TWENTY-SECOND ANNUAL MEETING — American Aging Association
SEVENTH ANNUAL MEETING — American College of Clinical Gerontology
Friday through Tuesday
October 16-20, 1992
St. Francis Hotel
335 Powell Street
San Francisco, California 94102

SUBMITTED PAPERS
Poster Session

1. Bains*, J. S., Kakar, R., and Sharma, S. P.: **Glutathione reductase activity in ageing *Zaprionus Paravittiger* (diptera: drosophilidae) fed on propyl gallate.**
2. Bains*, J. S., Kakar, R., and Sharma, S. P.: **Age-related analysis of NADH dehydrogenase in banana fruit fly fed on propyl gallate.**
3. Kakar*, R., Bains, J. S., and Sharma, S. P.: **Changes in longevity, antioxidant enzymes and thiobarbituric acid reactive substances in vitamin E fed Z *paravittiger.***
4. Robbins*, S., McClaran, J., and Waked, E.: **Do footwear influence proprioception in older men?**
5. Gerst, P. H., Katter, H., and Rosario*, P. G.: **Cholecystocolic fistula masquerading as colon cancer.**
6. Khokhlov*, A. N., and Ushakov, V. L.: **On the problem of relationship between cellular aging and proliferation: how broadly applicable the commitment theory is?**
7. Khokhlov*, A. N., and Prokhorov, L. Y.: **Cell colony-forming ability analysis and cytogerontological studies.**
8. Ushakov, V. L., and Khokhlov*, A. N.: **Cyanobacteria (blue-green algae) in investigations of cellular aging mechanisms.**
9. Khalsa, D. S.: **Successful brain aging and the relaxation response: a new perspective.**
10. Farrell, C., Spoto, V., Fuller, S., Gergen, G., Sinnott, J., and Ganguly*, R.: **Hepatitis B vaccination among the older health care provider.**

MINISYMPOSIUM:
"Immunogerontology"

11. **Busbee, D. L.: Biological modulators of immune function in the aged.**
12. Effros, R.: **Influenza, Immunity and aging: T cell and macrophage defects.**
13. Meydani, S.: **Dietary antioxidant modulation of the immune response in the aged.**
14. Rabinovitch, P.: **Relationship of redox status and glutathione to signal transduction and aging in T lymphocytes.**
15. Kelsoe, G.:**Evidence for the absence of somatic mutation in germinal centers of aged C57BL/6 mice.**
16. Murasko, D.: **Heterogeneity of immune response in the elderly.**

MINISYMPOSIUM:
"Neurobiology of Aging and Alzheimer's Disease"

17. De Armond, S.: **Multiple pathogenic mechanisms in prion diseases.**
18. Leuchter, A.: **What's new in neuroimaging.**
19. Matsuyama, S.: **Looking at microtubule function.**
20. Mobley, W.: **What's happening with nerve growth factors.**

    Jarvik, L.F.: **Discussant**

SUNDAY, OCTOBER 18, 1992
MINISYMPOSIUM:
"Antioxidant Vitamins"

21. **Meydani, M.: Role of dietary antioxidants in aging.**
22. Ames*, B. N., Hagen, T., and Shigenaga, M.: **DNA oxidation, aging, and cancer.**
23. Packer, L.: **Oxidants and antioxidant defenses in metabolism and aging.**
24. **Urano*, S., and Matsuo, M.: Vitamin E and the susceptibility of synapses to oxidative stress in aging.**
25. Cutler, R.: **Biology of human aging and longevity: possible role of oxidative state.**
26. Johnson, E. J.: **The effect of aging on B-carotene status.**
27. Block, G.: **Vitamin C, cancer and aging.**

SUBMITTED PAPERS
Oral Presentations

28. Carrillo, M.-C., Kanai, S., Sato, Y., Ivy, G. O., Kitani*, K.: **Sequential changes in activities of superoxide dismutase (SOD) and catalase (CAT) in brain regions and liver during (-)deprenyl infusion in male rats.**
29. Bickford*, P. C., Heron, C., Rose, G. M., Lin, A. M-Y., Friedemann, M., and Gerhardt, G. A.: **Effects of long term deprenyl administration on behavior and noradrenergic neurotransmission in aged rats.**
30. Bennett*, M. C., Fordyce, D., Diamond, D. M., Rose, G. M., and Wehner, J.: **Chronic cytochrome oxidase inhibition by sodium azide infusion alters hippocampal protein kinase C activity.**
31. Gerhardt*, G. A., Friedemann, M. N., and Maloney, R. E.: **Microdialysis studies of age-related changes in basal levels and potassium-evoked overflow of dopamine in the striatum of the Fischer 344 rat.**
32. Fahim, M. A.: **Exercise affects presynaptic age-related changes at mouse neuromuscular junctions.**
33. Nandy*, K., Mostofsky, D. I., and Nandy, S.: **Aging brain and role of nutrition.**

34. Sobel, H., **Alzheimer's disease: a supply-delivery failure for a system of the CNS?**

35. Srivastava, V., Schroeder, M., and Busbee*, D.: **DNA polymerase from normal and pSV3. neotransformed cells.**

36. Lee*, A. T., Cerami, A., and Bucala, R.: **Age-related DNA mutations of the integrated 1acI gene in transgenic mice.**

37. Capasso*, J. M., Bruno, S., Li, P., Zhang, X., Darzynkiewicz, Z., and Anversa, P.: **Myocyte DNA synthesis with aging and biventricular dysfunction in rats.**

38. Chang*, E., and Harley, C. B.: **Telomere length as a biomarker for *in vitro* and *in vivo* age of intimal and medial tissue.**

MINIYSYMPOSIUM:

"Unique Models for Gerontological Research"

39. Jazwinski, M.: **Genes of youth: genetics of aging in yeast.**

40. Austad, S.: **Evolutionary determinants of longevity: the logic of selecting animal models for gerontological research.**

41. Cordell, B.: **Transgenic animal models of Alzheimer's disease amyloidosis.**

42. Smith, G.: **Peromyscus as a comparative model of aging.**

43. Flood, J.: **Age-related changes in learning, memory and in the pharmacotherapy of retention in senescence accelerated mouse (SAM) model.**

44. Goss, J.R.: **Walter Nicolai Lecture: The effect of age on glial fibrillary acidic protein RNA induction by fimbria/fornix transection in the mouse brain.**

Luncheon

Annual Awards:

Excellence in Journalism Award —
Ronald Kotulak and **Peter Gorner**

"In recognition of their outstanding contributions, through journalism, to the general public's knowledge and understanding of current biomedical aging research and the potential benefit of such research to all Americans, Peter Gorner and Ronald Kotulak are awarded the American Aging Association's first Excellence in Journalism Award."

Walter Nicolai Prize —
James R. Goss

"James R. Goss is the 1992 recipient of the Walter R. Nicolai Prize in Biomedical Gerontology. This prize is presented to Mr. Goss for his manuscript, 'The Effect of Age on Glial Fibrillary Acidic Protein RNA Induction by Fimbria/Fornix Transection on the Mouse Brain.' "

Research Award —
Earl R. Stadtman, Ph.D.

"This award is presented to Dr. Stadtman for an extensive series of studies which demonstrated that metal catalyzed oxidation of enzymess is a marking step in protein turnover and that the accumulation of oxidized protein is likely implicated in aging."

Distinguished Achievement Award —
Milton Friedman, Ph.D.

"The Distinguished Achievement Award for 1992 is presented to Dr. Milton Friedman. Dr. Friedman epitomizes the purpose of this award, established to call attention to the fact that chronological age is not a barrier to a full and productive life. At 80 he has just published his 24th book, *Money Mischief.*

"His writings and teachings have contributed to the improvement of economic theory as an engine for analyzing the real world, and to the understanding of monetary history and the analysis of monetary policy. Dr. Friedman is author of *Capitalism and Freedom* and other books and columns devoted to promoting a free society, as well as a major presenter of the television program "Free to Choose." Dr. Friedman is a participant in public policy; his proudest achievement is the role he played in ending the military draft."

"Increasing the Functional Life Span:

What Can We Do Now?"

45. Dean, W.: **Review of cognitive-enhancing substances.**

46. Thomas, Charles: **Assessing the antioxidant status of individuals.**

SUBMITTED PAPERS

Oral Presentations

48. Kitani*, K., Kanai, S., Sato, Y., Ivy, G. O., and Carrillo, M.-C.: **Chronic treatment of (–)deprenyl prolongs the life span of male Fischer 344 rats.**

49. Massie*, H. R., Aiello, V. R.: **Effect of vitamin A on longevity.**

50. Leutzinger, Y., Parthasarathy, S., Molloy, V., Zimmerman, J. A., and Richie, Jr.*, J. P.: **High blood glutathione in aging F344 rats fed a methionine restricted diet.**

51. Evans*, G. W., and Meyer, L.: **Chromium picolinate increases longevity.**

52. Feuers*, R. J., Chen, F., Oriaku, E. T., and Hart, R. W.: **Age associated changes in glucagon binding, glucose response and the impact of caloric restriction.**

53. Zimmerman*, J. A., Malloy, V., Parthasarathy, S., and Orentreich, N.: **The role of calories in life span extension produced by reduced methionine intake.**

54. Reiser*, K. M., and Amigable, M.: **Nonenzymatic glycation of type I collagen: the effects of age on sites of adduct formation.**

SUBMITTED PAPERS

Poster Session

55. Duffy*, P., Aly, K., Divine, B., and Hart, R.: **Effect of chronic caloric restriction, age and isoproterenol on treadmill performance and cardiovascular function in rats.**

56. Pipkin*, J. L., Hinson, W. G., Lyn-Cook, L. E., Burn, E. R., Feuers, R. J., Hart, R., and Casciano, D. A.: **The temporal relationships of synthesis and phosphorylation in stress proteins 70 and 90 in aged caloric restricted rats exposed to bleomycin.**

57. Lee, C., Chung, S., McKenzie, D., and Aiken*, J.: **Development of animal models for age- associated mitochondrial DNA deletions.**

58. Tsuchiya, T., Ishizuka*, J., Shimoda, I., Townsend, Jr., C. M., and Thompson, J. C.: **Effect of aging on the intestinal mucosal growth after ileo-jejunal transposition in rats.**

59. Khokhlov, A. N.: **Stationary cell culture as a model for experimental gerontology.**

60. Koltover, V. K.: **Free radical theory of aging: a survey from the reliability theory point of view.**

61. Reddy*, K. K., Ramachandraiah, T., Kumari, K. S., Reddanna, P., and Thyagaraju, K.: **Serum lipid peroxides and antioxidant defence components of rural and urban populations and aging.**

62. Singh*, B., Sharma, S. P., Goyal, R., and James, T. J.: **Efficacy of geriforte, a herbal formulation in superoxide dismutase formation in the cerebella and spinal cords of Wistar male rats.**

63. Singh*, B., Sharma, S. P., Goyal, R., Patro, I. K., and James, T. J.: **Effect of dietary selenium on cerebella and spinal cords glutathione peroxidase in the rats.**

64. Walker*, R., and Bercu, B. B.: **Responses of aged female rats to GH secretagogues.**

65. Huo*, Y.-S., and Utsuyama, M.: **Effect of ginseng saponin on the immunity of young and old mice.**

MINISYMPOSIUM:

"Mitochondria and Aging"

66. Miquel*, J., and de la Fuente, M.: **An update on the mitochondrial oxidation-damage theory of cell aging.**

67. Floyd, R. A.: **Age-dependent impairment of the ability of brain mitochondria to handle oxidative stress.**

68. Ames, B.: **Oxidative damage to mtDNA and aging.**

69. Linnane*, A. W., Martinus, R. D., Nagley, P., Zhang, C.-F., Baumer, A., Maxwell, R. J., and Vaillant, F.: **Mitochondria and ageing: mt. DNA mutation and the universality of bioenergetic disease.**

70. Arnheim, N.: **Age-related accumulation of a specific mitochondrial DNA deletion in human heart and brain.**

71. Colburn, N.: **Gene regulation involved in induced apoptosis.**

72. Funahashi, T., Floyd, R. A., and Carney, J. M.: **The pH dependence of brain peroxidation in vitro and its relation to ischemic/reperfusion injury in vivo.**

SPECIAL LECTURE

73. Drexler, K. E.: **Nanotechnology and Aging.**

SUBMITTED PAPERS

Oral Presentations

74. Recasens, J. F.: **Age-related changes in ocular antioxidant enzyme activity in inflammation.**

75. Starnes*, J. W., Bowles, D. K., Seiler, K. S.: **Hypoxia/reoxygenation response in immature, adult and aged rat hearts.**

76. Niedzwiecki*, A., and Fleming, J.: **Effect of oxidative stress and antioxidants on heat shock response in aging *Drosophila*.**

77. Ishizuka*, J., Murakami, M., and Thonpson, J. C.: **Effect of aging on contractile mechanism in guinea pig gallbladder.**

78. Brunk*, U., and Sohal, R.: **Mechanisms of lipofuscinogenesis, studies on a model system of cultured rat neonatal myocardial cells.**

79. McClaran*, J., Robbins, S., and Waked, E.: **Relation between stability and proprioception in older men when barefoot and shod.**

80. Engelberg, H.: **Heparin and aging.**

81. Nakamura, E.: **Biological vigor age and its factor structure.**

82. Phillips., J. M.: **For the elderly: administrative and town planning.**

83. Dilman*, V. M., and Toniolo, P. G.: **Age-dependent hyperlipidemia as a cancer-prone condition.**

1

GLUTATHIONE REDUCTASE ACTIVITY IN AGING *ZAPRIONUS PARAVITTIGER* (DIPTERA: DROSOPHILIDAE) FED ON PROPYL GALLATE. *J.S. Bains, R. Kakar* and S.P. Sharma,+* Dept. of Pathol., Health Sciences Ctr., Univ. of Calgary, Calgary, AB, Canada T2N 4N1. *Dept. of Pathol., Univ. of Saskatchewan, Saskatoon, Canada S7N OWO, and +Dept. of Zoology, Guru Nanak Dev Univ., Amritsar-143 005, India.

Glutathione reductase (GR) maintains the redox potential of the cell and hence protects the organism from the toxic effect of free radicals. Propyl gallate, an inhibitor of superoxide radical, was fed to *Zaprionus paravittiger* using a wide range of concentrations (0, 2.5, 25, 250, 2500, 5000 and 7500 µg/ml). The optimal concentration, i.e. 25 µg/ml (which increased the median and maximum life span maximally), was calculated on the basis of life span studies. The cultures were maintained on this concentration to observe its effect on GR activity. The enzyme activity was measured in whole body as well as mitochondrial fraction of male and female flies at 1, 8, 15, 22, 29, 36 and 43 days of age. The maximum activity was found on the 15th day and it declined during senescence. A similar pattern of GR activity was revealed in mitochondrial homogenates. However, propyl gallate enhanced the activity significantly in both the sexes. In conclusion, the increased GR activity on propyl gallate feeding is indicative of more efficient defense system that may be attributed to prolonged longevity.

2

AGE-RELATED ANALYSIS OF NADH DEHYDROGENASE IN BANANA FRUIT FLY FED ON PROPYL GALLATE. *J.S. Bains, R. Kakar* and S.P. Sharma,+* Dept. of Pathol., Health Sciences Ctr., Univ. of Calgary, Calgary, AB, Canada T2N 4N1; *Dept. of Pathology, Univ. of Saskatchewan, Saskatoon, Canada S7N OWO, and +Dept. of Zoology, Guru Nanak Dev Univ., Amritsar-143 005, India.

NADH dehydrogenase forms complex I of the respiratory chain. To know the effect of age and antioxidant, the activity of this enzyme was studied in banana fruit fly, *Zaprionus paravittiger* (Diptera: Drosophilidae). The flies were reared at 26±2°C on standard cornmeal agar (CMA) medium mixed with 25 µg/ml concentration of propyl gallate as found optimal from life span studies. The NADH dehydrogenase activity was analysed in mitochondrial and whole body homogenates of both sexes at weekly intervals starting from 1 day to 43 days of age. The peak of enzyme activity was observed during reproductive phase, and it declined gradually thereafter with age. The propyl gallate decreased the NADH dehydrogenase activity in all age-matched cohorts. More pronounced activity was revealed in mitochondrial fractions as compared to whole body homogenates. The decreased activity of NADH dehydrogenase on propyl gallate feeding suggests that the flies fed on control diet might need more energy to cope with the higher production of free radicals.

3

CHANGES IN LONGEVITY, ANTIOXIDANT ENZYMES AND THIOBARBITURIC ACID REACTIVE SUBSTANCES IN VITAMIN E FED *Z. PARAVITTIGER.* *R. Kakar, J.S. Bains, and S.P. Sharma,** Dept. of Pathol., Coll. of Med., Univ. of Saskatchewan, Saskatoon, Canada S7N OWO. *Dept. of Zoology, GND Univ., Amritsar 143 005, India.

