTRICTION. B.P. Yu, Dept. of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX

We have previously proposed that the well-diversified anti-aging and life-prolonging action of dietary restriction depends on its antioxidative mechanisms and the modulation of free radical metabolism. This proposal was made based on available data on the suppression of free radical generation, the maintenance of cytosolic antioxidant defense systems, the enhancement of eliminating cytotoxic substance, and the maintenance of efficient repair process.

Our recent findings on mitochondrial lipid peroxidation - another major source of oxidative stress - have shown that endogenously reactive aldehydes generated from age-related lipid peroxidation can produce a variety of alterations in both mitochondrial structure and function. Our results show that both major aldehyde products, 4-hydroxynonenal (HNE) and 4-hydroxyhexanol (HHE) can readily modify the integrity of mitochondria. For instance, the interaction of HNE with mitochondria causes inactivation of adenosine nucleotide translocator by binding to the SH group of the enzyme protein. Mitochondrial respiration is significantly suppressed by HNE, leading to reduced respiratory control ratio (RCR). Mitochondrial Ca channel (permeability transition pore) can be activated by picomoles of HHE. Dietary intervention as shown in mitochondrial aging was remarkably well exhibited in several parameters measured: inhibited HNE binding, increased ANT, increased resistance to HNE-induced membrane rigidity, and well-maintained RCR. Based on these findings, it is proposed that the suppression of mitochondrial lipid peroxidation is an important mechanism by which dietary restriction preserves the mitochondrial function during aging.

83

THE EFFECT OF DIETARY RESTRICTION ON ELECTRON TRANSPORT IN AGING. R.J. Feuers, National Center for Toxicological Research (NCTR), Jefferson, AR 72079.

Investigations concerning the "Free Radical Theory of Aging" have begun to focus on mitochondria, and a potential site of free radical generation would be within the electron transport system (ETS). Age-associated alterations in ETS may lead to increased rates of free radical generation which contribute to aging, and dietary restriction's (DR) life-extending properties may, in part, be due to ameliorating degeneration of ETS function. When the activities of the complexes of ETS were monitored from muscle preparations of young and older B6C3F1 female mice fed *ad libitum* (AL) or a calorically restricted diet (60% of AL), age associated decreases which were offset by DR were noted for complexes I, III, and IV. Complex II was not affected by age or diet, and it is the only complex of ETS which is totally of nuclear origin. To investigate the relationship of these activity losses with potential for increased free radical generation, Michaelis-Menton kinetic studies were performed. Age associated increases in Km values for complex III, did not occur in DR animals. This suggests a loss of function with age and a decline in normal binding of substrate may be associated with not only inhibition of ETS, but also premature release of product as a free radical. Complex IV possesses a high and low affinity binding site. The proportion of high to low affinity binding sites was 69% in young AL animals, and 80% in young DR animals. In older AL animals the percentage of low affinity sites increased to 55%, but remained 80% in older DR animals. As was the case with complex III, this loss of function with age is suggested as a potential mechanism for age associated increased levels of free radical production which is retarded by DR.

84

DIETARY FATTY ACID MODULATION OF CARDIAC MEMBRANE COMPOSITION: CARDIAC PERFORMANCE AND MITOCHONDRIAL FUNCTION IN AGING. S. Pepe, LCS, Gerontology Research Center, NIA, NIH, Baltimore, MD 21224.

The composition of myocardial membrane phospholipid fatty acids can be altered by modification of the type of dietary fat intake. By increasing the membrane phospholipid content ratio of polyunsaturated fatty acids (PUFA) vs saturated fatty acids, and increasing the content ratio of omega-3 PUFA vs omega-6 PUFA, a significant increase in membrane fluidity occurs. This alters cellular function as a consequence of influence on many effectors such as: ion flux, respiratory electron transport, membrane-bound enzyme activity, receptor-activated intracellular transduction systems, eicosanoid metabolism, lipid peroxidation and subsequently alters their response in the etiology of cardiovascular pathology.

