TWENTY-FIRST ANNUAL MEETING — American Aging Association
SIXTH ANNUAL MEETING — American College of Clinical Gerontology
Wednesday through Saturday
October 9-12, 1991
Hyatt Regency Hotel
1750 Welton Street
Denver, Colorado 80202-3999

WEDNESDAY, OCTOBER 9, 1991

CLINICAL GERONTOLOGY
A: "Mechanisms of Cardiovascular Aging"

1. Anversa*, P., and Capasso, J. M.: **Mechanisms of altered cardiovascular function with aging.**
2. **Lakatta, E. G.: Biophysical, biochemical and molecular changes in the senescent rat heart.**
3. Wei, J. Y.: **Chronic exercise effects on calcium and contraction in cardiac muscle.**
4. Tate, C. A.: **Chronic exercise effects on sarcoplasmic reticulum CA^{2+} pump.**
5. Scarpace*, P. J., and Borst, S. E.: **$Alpha_1$- and beta-adrenergic signal transduction in the aged myocardium.**
6. Duckles, S. P.: **Maintenance with age of vascular adrenergic reactivity: reallocation of receptor function.**

B: "Antihypertensive Treatment in the Elderly"

7. Moser, M.: **Clinical trials.**
8. Cushman, W. C.: **VA experience.**
9. Tuckman, J.: **Antihypertensive medications.**

THURSDAY, OCTOBER 10, 1991

MINISYMPOSIUM:
"Stress and Aging"

10. Morimoto, R. I.: **The heat shock response as a molecular sensor of cell damage.**
11. Liu, A. Y.-C.: **Transcriptional regulation of heat shock genes and cell aging.**
12. Niedzwiecki*, A., Kongpachith, A., and Fleming, J. E.: **Role of abnormal proteins in the heat shock response in aged *Drosophila*.**
13. Holbrook, N. J.*, Udelsman, R., and Blake, M. J.: **Age-related alterations in the response to physiologic stress: decline in expression of heat shock protein 70 (HSP70).**
14. Heydari, A. R., Wu, B., Conrad, C. C., and Richardson*, A.: **The ability of dietary restriction to change the age-related alteration in the expression of heat shock genes.**
15. Hallgren, H. M.*, and O'Leary, J. J.: **Heat shock protein expression in lymphocytes from aging humans.**

"Increasing the Functional Life Span: What Can We Do Now?"

16. Blumberg, G.: **Antioxidant vitamins.**
17. Dean, W.: **Review of cognitive-enhancing substances.**
18. Ingram, D.: **Caloric restriction.**
19. Fielding, R.: **Exercise and aging.**

SUBMITTED PAPERS
Oral Presentations

20. Feuers*, R. J., Weindruch, R., Leakey, J. E. A., Duffy, P.H., and Hart, R. W.: **A comprehensive program for the evaluation of the effect of caloric restriction (CR) on free radical detoxification and its effect on aging.**
21. Leakey*, J. E. A., Harmon, J. R., Bazare, Jr., J. J., Chen, S., Manjgaladze, M., Feuers, R. J., Duffy, P. H., and Hart, R. W.: **Role of corticosteroids and "caloric stress" in modulating the effects of caloric restriction (CR) in rodents.**
22. Busbee*, D., Srivastava, V., Schroeder, M., and Miller, S.: **An isoform of DNA polymerase from SV-40 transformed human fibroblasts.**
23. Busbee*, D., Tilley, R., and Miller, S.: **The response of senescent human fibroblasts to DNA damage.**
24. Katz*, M., and Norberg, M.: **Retinoid deficiency prevents leupeptin-induced autofluorescent pigment accumulation in the retinal pigment epithelium.**
25. Shapiro, S., and Gershon*, H.: **Immune complexes and complement and the turnover of red blood cells from young and old human donors.**

FRIDAY, OCTOBER 11, 1991

MINISYMPOSIUM:
"Genetics and Aging"

26. Ebert, R. H., Egilmez, N. K., Chen, J. B., and Shmookler-Reis*, R. J.: **A strategy for the identification of lifespan-determining genes in *C. elegans*.**
27. Johnson, T., Fabian*, T., Tedesco, and Hutchinson, T.: **Molecular genetic approaches to the analysis of aging in *Caenorhabditis Elegans*.**
28. Rose*, M. R., Ayala, F. J., Spicer, G. S., Tyler, R. H., and Fleming, J. E.: **Genetics of postponed aging in *Drosophila Melanogaster*.**
29. McClung*, J. K., Walker, L., Friedman, V., Nuell, M. J., Danner, D. B., and Dell'Orco, R.: **Expression of an antiproliferative protein, prohibitin.**
30. McCleam, G. E.: **The Swedish adoption/twin study on aging: a review.**
31. St. George Hyslop, P.: **Mapping of Familial Alzheimer's Disease: role of the B-amyloid gene.**

Luncheon (Friday)

Annual Award:

Walter R. Nicolai Prize —
Marilyn N. Friedmann

"Marilyn N. Friedemann is the 1991 recipient of the Walter R. Nicolai Prize in Biomedical Gerontology. This prize is presented to Ms. Friedemann for her manuscript, "*In Vivo* Electrochemical Studies of Dopamine Diffusion and Clearance in the Striatum of Young and Aged Fischer 344 Rats."