Oxygen-free radicals (OFR) are implicated as a contributory factor in aging. Essential nutrients such as vitamin E (a chain-breaking antioxidant) constitute a strong line of defense against free radical-induced cellular damage. The objective of the present work was to see the role of OFR and the effect of vitamin E on the life span of *Zaprionus paravittiger* (Diptera). Insects were fed on cornmeal agar medium (CMA) mixed with different concentrations (1, 5.10, 25 and 50 µg/ml) of vitamin E. Their longevity was recorded in comparison to control at at 26±2°C. The maximum increase in median and maximum life span was 33.59% and 15.67% in males and 17.19% and 21.13% in females at 5 µg/ml concentration of vitamin E. The activity of enzymes Catalase (Cat), peroxidase (Px) and Thiobarbituric acid reactive substances (TBARS) were measured at 1, 8, 15, 22, 29, 36, and 43 days of age in control and vitamin E (5 µg/ml) fed insects. Cat and Px decreased in post-reproductive phase, whereas TBARS content increased throughout the life span. The females exhibited lower TBARS content and higher activities of Cat and Px in comparison to males in control and vitamin E group. Vitamin E feeding caused a significant increase in Cat and Px and decrease in TBARS when compared with age- and sex-matched cohorts. These results suggest that OFR-mediated processes are involved in aging, and antioxidants such as vitamin E may act as protective agents.

4

DO FOOTWARE INFLUENCE PROPRIOCEPTION IN OLDER MEN? *S. Robbins*, J. McClaran, and E. Waked,* Div. of Geriat., Dept. of Med., Montreal Gen. Hosp., Montreal, Quebec, Canada H3G 1A4.

Gerontologists have suggested that soft-soled shoes destabilize the elderly through impairing proprioception, which is relied upon for maintaining stable equilibrium. One assertion contained in this statement is that footwear can affect proprioception, a notion which has never been examined. We tested this hypothesis in 15 older men (mean age = 73.9, SE 1.2). We used a method whereby the blindfolded subjects were asked to estimate the perceived direction and amplitude of ankle angle after they applied full body weight to a series of blocks when wearing athletic shoes and when barefoot. The top surface of the blocks was angled so as to vary between 0° and 25° inversion, eversion, plantar and dorsiflexion. They were presented in random order. Estimates of ankle angle were made on an ordinal scale. Subjects were given reference values every 11 estimates. Subjects overestimated ankle angle similarly when wearing athletic shoes and when barefoot; however, the mean error was significantly greater when wearing athletic shoes (athletic shoe 6.58°; barefoot 5.13°; $p<0.001$). Furthermore, athletic footwear produced the greatest errors in estimates of ankle angle in the high angle range, the range presumably associated with injuries such as inversion injuries which often precipitate falls (range 15-25°; barefoot mean error = 5.7° SE 0.15; athletic shoe mean error; 7.3° SE 0.18; $p<0.02$). This supports the hypothesis that shoes with soft soles, such as athletic shoes, significantly impair kinesthetic sense in older individuals.

5

CHOLECYSTOCOLIC FISTULA MASQUERADING AS COLON CANCER. *P.H. Gerst, H. Katter, and P.G. Rosario*;* Bronx Lebanon Hosp. Ctr., Bronx, NY 10457.

A 71-year-old female patient was investigated after being detected to have guaiac positive stools. She denied jaundice, biliary colic, weight loss, or melena. Examination revealed no hepatosplenomegaly or abdominal masses. Liver function tests were normal. Barium enema showed a 3 cm "apple core" lesion at the hepatic flexure with "shouldering." Colonoscopy detected a nodular mass; the biopsy was consistent with a tubular adenoma. At surgery, dense adhesions were found between the gall bladder and colon. There was a 3 cm defect in the colon wall and a large cholesterol gallstone encased in a pouch of gallbladder was protruding into the lumen. Following cholecystectomy and segmental resection of the hepatic flexure, the patient made an uneventful recovery. Histopathological evaluation of the specimen failed to reveal cancer.

Due to early detection and treatment of cholecystitis, cholecystocolic fistula has become a disease of the elderly with neglected biliary tract disorders. Generally asymptomatic, these fistulae may present in a bizarre manner, mimicking other disorders. The low morbidity and mortality associated with cholecystectomy and excision of the fistula make it the operation of choice even in the elderly.

6

ON THE PROBLEM OF RELATIONSHIP BETWEEN CELLULAR AGING AND PROLIFERATION: HOW BROADLY APPLICABLE IS THE COMMITMENT THEORY? *A.N. Khakhlov* and V.L. Ushakov,* Evolutionary Cytogerontol. Sector, Biolog. Faculty, Moscow State Univ., 119899, Russia.

The commitment theory of aging *in vitro* (Kirkwood & Holliday, 1975) was elaborated to explain the slowing down of cell proliferation with "age" (a population doubling level) of normal human fibroblast culture. It postulates that some portion of cells spontaneously become "committed" and their offspring, after the strictly determined number of divisions (M), stops to divide. Since the theory was proposed there was a great variety of both its confirmation and criticism in literature. However, the fact that it explains the proliferative structure of the mortal

(normal) and immortal (transformed) mammalian cell cultures in terms of the single, general mathematical model (which is applicable to the multicellular organism cell populations *in vivo* also) is one of the advantages the theory provides. And perhaps it is even more universal. We recently undertook the comparative analysis of the commitment theory and the model suggested yet in 1962 by V.D. Fedorov of Moscow St. Univ. (Russia) to explain the proliferative structure of blue-green algae (cyanobacteria) cell culture during exponential growth phase. The base assumptions of the Fedorov model, with some reserves, remind closely those of the commitment theory. It is apparent (as our analysis showed) that the mathematical expression of the commitment theory reduces to that of the Fedorov model if the "incubation period" of the former, M, equals zero. It should be noted that at this value of M the cell population has to be immortal, as is the case for blue-green algae. On the other hand, if proliferation is slowed down or arrested, blue-green algae undergo the aging process. All these lead us to the suggestion that there exists a general regularity (commitment theory being the more or less adequate approach to it) controlling the intrinsic proliferative status of population of any cells regardless whether they are pro- or eukaryotic, unicellular organisms or parts of the multicellular organism. At some parameters of the mathematical expression describing this regularity, endogenous arresting of cell proliferation leading to cell aging and death occurs; at others the cell population is intrinsically immortal but do grow old at the exogenous limitations of the growth.

7

CELL COLONY-FORMING ABILITY ANALYSIS AND CYTOGERONTOLOGICAL STUDIES. A.N. Khokhlov* and L.Yu. Prokhorov, Evolutionary Cytogerontol. Sector, Biolog. Faculty, Moscow State Univ., 119899, Russia.

In recent years the evaluation of ability of cultured cells to form colonies has been used rather often for studies of *in vitro* aging ("Hayflick's phenomenon"). In fact, it allows one to get information about proliferative ability of single cells, i.e., to analyze the cells' distribution (based on this ability) between corresponding classes. We used this method for investigation of peculiarities of "stationary phase aging" (cell changes during stationary phase of growth similar to those of aging organism cells) of cultured transformed Chinese hamster cells and normal diploid human fibroblasts. It was shown that the relative number of colony-forming cells as well as the size of the colonies decreases with increasing of time of cell cultivation in the stationary phase of growth. Besides, we evaluated the effect of some biologically active preparations (geroprotectors-antioxidants epigid and butylated hydroxytoluene, immunostimulating agent T-activin, phospholipid compounds a.o.) on colony-forming ability of cultured Chinese hamster cells. In particular, we found that the treatment by liposomes containing cholesterol alone decreases the colony-forming ability of Chinese hamster cells by 10-fold, and the cholesterol- and 7-ketocholesterol-containing preparation decreases it down to zero (colonies are fully absent on dishes). The liposomes containing only phosphatidylcholine did not influence the colony-forming ability. It is also interesting that cholesterol-containing liposomes not only decrease the absolute number of colonies appearing (i.e., colony-forming ability) but also decrease the "greater than 255 cells" subclass, and increase the middle-size subclasses ("128-191 cells" and "191-255 cells"). The sizes of the two classes with the smallest colonies ("0-63 cells" and "64-127 cells") remain unchanged. It means that the cell subpopulation with the greatest proliferative capacity ("greater than 255 cells" class) is the most sensitive to cholesterol action. After the preparation treatment, a part of this cell subpopulation moves to neighboring subclasses with the lesser cell-proliferative activity. All the data obtained led us to consider the method of colony-forming ability analysis a very useful one for studies of cellular aging mechanisms and testing geroprotectors, geropromotors and other biologically active compounds interesting from the gerontological point of view.

8

CYANOBACTERIA (BLUE-GREEN ALGAE) IN INVESTIGATIONS OF CELLULAR AGING MECHANISMS. *V.L. Ushakov and A.N. Khokhlov*,* Evolutionary Cytogerontol. Sector, Biolog. Faculty, Moscow State Univ., 119899, Russia.

It is obvious that studies of cellular aging as the general biological phenomenon require comparative and evolutionary approaches. Since such studies up to the present have been carried out as a rule on mammalian cells, it is desirable to study now the organism(s) which is physiologically and phylogenetically far from them, and evolutionary primitive. We think that cyanobacteria (CB) meet these requirements well enough. Our analysis of available literature data revealed that CB do undergo a kind of aging. They degenerate with time ("age") in the stationary phase culture ("stationary phase aging" phenomenon), and there are two model systems in which the degeneration occur: in light conditions (when CB transform energy in an autotrophic way) and in darkness (when some of the CB species are able to perform heterotrophic metabolism). We call these two processes "light aging" and "darkness aging," correspondingly. The alterations of CB cells during the first one are very similar to those during "stationary phase aging" as well as during *in vivo* aging of mammalian cells. However, it is rather possible that the principal mechanism of macromolecular damage during the "light aging" of cyanobacteria is photooxidation in contrast to free radical damage as the putative mechanism of mammalian cell aging. Nevertheless, free radical reactions also occur in cyanobacteria and play their own role in primary cell damage. As a result, some peculiarities of cyanobacteria defense systems exist. One one hand, CB express superoxide dismutase and catalase activities, protecting the cells from superoxide anion radical and hydrogen peroxide, correspondingly. On the other hand, this defense system can not protect them against photooxidation, so CB must have some other defense "devices." The literature data analyzed indicate that a pigment, phycocyanin, may be a component of such system, absorbing abundant light energy and playing the role analogous to that of antioxidants — free radical "scavengers," during free radical damage of mammalian cells. All this allows one to consider CB culture a very promising model system for comparative and evolutionary studies on cellular aging, particularly because of the presence in CB of a damage process (probably playing an important role in their aging) which is absent in mammalian cells.

9

SUCCESSFUL BRAIN AGING AND THE RELAXATION RESPONSE: A NEW PERSPECTIVE. *D.S. Khalsa,** Dept. of Anesthesiol., Maricopa Med. Ctr., Phoenix, AZ 85010.

The deleterious effects of stress are well known. The cardiovascular system and immune system have been most extensively studied. Additionally, chronic stress can produce hippocampal neuronal degeneration especially in the aging brain, leading to a breakdown of negative feedback systems which further accelerates brain aging perhaps leading to early dementia with its negative social and economic impact.

The relaxation response is an innate physiologic event that can be elicited by a number of techniques including biofeedback, autogenic training, yoga/meditation and hypnosis.

Hess has demonstrated a distinct hypothalamic area responsible for inducing the relaxation response changes of ↓BP, ↓P, ↓RR, and ↓O_2 consumption. Clinically, Benson and others have shown multiple salubrious effects of the regular elicitation of the response including an increase in life span in nursing home occupants compared to controls.

Additionally, the relaxation response decreases serum cortisol under stressful conditions, and thus may protect the brain against the harmful effects of stress and prolong functional life span.

10

HEPATITIS B VACCINATION AMONG THE OLDER HEALTH CARE PROVIDERS. *C. Farrell,* V. Spoto, S. Fuller, G. Bergen, J. Sinnot, and R. Ganguly,* Univ. of South Florida and James A. Haley Veterans' Hosp., Tampa, FL 33612.

Hepatitis B Virus (HBV) vaccine is highly recommended for the health care professionals (HCP), but its acceptance among the older health care workers remains suboptimal in medical settings. This study analyzed the immune response, vaccination status and reasons for nonimmunization of the HCP (predominantly nurses) ≥50 years old in a university-affiliated hospital. A total of 248 high-risk subjects were identified by random review of 1:10 employees' health records. Of these, 130 were ≥50 years old (test group) and 145 were younger (control group). Antibody response to the vaccine was determined by RIA technique and protective levels were defined as ≥10 IU. A survey questionnaire inquiring into the circumstances which may have influenced their decision to be immunized against HBV infection was mailed to the HCP and factors affecting compliance were analyzed. The immunization rates of the test and control groups were 67% and 79.3% respectively ($P>0.05$). In both groups, 25 subjects (24.3% of test and 17.2% of controL) remained unimmunized, indicating decreased vaccine compliance among the older HCP. An inadequate antibody response was obtained in 12 of 67 test subjects (17.9%) and in 5 of 72 control subjects (6.9%) ($0.05>P>0.02$). The older HCP were more frequently misinformed regarding HBV vaccine benefits than the younger HCP (50% vs. 38%, $P>0.05$). They disliked shots and side effects of immunization, and often doubted the safety of the vaccine product. Data suggest that age interferes with development of immunity to the HBV vaccine. Modified immunization schedule and health education intervention measures are necessary to protect the older HCP in high-risk settings.

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BIOLOGICAL MODULATORS OF IMMUNE FUNCTION IN THE AGED. *D. Busbee* and E. Merriam,* Div. of Cell Biol., Dept. of Anat. and Public Health, Coll. of Veter. Med., Texas A&M Univ., College Station, TX 77843.

An extract from the parenchyma of *Aloe barbadensis Miller* containing long chain polydispersed β(1,4)-linked mannan polymers with random O-acetyl groups (ACM, acemannan) was found to stimulate macrophage production of cytokines that supported antibody-dependent cellular cytotoxicity and stimulated blastogenesis in thymocytes and in peripheral blood and spleen lymphocytes. In a non-aged model system using female CFW and C57BL/6 mice ACM stimulated IL-1 and TNFα secretion by peritoneal macrophages (PM) at a level higher than was found for LPS-treated mice. Antibody dependent cytotoxic lysis of EL4 tumor cells by C3H/HeJ thymocytes increased significantly in the presence of murine IL-2 and increasing concentrations of medium conditioned by ACM-stimulated PM, indicative of TNFα in the medium. C57BL/6 female mice across a 3 mo-24 mo age continuum were treated IP with ACM at 1 mg/Kg and sacrificed 4 hr later for evaluations of splenocyte blastogenesis. Elevated levels of blastogenesis were seen in cells from 3 mo (4-fold), 9 mo (1.4-fold), and 17 mo (1.2-fold) animals, correlating with elevated levels of PM secreted IL-1. Blastogenesis was not elevated in the 24 mo animals. Acemannan, which is not toxic to animals at levels up to 100 times the concentrations used in this study, appears to stimulate a general secretion of cytokines by macrophages. This complex plan polysaccharide may be extremely useful since it stimulates macrophage secretion of a variety of cytokines. However, it remains to be seen whether the presence of increased macrophage-derived cytokine addresses the functional decline in the aging human immune system.

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RELATIONSHIP OF REDOX STATUS AND GLUTATHIONE TO SIGNAL TRANSDUCTION AND AGING IN T LYMPHOCYTES. *P.S. Rabinovitch*, J.A. Ledbetter and A. Grossman,* Dept. of Pathol., Univ. of Washington and Oncogen, Inc. (JAL), Seattle, WA.

In T lymphocytes, impairment of transmembrane signal transduction has been postulated as a factor in the age-related decline in proliferative response. To determine whether this may be related to altered intracellular redox state, we examined the effects of oxidation and the importance of glutathione (GSH) content in signal transduction. Treatments that deplete GSH result in reduced $[Ca^{2+}]_c$ mobilization and impaired tyrosine phosphorylation following stimulation of the CD3 receptor. Phosphorylation of phospholipase C gamma-1 in particular is sensitive to altered redox state. The physiologic importance of intracellular GSH is suggested by experiments in which T cells are viably sorted according to GSH content: upon stimulation, cells sorted for high GSH exhibit markedly greater $[Ca^{2+}]_c$ response and proliferation than cells with low GSH. While human CD4 T cells show little change in this GSH dependence with age; CD4 cells from old mice show a greater GSH dependence than cells from young mice. Transmembrane signal transduction is affected by redox state, and this might be an aspect of the free radical theory of aging.

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EVIDENCE FOR THE ABSENCE OF SOMATIC MUTATION IN GERMINAL CENTERS OF AGED C57BL/6 MICE. *G. Kelsoe,* Dept. of Microbiol. and Immunol., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

While the aged mouse can produce substantial quantities of high-affinity antibody to antigens first encountered in its youth, the response to antigens administered late in life is comparatively decreased in amount and little or no affinity maturation is observed. Antibody affinity maturation and the generation of memory B cells takes place within the germinal centers of secondary lymphoid tissues and depends upon the processes of the somatic hypermutation of Ig-V region genes and the affinity-driven selection of mutant B cells. Using a combination of histologic and molecular genetic techniques, germinal centers in 24-month-old C57BL/6 mice were shown to be structurally grossly abnormal and to contain few mutant B lymphocytes. These findings are significant in that they suggest that although B cells are capable of responding in these aged animals, the differentiation pathways necessary for the high-affinity memory responses associated with protective immunity may be blocked.

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ROLE OF DIETARY ANTIOXIDANTS IN AGING. *M. Meydani*, Antioxidant* Res. Lab., USDA Human Nutr. Res. Ctr. on Aging, Tufts Univ., Boston, MA 02111.