Age is a major risk factor for the occurrence of ventricular fibrillation in humans. It has been shown in studies with rats that the vulnerability to arrhythmic stimuli increases with age and that fish oil diet rich in omega-3 PUFAs (FO) was shown to abolish this effect, whereas a diet rich in saturated fat (SAT) exacerbated arrhythmogenesis. In isolated working rat heart studies developed to evaluate the influence of dietary lipids on mechanical and biochemical responses to ischemia/reperfusion (I/R) it was shown that dietary lipids produced marked effects on myocardial oxygen demand that were independent of contractile function under control conditions. The consequences of I/R were more severe in hearts from the SAT group compared to FO group hearts, in terms of decreased work output with increased MVO_2, K^+ release, lactate production, venous acidosis, creatine kinase release and arrhythmias. MVO_2, especially after I/R was distinctly high in SAT hearts but markedly reduced in FO hearts that had high O_2-energy utilization efficiency. This was not due to any change in basal O_2 metabolism but rather was indirectly found to be related to altered intracellular

Ca^{++} homeostasis as when hearts were perfused with ruthenium red to block mitochondrial Ca^{++} entry the thermodynamic efficiency increased in SAT hearts. Fatty acid analysis of myocardial membranes in this dietary model indicated that with increased age (6 vs 24 mo) arachidonic acid (omega-6 PUFA) markedly increased whereas omega-3 PUFAs were markedly reduced. SAT diet augmented this age effect whereas no major change in the proportion of these fatty acids with increased age occurred with FO.

Recently, studies were conducted to define the less efficient use of O_2 at the mitochondrial (MITO) level and test whether this was related to increased Ca^{++} handling by MITO in SAT hearts compared to FO. The respiratory control ratio was raised significantly in MITO from FO hearts with the oxidative substrates utilized. Ca^{++}-dependent activation of MITO pyruvate dehydrogenase and $[Ca^{++}]_{MITO}$ was significantly higher in preps from 24 mo rats vs 6 mo and this effect was augmented in SAT groups vs FO. It is concluded that decreased membrane fluidity with aging or SAT diet contributes to increased MITO H^+ and Ca^{++} cycling with decreased thermodynamic efficiency. The effect of increased omega-3 PUFA content of phospholipids may thus have important beneficial consequences on cardiac mechanical and metabolic function with aging.

85

EFFECTS OF AGE, DIETARY RESTRICTION AND ADHERENCE ON HSP70 EXPRESSION IN FISHER 344 RAT ALVEOLAR MACROPHAGES. S.A. Moore*, A. Lopez, A. Richardson, University of Texas Health Science Center, & the Audie Murphy Veterans Hospital, San Antonio, TX.

It has been well documented that with age there is a decline in the ability to respond to injury, inflammation and pollutants. It is conceivable that age-related changes in the expression Hsps in macrophages, which respond to these environmental challenges, could contribute to this decline in immune function. In this study, alveolar macrophages (AM) were isolated from the lungs of young (4 to 6 months), middle aged (12 to 13 months), and old (24 to 25 months) Fisher rats fed ad libitum and calorie restrictive (60% of ad libitum) diets Fisher 344 rats. There was no variation in cell number, differential (>85% macrophage), viability, or adherence between the groups. The induction of hsp70 was measured at the mRNA level by northern analysis on total RNA extracted from AM cultured a total of 2 hours in suspension (unactivated), or adherent in tissue culture dishes (activated) at 37°C or at heat shock (43°C for 1 hour) in serum-free media. The induction of adherence stimulated hsp70 in AM was defined to be insensitive to cycloheximide, cytochalasin B, indomethacin, and nitric oxide synthetase inhibitors, but extremely sensitive to the antioxidant, beta-2-mercaptoethanol. Similar inhibitory effects were also seen with dithiothreitol. In AM suspension cultures, hsp70 mRNA was undetectable at 37°C. However, adherent AM cultured at 37°C decreased significantly with age the expression of hsp70. For example, hsp70 mRNA levels in AM from young, ad libitum-fed rats were 3 fold greater than hsp70 mRNA levels in AM isolated from old ad libitum fed rats. In addition, the induced levels of hsp70 mRNA levels by heat shock decreased with age in ad libitum-fed rats. Caloric restriction also had a significant effect on the expression of hsp70 in adherent AM with a response in the old group resembling the response of AM from young rats, meaning an increase of 3 fold expression over the old ad libitum response. Interestingly, heat shocked AM cultured in suspension, exhibited no age related alterations in the expression of hsp70. The data presented here indicate that the age-related changes in the expression of hsp70 in AM may be due to a thiol-sensitive step in the pathway of signal transduction of adherence activation.

86

LIPOFUSCIN ACCUMULATION IN MOUSE BRAIN WITH AGE: EFFECTS OF CALORIC RESTRICTION. W.A.L. Moore, V.A. Davey, R. Weindruch & G.O. Ivy, Office of Post Graduate Education, Clarke Institute of Psychiatry, Toronto, ON, Canada M5S 1R8; Life Sciences Division, University of Toronto, Scarborough, ON, Canada M1C 1A4.