SUBMITTED PAPERS

Oral Presentations

32. *Walter Nicolai Award Lecture:* Friedemann*, M. N., and Gerhardt, G. A.: **The effects of aging on dopamine diffusion and clearance in the striatum of the Fischer 344 rat.**

33. Carrillo*, M.-C., Kitani, K., Kanai, S., Sato, Y., and Ivy, G. O.: **Tissue and region selectivity of the effect of (-)deprenyl to increase superoxide dismutase (SOD) activities in the rat.**

34. Kitani*, K., Carrillo, M.-C., Kanai, S., Sato, Y., and Ivy, G. O.: **The optimal dosage of (-)deprenyl to increase superoxide dismutase (SOD) activity in the striatum is sex and age dependent in F-344 rats.**

35. Poth, K., Heron*, C., Miniclier, N., and Bickford-Wimer, P.: **Effects of long term deprenyl administration on motor function in F344 rats.**

36. Rose*, G. M., Engstrom, D. A., Humphreys, A. G., and Bickford-Wimer, P. C.: **Differential effects of aging upon hippocampal pyramidal cell responsiveness to muscarine and nicotine.**

37. Cardarelli, N.: **Senescence as a programmed phenomenon.**

38. Massie*, H., Ofosu-Appiah, W., and Aiello, V.: **High serum copper results in impaired immune response.**

39. Prabhakaram, M., and Singh*, S. N.: **Differential inhibition of the rat liver glutamate dehydrogenase of various ages by metal ions.**

40. Kaats*, G. R., Fisher, J. A., and Blum, K.: The effects of chromium picolinate supplementation on body composition in different age groups.

41. Leblhuber*, F., Windhager, E., Weber, J., Steinparz, F. X., Reisecker, F., and Dienstl, E.: **Antiglucokorticoid effects of dehydroepiandrosteronesulfate (DHEAS): serum levels of cortisol and DHEAS in normal controls.**

42. Chodzko-Zajko*, W. J., Caruso, A. J., and Sothmann, M. S.: **The effect of age on physiological and behavioral responses to cognitive stress.**

43. Rosario*, P. G., Greenstein, S., Schechner, R. S., and Tellis, V.: **Too old for a transplant?**

44. McClaran*, J., and Robbins, S.: **Stability in elderly men in relation to footwear midsole hardness and thickness.**

FRIDAY EVENING, OCTOBER 11, 1991

Submitted Papers

Poster Session

45. **Khokhlov, A. N.: Testing of geroprotectors and geropromoters with the cell kinetics model.**

46. Reddy, K. K.: **Lipid peroxidation and antioxidant defenses among the aged people.**

47. Brooks*, A., Wold, R., and Johnson, T.: **The interaction between hermaphrodite fertility and life span.**

48. Wallace*, D. R., and Dawson, R.: **Regional age-related changes in ammonia regulation of phosphate-activated glutaminase (PAG).**

49. Wallace*, D. R., and Dawson, R.: **Age-related alterations in both phosphate and calcium regulation of regional phosphate-activated glutaminase (PAG).**

50. Duhon*, S., and Johnson, T.: **Movement and pharyngeal pumping as biomarkers of longevity in *Caenorhabditis Elegans.***

51. Duffy*, P. H., Feuers, R. J., Leakey, J. E. A., and Hart, R. W.: **Effect of chronic caloric restriction in rodents: physiological adaptations associated with a rapid change in the level of food consumption.**

52. Chen*, S., Leakey, J. E. A., Bazare, Jr., J. J., Harmon, J. R., Manjgaladze, M., Feuers, R. J., Duffy, P. H., and Hart, R. W.: **Acute and long-term effects of caloric restriction (CR) on serum hormone profiles and responsive enzymes in the Fischer 344 rat.**

53. Bazare, Jr.*, J. J., Leakey, J. E. A., Harmon, J. R., Chen, S., Manjgaladze, M., Feuers, R. J., Duffy, P. H., and Hart, R. W.: **Acute and long-term effects of caloric restriction (CR) on blood glucose, serum insulin, and hepatic glycogen profiles in the Fischer 344 rat.**

54. Manjgaladze*, M., Leakey, J. E. A., Harman, J. R., Bazare, Jr., J. J., Chen, S., Feuers, R. J., Duffy, P. H., and Hart, R. W.: **Acute and long-term effects of caloric restriction on hepatic conjugating enzyme expression in the Fischer 344 rat.**

55. Undie*, A. S., and Friedman, E.: **Food restriction prevents aging-induced decrements in brain phosphoinositide metabolism.**

56. Harmon*, J. R., Leakey, J. E. A., Bazare, Jr., J. J., Chen, S., Manjgaladze, M., Feuers, R. J., Duffy, P. H., and Hart, R. W.: **Effect of caloric restriction on cytochrome P450 (CYT P450) isoform expression in Fischer 344 rats.**

57. Chen*, F., and Feuers, R. J.: **The effect of caloric restriction on reduction potential for maintenance of free radical detoxification.**

58. Oriaku*, E. T., and Feuers, R. J.: **Effects of diet on hydrogen peroxide detoxification in Fischer 344 rats.**

59. Turner*, N., Lowenthal, D. T., and Scarpace, P. J.: **Influence of exercise and age on myocardial β-adrenergic receptor properties.**

60. Shaddock*, J. G., Feuers, R. J., and Casciano, D. A.: **Aging and enzyme induction: effect on chemically-induced DNA repair in non-proliferating rat hepatocytes.**

61. Ho*, L.-T., Cheng, Y. C., and Shiao, M.-S.: **The age related aberrations in lipid metabolism of rat adipocytes.**

62. Ganguly*, R., Webster, T. B., Sinnott, J., and Chmel, H.: **Immunization of the institutionalized elderly against pneumococcal infection.**

63. Bennett*, M. C. and Rose, G. M.: **Cytochrome oxidase inhibition by sodium azide infusion impairs learning in the Morris Water Maze.**