Free radicals have been indicated to contribute to the aging process and several age-related disorders. Reactive oxygen species are formed during the univalent reduction of oxygen which occurs in tissues and can damage DNA, proteins and lipids. There are multiple enzymic and non-enzymic antioxidant defense systems in cells which eliminate prooxidants and scavenge free radicals. Experimental and epidemiological data suggest that antioxidant vitamins and carotenoids are important dietary micronutrients that body depends on to protect against free radical damage. Vitamin C is an effective antioxidant against lipid

peroxidation in human plasma and can prevent oxidative damage to DNA. Vitamin E, the major lipid-soluble antioxidant, is responsible for protecting the polyunsaturated fatty acids in membrane against lipid peroxidation. Deficiency of vitamin E produces free radical injury and age-related pathological changes. Supplementary intake of vitamin E inhibits peroxide-free radicals accumulation, decreases oxidizability of low density lipoproteins, and decreases age-related neurological and immunological impairment. The antioxidant properties of carotenoids such as β-carotene and lycopene contribute to their protective effect in cardiovascular disease and cancer. Exposure to free radical reactions during the life span is inevitable and can be detrimental to health and aging. Adequate dietary antioxidant protection is essential to eliminate oxidative injuries and minimize the risk of degenerative diseases.

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DNA OXIDATION, AGING, AND CANCER. *B.N. Ames,* T. Hagen and M. Shigenaga,* Univ. of Calif. at Berkeley, Berkeley, CA 94720.

Endogenous oxidative damage to DNA is extensive.[1] Analyses have been made of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (oxo^8dG), one of the approximately 20 known major oxidative adducts in DNA. A normal young rat cell has about 10^6 oxidative lesions in its DNA, and this number increases with age. About 10^5 new oxidative lesions per cell are formed and repaired each day.

The level of oxo^8dG in rat liver mtDNA was found to be about 16 times higher than in nuclear DNA from the same tissue. This suggests that the rate of somatic mutation may be higher in the mitochondrial genome than in the nuclear genome. Moreover, the amount of oxo^8dG in mtDNA was found to increase roughly three-fold during aging of the rat.

Gladys Block[2] has shown that insufficient fruit and vegetable consumption will increase the rate of most types of cancer about two-fold. This in part appears to be due to their content of the antioxidants ascorbate, tocopherol, ubiquinol, and carotenoids. We will present evidence that men eating insufficient ascorbate are mutating DNA in their sperm.[3] Since only 9% of the U.S. population eat sufficient fruit and vegetables, the prospect for prevention of cancer and degenerative diseases is encouraging.

1. B.N. Ames and M.K. Shigenaga, Oxidants are a Major Contributor to Aging. In: *Ann. N.Y. Acad. Sci.,* C. Franceschi et al. eds. N.Y. Acad. Sci., N.Y., in press, 1992.
2. G. Block, et al., *Nutr. Cancer 18: 1-29, 1992.*
3. *C.G. Fraga, et al., Proc. Natl. Acad. Sci. USA* 88, 11003-11006, 1991.

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OXIDANTS AND ANTIOXIDANT DEFENSES IN METABOLISM AND AGING. *L. Packer,* Dept. of Molec. & Cell Biol., 251 Life Sciences Addition, Univ. of Calif., Berkeley, CA 94720.

The role of oxidants generated in biological systems in the aging process and how effective antioxidant protection mechanisms may be in adding "life to your years and years to your life" in aging processes are still unclear.

We will report on indices of oxidant damage at the molecular level in aging animals and in exercise, during which metabolism is accelerated. Studies of the effects of antioxidants on indices of oxidative damage will be reviewed. An important feature of the antioxidant defense mechanisms which has not yet been adequately evaluated is the interacting series of redox-based antioxidant cycles and non-redox based antioxidants, which act additively and synergistically in protection. In order to achieve a comprehensive understanding of effectiveness of antioxidant protection, it is essential to measure all the major antioxidant substances and antioxidant enzymes. Recent studies provide a conceptual basis of how one can think about the importance of these antioxidant defense mechanisms in metabolism and aging.

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VITAMIN E AND THE SUSCEPTIBILITY OF SYNAPSES TO OXIDATIVE STRESS IN AGING. *S. Urano* and M. Matsuo,* Tokyo Metropol. Inst. of Gerontol., Tokyo 173.

Since it has been found that dysfunctions of brain and nervous system with age are similar to those observed in vitamin E deficiency, it can be presumed that these phenomena caused by free radicals generated *in vivo.* The mechanism of the dysfunctional manifestation is, however, still unclear. In order to verify whether free radicals induce the oxidative damage into nervous systems, and whether vitamin E protects nerves against the oxidative stress, the effect of oxygen on the functions of nerve terminal membranes in rat and the protective effect of vitamin E have been investigated.

Synaptosomes from 10, 15, and 25 month old Fischer 344 rats which were exposed by 100% oxygen at 20°C for 48 hours were isolated. The TBARS values of the synaptosomes were significantly higher than that of the unexposed rat. The values were increased with age. The contents of unsaturated fatty acid ($C_{22:6}$) and vitamin E decreased through oxygen and with age. The permeability of membranes to glucose and the membrane fluidity were increased significantly. From these findings, it may be concluded that free radicals derived from oxygen attacked to the nerve terminal membranes, and hence, peroxidized the membrane lipids, resulting in the membrane damage. The susceptibility of rat synapses to the oxidative stress were significantly increased with age and vitamin E may protect the oxidative damage of nerve terminals.

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THE EFFECT OF AGING ON B-CAROTENE STATUS. *E.J. Johnson,** Human Nutr. Res. Ctr. on Aging at Tufts Univ., Boston, MA 02111.

There is a growing interest in B-carotene (BC) because of an inverse relationship between the incidence of cancer and the consumption of BC and BC serum levels, which has been seen in many epidemiologic studies. The protective effect of BC may be particularly important in the elderly given that cancer is a major cause of death in this age group. Serum BC levels have been reported to increase with age (Johnson and Russell, *Nutrition and the Elderly: The Nutrition Status Survey.* Smith and Gordon, 1992) which may be partially due to an increased intestinal absorption (Morbarhan et al., Age 14:13, 1991). The rate of intestinal absorption of BC is regulated by the amount of fat, protein and calories in the diet (Shiau et al., J. Am. Coll. Nutr. 9:44, 1990) and age differences in BC absorption, in part, may be related to intakes of these dietary components. Moreover, aging appears to cause a change in character of the water diffusion barrier, which overlies the small intestinal epithelial cell (Hollander and Morgan, Exp. Geront. 14:201, 1979). This could allow easier access of a lipid molecule to the epithelial membrane. The appearance of BC in the serum after an oral dose of BC is not only related to its absorption but also to tissue uptake and metabolism by peripheral tissues. Age differences in BC status appear to be due to several factors including diet and physiological changes in the intestine and peripheral tissues. Furthermore, it is not known how aging may affect the metabolism of BC to retinoids. Given the protective effect of BC in carcinogenesis and the fact that our population as a whole is aging, the study of BC metabolism in the elderly is necessary.

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SEQUENTIAL CHANGES IN ACTIVITIES OF SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) IN BRAIN REGIONS AND LIVER DURING (–)DEPRENYL INFUSION IN MALE RATS. *M.-C. Carrillo[1], S. Kanai[1], Y. Sato[1], G.O. Ivy[2], and K. Kitani*[1],* [1]Dept. of Clinical Physiology, Tokyo Metropool. Inst. of Gerontol., Itabashiku, Tokyo-173, JAPAN; 2. Div. of Life Sci., Univ. of Toronto, Scarborough, Ontario M1C 1A4, CANADA.

Previous studies including those of our own have shown that (–)deprenyl causes increases in SOD and CAT activities in striatum in rats. Our own study further has shown that this effect is selective for certain brain regions. Thus far, all studies adhered to the duration of deprenyl administration for 3 weeks. The present study aimed to clarify sequential changes in these enzyme activities during deprenyl treatment. A continuous s.c. infusion of (–)deprenyl in young male rats at a dose of 2.0 mg/kg/day for 1 week significantly increased

SOD activities (both Cu and Zn-SOD and Mn-SOD) in certain brain regions such as substantia nigra and striatum but not in hippocampus or cerebellum or in the liver. With continuing infusion, SOD activities were further increased in the following weeks, reaching a plateau at 3 weeks. In cerebral cortices the increase became significant at 3 weeks. In contrast, an increase in CAT activity became significant only after 2 weeks of infusion, and only in the same brain regions where SOD activities were increased earlier. The delay in the increase of CAT activity following deprenyl infusion suggests a possibility that this is an adaptive response to the earlier increase in deprenyl-induced SOD activities rather than a direct effect of deprenyl on CAT, although the latter possibility cannot be excluded.

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EFFECTS OF LONG-TERM DEPRENYL ADMINISTRATION ON BEHAVIOR AND NORADRENERGIC NEUROTRANSMISSION IN AGED RATS. *P.C. Bickford,* C. Heron, G.M. Rose, A.M-Y. Lin, M. Friedemann, and G.A. Gerhartdt,* Med. Res. Svc., VAMC, Depts. of Pharmacol. and Psychiat., and Neurosci. Training Prog., USHSC, Denver, CO 80220.

Deprenyl has been shown to increase the longevity of rats. This study was undertaken to examine the effects of deprenyl on behavior and brain function. Twenty F344 rats were treated with 1-deprenyl (0.5 mg/kg/day) via the drinking water starting at 12 months of age; 20 age-matched rats served as controls. The mean life span was extended in the deprenyl group from 51.5±3.7 weeks to 59.0±1.4 weeks ($p<0.005$ two-tailed Student's t-test). At 18 months of age, the rats were tested on a series of motor coordination tasks and a motor learning task that involves learning to negotiate unevenly spaced pegs on a runway. Performance on these motor coordination and learning tasks was not different between 1-deprenyl and control aged rats. There was also no difference when the rats were tested for spatial learning in the Morris water maze at 24 months of age. At 28 months of age the remaining rats (6 deprenyl and 4 controls) were studied with *in vivo* electrochemistry using carbon fiber electrodes coated with nafion. The clearance of exogenously applied norepinephrine (NE) from the extracellular space of the cerebellar cortex was enhanced in the aged deprenyl treated group compared to age-matched controls.

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CHRONIC CYTOCHROME OXIDASE INHIBITION BY SODIUM AZIDE INFUSION ALTERS HIPPOCAMPAL PROTEIN KINASE C ACTIVITY. *M. C. Bennett,[1]* D. Fordyce,[2] D.M. Diamond,[1,3] G.M. Rose[1,3] and J. Wehner.[2]* [1]Dept. of Pharmacol., UCHSC, 80262; [2]Inst. Behav. Genet., UC Boulder; and [3]Med. Research, VAMC, Denver, CO 80220.

There is a growing body of evidence that Alzheimer's disease (AD) patients are deficient in the mitochondrial enzyme cytochrome oxidase. In previous work, we reported that chronic cytochrome oxidase inhibition by sodium azide treatment causes deficits of learning and memory and of hippocampal long-term potentiation in young adult rats (*J. Geriat. Psychiat. Neurol.*, 5: 93-101, 1992).

Several investigators have reported that protein kinase C (PKC) activity is decreased in the particulate fraction and increased in the cytosolic fraction of brain tissue from AD patients (*Br. Res.*, 453: 165-159, 1988; *J. Neur. Trans.*, 30: 69-78, 1990). We therefore investigated PKC activity in rats with chronic and selective inhibition of cytochrome oxidase induced by continuous azide infusion.

Adult male Sprague-Dawley rats (375-425g) were implanted (SC) each with an Alzet osmotic minipump (2ML4) containing 0.9% NaCl or sodium azide (160 mg/ml of 0.9% NaCl). Two weeks later, rats were sacrificed and the hippocampi were assayed for PKC activity (*Br. Res.*, 523: 181-187, 1990). PKC activity was decreased in the particulate fraction, which includes mitochondria, and increased in the cytosolic fraction. Thus, alterations in PKC activity induced by azide treatment parallel those found in AD. These data are consistent with the hypothesis that a defect in mitochondrial metabolism may be pathogenic in AD.

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MICRODIALYSIS STUDIES OF AGE-RELATED CHANGES IN BASAL LEVELS AND POTASSIUM-EVOKED OVERFLOW OF DOPAMINE IN THE STRIATUM OF THE FISCHER 344 RAT. *G.A. Gerhardt,* M.N. Friedemann and R.E. Maloney,* Depts. of Psychiat. & Pharmacol., Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.

We have previously shown using *in vivo* electrochemical recordings that extracellular dopamine (DA) in the rat striatum following K^+ stimulation is reduced in aged rats as compared to young animals. In this study, the technique of *in vivo* microdialysis was used to further investigate both basal and K^+-evoked overflow of DA in the young and aged rat striatum. Eleven Fischer 344 rats were used: n=6 - 2-5 months (young) and n=5 - 24-25 months (old). Microdialysis probes that were 4 mm long and 300 microns in diameter were stereotaxically implanted into the striatum of urethane anesthetized rats. Probes were perfused with artificial CSA at a flow rate of 1.2 microliters/min and samples were taken every 10 min and analyzed by HPLC with electrochemical detection. When the variability of 5 consecutive DA sample peak heights was <10%, the perfusion medium was changed to 100 mM K^+ CSF for 10 min. The K^+ stimulation produced a robust change in extracellular DA levels without producing increases in dopamine metabolites. The percent increase in extracellular DA levels was significantly greater in aged rats (4119%) as compared to the DA levels measured in the young rats (1509%). Aged rats also had significantly lower baseline amounts of DA and dopamine metabolites as compared to young rat striatum. These data further support age-related changes in the dynamics of the dopamine system of the rat striatum.

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EXERCISE AFFECTS PRESYNAPTIC AGE-RELATED CHANGES AT MOUSE NEUROMUSCULAR JUNCTIONS. *M.A. Fahim,* Dept. of Physiol., Fac. of Med. Health Sci., UAE Univ., Al-Ain, U.A.E.

During an animal's life span, its neuromuscular junction (NMJ) undergoes dynamic structural and functional remodeling from early development through maturity until aging introduces new perturbations. Questions have been raised as to whether the post-maturity changes result from reduced physical activity or pure aging *per se*. To address this question and to determine the effects of endurance exercise on age-related changes at C57/BL mouse NMJ, synaptic structure and function were studied in extensor digitorum longus muscles which typically work against little load in adult (10 months) and old (28 months) mice. Mice were exercised at 28m/min for 60 min/day for three months. Electrophysiological properties were studied with conventional intracellular recording techniques. The safety margin of synaptic transmission was studied by measuring the ratio of the indirect isometric twitch tension in low Ca^{2+}, high Mg^{2+} concentrations to the tension in the standard Krebs solution. Morphometry of zinc iodide osmium-stained NMJ was measured with an image analyzer. Junctional receptors were assayed utilizing ^{125}-bungarotoxin binding. Repeated measures multivariate analysis of variance was used to test for differences due to aging and/or exercise. Measured quantal content, safety margin and nerve terminal areas increased significantly during aging. Training induced a significant (25%) increase in transmitter release in young and prevented the measured physiological age-related changes. However, there were no postsynaptic changes (the number and distribution of junctional acetylcholine receptors, muscle input resistance, muscle fiber diameter and membrane capacitance) either during aging or after exercise. The data confirm our previous investigations which suggested that physical activity at any age can modulate the structure and function of presynaptic and not postsynaptic mouse NMJ.

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AGING BRAIN AND ROLE OF NUTRITION. *K. Nandy,* D.I. Mostofsky and S. Nandy,* Dept. of Anat. and Neurobiol, and Psychol., Boston Univ. Sch. of Med., Boston, MA 02118.

One of the most consistent cytological changes in aging mammals is the deposition of lipofuscin age pigment in the neurons. The nature of the pigment formation is consistent with the biological properties of mammalian aging process. The studies in our laboratory have consistently shown that its formation can be altered by nutritional manip-

ulations. When mice of different ages were subjected to repeated and alternate vitamin E deficient and E excess diets, the pigment formation was alternately increased or decreased respectively in the neurons of hippocampus and frontal cortex. These results were also positively correlated with the decrease and increase in the learning ability of the animals. Similar results were noted in the pigment and learning when animals were fed on normal and restricted diets. It appears from these studies that lipofuscin is a reliable marker of neuronal aging and nutrition plays an important role in the aging process of the nervous system.

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ALZHEIMER'S DISEASE: A SUPPLY/DELIVERY FAILURE FOR A SYSTEM OF THE CNS? *H. Sobel,* IGPP UCLA, Los Angeles, CA 90024.