This study was designed to further elucidate the relationship between lipofuscin (LF) accumulation and the aging process by examining the effect of lifelong caloric restriction (CR) on LF accumulation in brain cells. CR has been shown to extend both average and maximum lifespan in rodents and other animals as well as to delay a wide variety of physiologic manifestations of aging. The results of previous experiments generally support the hypothesis that CR leads to decreased LF with age; however, methodological problems within these studies have precluded a clear resolution of this issue.

The present study was thus designed to address the previous methodological problems. Specifically, 1) our study included three age groups of CR and control mice including a group at maximum lifespan; 2) CR was the major dietary manipulation; 3) LF was identified using EM, the most accurate method; 4) LF was quantified by a real measurement; and 5) the results were analyzed by inferential statistics.

We quantified both % of cytoplasm containing LF and absolute amount of LF in 50 granule cells per mouse and found that 1) LF increased with age and 2) that animals on CR had significantly less overall LF in the perikarya of the granule cells of the dentate gyrus of hippocampus when compared to control animals at equivalent ages. In addition, CR mice at maximum lifespan (45 mo.) had slightly less LF than did control mice at their maximum lifespan (36 mo.). Therefore our results clearly demonstrate that CR retards the overall accumulation of LF with time and, further, suggest that LF accumulation is not simply a linear function of age.

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87

REGULATION OF THE RAT XANTHINE DEHYDROGENASE/OXIDASE AND URIC ACID FORMATION BY AGING AND DIETARY RESTRICTION (DR). H.Y. Chung* & B.P. Yu, Dept. of Pharmacy, Pusan National University, Pusan, Korea, & Dept. of Physiology, University of Texas Health Science Center at San Antonio, TX.

Xanthine oxidoreductase (XD) is an enzyme complex that catalyzes oxidation of hypoxanthine to xanthine and subsequently to uric acid. The enzyme exists in separate but interconvertible forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XOD). With respect to free radical metabolism, XOD is one of the major cellular sources of superoxide production, and its participation as a causative factor is well known in ischemia/reperfusion condition. However, the status and the regulation of XOD are almost unknown at present. Another important consideration of XD/XDH system is the formation of a potent antioxidant, uric acid.

In the present study, we investigated the age effect on hepatic XOD/XDH activities and uric acid formation, and modulation by dietary restriction. All rats were fed *ad libitum* until 6 weeks of age, then they were separated into two groups: *ad libitum* fed control (AL) and dietary restricted group (DR). In the DR group, whole food intake was restricted by 60% of the food intake of the control group. Liver tissue was homogenized with 100 mM Tris buffer containing 0.2 mM PMSF, 1 μM pepstatin, 2 μM leupeptin, trypsin inhibitor (80mg/l), 1 mM EDTA and 10 mM DTT, centrifuged at 15,000 g for 1 hr. The supernatants were passed over Sephadex G25 for the enzyme isolation. The activity of isolated enzyme is based on the formation of uric acid read at 295 nm. The assay was carried out in a medium containing 50 μM xanthine, 100 μM EDTA and 50 mM K-phosphate (pH 7.8) at 25°C. XOD was assayed in the absence of NAD, while total XD (XDH and XOD) was measured in the presence of 500 μM NAD. The extent of conversion (% XOD) was calculated from XOD divided XD.

Our results show that XOD activity changed little with age. However, an unexpected but significant increase (76%) was observed in DR rats, which peaked at 24 months. On the other hand, XDH activity decreased in AL rats with age. Although age was shown not to be a factor in DR rats, this group sustained higher XDH levels than AL rats at 18 and 24 months of age. Conversion of XDH to XOD expressed as ratio of XOD/XD showed a slight increase during aging in both groups. Interesting findings were found in uric acid levels which showed no age-related changes in AL rats; but DR rats showed consistently higher uric acid levels at all ages studied. We propose that the high levels of uric acid in DR rats may be a significant factor contributing to enhanced antioxidant defense systems through DR.

88

LONG-TERM CALORIE RESTRICTION ALTERS THE AGE-RELATED DECLINE IN DHEA AND DHEA-SULFATE IN RHESUS MONKEYS. M.A. Lane*, D.K. Ingram & G.S. Roth, Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224.