64. Weiss*, A., Livne, E., and Silbermann, M.: **The *in vitro* effects of various hormones on the synthesis of collagen and noncollagen proteins in the articular cartilage of aging mice.**

65. Fabian*, T. J., and Johnson, T. E.: **Differential gene expression during the adult life span of *C. elegans.***

OCTOBER 12, 1991

MINISYMPOSIUM

"Protein Glycation and Aging"

66. Monnier*, V. M., Miyata, S., Sell, D. R., Grandhee, S., and Nagaraj, R.: **Pyrraline and pentosidine as biomarkers of aging.**

67. Charonis*, A., Kouzi-Koliakos, N., Haitoglou, C., Anderson, S., and Tsilibary, E.: **Structural/functional changes of matrix proteins by glycation.**

68. Lee*, A. T., and Cerami, A.: DNA glycation and aging.

69. Bucala, R.: **Advanced glycation and hypertension in aging: role of nitric oxide.**

70. Vlassara*, H., Fuh, H., Makita, Z., and Morgello, S.: ***In vivo* advanced glycosylation-induced vascular permeability and monocytic migration is inhibited by aminoguanidine.**

71. Brownlee, M.: **Pharmacologic intervention of nonenzymatic glycation.**

72. Dyer, D. G., Dunn, J. A., Blackledge, J. A., Lyons, T. J., McCance, D. R.,Thorpe, S., and Baynes*, J.,: **Accumulation of glycoxidation products in skin collagen in aging and diabetes.**

1

MECHANISMS OF ALTERED CARDIOVASCULAR FUNCTION WITH AGING. *P. Anversa and J.M. Capasso,* Dept. of Med., New York Med. Coll., Valhalla, NY 10595

To determine the effects of age on the myocardium, the hemodynamic performance and structural characteristics of the heart were studied in Fischer 344 rats at 4, 12, 20, and 29 months of age. *In vivo* assessment of cardiac pump function showed no change up to 20 months, whereas left ventricular end-diastolic pressure was increased at 29 months. Moreover, peak rates of pressure rise and decay, stroke volume, ejection fraction, and cardiac output were depressed at the later age interval, demonstrating the presence of ventricular failure at this time. The measurements of chamber size and wall thickness showed that ventricular end-diastolic and end-systolic volumes progressively increased with age with the greatest change occurring at 20-29 months. In the period between 4 and 12 months, a reduction of 19% in the total number of myocytes was measured in both ventricles. In the subsequent ages, similar decreases in myocyte cell number were found in the left ventricle, whereas in the right ventricle, the initial loss was fully reversed by 20 months. Moreover, from 20 to 29 months, a 59% increase in the aggregate number of myocytes occurred in the right ventricular myocardium. In the left ventricle, a 3% increment was also seen, but this small change was not statistically significant. These estimations of myocyte cellular hyperplasia, however, were complicated by the fact that cell loss continued to take place with age leading to an augmentation in the volume fraction of collagen in the myocardium. In conclusion, myocyte cellular hyperplasia tends to regenerate the ventricular mass being lost with age in the adult mammalian rat heart.

2

BIOPHYSICAL, BIOCHEMICAL AND MOLECULAR CHANGES IN THE SENESCENT RAT HEART. *E.G. Lakatta,* Lab. Cardiovasc. Science, Gerontol. Res. Ctr., Natl. Inst. on Aging, Natl. Inst. Health, Baltimore, MD 21224.

Aging in rodents is associated with a characteristic pattern of change in several cardiac excitation-contraction coupling mechanisms. The trans-membrane action potential (TAP) is prolonged by about twofold in senescent (S) (24 mo) vs that from younger adult (A) (6-8 mo) rats. The L type sarcolemmal Ca current is not substantially increased in magnitude but inactivates more slowly and could possibly account, in part, for the prolonged TAP. The cytosolic Ca^{2+} transient following excitation is prolonged in S and appears to be due to a reduction in the rate of Ca sequestration by the carcoplasmic reticulum. A reduction in the mRNA coding for the SR Ca^{2+} ATPase may indicate a decrease in the SR pump site density. The myofilament force response to Ca is not altered by age. The myosin Ca^{2+} ATPase activity declines with age and is due to a marked increase in expression of the β or V_3 myosin heavy chain and a marked reduction in α or V_1 isozyme in S (85% β vs 15% α). These shifts are due to changes in the expression of the gene coding for these proteins. The increase in cell Ca^{2+} and contraction following β-adrenergic stimulation are reduced with age. The altered cellular profile, which results in a contraction that exhibits a reduced velocity and a prolonged time course, can be considered to be adaptive rather than degenerative in nature because the reduced velocity is energy efficient and prolonged contraction permits continued ejection for a prolonged period.

3

CHRONIC EXERCISE EFFECTS ON CALCIUM AND CONTRACTION IN CARDIAC MUSCLE. *J.Y. Wei,* Beth Israel Hosp., Harvard Med. School, Brockton/West Roxbury VAMC, Boston, MA 00215.

The effect of chronic exercise on cardiovascular function is an area of current interest. It is known that isometric twitch and action potential duration are prolonged with advancing age in rats. To investigate whether chronic exercise might reverse the age-related changes in excitation contraction coupling, we studied aged (24-26 mo) Fischer 344 rats after they underwent eight weeks of moderate exercise conditioning. Right ventricular papillary muscles were loaded with the calcium indicator aequorin. Electrophysiological recordings were also performed.