An hypothesis originally designated "the diffusion theory of aging" and relating to the insterstitial space was developed in 1952. It has been modified to include recent knowledge of the plasma membranes and now relates to the system: interstitial space (S)-basement membranes (BM)-pericellular (PC) region-plasma membranes (PM) which control the delivery and departure of molecules within the cytoplasm. The Barrier Dynamics Hypothesis of physiological aging was proposed: Changes in the rate of deposition of the constituents of the IS-BM-PC-PM over time as a result of differences in the net rate of their deposition and turnover and as a consequence of the history of the organism causes dyscoordination in the rate of delivery of molecules into the cells (cellular dysprositosia), so that, following maturity, physiological decline and its consequences ensue. Application to late onset diseases of the CNS was considered. A model for Alzheimer's disease is proposed in that supply/delivery failure resulting from the above events would certainly affect composition and energy supply to regions of the CNS and gradually lead to failure of certain systems of the brain. D.C. Wallace's OXPHOS paradigm (Science 256: 628-632, 1992) applies. While the latter does not address the amyloid precursor protein-beta amyloid problem and lysosomal involvement supply/delivery failure may affect membrane phospholipids (R.M. Nitsch, et al., PNAS 89: 1671-1675, 1992) or calcium influx control (M.P. Matson, et al., J. Neurosci. 12: 376-389, 1992). The role of supply/delivery failure as a final initiator of Alzheimer's disease should be investigated.

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DNA POLYMERASE α FROM NORMAL AND pSV3.NEO-TRANSFORMED CELLS. *V. Srivastava, M. Schroeder, and D. Busbee,** Dept. of Anat. and Public Health, Coll. of Vet. Med., Texas A&M Univ., College Station, TX 77843.

DNA polymerase α (pol α) was isolated from untransformed and pSV3.neo-transformed human fibroblasts in order to evaluate the differences in enzyme from fibroblasts derived from an old donor (GM3529) or from the transformed GM3529 corollary (2-1). The activity of pol α isolated from 2-1 cells was more than 9-fold higher than pol α from GM3529 cells. Enzyme from 2-1 cells exhibited a higher sedimentation coefficient and showed an increased relative ability to copy a variety of template-primers compared to enzyme from GM3529 cells. Western blot analysis, using anti-large T antigen, of both a crude cellular extract and a partially purified pol α from 2-1 cells revealed a single 76 kda immunoreactive band not found in either crude extracts or pol α from GM3529 cells. Enzymes from GM3529 and 2-1 cells differed in their sensitivity to various inhibitors and in their kinetics of substrate utilization. These data suggest that pol α isolated from cells transformed with the SV40-derived plasmid, pSV3.neo, demonstrates altered physical and catalytic characteristics, compared with its untransformed counterpart, that are associated with the increased genomic replication of virally transformed aged cells.

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AGE-RELATED DNA MUTATIONS OF THE INTEGRATED lacI GENE IN TRANSGENIC MICE. *A.T. Lee,* A. Cerami and R. Bucala,* The Picower Inst. for Med. Res., Manhasset, NY 11030.

The effects of biological aging are evident in all eukaryotic organisms. There have been a number of observations of age-related increase in DNA damage; however, the underlying mechanisms have not yet been determined. The recent development of transgenic mice containing an excisable marker gene offers an efficient and sensitive method to determine age-related DNA damage in a complete animal. We have been investigating age-related DNA damage in transgenic mice containing integrated copies of the well-defined mutagenesis reporter gene lacI. Since all cells of these mice carry the lacI gene, both the frequency and type of mutation in different organs in mice of increasing age can be determined. The lacI gene is contained within a lambda phage construct that enables it to be excised from genomic DNA, efficiently packaged into phage particles, and analyzed for lacI mutations. Mutational analysis of lacI DNA isolated from splenic tissue of transgenic mice demonstrates an increase in mutations as a function of age. Additional studies suggest that these mutations appear to be comprised of a variety of sequence changes including insertions and deletions. The use of transgenic animals to investigate age-related DNA damage and mutations will provide valuable molecular information regarding the extent and patterns of DNA damage which occur during the aging process of whole animals.

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MYOCYTE DNA SYNTHESIS WITH AGING AND BIVENTRICULAR DYSFUNCTION IN RATS. *J.M. Capasso,* S. Bruno, P. Li, X. Zhang, Z. Darzynkiewicz, and P. Anversa,* Dept. of Med., New York Med. Coll., Valhalla, NY 10595.

To determine whether the detrimental mechanical and anatomical changes which are seen to occur biventricularly with normal aging are associated with activation of DNA synthesis, flow cytometric analysis was performed on myocyte nuclei prepared from the left and right ventricles of male Fischer 344 rats at 4, 12, 20 and 29 months of age. Although body weight ceased to increase from 12 to 20 months and actually decreased to 4 month levels at 29 months, heart weights increased significantly at each age interval from 4 to 29 months of age. The development of left ventricular failure and right ventricular dysfunction as aging occurred were evidenced from hemodynamic performance profiles which revealed an age-related progressive decrease in arterial and ventricular systolic pressures and a significant increase in cavitary diastolic pressures on both sides of the heart. This increase in diastolic Laplace stress on the aging myocardium was associated with marked increase in the number of myocyte nuclei in the $S+G_2M$ phase of the cell cycle, biventricularly. Moreover, an excellent correlation was found with the degree of left ventricular dysfunction and the number of myocyte nuclei entering the cell cycle. Interestingly, a greater percent of myocyte nuclei was found in the right ventricle of 4, 12, and 20 month old animals with this situation completely reversing at 29 months of age. In conclusion, myocyte nuclear mitotic division occurs as a function of age and may represent an important adaptive component of the myocardial response to the functional and structural alterations associated with this progressive disease state.

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TELOMERE LENGTH AS A BIOMARKER FOR *IN VITRO* AND *IN VIVO* AGE OF INTIMAL AND MEDIAL TISSUE. *E. Chang* and C.B. Harley,* Dept. of Biochem., McMaster Univ., Hamilton, ON, Canada, L8N 3Z5.

Repeated denudation of the endothelium and consequent senescence of intimal cells may contribute to atherogenesis. Our previous work has shown that telomeres, the G-rich ends of the eukaryotic chromosomes, shorten with *in vitro* and *in vivo* age of human skin fibroblasts. Our aim was to determine if telomere length serves as a biomarker of cellular turnover in tissues implicated in atherogenesis. Telomere lengths were assessed by southern analysis of terminal restriction fragments (TRF) in *Hinf*1/*Rsa*1-digested genomic DNA. Mean TRF length decreased with *in vitro* age in endothelial cell cultures from human umbilical veins (slope (m) = -190 base pairs (bp)/ Population Doubling (PD), r=-0.98, P=0.01), iliac arteries (m = -120 bp/PD, r=-0.90, P=0.09) and iliac veins (m = -180 bp/PD, r=-0.99, P=0.02). Preliminary data also show a significant decrease in TRF length of intimal (P=0.03) and medial (P=0.05) tissue as a function of donor age. Endothelial cells from iliac arteries had shorter TRFs than cells from iliac vein which is consistent with increased cellular turnover *in vivo* and reduced life span *in vitro* of endothelial cells from

iliac artery. These observations suggest that telomere length may be a biomarker for the replicative history and replicative capacity of intimal and medial tissue.

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AGE-RELATED CHANGES IN LEARNING, MEMORY AND IN THE PHARMACOTHERAPY OF RETENTION IN SENESCENCE ACCELERATED MOUSE (SAM) MODEL. *J.F. Flood,* J.E. Morley and R. Strong,* St. Louis Univ. Med. Ctr. and GRECC, VA Med. Ctr., St. Louis, MO 63106.

Recently, mouse strains were developed that appear to exhibit characteristics of accelerated aging. Different sub-lines exhibit different phenotypic characteristics of aging. One is prone to an early onset of osteoporosis, some develop ophthalmic disease and others show a premature deposition of amyloid. The primary phenotypic characteristic of the P/8 line is impaired learning and memory beginning as early as 8 months of age. This deficit which occurs with aversive and appetitive, spatial and discrimination learning progressively worsens up to 12 months of age. A battery of memory enhancing compounts, administered peripherally immediately after training, alleviated a retention deficit for footshock avoidance conditioning. However, cholinomimetics had to be administered at higher doses in 8-month-old mice than in 4-month-old mice and at still higher doses in 12-month-old mice than in the 8-month-old mice. Neurochemical studies found that choline acetyltransferase decreased 50% in the septum and hippocampus (HPC) of 8-month-old P/8 mice and decreased an additional 33% in the HPC of 12-month-old mice; such changes were not found in other limbic structures. Compounds improving retention by acting on other neurotransmitters did so in 4- and 8-month-old mice at the same dose. Pending determination of the related pathological changes, this strain appears to be a useful model for studying drug therapy aimed at alleviating dementia associated with aging.

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CHRONIC TREATMENT OF (–)DEPRENYL PROLONGS THE LIFE SPAN OF MALE FISCHER 344 RATS. *K. Kitani,[1] S. Kanai,[1] Y. Sato,[1] G.O. Ivy[2] and M-C. Carrillo,[1]* [1]Dept. of Clinical Physiol., Tokyo Metropol. Inst. of Gerontol., Itabashiku, Tokyo-173, Japan; [2]Div. of Life Sci., Univ. of Toronto, Scarborough, Ontario M1C 1A4, Canada.

Previous two studies have shown that the chronic administration of (–)deprenyl starting from 24 months of age significantly prolonged the life span of male rats (1, 2). We wanted to confirm results of these previous studies. Thirty-five male Fischer 344 (F-344) rats started to be treated with s.c. injection of (–)deprenyl (0.5 mg/kg) 3 times a week at the age of 18 months. Thirty-five control animals of the same age were treated with a physiological saline solution injection. The mean survival times after the start of treatment (18 months) and after 24 months were 378.3±97.4 days (mean±SD) and 196.3±97.4 days respectively in deprenyl-treated rats and both 328.7 days, 146.7±108.7 days in control rats. Increases in average life expectancies (15% from 18 months and 34% from 24 months) caused by deprenyl treatment were both statistically significant ($P<0.05$, two-tailed t-test). The average body weights were comparable for both groups excluding the possibility that the effect of deprenyl is secondary from the reduced diet intake caused by this treatment. The results confirm the previous two studies (1, 2) which reported a significant life-prolonging effect of this drug in aged rats, and support more strongly the result of a study on male F-344 rats where the effect was only marginally significant (16% increase after 24 months, $P=0.048$ by one-tailed t test) (2).

1. Mech Ageing Dev 46: 237, 1988.
2. Life Sci. 47: 415, 1990.

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EFFECT OF VITAMIN A ON LONGEVITY. *H.R. Massie* and V.R. Aiello,* Masonic Med. Res. Lab., Utica, NY 13501.

The purpose of this study was to determine a possible mechanism for why vitamin A both prolongs and reduces life span.

Increasing the dietary content of vitamin A during the developmental stages of *Drosophila* increased the median life span by as much as 17.5%. The optimum dietary concentration of vitamin A for increasing the life span of *Drosophila* was found by extrapolation to be 8 I.U./gm of food. The maximum life span was reduced as dietary concentrations of vitamin A exceeded this value. Vitamin A palmitate and retinal inhibited the peroxidation of linolenic acid induced by the generation of superoxide radicals from acetaldehyde. Other forms of vitamin A, such as retinol and retinoic acid, moderately inhibited lipid peroxidation at low concentrations but stimulated peroxidation considerably when present at high concentrations. Based upon the ability of these retinoids to inhibit the reduction of cytochrome c by superoxide radicals, we propose that retinoids can both inhibit and stimulate lipid peroxidation depending upon their concentration by reacting with superoxide radicals. We also propose that this reaction is the basis for the apparent ability of vitamin A to both prolong and shorten life span depending upon the dietary intake.

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HIGH BLOOD GLUTATHIONE IN AGING F344 RATS FED A METHIONINE-RESTRICTED DIET. *Y. Leutzinger, S. Parthasarathy, V. Malloy, J.A. Zimmerman, N. Orentreich and J.P. Richie, Jr.,** Amer. Health Fndn., Valhalla, NY 10595; St. John's Univ., New York, NY 11439; and Orentreich Fndn. for the Advancement of Sci., Cold Spring-on-Hudson, NY 10516.

Glutathione (GSH) deficiency appears to be a general phenomenon of senescent tissues and may play a key role in the aging process. Although previous results suggested that aging could be delayed when GSH levels were increased by feeding GSH precursors, recent findings demonstrated an extended life span in mice and rats fed diets low in the GSH precursor methionine (Met). The objective of the present study was to examine the role of GSH in the enhancement of longevity by determining GSH and cysteine (Cys) status in the blood of rats fed a low Met diet. Male F344 rats, beginning at 6 weeks of age, were fed a defined amino acid diet containing either 0.17% (low-Met) or 0.86% (control) Met, as the sole source of sulfur amino acid. After 20-25 months, blood was obtained from the suborbital vein, extracted with 5% metaphosphoric acid, and analyzed for GSH and Cys by HPLC with electrochemical detection. As observed previously, a 30% increase in median life span was observed in animals fed the low-Met diet as compared to control animals. The blood GSH levels of rats fed the low-Met diet were 81% greater than in control animals ($P<0.0001$). In contrast, blood Cys levels were 86% lower in low-Met animals than in controls ($P<0.0001$). The enhanced blood GSH levels in the low-Met group were also seen after the rats were fasted overnight prior to sampling. These results suggest that drastic changes in the metabolism of Met, Cys and GSH occur in response to a chronic diet low in Met. These changes result in the elevation of blood GSH concentrations and, thus, strongly support our hypothesis on the role of GSH in the aging process.

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CHROMIUM PICOLINATE INCREASES LONGEVITY. *G.W. Evans* and L. Meyer,* Chemistry, Bemidji State Univ., Bemidji, MN 56601.

Chromium picolinate, a complex that has been shown to be efficacious in maximizing insulin function, was tested to determine its effect on protein glycation and longevity. Weanling, male Long-Evans rats were housed individually in plastic cages and fed *ad libitum* a purified diet (0.8 µg/Cr/g diet) to which chromium chloride (CrCl), chromium nicotinate (CrNic) or chromium picolinate (CrPic) was added at a level of 1 µg Cr/g diet. At various ages, 50 µL blood was drawn from the tail of each rat between the hours of 1400 and 1500 and plasma glucose and glycated hemoglobin (HbA_{Ic}) were measured. At 200 days, plasma glucose was 6.6±0.12 mM (means±S.E. of 10 rats) in rats fed CrPic, 7.7±0.12 mM in rats fed CrNic and 7.8±0.11 mM in rats fed CrCl. At 200 days, HbA_{Ic} was 3.31±0.08% in rats fed CrPic, 5.19±0.09% in rats fed CrNic and 5.39±0.08% in rats fed CrCl. At 1000 days, plasma glucose was 6.5±0.14 mM in rats fed CrPic, 8.3±0.17 mM in rats fed CrNic and 8.2±0.18 mM in rats fed CrCl. At 1000 days HbA_{Ic} was 3.41±0.09% in rats fed CrPic, 8.29±0.19% in rats fed CrNic and 8.43±0.28% in rats fed CrCl. After 41 months, all of the animals fed either CrNic or CrCl had died while 80% of the rats fed CrPic were alive. These results demonstrate that chromium picolinate increases longevity possibly by retarding hyperglycemia and protein glycation.

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AGE-ASSOCIATED CHANGES IN GLUCAGON BINDING, GLUCOSE RESPONSE AND THE IMPACT OF CALORIC RESTRICTION. *R.J. Feuers,* F. Chen, E.T. Oriaku, and R.W. Hart,* Natl. Ctr. for Toxicolog. Res., Jefferson, AR 72079.

The role of glucagon and its receptor in the life-extending capacity of caloric restriction (CR) was investigated by measuring 125I-Glucagon binding in the liver and glucose response to glucagon in 12- and 24-month-old B6C3FI male mice. The mice were fed either *ad libitum* (AL) or a diet with the total caloric intake reduced by 40% beginning at 14 weeks of age. Basal blood glucose increased with age in AL mice, but was lower and remained constant across age in CR mice. Maximum binding occurred during the dark after the animals ate and was 2-fold higher in CR mice. Binding decreased with age in both AL and CR mice, but temporal patterns and total binding were maintained to a significantly higher degree in CR mice. Glucagon-stimulated glucose increases in most cases. CR and AL mice were most sensitive to glucagon at the time of transition from dark to light. Response to glucagon decreased with age in AL mice, but not in CR mice. The increased binding seen in CR mice suggests increased receptor number or affinity. The increased response due to CR and maintenance of response across age suggests basic increases in efficiency in regulation of metabolic pathways. Increased sensitivity to insulin and glucagon seems to play a role in the ability of CR to maintain low, well-regulated blood glucose levels and metabolic rates. These endocrine systems may play a key role in the mechanism through which CR exerts its life span extending effects.

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THE ROLE OF CALORIES IN LIFE SPAN EXTENSION PRODUCED BY REDUCED METHIONINE INTAKE. *J.A. Zimmerman,* V. Molloy, S. Parthasarathy and N. Orentreich,* St. John's Univ., Jamaica, NY 11439; and The Orentrich Fndn. for the Advancement of Sci., Cold Spring-on-Hudson, NY 10516.

Previous studies from our laboratories showed that reduced methionine (met) ingestion in rats leads to extended life span, reduced body growth and increased caloric consumption per unit of body mass. Nevertheless, since restricted animals are smaller their caloric consumption per animal is slightly reduced, leading to the possibility that the life-extending effect of met restriction is a result of caloric, and not of met, restriction. To test this possibility we limited Fischer 344 male rats to a normal met ration (0.86% met, 0% cys) in the reduced quantities consumed by rats receiving a life-extending concentration (0.17%) of met in their diet. Growth was slowed in these pair-fed rats for approximately one month after pair-feeding began, and then accelerated, so that by the 45th day of feeding there was no difference between pair-fed rats and those fed 0.86% met *ad libitum*. During the initial lag in growth total caloric intake was reduced somewhat in pair-fed rats, but by day 35 of feeding total intake was similar for all groups, while food intake expressed in terms of body weight did not vary between groups at any time during the study. These results indicate that the slight reduction in total caloric intake seen in met restriction is insufficient to significantly interfere with growth, and is probably insufficient to produce life span extension.