Among the most abundantly produced adrenal steroids, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), have attracted much attention for their ability to modify tumorigenesis, diabetes mellitus, obesity and immune function. Many of the apparently beneficial effects of DHEA are also manifested by rodents on a calorie restriction (CR) regimen. It has been suggested that DHEA and CR might affect age-related disease through similar mechanisms. Little is known concerning the effects of CR on DHEA and DHEAS; however, some studies suggest that CR enhances adrenocortical activity. In the present study thirty male and sixty female rhesus (Macaca mulatta) monkeys of different ages were fed approximately *ad libitum* or subjected to 30% CR for either 3 (females) or 6 (males) years. Although life span extension by CR has not been demonstrated in this species, many physiological effects known to occur in rodents, such as altered glucoregulation, delayed sexual and skeletal maturity and effects on body temperature and metabolic rate, have been demonstrated in rhesus monkeys subjected to CR. Circulating DHEA and DHEAS levels were determined in blood samples collected in the morning hours following an overnight fast. Significant gender differences in both DHEA and DHEAS were noted as females had consistently higher levels compared to males. DHEA and DHEAS

exhibited developmental increases and cross-sectional and longitudinal declines with aging. The early decline to adult levels was delayed in the youngest groups of monkeys subjected to CR. The physiological significance of this delay is unknown, but we have reported similar delays in sexual (testosterone) and skeletal (alkaline phosphatase) maturation for rhesus monkeys on CR. Slight elevations in DHEA and DHEAS might relate to CR-induced lowering of insulin levels. It has been proposed that hyperinsulinemia reduces DHEA and DHEAS by increasing metabolic clearance of these substances. Preliminary findings in our lab suggest that DHEAS but not DHEA levels were negatively correlated with blood insulin levels.

89

EFFECTS OF GROWTH HORMONE ON BONE LOSS AND KNEE JOINT DAMAGE CAUSED BY DENERVATION IN AGED MICE. A. Weiss*, A. Kalemen, R. Shofty, & A. Reznick, The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel.

The aim of the study was to examine the effects of growth hormone on changes in skeleton caused by immobilization in aged mice. The right legs of 14 months old male ICR mice were immobilized by dual, sciatic and femoral denervation, while the left, intact limbs served as control. Animals were treated by daily sc injections of either 1 mg/kg bovine rGH or vehicle for a period of one month. GH had no effect on body weight of the animals. In the vehicle treated animals denervation caused a 10% decrease in the wet weight of tibiae, 51% decrease in trabecular bone volume of the proximal condyle of tibia and 16% decrease in the cortical thickness of tibiae ($p<0.05$ in comparison to control leg). Also, the contents of mineral, DNA and protein in the tibiae from denervated limbs were decreased by 41%, 33% and 16% respectively. Denervation was found to cause a severe damage to the knee joint, such as degenerative changes in the articular cartilage, ossification and blood invasion of the menisci and inflammation of the synovium. Administration of GH significantly improved the morphological appearance of the tibia and knee joint and prevented bone loss. However, the atrophy of the triceps surae muscle, which mass was decreased by 66% in the disused leg, was not affected by GH.

In summary, the findings of this study reveal that in mice systemic growth hormone can prevent bone loss and joint degeneration due to prolonged immobilization.

90

THE EFFECTS OF AGE AND PHYSICAL ACTIVITY ON MYOCARDIAL CAPILLARY RECEPTOR ULTRASTRUCTURE. A. Cosmas* & T. Manfredi, University of Connecticut, School of Allied Health, U101, Storrs, CT. 06269; University of Rhode Island, Exercise Science, Independence Square, Kingston, RI 02881-0810.

In an exercise study we reported that hearts examined from rats that exercise throughout life have a larger concentration of smaller size mitochondria and a greater capillary to fiber ratio. Exercise and aging have been shown to have opposite effects on capillary numbers and mitochondrial concentrations in the rat myocardium.