The time to peak isometric tension in muscles from exercised aged rats was shorter than in those from unexercised aged rats. Time to 50% relaxation from peak isometric tension was also shorter in exercised animals. There was a trend toward a decrease in time to peak light in the exercised group. Action potential amplitude was smaller in the exercised hearts; however, action potential duration was longer with exercise. Right ventricular-to-body weight ratios revealed no evidence of hypertrophy in the exercised compared with the unexercised group. Cardiac tissue norepinephrine content was significantly higher in the exercised hearts.

Thus, exercise training reversed the age-related prolongation of isometric contraction and associated intracellular calcium transient in the aged rat while it prolonged the transmembrane action potential duration. In addition, exercise training in aged rats resulted in an increase in cardiac norepinephrine content.

4

CHRONIC EXERCISE EFFECTS ON SARCOPLASMIC RETICULUM CA^{2+} PUMP. *C.A. Tate,* Dept. of Pharmacol., Univ. of Houston, Houston, TX 77205-5515.

The impaired relaxation of the old rat heart is related, in part, to a decreased ability of the sarcoplasmic reticulum to transport calcium. Calcium transport is an active process using the hydrolysis of ATP by the Ca^{2+} pump (CaATPase). Recent work from our laboratory showed that the slower calcium transport by the sarcoplasmic reticulum isolated from old rat hearts is caused by a decreased CaATPase. This decreased CaATPase is matched by a decrease in the mRNA-specific for the CaATPase, implicating alterations of transcription. When the previously sedentary old rats undergo a chronic aerobic-like exercise training program, the content of the CaATPase increases, leading to a faster rate of calcium transport by the isolated sarcoplasmic reticulum. This in turn results in an improved relaxation of the isolated heart muscle from the old, trained rats. Although the CaATPase content is increased, the mRNA-CaATPase is not increased with the exercise program when both the protein and mRNA contents are examined 24 h. after the last exercise bout. A number of factors may be involved; e.g., the turnover of the mRNA may be faster than the protein.

5

$ALPHA_1$- AND BETA-ADRENERGIC SIGNAL TRANSDUCTION IN THE AGED MYOCARDIUM. *P.J. Scarpace* and S.E. Borst,* GRECC, VA Med. Ctr. & Dept. of Pharmacol., Univ. of Florida, Gainesville, FL 32610.

Regulation of cardiac function involves both adenylate cyclase and phosphatidyl inositide signaling mechanisms. Inotropic and chronotropic responses mediated by the β-adrenergic-adenylate cyclase signaling mechanism are decreased with age. The role of the α_1-adrenergic-phosphatidyl inositide signaling mechanism in cardiac function has been implicated in the positive inotropic response in the ventricle and in regulation of atrial rhythm. We investigated the α_1-stimulated phosphatidyl inositide (PI) hydrolysis in atrial slices from female F-344 rats of 5 and 25 months of age. Epinephrine (1 µM to 300 µM) in the presence of 5 µM propranolol resulted in a pure α_1-stimulation of PI hydrolysis and the maximum response was reduced 70% at 25 months compared with 4 months (2.7 fold increase over basal vs 8.6 fold). The sensitivity to epinephrine (2.42±0.72 µM) was unchanged with age. We also investigated forskolin stimulation of adenylate cyclase (AC) activity in the presence and absence of activated Gs in ventricle membranes. Forskolin AC activity decreased by 42% in senescence compared with either 12- or 3-month-old rats. The age-related decline persisted whether forskolin AC activity was assessed in presence of isoproterenol and β, γ-imidoguanine 5'-triphosphate (Gpp[NH]p) to activate Gs or in the presence of guanosine 5'0'(2-thiodiphosphate) (GDP-βS) to deactivate Gs. There was no change in the EC_{50} (3.2±0.4 µM) for forskolin activation with age or in the presence or absence of activated Gs. These data indicate that there is reduced adenylate cyclase catalytic unit activity in cardiac ventricle and reduced α_1-adrenergic signal transduction in atrial slices with age.

6

MAINTENANCE WITH AGE OF VASCULAR ADRENERGIC REACTIVITY: REALLOCATION OF RECEPTOR FUNCTION. *S.P. Duckles,* Dept. of Pharmacol., Coll. of Med., Univ. of California, Irvine, CA 92717.

There is an age-related increase of stimulation evoked norepinephrine (NE) release from the isolated tail artery of the Fischer-344 rat which is correlated with a decline in sensitivity to both the α_2 adrenoceptor antagonists, yohimbine and idazoxan, as well as the α_2 agonist, UK 14304. However, there is no change in maximal effect of idazoxan, suggesting that endogenous activation of presynaptic α_2 adrenoceptors does not change with age. Alterations in sensitivity to agonists and antagonists may be due to competition with the higher biophase concentration of endogenous NE in older animals. Age-related increase in NE release may be due to a change in control of neuronal intracellular calcium. In spite of increased NE release, vascular contractile responses to adrenergic nerve stimulation do not change with age. This may be due to a loss of postsynaptic α_2 adrenoceptor function, as idazoxan causes less shift of NE concentration response curves in older animals. Compensatory decreases in postsynaptic α_2 receptor function in the face of increased transmitter release may maintain overall vascular function; however, the predicted effects of pharmacological agents acting on component parts of the system may be profoundly altered with advancing age.

7

CLINICAL TRIALS. *M. Moser.* Abstract appears on page 144.

8

VA EXPERIENCE. *W.C. Cushman.* Abstract appears on page 145.

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ANTIHYPERTENSIVE MEDICATIONS. *J. Tuckman.* Abstract appears on page 145.

10

THE HEAT SHOCK RESPONSE AS A MOLECULAR SENSOR OF CELL DAMAGE. *R.I. Morimoto,* Dept. of Biochem., Molecular and Cell Biol., Northwestern Univ., Evanston, IL 60208.