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NONENZYMATIC GLYCATION OF TYPE I COLLAGEN: THE EFFECTS OF AGE ON SITES OF ADDUCT FORMATION. *K.M. Reiser* and M. Amigable,* Sch. of Med., UC-Davis, Davis, CA 95616-8542.

The present study was designed to investigate the effects of aging on preferential sites of glucose adduct formation on type I collagen chains. Two CNBr peptides, one from each type of chain, were investigated in detail: α1(ICB3 and α2CB3-5. The CNBr peptides were purified from 6-, 18-, and 36-month-old rat tail tendon by ion exchange chromatography, gel filtration and HPLC. Sugar adducts were radiolabelled by reduction with NaB^3H_4. Glycated tryptic peptides were purified from CNBr peptides by boronate affinity chromatography and HPLC, and were identified by sequencing and by compositional analysis. Preferential sites of glycation were observed in both CB3 and α2CB3-5. Of the 5 lysine residues in CB3, Lys 434 was the favored glycation site. Of the 18 lysine residues and 1 hydroxylysine residue in α2CB3-5, three residues (Lys 453, Lys 479, and Lys 924) contained more than 80% of the glucose adducts on the peptide. Preferential glycation sites were highly conserved with aging. Under hyperglycemic conditions, the relative distribution of glucose adducts in old animals differed from those of young animals. *In vitro* experiments suggest that primary structure is the major determinant of preferential glycation sites but that higher order structure may influence the relative distribution of glucose adducts among these preferred sites. We conclude that the information present in primary structure alone is sufficient to regulate the first step in nonenzymatic glycation of collagen during aging, even if there is a decrease in order of the fibrillar arrays.

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EFFECT OF CHRONIC CALORIC RESTRICTION, AGE AND ISOPROTERENOL ON TREADMILL PERFORMANCE AND CARDIOVASCULAR FUNCTION IN RATS. *P. Duffy,* K. Aly, B. Divine and R. Hart,* Natl. Ctr. for Toxicol. Res., Jefferson, AR 72079.

The purpose of the study was to determine the effect of caloric restriction (CR), age and drugs on physiological performance in rats under conditions of maximum metabolic output. Cardiovascular and physical fitness were assessed in different age groups of control rats that were fed *ad libitum* (AL) and CR rats (60% of AL). Body temperature, heart rate and ECG were monitored before, during and after treadmill exercise. Physical endurance was better in CR rats than AL rats. CR rats had a lower heart rate and body temperature than AL rats during and immediately after exercise, indicating increased thermal conductivity in CR rats. In a second experiment, AL and CR rats from various age groups were given a single IM injection of the drug isoproterenol (IPR) in doses ranging from .2mg/kg to 500mg/kg, and physiological performance was monitored as before. IPR accelerated heart rate and produced cardiovascular effects similar to those resulting from occlusions of the coronary artery. CR dramatically reduced IPR-induced mortality (100-fold) and myocardial damage. The rapid reduction in core temperature that occurred in CR rats immediately after exposure to IPR may protect the heart from damage, thereby increasing survival potential. CR slowed the age-related decline in physical performance and cardiac function.

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THE TEMPORAL RELATIONSHIPS OF SYNTHESIS AND PHOSPHORYLATION IN STRESS PROTEINS 70 AND 90 IN AGED CALORIC RESTRICTED RATS EXPOSED TO BLEOMYCIN. *J.L. Pipkin,* W.G. Hinson, L.E. Lyn-Cook, E.R. Burns, R.J. Feuers, R. Hart and D.A. Casciano,* Natl. Ctr. for Toxicolog. Res., Jefferson, AR 72079.

A single intraperitoneal injection of the human therapeutic drug bleomycin (BL) was administered to three groups of male Fischer 344 rats at time 0. The synthesis (^{35}S) and phosphorylation (^{32}p) of stress proteins (sps) were analyzed over time by two-dimensional

electrophoresis and fluorography as biomarkers of aging. The peptides were analyzed to determine the effects of caloric restriction (CR) on aging. Two groups of rats, young *ad libitum* (Y/AL – 1½ mo) and old *ad libitum* (O/AL — 28 mo), had free access to rat chow, and a third group of old rats (O/CR — 28 mo) were maintained on a CR intake (60% of the AL diet). Synthesis of sp 90 was significantly increased in Y/AL as compared with O/AL and O/CR, and the incorporation pattern of O/CR was intermediate to Y/AL and O/AL animals following BL dosing. Stress protein 90 reached peak ^{35}S-incorporation at 4 hr. Stress protein 70x assumed a similar pattern of ^{35}S-incorporation but was depressed below that of sp 90. Phosphorylation of sp 90 which peaked at 2 hr after BL treatment in Y/AL was significantly increased over O/AL, and O/CR had an intermediate level. The demonstration of sps 70 and 90 as biomarkers of aging and the metabolic shifts orchestrated by limited caloric consumption suggests a general homeostasis in O/CR rats following a prolonged regime of caloric restriction.

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DEVELOPMENT OF ANIMAL MODELS FOR AGE-ASSOCIATED MITOCHONDRIAL DNA DELETIONS. *C. Lee, S. Chung, D. McKenzie and J. Aiken,* Dept. of Animal Health and Biomed. Sci., Univ. of Wisconsin, Madison, WI 53706.

Large deletions of the mitochondrial DNA genome have been characterized in humans. These deletions appear to increase in frequency with normal aging. We are using animal models, specifically Rhesus monkeys and mice, to characterize deletions of the mitochondrial genome. In the Rhesus monkey we are screening for deletions in the frontal cortex, parietal, and occipital regions of the brain as well as cardiac, skeletal muscle and liver. We are in the process of analyzing such tissue samples from animals 6-28 years of age. By using the polymerase chain reaction (PCR), we have amplified several mitochondrial fragments which are smaller than the expected size. By combining various PCR techniques with dideoxy DNA sequencing, we have confirmed several deletions in the older animals. We conclude that these animals will serve as useful models for studying aging.

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EFFECT OF AGING ON THE INTESTINAL MUCOSAL GROWTH AFTER ILEO-JEJUNAL TRANSPOSITION IN RATS. *T. Tsuchiya, J. Ishizuka, I. Shimoda, C.M. Townsend Jr., and J.C. Thompson,* Dept. of Surg., University of Texas Med. Branch, Galveston, TX 77555.

Growth of intestinal mucosa is stimulated after ileo-jejunal transposition (IJT) in young rats. The purpose of this study was to examine whether aging affects growth of intestinal mucosa in the IJT model, using 4- and 24-mo-old male Fischer 344 rats. In the IJT group (T), the distal ¼ of the small intestine was interposed into the jejunum 8 cm distal to the ligament of Treitz in an isoperistaltic fashion, and continuity was restored by end-to-end anastomosis. In the control group (C), the bowel was only transected. Twenty-one days later, rats were sacrified and the small intestine was removed and divided into 3 segments [duodeno-jejunal (DJ), jejuno-ileal (JI) and ileal (I)]. Weight, DNA and protein contents of mucosa were measured.

Mucosal Weight (mg/cm)

		DJ	JI	I
4 mo	C	17.9±0.8	16.6±0.6	15.5±0.7
	T	28.1±1.4*	27.2±1.2*	38.1±5.4*
24 mo	C	22.6±1.0	17.1±1.0	17.0±2.0
	T	36.5±1.8*	32.0±1.7*	47.8±3.9*

(n = 10-12, * = $p<0.05$ vs control)

Mucosal weight, DNA and protein contents of all 3 segments tested significantly increased in both age groups after IJT, with the greatest in the ileal segment. The % increase of mucosa weight was significantly greater in aged than in young rats. These findings suggest that in aged rats the ability of intestinal mucosa to respond to trophic stimuli is preserved and may even be greater than in young rats.

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STATIONARY CELL CULTURE AS A MODEL FOR EXPERIMENTAL GERONTOLOGY. *A.N. Khakhlov,* Evolutionary Cytogerontol. Sector, Biolog. Faculty, Moscow State Univ., 119899, Russia.

The studies of aging mechanisms are very often carried out on cells senescing *in vitro* ("Hayflick's model"). However, some data allow one to suppose that the results obtained on this model are not in accordance in some cases with the results of *in vivo* aging investigations. Besides, such experiments are comparable by labor-consuming character with ones on laboratory animals. L. Hayflick himself notes that cells *in vivo* never realize all their proliferative potential. In other words, an organism never ages because of cells' "Hayflick's limit." It ages, we think, because of accumulation in cells of various damage due to cell proliferation restriction during formation (in the process of differentiation) of populations of specialized resting, or very slowly dividing, cells. The rate of any type damage accumulation in cell population (not in a single cell!) depends on the ratio of rates of three processes: 1) cell proliferation, 2) spontaneous damage appearance, and 3) damage repair. During cell proliferation a "dilution" of the damage occurs. Our data about the direct relationship between the average proliferative activity of cell line or strain and the average DNA molecular weight support the hypothesis. Bearing all this in mind, we suppose it is more advisable to study cellular aging mechanisms using the "stationary phase aging" model. It is based on the assumption that in the cells of stationary cultures various changes similar to those of aging organism cells have to appear. Recent years we and other investigators obtained many experimental results confirming this assumption. Really, "age" changes on different levels (accumulation of DNA breaks and DNA-protein cross-links, DNA demethylation, changes of spontaneous sister chromatid exchange level, plasma membrane changes, nuclear structure modulations, decrease of the rate of mitogen-stimulated cell cycling and of cell colony-forming ability a.o.) were shown to occur in stationary cell cultures. Such experiments can be carried out on nearly any type cells including normal and transformed human and animal ones, plant cells, bacteria, mycoplasms, yeasts, etc. Thus the evolutionary approach to analysis of the data is provided. Moreover the changes in the stationary cell cultures become detectable very soon — as a rule, in 2-3 weeks after beginning of the experiment. All this allows us to suppose that the "stationary phase aging" model is a rather good alternative to the Hayflick's model.

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FREE RADICAL THEORY OF AGING: A SURVEY FROM THE RELIABILITY THEORY POINT OF VIEW. *V.K. Koltover,* Inst. of Chem. Physics, Russian Acad. of Sci., Moscow Dist., Chernogolovka, 142432, Russia.

The results of our measurements of production of the oxygen-free radicals in tissues of rats of different ages as well as the analysis of the data available from the literature evidence that intensification of free radical chain reactions as combustions or outbreaks seems to be unthinkable in a living system with age. Meanwhile, the kinetics of mortality rate growth for animals and humans follows the exponential law (the so-called Gompertz's law of mortality). The reliability theory approach to the free radical theory of aging has been developed. In terms of this approach it becomes possible to explain now the linear kinetics of accumulation of free radical damages in the cellular targets (chromosomes) can lead to the exponential mortality rate growth with age, as well as to explain the nature of the well-known empiric interspecies correlations between the maximal life span values and the metabolic factors and to estimate species-specific life span potentials of animals and humans (120 y for humans). It has also been estimated that the life span of primates could run up to 250 y, but for the damages due to the oxygen-free radicals.

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SERUM LIPID PEROXIDES AND ANTIOXIDANT DEFENSE COMPONENTS OF RURAL AND URBAN POPULATIONS AND AGING. K.K. Reddy*, T. Ramachandraiah, K. Soorya Kumari, P. Reddanna and K. Thyagaraju, Dept. of Phys. Anthropol. and Biochem., S.V. Univ., Tirupati-517502, India.

Serum lipid peroxides, antioxidant vitamins such as vitamin C, vitamin E and an antioxidant enzyme glutathione peroxidase activity were determined among urban (175M + 139F) and rural (135M + 87F) inhabi-

tants, with an age range of 40-78 years. Serum lipid peroxide (LPO) levels show no significant difference in 40-49 and 50-59 age groups between rural and urban populations; however, these levels were elevated in >60 age group in urban individuals. The main cellular defense systems against free radical-mediated oxidative stress functioned effectively until the concentration of LPO attained 4.0 nmol/ml, in both groups. Further increase in LPO resulted in depletion of antioxidants, and the percentage of individuals possessing >4.0 nmol/ml of LPO with age was increased in urban population when compared to rural population. The data indicate that an impairment of the antioxidant system would render the older individuals in urban populations more susceptible to peroxidative stress.

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EFFICACY OF GERIFORTE, AN HERBAL FORMULATION, IN SUPEROXIDE DISMUTASE FORMATION IN THE CEREBELLA AND SPINAL CORDS OF WISTAR MALE RATS. *B. Singh*, S.P. Sharma, R. Goyal and T.J. James,* Lab. of Nutr. Histopathol. and Ageing, Dept. of Zool., Kurukshetra Univ., Kurukshetra-132119, India.

Geriforte, an herbal formulation, is an ideal geriatric tonic to solve the problems of aging. Geriforte is being used as a restorative tonic in old age in India. In the present study, this antistress adaptogenic Ayurvedic drug has been evaluated for an antioxidant enzyme, superoxide dismutase (SOD), production in the cerebella and spinal cords of Wistar male rats. Since aging is associated with free-radical damage, SOD plays a vital role in scavenging the free radicals. Therefore, in the present experiment, the efficacy of Geriforte was assessed on SOD content in the cerebella and spinal cords of rats. Rats were given Geriforte powder (900 mg/kg body weight) orally once a day for two months. The Geriforte-administered cerebella and spinal cords showed a greater increase in SOD level than that registered in control rats. The cerebella of Geriforte-treated rats showed significantly ($P<0.01$) higher SOD activity. They were reported to have 20.26% more SOD content. The level of SOD in the spinal cords of Geriforte-treated animals also tended to increase; however, this elevation in SOD content was insignificant. The spinal cords had only 3.15% more SOD activity in the drug-treated rats. Thus, it appears that Geriforte has a marked potency to produce large amounts of SOD.

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EFFECT OF DIETARY SELENIUM ON CEREBELLA AND SPINAL CORDS GLUTATHIONE PEROXIDASE IN THE RATS. *B. Singh,* S.P. Sharma, R. Goyal, I.K. Patro[1] and T.J. James,* Lab. of Nutr. Histopathol. and Ageing, Dept. of Zool., Kurukshetra Univ., Kurukshetra-132119, India; [1]School of Studies in Zool., Jiwaji Univ., Gwalior-474001, India.

Selenium (Se) is an integral part of the enzyme glutathione peroxidase (GPx) and the magnitude of GPx in a tissue reflects Se status of that tissue. GPx plays an important role in the antioxidant defense system, eliminating H_2O_2 and lipid hydroperoxides yielded during lipid peroxidation, when superoxide radicals are acted upon by superoxide dismutase. In the present study, one-month-old Wistar male rats were taken and they were maintained on selenium-deficient (-Se) and control (+Se) Altromin purified diets for 3, 6 and 9 months. After completion of each parameter, the rats were sacrificed and enzyme activity was determined. Level of GPx in the cerebella and spinal cords decreased significantly ($P<0.001$) in all 3 groups of animals fed -Se diet. The cerebella of -Se and +Se rats were found to have greater activity than that recorded in the spinal cords of all 3 animal groups. The results confirm the earlier observations that selenium deficiency decreases GPx content.

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RESPONSES OF AGED FEMALE RATS TO GH SECRETAGOGUES. *R.F. Walker and B.B. Bercu,* Dept. of Pediat., Univ. So. Florida College of Medicine, Tampa, FL 33620.

The purpose of this study was to examine the effects of growth hormone (GH) releasing hormone (GHRH) and GH releasing hexapeptide (GHRP-6), an efficacious combination of GH secretagogues, on maladaptive, anatomical and physiological changes associated with aging in female Fischer 344 rats. Interim analysis showed that GH hypersecretion in 24-month-old rats was attenuated within the first 5 days of treatment, correlating with a significant increase in plasma insulin-like growth factor (IGF-1), a negative feedback modulator of GH secretion. Co-administration of GHRH and GHRP-6 to the old rats for sixty consecutive days was associated with significantly lower pituitary, adrenal and kidney weights, a reduced incidence of pituitary adenomas, and higher thymus weights compared to saline-treated, age-matched controls. Structural changes were accompanied by significantly increased concentrations of pituitary GH, plasma IGF-1, and reduced concentrations of plasma prolactin and cholesterol. However, plasma corticosterone was lower and insulin was higher in old rats than in young rats, suggesting that age-related adrenal and pancreatic dysfunctions were not reversed by daily administration of GHRH and GHRP-6. In conclusion, the results of this study suggest that low pituitary and plasma GH concentrations in old age can be attributed to inappropriate or inadequate trophic signals rather than to inherent defects in pituitary function. The data also suggest that pharmacological stimulation of endogenous GH secretion may correct certain maladaptive changes of aging.