Myocardial capillaries contain specific membrane receptor proteins which can cause the membrane to form endocytotic vesicles and provide a mechanism for taking in extracellular fluid and specific proteins that could not normally enter the cell by any other means. Previous research has presented evidence that capillary vesicle numbers, rather than vesicle size provide valuable morphometric information regarding receptor transport. The purpose of this study was to examine a subset of these animals taken from an exercise/aging study in order to determine if physical activity has any effect on the myocardial capillary ultrastructure and endocytotic vesicle concentration and size distribution in young control (YC; age =195 days), old control (OC; age = 930 - 1095 days) and old exercise trained (OE; age=850-1095 days) Sprague Dawley rats. Animals were trained on a motor driven treadmill 5 days a week, 20 minutes a day and ran 200 meters at the end of each training session. Diets were carefully controlled to maintain constant body weights between groups. Left ventricular tissue samples were fixed for conventional electron microscopy and capillary vesicle sizes were measured using a Ziess particle size analyzer. A Dietzen compensating polar planimeter was used to measure capillary luminal and endothelial areas. The % of the total capillary area taken up by the endothelium of the YC and OE animals were similar whereas the & endothelial area of the OC animals was smaller and the endothelial walls were thinner ($p<05$). The capillaries of the young animals also had greater endothelial area than the OC and OT groups. Age and exercise had no effect on the mean vesicle area and concentration (vesicles per unit area). However, the vesicle size distributions were similar in the OC and OT groups, which differed from the YC animals and which had a larger percentage of vesicles in the mid-range of the 5-size categories. Also, the endothelium of the YC animals had a greater total number of vesicles due to the larger surface area. We conclude that age effects the

morphometry of myocardial capillaries which may alter capillary receptor mediated transport. Furthermore, exercise training alters the capillary endothelium without changing the morphometry of the vesicles.

91

LIPOFUSCIN ACCUMULATION AND CATHEPSIN B ACTIVITY IN RAT BRAIN AND HEART DURING DEVELOPMENT AND AGING. E.A. Porta*, S. Llesuy, A.J. Monserrat, S. Benavides & M. Travacio, Dept. of Pathology, School of Medicine, University of Hawaii, Honolulu, HI 96822, & Dept. of Chemistry and Pathology, University of Buenos Aires, Argentina.

Although it has been hypothesized that a decline in the activity of thiol proteases with age may be responsible for the progressive accumulation of lipofuscin (age pigment) in animal tissues (Ivy et al., 1989), no direct evidence for this age-wise enzymatic decline has been presented. In the present study Wistar female rats, maintained from weanling on a commercial ration *ad lib.*, were killed at the ages of 5, 14, and 24 months and the brain and heart activities of cathepsin B, the most abundant of lysosomal thiol proteases, were determined by enzymatic histochemistry and biochemistry, while the amounts of lipofuscin from the same organs were histologically quantitated by autofluorescence and by Ziehl-Neelsen stain. The results indicated that during development (between 5 and 14 months) cathepsin B activities in brain and heart remained statistically unchanged, but they significantly decreased in both tissues during aging (between 14 and 24 months). On the other hand, and in line with numerous previous findings, the amounts of heart and brain lipofuscin increased almost linearly during development and aging. These results suggest that the age-wise changes in the enzymatic activities of at least brain and heart cathepsin B may not correlate with the progressive increase of lipofuscin.

92

EFFECT OF ESTROGEN AND PROGESTERONE ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN CEREBELLA AND CEREBRA OF FEMALE WISTAR RATS. B.N. Reddy*, S.P. Sharma, T.J. James & B.P.S. Vohra, Dept. of Zoology, Kurukshetra University, Kurukshetra, Haryana-132 119, India.

Two major female reproductive hormones, i.e., estrogen (17β Estradiol) and progesterone (4-Pregnene-3, 20-dione) were used in the present study to register their effects on the cytosolic and mitochondrial lipid peroxidation and the enzymes associated with antioxidant defense mechanism, viz., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GRD) in cerebella and cerebra of female Wistar rats of 3 age groups. The hormones, individually and in combination, were administered at a dosage of 10 μg/100g body weight daily at 11:00hr for 21 days. After completion of hormonal treatment the animals were killed and their brains were removed and subsequently the 2 regions, i.e., cerebella and cerebra, were fractionated into mitochondrial and cytosolic ones. Lipid peroxidation was measured by thiobarbituric acid (TBA) method, while the antioxidant enzymes were estimated by well established methods. The quantity of thiobarbituric acid-reactive substances (TBARS) was found to have increased substantially with age as well as in the ovariectomized animals. The hormonal administration was found to have reduced the formation of TBARS while it enhanced the antioxidant enzymes in all the groups and in both the regions. The results indicate that administration of reproductive hormones in post-reproductive phase could effectively check the increased oxidative damage in old age.

93

AGE-RELATED MODULATION OF SYNAPTIC PLASTICITY IN LESIONED RATS. Carlo Bertoni-Freddari*, Patrizia Fattoretti, Ugo Caselli & Roberta Paoloni, Neurobiology of Aging Laboratory, "N. Masera" Research Dept., 60121 Ancona, Italy.