Common among all organisms is an essential, highly conserved and exquisitely regulated cellular response to physiological stress. This adaptive mechanism involves the induced synthesis of a family of heat shock or stress-induced proteins including HSP100 which has a role in thermotolerance, HSP90 which is a component of receptor complexes, HSP70 involved in folding of cytosolic and lumen proteins, HSP60 which is involved in transport and folding of mitochondrial proteins and HSP 20 or the alpha-crystalline related proteins. There is a commonly held expectation that heat shock proteins recognize a common structural motif transiently maintained in nascent protein chains and for an extended period in covalently modified or malfolded proteins. It would therefore not be surprising if the balance of interactions between normal and abnormal proteins with stress proteins could influence cell growth, differentiation, senescence and cell death. In addition to stress proteins as sensors, a second component known as heat shock transcription factor responds to protein damage by activation of heat shock gene transcription. The mechanisms by which cell damage leads to selective gene activation and binding to malfolded proteins offer an interesting and potentially relevant paradigm.

11

TRANSCRIPTIONAL REGULATION OF HEAT SHOCK GENES AND CELL AGING. *A. Y.-C. Liu*, Y.K. Lee and H.-S. Choi,* Dept. of Biolog. Sciences, Rutgers Univ., Piscataway, NJ 08855-1059.

We examined the effects of cell aging on the regulation of heat shock gene expression in IMR-90 human diploid lung fibroblasts. We showed that the induction of synthesis of HSPs was inversely related to the age (i.e., population doubling level) of the cells used. Analyses of the abundance and translatability of mRNA of hsps, as well as the transcription rate and promotor activity of the hsp 70 gene, provided evidence that this attenuated induction of HSPs in senescent cells was attributable to a transcriptional mechanism. This conclusion was corroborated by quantitation of the DNA-binding activity of the heat shock gene transcription factor (HSTF). In addition to the decreased magnitude of induction, we noted that a higher temperature as well as a longer duration of heat shock were required to activate the HSTF DNA-binding activity in senescent cells as compared to that of the young cells. Experiments of mixing extracts from young and old cells provided evidence of a dominant inhibitor of the HSE-binding activity in old cells. We conclude that there is an age-dependent attenuation in transcriptional activation of heat shock genes, and this is due, at least in part, to the increased expression of a negative regulatory factor of HSTF.

12

ROLE OF ABNORMAL PROTEINS IN THE HEAT SHOCK RESPONSE IN AGED *DROSOPHILA*. *A. Niedzwiecki*, A. Kongpachith, and J.E. Fleming,* Linus Pauling Inst. of Science and Med., 440 Page Mill Rd., Palo Alto, CA 94306.

The increased denaturation of proteins under heat stress is important regarding the mechanism of molecular senescence since a higher level of abnormal proteins accumulate with age. We suggested that increased accumulation of abnormal proteins generated in heat stressed old organisms play a role in the regulation of the heat shock response. The expression of hsp70 in heat shocked old flies was higher than in young *Drosophila;* however, this did not result in their increased resistance to heat. The expression of ubiquitin, a protein involved in targeting abnormal proteins for degradation, increases during heat stress. Also, the level of polyubiquitin RNA in heat stressed flies increases with age. Moreover, old flies expressed more polymorphic ubiquitin variants than young *Drosophila* under the same stress conditions. The enhanced expression of polyubiquitin gene in heat shock flies with age was accompanied by the increased level of protein-ubiquitin conjugates formed in heat stressed aging *Drosophila.* Regardless of age, proteins synthesized before heat shock were more ubiquitinated than the ones synthesized during and after heat stress. The role of different factors affecting the stability and heat-induced denaturation of proteins on the heat shock response and thermal sensitivity will be discussed.

13

AGE-RELATED ALTERATIONS IN THE RESPONSE TO PHYSIOLOGIC STRESS: DECLINE IN EXPRESSION OF HEAT SHOCK PROTEIN 70 (HSP70). *N.J. Holbrook*, R. Udelsman, and M.J. Blake,* Lab. Mol. Genetics, Natl. Inst. Aging, Baltimore, MD 21224.

Exposure of cells *in vitro* to a variety of stresses results in the expression of HSP70, thought to be a protective/reparative response to the stress. We have found the HSP70 is likewise expressed in rats *in vivo* in response to physiologic stresses including temperature extremes, ether anesthesia, surgery and restraint; the expression being tissue-specific dependent on the stress administered. In particular, restraint induces HSP70 expression selectively in the adrenal gland and vasculature. Since restraint is also known to activate the hypothalamic-pituitary-adrenal (HPA) axis and increase sympathetic nervous system activity, we have explored the possible interrelationship between cellular HSP70 mRNA expression and the neuroendocrine stress response in these tissues. Hypophysectomy prevented the restraint-induced HSP70 expression in adrenal tissue. Administration of adrenocorticotropic hormone (ACTH) to hypophysectomized animals restored the response and itself induced HSP70 expression in the adrenal. In contrast, pertubations of the HPA axis had no effect on vascular HSP70 expression. Prazosin (1 mg/kg i.p.), an alpha adrenergic receptor antagonist, completely prevented restraint-induced HSP70 expression in aortic vessels, but had no effect on adrenal expression. These findings suggest that restraint-induced HSP70 expression in the adrenals is regulated by the HPA axis, while in the vasculature it is controlled by the sympathetic nervous system. Adrenal HSP70 mRNA expression in response to restraint declines more than 5-fold as animals age from 6 to 24 months. Aortic HSP70 expression declines greater than 20-fold over the same period. We hypothesize that a general decline in HSP70 expression renders the aged animal more susceptible to the adverse effects of stress, thereby further contributing to the aging process.