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EFFECT OF GINSENG SAPONIN ON THE IMMUNITY OF YOUNG AND OLD MICE. *Y-S. Huo and M. Utsuyama,** Inst. of Med. & Pharmaceut. Sci. of Dalian, 21 Chun-Yang St. Dalian 116013, China P.R.; *Dept. of Pathology, Tokyo Metropol. Inst. of Gerontology.

Ginseng Saponin (GS) was extracted from Panax Ginseng, which is famous Chinese traditional herb medicine. It may help maintaining homeostasis were named as "Adaptogens;" immune function is important for these. In this report, we used C57BL/6 male mice [7 weeks (young) and 25 months (old)] in experiments. *In vivo* experiment: GS was given to mice by gastric tube at 0.06 mg/g body w. for 7 days. Number of splenic T cells (Thy-1+ cells). T cell subsets (L3T4+ cell and Lyt-2 cells) and B cells (surface immunoglobulin+ cells) were assessed by a flow cytometer (FCM-1.JASCOL). The result was significant. This effect was observed only in the percentage of splenic T cells of old mice; a trend of increase was also observed in L3T4+ cells in the spleen as well as in the ratio of L3T4/Lyt-2(CD4/CD8) of old mice only. *In vitro* in old mice, GS showed the spleen cells an enhancing effect in PHA responses (PHA was used 2.5 ug/ml). Those results showed that GS could bring about a trend of immunological restoration in spleen cells of old mice, both *in vivo* and *in vitro.*

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Busbee, D. Abstract appears on page 19.

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AN UPDATE ON THE MITOCHONDRIAL OXIDATION DAMAGE THEORY OF CELL AGING. *J. Miquel,*[1,2] and M. de la Fuente,[3],* [1]Facultad de Medicina, 03080 Alicante, Spain; [2]Linus Pauling Inst., Palo Alto, CA and [3]Facultad de Ciencias Biológicas, Universidad Complutense, 28040 Madrid, Spain.

The finding of age-related mitochondrial breakdown (with resulting age pigment accumulation) in the somatic tissues of insects[1] as well as in the fixed postmitotic cells of mouse testis[2] led to the hypothesis that the aging of metazoans may be rooted in oxyradical injury to the mitochondria of their differentiated cells. It was further proposed, in agreement with Harman,[3] that mitochondrial DNA could be the primary target of free radical attack.[1,4,5] More recently, the concept of intrinsic mitochondrial aging has been used to link the classic views of Minot and Pearl on the role of cell differentiation (as the foundation of aging) and metabolic rate (as the main determinant of rate of aging and life span). This integrated theory,[6] which shares concepts of both programmed and wear-and-tear hypotheses of aging, provides a logical explanation of senescence from the molecular to the systemic levels.

Presently, there is a surge of interest in the subject of mitochondrial aging, as evidenced by the number of theoretical articles and meetings dealing with this subject. Further, recent data from a number of laboratories, including our own, suggest that mitochondrial membrane changes and decreased respiratory enzyme activity may play a key role in the senescent decline of CNS, neuroendocrine and immune functions.

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AGE-DEPENDENT IMPAIRMENT OF THE ABILITY OF BRAIN MITOCHONDRIA TO HANDLE OXIDATIVE STRESS. *R.A. Floyd,* Oklahoma Med. Res. Fndn., Oklahoma City, OK 73104.

The diminished ability of mitochondria to mount an adequate response to a large oxidative challenge may explain age-associated problems that develop in brain. Experimental data will be reviewed which support this notion. We have found that an ischemia/reperfusion insult (IRI) to brain is more lethal to older Mongolian gerbils than to younger animals. Experimental data obtained implicate the age-associated lesion to be localized in mitochondria. The data include: (A) significantly decreased pyruvate driven oxygen consumption in synaptosomes from brain of older gerbils compared to younger gerbils after the nerve endings have been damaged by a large peroxidative insult; and (B) a significantly decreased time comparing older gerbils with younger animals for brain to recover to the normal intracellular pH and high energy phosphate levels after they have experienced an IRI. Brain cortical pH and high energy phosphate levels were assessed *in vivo* using ^{31}p-NMR spectroscopy. The brain intracellular pH fell from 7.4 to 6.3 as the 10 min ischemia period developed, but recovered to nearly normal levels within about 30 min of reperfusion in younger gerbils but had not fully recovered to normal pH values after one hour in older gerbils. Restoration of intracellular pH and high energy phosphates are indices of mitochondrial effectiveness *in vivo* and suggest that the energy needed to offset preturbations induced by the large oxidative stress brought on by an IRI is not produced as readily in older gerbil brain as in younger gerbil brain. Supported in part by NIH grants NS 23307 and AG 09690.

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OXIDATIVE DAMAGE TO mtDNA AND AGING. *B.N. Ames,* K. Beckman and T.M. Hagen,* Univ. of California at Berkeley, Berkeley, CA 94720.

Studies in our laboratory show that oxidative DNA damage is enormous. Using 8-oxo-2'-deoxyguanosine (oxo^{8}dG) as a damage marker, we determined that oxo^{8}dG levels increase in nuclear DNA from 1 per 100,000 bases in young (4 month) rats to 1 per 55,000 in senescent (24 month) animals. Damage to mtDNA is more extensive. Steady-state oxo^{8}dG in young rats is $\approx$0.3 pmol per µg mtDNA (or $\approx$1 per 10,000 bases) and increases to $\approx$1 pmol per µg in 24-month-old rats. This corresponds to one in every 3,000 bases oxidatively damaged in senescent animals. Thus, mtDNA becomes increasingly damaged by reactive oxygen as a function of age.

The extensive oxidative damage of mtDNA suggests that it may be a critical site of somatic mutation, and that somatic mutation of mtDNA may contribute to the losses of function that are observed during aging. The mitochondrial genome is not as actively repaired as nuclear DNA. It is also well established that the mitochondrial genome mutates about ten times faster than the nuclear genome over evolutionary time spans, which is suggestive of a higher rate of mutation in general. Indeed, many workers have reported an increase in somatic deletions of mtDNA with age in humans. We are therefore developing techniques for the measurement of somatic point mutations in mtDNA which will be useful in testing the extranuclear somatic mutation theory of aging.

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Linnane, A.W. Abstract appears on page 19.

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AGE-RELATED ACCUMULATION OF A SPECIFIC MITOCHONDRIAL DNA DELETION IN HUMAN HEART AND BRAIN. *N-W. Soong, D.R. Hinton, G. Cortopassi and N. Arnheim,** Univ. of So. California, Los Angeles, CA 90089-1340.

A specific mitochondrial DNA deletion mutation, discovered in patients with the rare neuromuscular diseases Kearns-Sayre syndrome and progressive external ophthalmoplegia, has been found in normal adults. Using a sensitive polymerase chain reaction (PCR) assay, tissues with high levels of nerve and muscle cells were found to have the highest proportions of the deletion. Studies on comparable fetal or neonatal tissues show little if any evidence of the mutation suggesting that the deletion accumulates with age. Recent quantitative PCR studies have shown that amoung 10-14 anatomical regions studied in the adult brain, the level of the deletion varies hundreds of fold. Oxidative damage of mtDNA is a possible origin of the deletion and the anatomical distribution pattern can be correlated with the potential of different brain regions for oxygen radical formation.

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Colburn, N. Abstract appears on page 19.

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THE pH DEPENDENCE OF BRAIN PEROXIDATION *IN VITRO* AND ITS RELATION TO ISCHEMIA/REPERFUSION INJURY *IN VIVO*. *T. Funahashi,*[1] R.A. Floyd[1] and J.M. Carney,[2]* Molec. Toxicol. Res. Prog., Oklahoma Med. Res. Fndn, Oklahoma City, OK 73104-5046 and [2]Dept. of Pharmacol., Univ. of Kentucky, Lexington, KY 40536-0036.

Older Mongolian gerbils are more sensitive to ischemia/reperfusion injury (IRI) than younger gerbils. *In vitro* lipoperoxidative activity of the homogenate of younger gerbil brain is greater than in older gerbil brain. *In vitro* peroxidation mediated by ADP/Fe/ascorbate is 1.1 fold higher in younger gerbil brain homogenate than from older gerbil brain homogenate. Decreasing the pH from 7.4 to 6.4 caused a two-fold stimulation of lipid peroxidation in each brain region (cortex, hippocampus, cerebellum). Decreasing the pH also caused an increase in peroxidation in the absence of ADP/Fe/ascorbate; younger gerbil brain homogenate peroxidized 40% more than older gerbil brain homogenate.

Use of *in vivo* monitoring showed that an IRI caused the intracellular pH to decrease to as low as 6.3 in the gerbil brain as assessed by ^{31}p-NMR. The pH of older gerbil brain took more time to recover back to the normal physiological conditions than younger after an IRI. In addition, the recovery of high energy intermediates recovered slower in older gerbil brain than in younger gerbil brain after an IRI. Supported in part by NIH grants NS23307 and AG09690.

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Drexler, K.E. Abstract appears on page 20.

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AGE-RELATED CHANGES IN OCULAR ANTIOXIDANT ENZYMES ACTIVITY IN INFLAMMATION. *J.F. Recasens,* Emory Univ. Eye Ctr., Atlanta, GA 30322.

Ocular antioxidant enzyme activity was examined following endotoxin-induced inflammation. Iridic, choroidal and retinal superoxide dismutase (SOD) was measured in adult (6 mo) and aged (4+ yr) rabbits before and after intravitreal endotoxin (lipopolysaccharide). Though no age-related decrease in SOD was noted in control animals, endotoxin was associated with significant SOD induction in irides, choroids and retinas of adult, but not of aged, animals. Iridic catalase, however, exhibited a 22% age-related decrease in control animals and a 45% decrease in aged animals after the inflammatory stimulus, though no change in adults was noted. Malondialdehyde, quantified by thiobarbituric acid reactivity as an index of lipid peroxidation, exhibited a linear relationship in tissues from young (6 wk), adult and aged animals in endotoxin-treated eyes (r=0.999) while not differing in controls. The data indicate that the propensity of ocular tissues from aged animals to experience greater damage may be due to both the age-related decrease in catalase activity and the inability to induce SOD in response to an inflammatory stimulus.

In order to elucidate the mechanism(s) by which endotoxin elicits these effects, isolated corneas were perfused with a physiological solution containing endotoxin and/or the protein kinase C (PKC) inhibitor, Retinal. While endotoxin produced marked corneal swelling, Retinal blocked this effect implicating PKC in endotoxicity. *In vitro* assay of PKC in the presence of endotoxin and/or Retinal revealed Retinal-inhibitable induction of PKC by endotoxin suggesting the involvement of PKC in inflammation protective mechanisms which may undergo age-related deficits.

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HYPOXIA/REOXYGENATION RESPONSE IN IMMATURE, ADULT AND AGED RAT HEARTS. *J.W. Starnes,* D.K. Bowles and K.S. Seiler,* Dept. of Kinesiol., Univ. of Texas, Austin, TX.

We evaluated the abilities of isolated perfused hearts from immature (IM) (2.5-3 mo), adult (11-13 mo) and old (24-26 mo) Fischer 344 rats

to tolerate and recover from oxygen deprivation. Hearts were perfused at 60 mmHg for a 30 min prehypoxic period with oxygenated buffer supplemented with 10 mM glucose (+insulin) and 2 mM acetate, then 30 min with substrate-free, hypoxic buffer gassed with 95% N_2:5% CO_2 and finally reoxygenated for an additional 45 min with the same buffer used during the prehypoxic period. During prehypoxia all groups were similar in ventricular mechanical function, the contents of glycogen, high-energy phosphates (HEP) and reduced glutathione (GSH), as well as mitochondrial state 3 rates. During the first 5 min of hypoxia, IM performed the most cardiac work as indicated by a greater lactate production. After hypoxia, glycogen levels were similar and almost completely depleted in all groups, HEP were lower ($P<0.05$) in adult vs other groups, mitochondrial state 3 rates were decreased (24%, $P<0.05$) only in adult and GSH was depleted by 34% in IM vs only 13% in old ($P<0.05$). After 45 min of reoxygenation IM and old had recovered 48 and 45% of their respective prehypoxic function which was two-fold greater than the 23% recovery by adult. Loss of cytosolic enzymes was estimated by measuring lactate dehydrogenase (LDH) release. Upon reoxygenation the amount released by IM was only about half of that released by adult or old. We conclude that immature and aged hearts tolerate and recover from hypoxia better than hearts from adults, and that the strategy employed to cope with oxygen deprivation and reoxygenation is affected by aging.

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EFFECT OF OXIDATIVE STRESS AND ANTIOXIDANTS ON HEAT SHOCK RESPONSE IN AGING *DROSOPHILA.* *A. Niedzwiecki* and J. Fleming,* Linus Pauling Inst. of Sci. and Med., Palo Alto, CA 94306.

The resistance of organisms to environmental stress is manifested on a cellular level by the synthesis of heat shock proteins (hsp's). We observed that aging *Drosophila* are more sensitive to heat than young flies and suggested that increased damage to cellular proteins in aging flies may play a role in this process. We have investigated whether preexposure of different-aged flies to oxidative stress before a subsequent heat shock affects the synthesis of hsp's. Another area of this study involved an investigation of the protective effect of antioxidants and protein stabilizers on the heat shock response. We observed that an increase in free radical damage by preexposure of young and old flies to H_2O_2, Fe^{+2} or H_2O_2/Fe^{+2} resulted in a dramatic decrease in the synthesis of hsp's, especially hsp 70, in young and old flies. Those kept on ascorbate, mannitol, GSH or glycerol before heat shock did not show substantial differences in hsp expression compared to control flies. The role of protein damage in the efficient response to heat stress in aging *Drosophila* will be discussed.

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EFFECT OF AGING ON CONTRACTILE MECHANISM IN GUINEA PIG GALLBLADDER. *J. Ishizuka,* M. Murakami and J.C. Thompson,* Dept. of Surgery, Univ. of Texas Med. Branch, Galveston, TX 77555.

The contractile sensitivity of gallbladder (GB) muscle to cholecystokinin (CCK) diminishes with aging, partly due to a decreased concentration of CCK receptors on GB muscle cells. However, whether mobilization of intracellular calcium $[Ca^{++}]_i$ (which plays an important role in muscle contraction) in response to CCK is affected by aging is unknown. We have examined the response of $[Ca^{++}]_i$ to CCK using guinea pig GB muscle. GB muscle strips prepared from 2-, 12- and 24-mo-old guinea pigs were loaded with 10 μM of Fura-2-acetoxy-methylester for 3 h at 37°C. $[Ca^{++}]_i$ and the force of contraction of GB muscle in response to CCK-8 (10^{-14} to 10^{-8} M) were simultaneously measured using spectrofluorometer (Spex) and force-displacement transducers. To evaluate the character of Ca^{++}-channel, we studied the binding ability of ^{125}I-diltiazem, a Ca^{++}-channel antagonist, on GB muscle membranes. GB muscle compliance was also compared. CCK-8 evoked a dose-dependent increase in $[Ca^{++}]_i$ and in contractile force. Sensitivities to CCK-8 were significantly decreased with aging, but the curve of relation between % increase of $[Ca^{++}]_i$ and that of force showed no significant differences between young and aged groups. The binding ability of ^{125}I-diltiazem did not differ, but the compliance of GB muscle decreased with aging. We conclude that the system of Ca^{++}-regulated muscle contraction is intact despite aging. Age-related decreased contraction is likely due to both decreased concentrations of CCK receptors and decreased compliance of GB muscle.

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MECHANISMS OF LIPOFUSCINOGENESIS. STUDIES ON A MODEL SYSTEM OF CULTURED RAT NEONATAL MYOCARDIAL CELLS. *U. Brunk* and R. Sohal,* Depts. of Pathol., Univ. of Linköping, Sweden; and Biology, SMU, Dallas, Texas.

Cultures of rat myocardial cells constitute a suitable model system for the study of lipofuscinogenesis. These postmitotic cells accumulate lipofuscin within their secondary lysosomes so that the mechanisms influencing this process may be evaluated within two weeks.

Oxidative stress, by culturing the cells at 40% oxygen, was found to be a dominating cause of enhanced lipofuscinogenesis. Inhibition of GSH synthetase by BSO also led to increased lipofuscin. The addition of iron also considerably enhanced lipofuscinogenesis, as did the inhibition of lysosomal proteolytic and lipolytic enzymes. Enhanced concentration of vitamin E in the medium, the addition of synthetic antioxidants, and 5% oxygen during culture decreased lipofuscinogenesis.

The results substantiate the hypothesis that (a) lipofuscinogenesis is strongly related to oxidative stress and dependent on intralysosomal oxidation due to Fenton reactions, and (b) lipofuscin forms as a result of processes involving incomplete degradation of heterophagocytosed and/or autophagocytosed material followed by peroxidation, fragmentation and polymerization processes.

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RELATION BETWEEN STABILITY AND PROPRIOCEPTION IN OLDER MEN WHEN BAREFOOT AND SHOD. *J. McClaran,* S. Robbins and E. Waked,* Div. of Geriat., Dept. of Med., Montreal General Hosp., Montreal, Quebec, Canada H3G 1A4.