The number of synapses/μm^3 (numerical density: Nv), the average area of the synaptic contact zones (S) and the total area of the synaptic junctions/μm^3 (surface density: Sv) were measured in the hippocampus of adult (11 months of age) and old (22 months of age) rats undergone partial transection of the fimbrial bundle. The lesioned animals were sacrificed at 3, 7, 15, 30 and 60 post-lesion (PL) days. Unlesioned controls (UC) were sacrificed at 11 and 22 months of age. Adult Nv decreases at 15 PL days to increase again at 30 and 60 PL days. In the old group Nv is very low at 30 PL days, while at 60 PL days a not significant increase was observed. In the adults S is the same at 3 and 7 PL days, it increases at 15 PL days, decreases significantly at 30 and increases again at 60 PL days. In old animals, at 3 PL days S is significantly larger than at 7 and 15 PL days, then it increases again at 30 PL days and decreases at 60 PL days. S in old rats is significantly larger than in adults at 3, 30 and 60 PL days, whereas at 7 and 15 PL days adult and old animals show the same value. The adult Sv increases at 7 PL days and remains constant up to 15 PL days. It decreases at 30 PL days and increases again at 60

PL days. Sv in old rats is not significantly different from 3 up to 60 PL days. Adult values are significantly higher than the old values at 3, 7, 15 and 60 PL days. At 60 PL days an age-paired comparison with UC animals shows a still consistent reduction of Sv in both the groups analyzed. In any adult PL group we found high percentages (from 25% at 15 PL days to 40% at 60 PL days) of small synapses (< 0.08 μm^2), while in the adult UC only 5% of the contacts accounts for junctional areas smaller than 0.08 μm^2. In the old animals, synapses smaller than 0.08 μm^2 are consistently found at any survival period following lesion, although at a lesser extent than in adults. Adaptive changes in the mature mammalian brain are currently reported to occur as a consequence of different environmental stimuli. Namely, morphological rearrangements of the synaptic contact areas are carried out through a sequence of steps involving enlargement, perforation and fragmentation of the contact zones. Our present findings support the assumption that some junctional areas have undergone splitting during the time course of recovery both in adult and old rats. However, at any adult or old PL day Sv was always significantly lower than in the UC age-matched animals, therefore the restoring of synaptic networks appears to be incomplete up to 60 PL days. The different size composition of synaptic population in the same adult and old PL groups supports the idea that synaptic structural dynamics are markedly modulated by age when accomplishing recovery from lesions.

94

LONG-TERM TREATMENT WITH DEPRENYL REDUCES THE OPTIMAL DOSE AS WELL AS THE EFFECTIVE DOSE RANGE FOR INCREASING ANTIOXIDANT ENZYME ACTIVITIES IN OLD MOUSE BRAIN. K. Kitani*[1], G.O. Ivy[2] & M.C. Carillo[3], [1]Radioisotope Research Institute, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan; [2]Division of Life Sciences, University of Toronto at Scarborough, Scarborough, ON, Canada M1C 1A4.

Chronic treatment with deprenyl has been reported to increase the life span of old rats. As one possible mechanism for the life extension effect of the drug, an up-regulation of antioxidant enzymes in selective brain regions has been suggested. In order to know the optimal dose for this effect, we treated old male mice for 3 months with different doses and compared the results with our previous data of short treatment of 3 wks. C57BL mice of the male sex received different doses of deprenyl (0.25, 0.15, 1.0 mg/kg per injection 3 times a week, s.c.) for 3 months beginning at the age of 26 months. At the age of 29 months, animals were sacrificed and superoxide dismutase (SOD) and catalase (CAT) activities were examined in several brain regions. The dose of 0.5 mg/kg (3 times a week) was most effective in increasing SOD and CAT activities in S. nigra, striatum and cerebral cortex but not in hippocampus or cerebellum. The dose of 0.25 mg/kg was also effective in increasing enzyme activities, but the effect was much lower than the dose of 0.5 mg/kg. The highest dose of 1.0 mg/kg had negligible effect. Together with the results from our previous study with short term deprenyl treatment in old mice, these results replicate our previous findings in old female rats. We showed that longer term treatment with deprenyl reduces the optimal dose for increasing antioxidant enzyme activities by a factor of 5 to 10. The present study further indicates that a longer term treatment with deprenyl also reduces the effective dose range of deprenyl as well as the magnitude of increase of enzyme activities. If the effect of deprenyl to increase these antioxidant enzyme activities in selective brain regions is causally related to its effect of increasing average life expectancies of rats, the selection of a proper dose of the drug may be a critical factor in life span studies in which the drug is administered for more than one year.