14

THE ABILITY OF DIETARY RESTRICTION TO CHANGE THE AGE-RELATED ALTERATION IN THE EXPRESSION OF HEAT SHOCK GENES. *A.R. Heydari, B. Wu, C.C. Conrad, and A. Richardson,* GRECC, Audie L. Murphy Memorial VA Hosp., and the Dept. of Med., Univ. of Texas Health Science Ctr., San Antonio, TX 78284.

We have compared the ability of hepatocytes isolated from young (5 to 7 months) and old (22 to 27 months) male Fischer F344 rats fed *ad libitum* or a caloric-restricted diet (60% of *ad libitum*) to express three genes in the HSP70 family: the heat-inducible hsp70, the constitutive hsc70, and grp78, which is constitutive and induced by glucose starvation. The induction of hsp70 expression (synthesis, mRNA levels and nuclear transcription) after a mild heat shock (42°C for 30 minutes) was approximately 50% lower for hepatocytes isolated from old rats. Using *in situ* hybridization, we found that a similar percentage of cells from young and old rats responded to heat shock; however, hepatocytes from the old animals expressed less hsp70 after heat shock. Dietary restriction significantly increased the induction of hsp70 expression, the expression of hsp70 by hepatocytes from old, caloric-restricted rats was similar to the level of hsp70 expression by hepatocytes isolated from young rats fed *ad libitum*. In contrast to hsp70, no age-related change in either the basal or heat-induced expression of hsc70 was observed. However, the heat-induced expression of hsc70 was significantly greater in hepatocytes isolated from rats fed the caloric-restricted diet. The levels of grp78 mRNA declined slightly with age; however, the levels of grp78 mRNA were reduced 40 to 50% by dietary restriction for hepatocytes isolated from both young and old rats. Thus, the expression of the genes in the HSP70 family change with age, and dietary restriction, which increases the survival of rats approximately 30%, alters the age-related changes in the expression of these genes. However, the effect of age and dietary restriction on the expression of these three genes varies considerably from gene to gene.

15

HEAT SHOCK PROTEIN EXPRESSION IN LYMPHOCYTES FROM AGING HUMANS. *H.M. Hallgren* and J.J. O'Leary,* Dept. of Lab. Med. and Pathol., Univ. of Minnesota, Minneapolis, MN 55455.

Mitogen activation of quiescent human T cells is associated with preferentially enhanced synthesis of a number of "cell cycle dependent" gene products during the prereplicative interval. The diminished proliferative response of T cells from older human subjects (>70 years) correlates with decreased synthesis of these products. We have demonstrated that two of the constitutively synthesized heat-shock proteins, HSP90 and HSC70, also show marked preferentially enhanced synthesis in the prereplicative interval of mitogen activated human T cells. Comparing lymphocytes from healthy older donors and young adults, we find that synthesis of HSP90 and HSC70 is reduced by about 50% in the older subjects' cells following PHA activation. This relative decrease is similar to that observed for c-myc, Interleukin 2 (IL-2) and its receptor (IL-R). Following heat shock, however, lymphocytes from older subjects show increases in the synthesis of HSP90, HSC70 and the major 70kD heat-shock protein, HSP 70, similar to that of the young adult control population. While the protein synthesis results are consistent with an impairment in PHA induced transmembrane signalling, an age-related generalized defect in the ability to activate transcription and translation cannot be ruled out. Experiments to evaluate these alternatives at the level of induction of mRNA for the heat-shock genes are currently under way.

16

ANTIOXIDANT VITAMINS. *G. Blumberg.* Abstract appears on page 145.

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REVIEW OF COGNITIVE-ENHANCING SUBSTANCES. *W. Dean.* Abstract appears on page 145.

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CALORIC RESTRICTION. *D. Ingram.* Abstract appears on page 145.

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EXERCISE AND AGING. *R. Fielding.* Abstract appears on page 145.

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A COMPREHENSIVE PROGRAM FOR THE EVALUATION OF THE EFFECT OF CALORIC RESTRICTION (CR) ON FREE RADICAL DETOXIFICATION AND ITS EFFECT ON AGING. *R.J. Feuers*, R. Weindruch+, J.E.A. Leakey, P.H. Duffy, and R.W. Hart,* Natl. Ctr. for Toxicolog. Res., Jefferson, AR 72029, and +Dept. Med., Sect. of Genetics and Gerontol., Univ. Wisconsin, Madison, WI 53706.

Aging may result as free radical induced damage at important macromolecules accumulates. Since CR extends lifespan, free radical production may be limited or detoxification may be stimulated. We have shown that the potential for lipoperoxidation is limited by CR and that P450 enzymes which produce free radicals have lower activity under CR during aging. Additionally, we have developed a comprehensive strategy for the evaluation of free radical detoxification. As $\cdot O_2^-$ is generated, superoxide dismutase rapidly converts it to H_2O_2. Then catalase (CAT) binds H_2O_2 and then in the presence of another H_2O_2, produces water, oxygen and free enzyme. However, CAT may also be oxidized to the inactive compound II. This inactive CAT can be recovered by enzymatic reduction with NADPH. We have shown that with age the rate of accumulation of compound II is decreased by CR. The NADPH used in reduction is produced by glucose 6 phosphate dehydrogenase. This NADPH is also used by glutathione reductase for reduction of GSSG. The resultant GSH Is then used for a second route of H_2O_2 detoxification by glutathione peroxidase. Measurement of each of these parameters yields the potential for understanding how CR affects the mechanism of free radical detoxification.