Gerontologists propose that soft-soled shoes impair proprioception and thereby destabilize the elderly. An untested hypothesis contained in this statement is that footwear induce instability in the elderly by their effect on proprioception. To test this, 10 healthy older men (mean age 70.0 years, SE 1.3) were tested for both stability and kinesthetic sense at the ankle when barefoot and when wearing athletic footwear with relatively soft soles. A validated balance beam method was used to measure stability. Ankle kinesthetic sense was examined via a method whereby blindfolded subjects estimated perceived direction and amplitude of ankle angle after they applied full body weight to a series of blocks which varied in angle amplitude and direction of the top surface. Mean balance failures (BF) when wearing the athletic shoe was 13.3 BF/100 m (SE 2.6) and when barefoot was 16.8 BF/100 m (SE 3.4, $p<.0001$). Mean error in ankle angle estimates when wearing athletic shoes was 5.9 degrees (SE 0.14) and when barefoot was 4.4 degrees (SE 0.13; $p<0.03$). Correlation between the stability measure and the kinesthetic measure was -0.668 ($p=0.015$). Poorer proprioception was associated with better stability, which is contrary to the hypothesis. We believe that shoes which can conform to the plantar surface, as used in this experiment, though impairing proprioception slightly, stabilize elderly men though acting as an interface between their typically rigid feet with undulating plantar surface, and the flat support surface. This distributes over a broader area, thereby giving them a more stable mechanical support base.

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HEPARIN AND AGING. *H. Engelberg,** California Arteriosclerosis Res. Fndn., Beverly Hills, CA 90210.

Some actions of heparin may retard aging. It has prophylactic effects in atherogenesis via actions in the blood, at the endothelial surface and in the arterial wall. It is an antioxidant. It enhances the effectiveness of superoxide dismutase. Somatomedin C (IGF-1) mediates growth hormone actions. Heparin releases IGF-1 from its circulation binding proteins, thus increasing the supply of IGF-1 to tissues.

Recently it has been shown in rats that oral heparin is very rapidly totally absorbed, and that it quickly attaches to the endothelial surface where it remains for 6-7 days. In man heparin is similarly very rapidly absorbed. In view of its beneficial effects in atherogenesis, and probably in the aging process, this route of administration of heparin presents new preventive possibilities.

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BIOLOGICAL VIGOR AGE AND ITS FACTOR STRUCTURE. *E. Nakamura,* Dept. of Health & Exercise Sci., Kyoto Univ.

The present study was conducted to estimate biological vigor through the application of principal component analysis, and to investigate the relative importance of each explanatory variable to biological vigor age.

In the present study, biological vigor was defined as an integrated capacity of physiological function, physical working capacity and physical fitness. Biological vigor age (VA) can, therefore, be viewed as an objective measure for a "normal person's biological vitality" at a certain chronological age.

The subjects of this study were 266 healthy Japanese women (aged 19-70), who participated in a community health promotion program. Twenty-two variables used for the assessment of VA characterize the age-related changes of particular organs and systems, one's physical working capacity and primary sub-domains of physical fitness. This variable set was then submitted to principal component analysis, and the 1st principal component obtained from this analysis was used as an equation for assessing one's biological vigor. To assess the adequacy of estimated VA, the relationships between VA and the self-reported physical fitness and health conditions were investigated. The finding showed that there was no relationship between the VA and fitness or health condition. To clarify the relative importance of each explanatory variable to the variance of 1st principal component was calculated, and in addition they were categorized into 5 sub-factors from the viewpoint of physiological functions. As a result, body fatness factor, circulatory-respiratory function factor, biochemical examination factor, blood examination factor and physical fitness factor showed 6.1, 17.0, 33.6, 6.9 and 36.4 percentage contributions, respectively. A clear explanation of the relationship between VA and physiological function or physical fitness would greatly assist the development of methods for measuring aging.

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FOR THE ELDERLY: ADMINISTRATIVE AND TOWN PLANNING. *J.M. Phillips,* Victoria, B.C. V8Y 1E6, Canada.

This study showed a disorganized distribution of care facilities and uncoordinated distribution of seniors' housing, services and institutional beds in twelve cities in the U.S. and Canada; showed some districts over-provided by factors of two to six, thus depriving elderly in other districts. Twelve services could have ten addresses and phone numbers. A new, routine approach is required. Care for seniors can be improved by bringing components of care closer to home. This allows our elderly to age in place and allows a better knowledge of all of the resources of the neighborhood, making best use of volunteers, relatives and neighbors as well as staff. The presentation describes a model which is a composite of all services on one campus; and a system to routinely provide forms of this model, by neighborhood in cities and in every village and town. Each neighborhood would have a mechanism for providing its fair share of housing, supported housing and services for ensuring the quality of life of seniors. The system can be organized and mandated by Area Agencies on Aging. Techniques for use in urban, sub-urban and rural areas are shown. No resource is proposed to be abandoned. Pre-planning, then the use of creative opportunism, is proposed. The system provides for accountability, and for the quality of life of all, locally.

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AGE-DEPENDENT HYPERLIPIDEMIA AS A CANCER-PRONE CONDITION. *V.M. Dilman* and P.G. Toniolo,* New York Univ. Med. Ctr., New York, NY 10010.

Disturbances of carbohydrate metabolism and increased insulin secretion are key elements in the etiology of age-dependent hyperlipidemia. These hormonal and metabolic shifts decrease both cell-mediated immunity and the activity of DNA repair systems and contribute to cell division, especially in tissues that are under the control of sex hormones. Thus, conditions are created that favor cancer development.

Epidemiologic data showing an increased risk of cancer in individuals with extremely low cholesterol levels and a decreased risk in individuals with extremely high blood cholesterol levels may be related to genetic characteristics of subjects in these two subsets of population. When accompanied by hypertriglyceridemia, hypocholesterolemia, rather than a marker of predisposition, should be regarded as a preclinical sign of a malignant tumor. On the contrary, age-dependent hypercholesterolemia and the accompanying hormonal and metabolic shifts should be considered conditions favoring cancer development and a prime target for interventions directed at cancer prevention, particularly considering the age-related increase in cancer incidence.

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INFLUENZA, IMMUNITY, AND AGING: T CELL AND MACROPHAGE DEFECTS. *R.B. Effros* and R.L. Walford,* UCLA Med. Sch., Dept. of Pathol., Los Angeles, CA 90024.

Infections are a major cause of morbidity and mortality in the elderly human population. As an experimental model to analyze the immunologic defects leading to this increased vulnerability, we have utilized the murine influenza system. Following intraperitoneal inoculation with influenza virus, the primary cytotoxic T lymphocyte (CTL) response of old (24 mos) compared to young (4 mos) mice was dramatically reduced. In addition, memory T cells from aged mice previously immunized with influenza were deficient in both proliferative activity and interleukin 2 (IL-2) production in response to a secondary *in vitro* challenge with the virus. Since the addition of exogenous IL-2 to the cultures only partially improved the proliferative defect and subsequent CTL function, we investigated possible involvement of additional components of the immune system. Using limiting dilution cultures to evaluate the precursor frequency of influenza-specific memory T cells, we demonstrated that the age of the mice from which the influenza infected antigen-presenting cells were derived dramatically affected the T cell frequency. Further analysis specifically showed that spleen adherent cells (macrophages) from aged compared to young mice were deficient in their capacity to process and/or present influenza to T cells. In addition, aged macrophages also showed reduced production of IL-1, IL-6 and tumor necrosis factor. Thus, the diminished immune response to influenza seen in aged mice is caused by a combination of T cell and macrophage defects. These findings have important implications for strategies aimed at vaccine protection of the elderly human population.

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DIETARY ANTIOXIDANT MODULATION OF AGING IMMUNE RESPONSE. *S.N. Meydani, M. Hayek, and D. Wu,* USDA-HNRCA at Tufts Univ., 711 Washington St., Boston, MA 02111.

Age-associated decline of the immune response is well documented. However, the biochemical changes leading to these alterations are not well-defined. The elderly are at greater risk for low consumption of several antioxidant nutrients known to have a role in maintaining immune response. In old mice and older adults, a series of experiments using dietary antioxidants were conducted to determine the contribution of eicosanoids and other products of lipid peroxidation to the age-associated changes of the immune response. Splenocytes from old mice had significantly higher production of prostaglandin E_2 (PGE_2), leukotreine (LT) B_4 and LTC_4 than young mice. No difference was observed in production of 12-hydroxyeicosatetraenoic acid (HETE), 15-HETE or H_2O_2 between the two age groups. **In vitro** inhibition of PGE_2, but not LTB_4 and LTC_4, resulted in increased mitogenic response. Similarly, peripheral blood mononuclear cells (PBMC) from older subjects synthesized more PGE_2 than young subjects and their plasma exhibited higher thiobarbituric acid (TBAR) levels compared to their young counterparts. Vitamin E supplementation of both old mice and older adults decreased PGE_2 synthesis and plasma TBAR while increasing interleukin (IL)-2 production, mitogenic response to Con A and the delayed-type hypersensitivity skin response (DTH). Old mice supplemented with another dietary antioxidant, glutathione (GSH) (0.1 to 1% by wt of diet for 4 wk) evidenced significantly improved mitogenic responses to Con A and PHA and DTH to DNFB. This effect was due to an increase in spleen GSH level. PBMC from older subjects had significantly lower GSH levels than young subjects. *In vitro* GSH supplementation significantly increased PBMC's GSH level, mitogenic response to Con A and PHA, and IL-2 production while decreasing PGE_2 production in both young and old subjects. However,

a significantly higher percent increase was observed in older subjects than young subjects. In conclusion, increased PGE_2 production contributes to the age-associated decrease in IL-2 production and mitogenic response. Supplementation with dietary antioxidants decreases PGE_2 production and increases immune response in the aged.

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HETEROGENEITY OF IMMUNE RESPONSE WITH INCREASING AGE. *D.M. Murasko,** Dept. of Microbiol. and Immunol., Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

It has been repeatedly demonstrated in humans, mice and rats that the ability of lymphocytes to proliferate in response to mitogenic or antigenic stimuli decreases with increasing age. Similar to the heterogeneity observed in general health status, level of lymphoproliferative response varies considerably among individual elderly subjects. In addition, the ability of exogenous lymphokines to enhance the decreased proliferation of the elderly is also heterogeneous; addition of IL-2 and/or IFN-g significantly increases proliferation of only one third of the elderly who demonstrated decreased proliferation. Since the heterogeneity observed in humans has been attributed to differences in genetic background, diet or environment, we investigated these factors using mice and rats maintained under rigorous conditions of breeding, nutrition and husbandry. Although the genetic background of the animals influenced the overall level of immune response of both young and aged mice and rats, heterogeneity was also observed among individual animals of the same genetic background maintained under identical environmental conditions. Some, but not all, of the variability was due to the method assessment. However, since cohorts of the same animals demonstrated mortality over a 12-month period, the residual variability may reflect the physiologic age of each animal.

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MULTIPLE PATHOGENIC MECHANISMS IN PRION DISEASES. *S.J. DeArmond,* Dept. of Pathol., Univ. of California, San Francisco, CA 94143.

There are three types of human prion disease: sporadic, exemplified by 85% of Creutzfeldt-Jakob disease (CJD) cases; familial, exemplified by Gerstmann-Sträussler syndrome (GSS) and a few CJD cases; and infectious, exemplified by iatrogenic CJD. All of these as well as the analagous animal disease, scrapie, regardless of their etiology, can be transmitted to other animals. The prion hypothesis proposes that the transmissible agents, termed prions, are composed of an abnormal form of a normal neuronal protein termed the prion protein (PrP); normal PrP is designated PrP^{C} and the abnormal infectious forms, PrP^{CJD}, PrP^{GSS} and PrP^{SC} respectively. A putative dimer composed of abnormal PrP in the form of a prion plus a PrP^{C} molecule is proposed as an essential step in the conversion of PrP^{C} to another abnormal, infectious form. Once started, the process is self-perpetuating and leads to the accumulation of abnormal PrP which causes the clinically relevant neuropathology. In familial cases, mutations in the PrP gene lead to synthesis of abnormal PrP. In infectious cases, exogenous prions initiate the conversion of PrP^{C} to more abnormal PrP. Eighty-five percent of human CJD cases have neither a familial nor infectious cause. They are age-related with a peak incidence at about 60 and may be the result of a somatic mutation in the PrP gene, perhaps in a single neuron.

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NEUROIMAGING OF NORMAL AND ABNORMAL FUNCTION IN THE AGING BRAIN. *A.F. Leuchter,** UCLA Neuropsych. Inst. and Hosp., UCLA Sch. of Med., Los Angeles, CA 90024-1759.

Aging is associated with many changes in brain structure and function. The most common structural changes are atrophy and white-matter lesions seen on magnetic resonance imaging (MRI). Functional imaging using positron emission tomography (PET), single-photon emission computed tomography (SPECT) and quantitative electroencephalography (QEEG) shows that there are changes in both global and regional metabolism and perfusion, and in functional connections between brain regions. Research suggests that some changes common in otherwise healthy elderly adults are associated with alterations in brain physiology; for example, even subtle white-matter disease seen on MRI may cause cortical deafferentation. This loss of afferent input is associated with decreased perfusion and metabolism on PET or SPECT, and diminished coherence or cordance seen on QEEG. These neuroimaging techniques will be increasingly helpful in understanding the distinction between healthy and suboptimal brain function in the elderly.

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CYTOSKELETAL PROTEINS AND ALZHEIMER DISEASE. *S.S. Matsuyama,* West Los Angeles VA Med. Ctr., Brentwood Div., Los Angeles, CA 90073 and Dept. of Psychiat. and Biobehav. Sci., UCLA, Los Angeles, CA 90024.

The etiology and pathogenesis of Alzheimer disease (AD) remains elusive. AD is most likely a heterogeneous disease reflecting a final common pathway of multiple etiologies and will require varied approaches to diagnosis, treatment and prevention. In addition to the intensely investigated amyloid of neuritic plaques, evidence from neuropathological studies also suggests abnormalities of the neuronal cytoskeleton. Immunohistochemical studies of neuropathological lesions observed in AD brains indicate the involvement of microtubules, microfilaments and intermediate filaments, the three major fibrous proteins of cells. We previously hypothesized that an impaired microtubule (MT) system may represent a basic underlying defect in AD. MTs are ubiquitous proteins with the highest concentration in the brain, the most prominently affected organ in AD, and MTs are involved in numerous vital cellular processes. Thus, an abnormality in the MT system can have far reaching consequences. Research in our laboratory focuses on MTs in skin fibroblasts and our investigations indicate a defect in the MT system in a group of AD patients. The presence of MTs in peripheral tissues together with the reports that cytoskeletal abnormalities are also expressed in these cells suggest that continued investigations may provide valuable insight into the biological basis of AD and lead to the development of a relatively noninvasive diagnostic test.

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NGF ACTIONS IN AN ANIMAL MODEL OF CHOLINERGIC NEURODEGENERATION. *D.M. Holtzman, Y.-W. Li, C. Epstein, F. Gage, and W.C. Mobley,** Depts. of Neurol. and Pediat., Univ. of Calif., San Francisco, CA 94143-0114.

Programmed cell death has an important role in creating the nervous system. Neurotrophic factors enhance neuronal survival by regulating cell death. How this occurs is uncertain, but a growing body of data indicates that neurotrophic factors produced in the target of developing neurons bind to specific receptors and suppress the activation of gene-directed programs that kill neurons. The genes responsible for neuronal cell death are yet to be defined. Though as yet only speculation, it is possible that one or more of these same cell death programs is activated in neurological disorders leading to death of selected neuronal populations. If so, neurotrophic factors may be used to prevent or retard the death of neurons in such patients. To test this hypothesis it is important to: 1) discover cell death genes, 2) create animal models of spontaneous, selective neuronal death to ask whether putative cell death genes are activated; and 3) examine the actions of neurotrophic factors on their activation and on neuronal viability. We report studies on TS16 basal forebrain cholinergic neurons in which cells transplanted to the normal hippocampus show selective age-related atrophy which is responsive to NGF treatment. We will discuss our experience with this model, its potential for defining neuronal death programs and for exploring NGF actions in degenerating neurons.

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BIOLOGY OF HUMAN AGING AND LONGEVITY: POSSIBLE ROLE OF OXIDATIVE STRESS. *R.G. Cutler,* Molec. Physiol. & Genet. Sec., Lab. of Cell. & Molec. Biol., NIA/NIH, Baltimore, MD 21224.

Although the process of human aging and age-dependent diseases is highly complex, comparative and evolutionary studies have lead to the hypothesis that considerably less complex processes may be operating in governing their aging rate. These mechanisms have been called longevity determinants and it has been predicted that most mammalian species share a common set — with aging rate in different species being governed largely as a result of differences in regulation of these genes. Another prediction of this longevity determinant gene hypothesis (LDGH) is that aging is a result of by-products of normal essential processes of development and metabolism. We have been testing the LDGH by investigating if possible oxidative stress is such an aging process and mechanisms acting to reduce oxidative stress represent a class of LDGs. Our results show a remarkably good positive correlation of antioxidant defense levels and an inverse correlation of DNA damage with life span of mammalian species.

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VITAMIN C, CANCER AND AGING. *G. Block,* Univ. of California–Berkeley, Berkeley, CA 94720.