95

EFFECT OF DOCOSAHEXAENOIC ACID (DHA) ON LEARNING AND MEMORY IMPAIRMENTS OF BRAIN IN THE SENESCENCE-ACCELERATED MOUSE (SAM-P8) STRAIN. J. H. Choi*[1] & H. S. Yoon[2], [1]Dept. of Nutrition & Food Science, National Fisheries, Univ. of Pusan and [2]Dongwon Food Research Institute, Korea.

We previously reported that administration of DHA suppressed triglyceride, LDL-cholesterol, 22:6/20:4 fatty acid fatty acid ratio, lipid peroxidizability, and hydroxyl radical formation, while it enhanced HDL-cholesterol and superoxide dismutase activity. In the present study, the effect of DHA feeding was assessed for cognitive function. Male SAM-P8 mice were fed diets containing lard (15%) for control group and lard (5%) plus fish oil (10%) containing 31.95% DHA for experimental group for 8 months. Changes in cognitive function of memory and passive avoidance were determined. The results show that the avoidance score was significantly higher in DHA fed group than in the control group. Other biochemical measurements, homovanillic acid, 5-hydroxyindole acetic acid, and acetylcholinesterase activity were significantly enhanced ($p<0.01$ 0.001) by DHA feeding. Also increased were superoxide dismutase and glutathione peroxidase activities. In addition, hydroxyl radical formation, malondialdehyde and

lipofuscin levels in brain membranes of DHA group were significantly lowered compared to the control group. These results suggest that DHA in fish oil could be beneficial in maintaining cognitive function.

96

EFFECT OF REED ROOT EXTRACT, ON LEARNING AND MEMORY IMPAIRMENTS IN THE SENESCENCE-ACCELERATED MOUSE (SAM-P8) STRAIN. J.H. Choi* & J.S. Choi, Dept. Of Nutrition & Food Science, National Fisheries University of Pusan, Korea.

Aging is accompanied with a neurodegenerative disorder, characterized by progressive loss of memory and cognitive function. Using SAM-P8 mice, a murine model of accelerated senescence, a study was conducted to evaluate the effect of reed root extract (RRE; Phragmites communis) on learning and memory impairments of SAM-P8. Male specific pathogen-free SAM-P8 strain was fed an experimental diet of RRE to 250 mg/kg body wt. for 8 months. Changes in memory and passive avoidance were measured. The results show that the passive avoidance test score was significantly higher in RRE group than in control group ($p<0.001$). Other biochemical changes, homovanillic acid, 5-hydroxyindole acetic acid, and acetylcholinesterase activity, all were remarkably increased in the RRE fed group ($p<0.05$ 0.001). Hydroxyl and superoxide radicals, and malondialdehyde (MDA) levels in brain membranes of the RRE group were significantly suppressed compared with those in the control group ($p<0.001$). Although lipofuscin levels showed little improvement, superoxide dismutase was significantly increased in the RRE group ($p<0.001$). The results suggest that RRE administration could effectively attenuate cognitive dysfunction of dementia. It is proposed that the major components of RRE, β-sitosterol and p-coumaric acid, may be responsible factors in modulating monoamine oxidase activity in the brain.

97

SOME CONSIDERATIONS ABOUT THE GENERAL MECHANISM OF AGING OF LIVING ORGANISMS. A.N. Khokhlov, Evolutionary Cytogerontology Sector, School of Biology, Moscow University, 119899 Russia.

Much of the data available allows one to consider that the aging process of different multicellular organisms is mainly determined by accumulation of molecular-genetic defects in their cells that, in turn, leads to deterioration of function for different physiological systems. We think the cause of the damage accumulation may be the restriction of cell proliferation during formation of differentiated cell populations in the process of development. Results of multiple experiments confirm that the proliferation restriction of cells of very different origin (normal and transformed animal and human cells, plant cells, cyanobacteria, mycoplasmas, bacteria, protozoa, etc.) leads to their "aging" we determine as appearing of various types of damage identical to the damage accumulating in senescing multicellular organisms (organisms for which the probability of death increases with time). The damage that appears ultimately ruin the "restricted" cells. It is also interesting that responses of all these cells to geroprotectors and geropromoters (factors prolonging and shortening life of experimental animals) are very similar when evaluated by analysis of different parameters of cell proliferation. All these facts lead us to think that for the overwhelming majority of unicellular (in this case we mean, of course, not a single cell but whole cell population) as well as multicellular organisms initiation and progress of the aging process occur as follows: 1) cell proliferation restriction for one reason or another; 2) accumulation in the cells of "senescence" defects; 3) deterioration of normal cell functioning followed by cell death (for unicellular organism it is the end of the story); 4) deterioration of normal functioning of physiological systems; 5) death of multicellular organism. And the greater the distance from the initiation stage, the greater the difference we can see between the same stages of the process in rather phylogenetically distant organisms. It seems quite possible that the disturbing variation in modern experimental gerontology data is related to these circumstances.