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ROLE OF CORTICOSTEROIDS AND "CALORIC STRESS" IN MODULATING THE EFFECTS OF CALORIC RESTRICTION (CR) IN RODENTS. *J.E.A. Leakey, J.R. Harmon, J.J. Bazare, Jr., S. Chen, M. Manjgaladze, R.J. Feuers, P.H. Duffy, and R.W. Hart,* Div. of Reproduct. and Develop. Toxicol., Genet. Toxicol., and Office of Director, NCTR, Jefferson, AR 72079.

Although it is well established that long-term CR in rodents results in increased longevity and a decreased rate of neoplasia and certain degenerative diseases, the biochemical mechanisms for these effects remain unclear. We have shown that one of the earliest effects of CR is the alteration of circadian profiles of serum corticosterone concentrations (C), resulting in increased levels at times when C are normally low. Similar changes in C profiles are produced by CR at later ages, in other strains of rats and in mice fed on alternate days. In all cases the changes in serum C were associated with induced hepatic tyrosine aminotransferase activity. Thus it appears that C are sufficiently elevated by CR to increase glucocorticoid-responsive enzymes. It is also possible that increased C mediates CR-induced changes in hepatic drug metabolizing enzymes (DME). The overall effect of CR on DME is to decrease male-specific DME isoforms in male rats and decrease female-specific DME isoforms in females. We have found that similar changes occur after glucocorticoid administration or surgical stress and that the latter effect is dependent on the presence of the adrenals. Thus it is probable that elevated or disrupted C profiles disrupt the pituitary secretions of growth hormone, which control the sexual differentiation of the liver. Stress or elevated C are also known to suppress immune function and testosterone secretion by the testes. Thus, it appears that a "caloric stress" condition may arise during CR which results in decreased metabolism for "peripheral" functions such as hepatic drug metabolism, immune function and reproduction. Decreased metabolism in these systems spares the associated organs from oxidative damage, and this, in turn, leads to increased longevity.

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AN ISOFORM OF DNA POLYMERASE α FROM SV-40 TRANSFORMED HUMAN FIBROBLASTS. *D. Busbee*, V. Srivastava, M. Schroeder, and S. Miller,* Dept. of Anat. and Cell Biol., Coll. of Vet. Med., TAMU, College Station, TX 77843.

Genomic DNA replication in eukaryotes is carried out by a putative multiprotein replication complex. DNA polymerase α, which is a key component in that complex, has a major role in the nucleotide selection

and polymerization processes, declines in specific activity and fidelity with increased age of the cell donor. DNA polymerases purified from normal vs transformed cells differed in their chromatographic characteristics, specific activities and molecular properties. Elution profiles of DNA polymerases showed a single peak from normal cells with two distinct enzyme peaks from transformed cells. Enzymes from transformed cells showed different binding affinities to DNA template and were in tight association with DNA primase, an oligoribonucleotide polymerase that synthesizes short ribonucleotide primers which can be extended by DNA polymerase α. Enzyme from transformed cells exhibited more than 3-fold higher specific activities than enzyme from normal cells. They also differed in molecular size, thermolability, sensitivity to polymerase inhibitors, and specificity in template-primer utilization. These data suggest that DNA polymerase α isoforms from virally transformed human cells co-elute with SV-40 derived large T antigen and may have modifications of common catalytic subunits or an altered primary structure which could play a different functional role in the cellular DNA replication machinery.

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THE RESPONSE OF SENESCENT HUMAN FIBROBLASTS TO DNA DAMAGE. *D. Busbee*, R. Tilley, and S. Miller,* Dept. of Anat. and Cell Biol., Coll. of Vet. Med., TAMU, College Station, TX 77843.

Post replicative senescent human fibroblasts (SHF) are unable to efficiently repair DNA lesions caused by ultraviolet light or mutagenic chemicals. The incorporation of ^{3}-thymidine as a measure of unscheduled DNA synthesis measures only one step in the complex DNA excision repair process. We have applied other methods of analysis to determine if senescent human cells respond to DNA damage. Proliferating cell nuclear antigen (PCNA), a protein detected in S phase cells by immunofluorescence microscopy, was not detected in the nuclei of SHF cultured under standard conditions. PCNA was readily detected in the nuclei of SHF following treatment with DNA damaging agents. Single cell gel DNA electrophoresis, with analysis utilizing the computer interactive scanning laser cytometer, was used to show that SHF respond to DNA damage by introducing nicks into DNA causing fragmentation of the genome in the absence of repair completion. These nicks are apparently initiated by the endonuclease and exonuclease steps of excision repair. However, the integrity of SHF DNA was not restored on continued repair incubation following DNA damage as is the case with early passage cells. These results indicate that although senescent fibroblasts are unable to perform the DNA resynthesis steps of excision repair, they respond to DNA damage by converting PCNA to an immunofluorescence detectable form and by introducing nicks at lesion sites in damaged DNA.

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RETINOID DEFICIENCY PREVENTS LEUPEPTIN-INDUCED AUTOFLUORESCENT PIGMENT ACCUMULATION IN THE RETINAL PIGMENT EPITHELIUM. *M.L. Katz* and M. Norberg,* Depts. of Ophthalmol. and Neurol., Univ. of Missouri Sch. of Med., Columbia, MO 65212.