Oxidative damage to DNA, proteins and lipids appears to be a major contributing factor in aging and the degenerative processes that accompany it, including cancer, heart disease, cataracts, and cognitive dysfunction. Numerous epidemiologic studies have found that persons with lower intake of antioxidant nutrients or the fruits and vegetables that provide them have a higher risk of almost every type of cancer. In many studies those with low intake had twice the risk of those with high intake. A large-scale follow-up study found that persons with a low vitamin C intake had a statisticaly significantly higher risk of heart disease mortality and total mortality over the subsequent 10 years. In addition, several studies have found that low intake of antioxidant nutrients such as vitamin C and carotene had significantly increased risk of developing age-related eye diseases such as cataracts. Although many older people consume more antioxidants than younger people, very substantial proportions of older persons have very low intakes and blood levels. Among white men 65-74 in the U.S., 15% have blood ascorbate levels at or below 0.4 mg/dl, the lower boundary of "normal." Among black men of that age, 25% have levels at or below 0.3 mg/dl.

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GENES OF YOUTH: GENETICS OF AGING IN YEAST. *S.M. Jazwinski,* N.P. D'mello, J. Sun, A.M. Childress, D.S. Franklin, N.E. Jeansonne and S.P. Kale,* Dept. of Biochem. & Molec. Biol., LSU Med. Ctr., New Orleans, LA 70112.

Yeasts possess a finite replicative life span, which is measured by the number of divisions an individual cell has undergone. We have instituted a molecular genetic approach to the study of yeast aging and longevity together with a mutational approach to identify those genes that may limit life expectancy as well as those that may postpone senescence. Several genes that are differentially expressed during the yeast life span have been cloned. Five of these have been at least partially sequenced, and this has revealed that they are novel genes. One of these, called *LAG*1, has been analyzed in detail. It appears to code for a membrane protein, and *LAG*1 mRNA levels decrease substantially with age. Disruption of this gene reduces the number of divisions available to the yeast cell. These studies suggest that *LAG*1 is a longevity-assurance gene in yeast. Overexpression studies, although not straightforward, are consistent with this conclusion. Sequences similar to yeast *LAG*1 have been found in the human genome. Another candidate for a longevity-assurance gene in yeast is *RAS2*. This gene is preferentially expressed in young yeast cells. Overexpression of *RAS2* or of its activated version, v-Ha-*ras*, increases the life expectancy of yeasts. Overexpression of *RAS2* also postpones the aging process, as evidenced by the delay in the increase in generation time during the life span of yeast cells overexpressing this gene. A genetic analysis of the cAMP pathway, one of the pathways through which the *RAS* functions in yeast, indicates that it is not likely to be involved in the effects of *RAS* on longevity. It is probable that *RAS* affects longevity through the alternate pathway related to inositol phospholipid metabolism, which is emerging as the pathway through which *c-ras* functions in mammalian cells.

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EVOLUTIONARY DETERMINANTS OF LONGEVITY: THE LOGIC OF ANIMAL MODEL SELECTION. *S.N. Austad,* Dept. of Organismic and Evolutionary Biol., Harvard Univ., Cambridge, MA 02138.

Animal models for research into the determinants of longevity are typically chosen by convenience or convention, without respect to their properties relative to longevity or mechanisms of aging. An evolutionary perspective on longevity suggests a specific logic to the choice of animal models, depending upon the sort of research question to be asked. If one is seeking to inquire into the generality of putative mechanisms of aging, models should be distantly related to one another. If one is seeking to identify and isolate specific determinants of longevity, one should use models as closely related as possible, but differing substantially in longevity. Ideally, one would use variation within a species in this instance. The caloric restriction paradigm has been useful here, but nature has also provided many examples of intraspecific variation in longevity which have yet to be exploited by researchers. Finally, if one is seeking to learn more about specific mechanisms thought to be important in extreme longevity, such as defenses against free radical damage, one should utilize species with demonstrably good free radical defenses. I will give specific examples for each of these cases.

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DEVELOPMENT OF A TRANSGENIC MOUSE MODEL WITH A PHENOTYPE OF EARLY ALZHEIMER'S DISEASE. *B. Cordell,* L. Higgins, R. Catalano, and D. Quon,* Scios Inc., Mountain View, CA 94043.

A small animal model representing the neuropathological alterations of Alzheimer's disease is desirable. Transgenic technology has been used to generate mice programmed for neuronal expression of either the 695 or 751 amino acid isoform of the human beta-amyloid precursor protein (APP). In a large survey of animals from both groups, only animals of the APP751 pedigree show features of early Alzheimer's disease including preamyloid-like deposits and cortical neurons displaying Alz50 immunoreactive soma. The quality and distribution of both structures revealed in the APP751 mouse brains closely resemble those seen in brains of young adults with Down's syndrome. These results suggest that increased levels of APP751 and/or preamyloid formation promote pathogenic alterations in the neuronal cytoskeletal protein tau.

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***PEROMYSCUS:* A GERONTOLOGIC ANIMAL MODEL.** *G.S. Smith,** Dept. of Pathol., UCLA Sch. of Med., Los Angeles, CA 90024.

Peromyscus leucopus has a lifespan of about twice that of the common laboratory mouse. The development of inbred lines of *Peromyscus* would result in a comparative gerontologic model between the two genus (*Peromyscus* vs genus *Mus*). We are inbreeding 10 lines of *P. leucopus* with two presently at F_{16} and two at F_{15}. Efforts toward defining the histocompatibility complex of *Peromyscus* by molecular biological methods will be presented.

The limited studies of aging changes in *Peromyscus* will be reviewed. These studies suggest that *Peromyscus* will be an important gerontological animal model for comparison to the laboratory mouse.

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THE EFFECT OF AGE ON GFAP RNA INDUCTION BY FIMBRIA/FORNIX TRANSECTIONS IN THE MOUSE BRAIN. *J.R. Goss* and D.G. Morgan,* Div. of Neurogerontol., Andrus Gerontol. Sch., Univ. of Southern Calif., Los Angeles, CA 90089-0191.

To better understand how age affects astrocytic responses to terminal degeneration within the hippocampus, unilateral fimbria/fornix transections were performed on 3-, 14-, and 23-month-old male mice. Glial fibrillary acidic protein (GFAP) RNA was examined using *in situ* hybridization analysis in non-lesioned control animals and 2, 5, and 10 days post-lesioned animals. GFAP RNA increased in both ipsilateral and contralateral hippocampi in response to the lesion in all age groups but with different latencies and magnitudes. GFAP RNA in the 3-month-old group increased by 2 dpl but returned to non-lesioned control values by 5 dpl. The increase in GFAP RNA in the 23-month-old group was delayed until 5 dpl but the magnitude of this increase was greater than that found in the 3-month-old group. The response in the 14-month-old group was intermediate between the 3- and 23-month-old groups.

This study shows that older animals have an increased astrocytic response to neuronal injury. This greater response may explain the differences in neuronal survival, regeneration, sprouting, and functional recovery often seen between young and aged individuals.

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REVIEW OF COGNITIVE ENHANCING SUBSTANCES. *W. Dean,* The Ctr. for Bio-Gerontol., P.O. Box 11097, Pensacola, FL 32524.

One of the most common manifestations of aging is a decline in cognitive function, ranging from "normal decline" to "Age-Associated Memory Impairment (AAMI)" and full-blown dementing illness like Alzheimer's disease. Mechanisms of memory and normal cognitive function are briefly reviewed, as well as the most likely causes of age-related cognitive impairment. Many cognitive enhancing agents

are now approved by pharmaceutical regulatory agencies around the world, and over 100 additional agents in this class are in various stages of development and evaluation. Studies evaluating the efficacy of many of these agents are reviewed, and the growing phenomena of the use of the agents by "normal" individuals for cognitive enhancement purposes and for prevention of age-related cognitive impairment are discussed. Because of legal and ethical questions regarding the "unapproved" use of these agents, U.S. regulatory agencies are employing various tactics and strategies to inhibit such use. Some of these tactics, which appear to be legally and ethically questionable, are examined. The prognosis for wider use of these agents to alleviate dementias and improve cognitive performance in normal subjects appears to be favorable.

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ASSESSING THE ANTIOXIDANT STATUS OF INDIVIDUALS. *C.A. Thomas, Jr.,* J.T. Huizenga, B. Frei, P.A. Cerutti, H. Sies, L. Packer, and K.F. Gey,* Pantox Laboratories, San Diego, CA 92109.

In view of the strong correlation of heart disease, cancer and other degenerative diseases with serum levels of antioxidant substances, it is of some interest for individuals to know their antioxidant status; since in most cases it is relatively easy to improve one's status. As a first approach to assessing the adequacy of an individual's extracellular antioxidant defense system, we have established a coordinated series of analyses of small molecules that include the lipid soluble antioxidants (retinol, two tocopherols, three carotenoids, coenzyme Q10), the water-soluble antioxidants (ascorbate, urate, bilirubin) as well as the iron status in terms of iron-binding capacity and serum ferritin. These results are combined with the more standard LDL, HDL, ApoB, ApoA analyses and certain relevant ratios are formed. The results are displayed in the form of a bar graph of percentiles which are determined by comparing the measured individual value against the cumulative frequency distribution of the general population as measured by us or others. All of these measurements must be taken as a whole because the small molecule antioxidant system is interacting and mutually regenerating. The results at this stage must be considered anecdotal and very preliminary. Among the approximately 800 samples that have been measured on a professional basis, we have identified a few individuals who have very low or no detectable carotenoids in their serum. Asymptomatic young men and women who are members of families with high risk for coronary disease have poor antioxidant profiles.

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DOES AGING AFFECT THE MICROSOMAL DRUG METABOLIZING SYSTEM IN FEMALE RATS? *M. Alterman, M. Carvan and D. Busbee,** Dept. of Anatomy and Public Health, Texas A&M Univ., College Station, TX 77843.

Investigations of the influence of aging on drug metabolism in male rats resulted in the conclusion that aging impairs metabolic function, though some reported data have been controversial with some evidence suggesting that age is not a major determinant in this process in man. The only well accepted data from these studies show that male-specific P-450 (CYP2C11) declines in expression with increased age, whereas the expression of female-specific P-450 (CYP2C12) does not change with age in female rats. A recent study utilizing monoclonal antibody (MAb) directed inhibition showed that CYP2C11 is completely responsible for at least 2 catalytic activities which are not specific for this particular isozyme, ethoxy- and pentoxyresorufin O-dealkylation (EROD and PROD, respectively, in uninfected animals). We have investigated the effects of aging and caloric restriction on drug metabolizing enzyme system in female Fischer 344 rats. Four cytochrome P-450 associated catalytic activities were analyzed: EROD, PROD, benzphetamine N-demethylation and aniline p-hydroxylation. Despite variations in the enzymes investigated, the changes in activity could not be characterized as having a marked age-related decline. MAb-directed phenotyping of EROD and PROD activities in microsomes demonstrates that, unlike CYP2C11, CYP2C12 does not display a clearly expressed ability to metabolize either ethoxy- or pentoxyresorufin in microsomes from *ad libitum*-fed (AL) or calorie-restricted (CR) rats of any age. The data suggest that the age-related decline in catalytic activities in aged male rats could be explained by a radical age-related decline in expression of CYP2C11. The levels of immunoinhibition of PROD activity differed widely between AL and CR female rats, especially between 26-month-old animals. Values from all MABs used varied about 2-fold. This indicates that long-term calorie restriction modulates the isozyme patterns of P-450 in female rats. In summary, our data, in conjunction with previously published data, suggest female rats may be a more suitable model for investigation of aging phenomena in humans.

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UBIQUINONE AND AGING. *L. Ernster,* Dept. of Biochem., Arrhenius Labs. for Nat. Sci., Stockholm Univ., 5-106 91 Stockholm, Sweden.

This presentation summarizes data which support the concept that a decreased capacity of the organism to maintain adequate ubiquinone levels may be an important contributory factor in degenerative diseases and aging. It is now well established that ubiquinone (coenzyme Q), in addition to its function as an electron and proton carrier in mitochondrial electron transport linked to ATP synthesis, acts in its reduced form (ubiquinol) as an antioxidant preventing the initiation and/or propagation of lipid peroxidation in biological membranes and in serum low-density lipoprotein (LDL). Recent evidence indicates that the antioxidant function of ubiquinol is independent of that of vitamin E but can also amplify the latter by regenerating the vitamin from its oxidized form which otherwise must rely on a water-soluble reducing agent such as vitamin C. In fact, ubiquinol is the only known lipid-soluble antioxidant that animal cells can synthesize *de novo* and for which there exist enzymic mechanisms that can regenerate it from its oxidized form, ubisemiquinone, which results from its antioxidant function. These features, together with its widespread occurrence in biological membranes and in LDL, mostly in the reduced from, suggest an important role of ubiquinone in cellular defense against oxidative damage leading to degenerative diseases and aging. Data summarized in this presentation show a substantial decrease in ubiquinone content in various rat and human tissues upon advancing age, reflecting a diminished rate of biosynthesis and/or an increased rate of breakdown, and resulting in a reduced capacity of the organism for antioxidant defense.

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THE UNIVERSALITY OF BIOENERGETIC DISEASE IN THE AGING PROCESS. *A.W. Linnane,* P. Nagley, A. Baumer, A. Boubolas, R.D. Martinus, R.J. Maxwell, F. Vaillant, and C. Zhang,* Ctr. for Molec. Biol. and Med., and Dept. of Biochem., Monash Univ., Clayton, Victoria 3168, Australia.

Aging is a complex biological process resulting in the loss of cell and tissue function; associated with the process is a decrease in bioenergy function. We have formulated a comprehensive hypothesis that the accumulation of somatic gene mutations in mitochondrial DNA throughout life is an important contributor to the aging process through the progressive loss of mitochondrial bioenergy capacity (Lancet 1989, 642). In addition to overt bioenergetic disease as a consequence of mtDNA mutation, it is suggested that a degree of bioenergetic decline which exists in all aged individuals will be interactive with, and contribute to, the pathology of some of the common diseases of aging. The evidence for the age-associated changes in (a) mtDNA (the occurrence of multiple sets of deleted mtDNA in individual cells) (b) tissue bioenergy capacity (formation of a tissue energy mosaic whereby individual cells of tissues and organs show a wide range of bioenergy capacities). Cells unable to meet their particular bioenergy demand will become non-functional leading to cell death (c) redox therapy for treatment of age-associated bioenergetic disease and the importance of the $NAD^+/NADH$ ratio in tissues will be discussed.

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GENES INVOLVED IN PHORBOL ESTER OR TNFα-INDUCED CELL DEATH. *N.H. Colburn,* Y. Sun, and N. Singh,* Natl. Cancer Inst., Frederick, MD 21702.

We have recently described variants of tumorigenic JB6-derived mouse epidermal cells that are differentially sensitive to putative apoptosis induced by protein kinase C activators (Ca. Res. 52, 1907, 1992). Our aim was to understand the genes whose expression is causally related to induced death. The mechanism of DNA damage induction does not involve differential expression of *c-jun, c-fos,* TRPM-2 or the mitochondrial bcl-2, genes which have been implicated as apoptosis mediators in other models. Also excluded are differential

induction of MnSOD or other antioxidant enzymes postulated to function as protectors against cell death. The JB6 variants are differentially sensitive to killing not only by C kinase activators, but also by TNFα. The phorbol ester *resistant* variants are, however, TNFα *sensitive*, suggesting differential biochemical pathways. The following possible mediators of killing cannot explain the TNFα sensitivity difference since they are not induced: bcl-2, TRPM-2, p53, and *fas*. Putative protector MnSOD was similarly induced by TNFα in sensitive and resistant JB6 tumor cells. The single difference found to date is a much greater induction of *c-jun* expression in the TNFα resistant than in the sensitive cells. This raises the possibility that transcriptional activation by AP-1 protects against TNFα induced killing.

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NANOTECHNOLOGY AND AGING. *K.E. Drexler*, Inst. for Molec. Mfg., Palo Alto, CA 94301.

Studies of nanotechnology based on standard models of molecular behavior indicate some of the capabilities that will result from the development of mechanosynthesis (that is, from the ability to build complex nanometer-scale structures by positioning and joining reactive *molecular building blocks*). *These studies use empirical potential* energy functions, quantum chemistry, statistical mechanics, and classical dynamics to analyze proposed nanomechanical devices, supplemented by reasoning based on the known capabilities of conventional engineering systems and of biological molecular machines. This body of analysis appears in *Nanosystems: Molecular Machinery, Manufacturing, and Computation* (Wiley-Interscience 1992). The chief conclusions of interest in the present context are that:

(1) Molecular machine systems can be built from components of protein-like complexity but greater stability.
(2) Molecular machine systems made of such components can be designed to operate in a nearly deterministic fashion in the presence of thermal fluctuations at physiological temperatures.
(3) The equivalent of a powerful modern computer, if built of such components, can be packaged in a volume that is small compared to that of a typical human cell.
(4) Computer-controlled molecular machine systems can be used to construct additional molecular machine systems.
(5) Implementation of such systems will be a long-term engineering effort requiring several stages of development, but intermediate steps beginning with present chemical capabilities have been identified.

The medical applications of advanced molecular machine systems will be broad. The scientific applications will include direct methods for probing and characterizing molecular structures and for characterizing cell and tissue structures in molecular detail. The clinical applications will include a wide range of therapies that exploit the feasibility of applying surgical control to molecular and subcellular structures. *The complexity of the systems necessary for the latter* applications will delay their development, but no barriers are apparent that would prevent the eventual emergence of molecular-scale repair of diffuse cellular damage, including that caused by aging.