98

A SUMMARY OF HUMAN LONGEVITY STUDIES. L.A. Gavrilov*, N.S. Gavrilova, V.G. Semyonova, A.L. Gavrilova, N.N. Evdokushkina, E.V. Lapshin & G.N. Evdokushkina, A.N. Belozersky Institute, Moscow State University, Moscow, 119899, Russia.

I. HUMAN LONGEVITY IS STABLE INDEED

The purpose of the study is to find out whether the historical increase in life expectancy is associated with real increase in human longevity or it is caused by trivial elimination of premature deaths at young ages.

Life span data for men (18,435 cases) and women (5,062 cases) born in 1700-1899 were extracted from Russian genealogical publications, biographic dictionaries and encyclopedias. The data were computerized, sorted by birth year (in decades, 1700-1709, 1710-1719, etc., up to 1890-1899) and 40

cohort life tables (20 for each sex) were constructed. Expectation of life for the oldest-old (those who reached age 85 years) was calculated for each cohort life table and was used as an indicator of any changes in human longevity.

Life expectancy at age 85 is equal to 4.1 years for men, 4.2 years for women and was not changed significantly during 2 centuries of Russian history. For comparison: these values, calculated for historical cohort life tables are close to modern data based on cross-sectional life tables: 4.3 years for men and 4.9 years for women in 1985 (former USSR).

The results support the idea that historical increase in life expectancy is not associated with significant increase in human longevity and is caused mainly by elimination of premature deaths at young ages.

II. Biomedical basis of gender gap in human longevity

The purpose of the study is to understand why women live longer than men. If gender gap is caused by higher genetic redundancy of women's genome (two X-chromosomes) then accumulation of mutational load in one of the female X-chromosomes should result in decrease of sex differential in human lifespan. Thus, the purpose of the study was to check the prediction of the above mentioned hypothesis that paternal age at reproduction should be associated with decrease of the gender gap in human longevity (through introduction of genetic load into female X-chromosome).

Data on longevity of men and women combined with data on paternal age at their birth were extracted from genealogical publications and biographic dictionaries. The data (for more than 3,000 males and 2,000 females) were computerized and sorted by paternal age at their birth. Then the mean gender gap in longevity was calculated for different paternal ages and the statistical analysis was made by standard methods (Student test).

It was found that gender gap in longevity is a function of paternal age: the highest gender gap is observed for the offspring of young fathers (20-29 years), while for the offspring of old fathers (50-59 years) women do not live longer than men.

The obtained results support the prediction of the hypothesis that gender gap in human longevity is caused by higher genetic redundancy of women's genome (two X-chromosomes).

III. Localization of human longevity genes

The purpose of the study is to locate the longevity genes in human genome. If these genes are located in X-chromosome, then age-related accumulation of mutational load in paternal germ cells should result in decrease of longevity among daughters only (since paternal X-chromosome is inherited by daughters only. Thus, the purpose of the study was to check the prediction of the above mentioned hypothesis that paternal age at reproduction should be associated with specific decrease of daughter's longevity.

Information on longevity of sons and daughters combined with information on paternal age at reproduction was extracted from genealogical publications and biographic dictionaries. The data (for more than 3,000 sons and 2,000 daughters) were computerized and sorted by paternal age at reproduction. Then the mean life span for sons and daughters was calculated for different paternal ages and the statistical analysis was made by standard methods (Student test).

It was found that longevity of the sons is the same for different paternal age subgroups. Quite different result is observed for daughters: mean life span of the daughters born by old fathers (50-59 years) was significantly lower than for daughters born by young fathers (20-29 years). The difference in mean life span between these two groups is more than 6 years and this difference is statistically highly significant ($p<0.01$).

The obtained results support the prediction of the hypothesis that human longevity genes are located in X-chromosome. That is why the accumulation of mutational load in germ cells of old fathers is detrimental for longevity of daughters only.

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