In mammals, aging is accompanied by a progressive accumulation of the autofluorescent pigment lipofuscin within the retinal pigment epithelium (RPE). Substantial evidence suggests that the fluorescent contents of RPE lipofuscin granules are at least partially derived from phagocytosed photoreceptor outer segments. The molecular components of the outer segments that are converted into RPE lipofuscin fluorophores have yet to be identified. Previous experiments have shown that RPE lipofuscin accumulation is greatly reduced in animals fed diets deficient in retinoids involved in the visual process. Those studies suggested that retinoid derivatives may be incorporated into RPE lipofuscin and contribute to its fluorescence. Autofluorescent pigment accumulation in the RPE can be greatly accelerated by intravitreal injections of the protease inhibitor leupeptin, which blocks degradation of phagocytosed outer segments by the RPE. Experiments were conducted to determine whether the fluorescence of the leupeptin-induced inclusions was dependent on the presence of retinoids in the retina. Rats were maintained on diets containing either retinyl esters, which can be metabolically converted to the retinoids involved in vision, or retinoic acid, which cannot be converted into forms that support the visual process. After rhodopsin levels had been reduced over 85% in the latter group, one eye from each animal was injected intravitreally with leupeptin, and the other eye was injected with saline vehicle. In the animals with normal visual pigment levels, leupeptin treatment caused a 25% increase in RPE autofluorescent pigment content. No such increase was observed in the animals with reduced retinal retinoid content. These findings suggest that retinoids may be directly involved in RPE lipofuscin fluorophore formation.

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IMMUNE COMPLEXES AND COMPLEMENT AND THE TURNOVER OF RED BLOOD CELLS FROM YOUNG AND OLD HUMAN DONORS. *S. Shapiro and H. Gershon*,* Technion Fac. of Med., Haifa, 31906, Israel.

RBC half-life is shortened in the circulation of old individuals. 10% of the RBC from old donors but not from young donors are phagocytosed *in vitro.* It has been proposed that low levels of IgG antibodies and/or C'3b are involved in RBC sequestration and that β-galactosides block this sequestration. The role of immune complexes (IC) and C'3b in the sequestration of human RBC was evaluated. Exposure of RBC from young donors to IC in the presence of fresh plasma leads to the binding of C'3b coated IC (IC-C'3b) to CRI and to galactose inhibitable phagocytosis of the RBC. Exposure of RBC from old donors to IC-C'3b barely increases their phagocytosis.. Inhibition of Factor I prevents release of IC-C'3b from the RBC membrane and doubles the level of phagocytosis of treated RBC from young donors with hardly any influence on RBC from old donors. Flow cytometric analysis shows more C'3b on RBC from old than young donors and an increment in C'3b bound to RBC from young donors after exposure to IC-C'3b with no change on cells from the elderly. Our experiments suggest that old RBC are defective in C'3b degradation thus leading to increased susceptibility to phagocytosis.

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A STRATEGY FOR THE IDENTIFICATION OF LIFESPAN-DETERMINING GENES IN *C. ELEGANS.* *R.H. Ebert, N.K. Egilmez, J.B. Chen, and R.J. Shmookler Reis*,* Dept. of Med. and Dept. of Biochem. and Molec. Biol., Univ. of Arkansas for Med. Sciences, & J.L. McClellan Vet. Med. Ctr., Little Rock, AR 72205.

The F_1 progeny of crosses between *C. elegans* strains N2 and BO have mortality curves similar to those of the parental strains, implying little or no heterosis (advantage to heterozygotes), and yet the F_2 and subsequent generations show significant increases in the variance of mortality (Johnson & Wood, PNAS **79**:6603, 1982; Johnson, PNAS **84**:3777, 1987). We have repeated these experiments crossing two non-mutator strains (N2 and DH424) with essentially the same result — marked and highly-significant increases in lifespan variance for the F_2 and F_{26} progeny of the cross, relative to the F_1 generation. These data imply the existence of multiple genes directly affecting lifespan, which are polymorphic between strains. We have begun to characterize such genes, by identifying alleles which are consistently present in the longest-lived recombinant-inbred progeny of N2xBO or N2xDH424 crosses. Tcl transposons present in one of the parental genomes (BO or DH424) but absent from the other (N2) will be identified by single-worm multiplex PCR (Williams *et al.,* WBG **11**:10, 1990) in order to score individual long-lived worms for chromosomal domains inherited from one or the other parental strain. This is a powerful technique, capable of identifying multiple loci which interact together to produce a polygenic phenotype, and is particularly suited to complex traits such as lifespan, fertility, temperature sensitivity, etc.

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MOLECULAR GENETIC APPROACHES TO THE ANALYSIS OF AGING IN *CAENORHABDITIS ELEGANS.* *T. Johnson, T. Fabian*, P. Tedesco, and T. Hutchinson,* Inst. for Behav. Genet., Univ. of Colorado, Boulder, CO 80309.

We are using classical, quantitative, and molecular genetic analyses to study the basic aging processes in the nematode *C. elegans.* Much of our work has centered on the characterization and cloning of the *age-1* gene where mutations result in a 70% extension of mean life span. The *age-1* gene has been mapped to the center of chromosome II and the mutant allele cosegregates with a four-fold reduction in

hermaphrodite self fertility (Brd) leading to the model that the *age-1* gene product specifies a fertility function that affects life span negatively. We have critically tested several predictions of this model. Using genetic analysis of deficiencies for *age-1* and a multipoint mapping strategy we find that the Brd phenotype results from a separable mutation several hundred kb proximal to *age-1* on chromosome II. *age-1* is being cloned by using the physical map to identify DNA polymorphisms near *age-1*.

A second strategy is to identify transcripts that are differentially expressed during the adult life span. We have identified such transcripts by differential screening of a nematode cDNA library. More than 30 transcripts were found to be more highly expressed in young animals and an additional 30 cDNA clones were expressed at higher levels in old animals. These clones represent several distinct genes and fall into several "classes" including embryonic specific and "vitellogenin-like" genes that are high in young worms and as yet unknown transcripts that are higher in old worms.

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GENETICS OF POSTPONED AGING IN *DROSOPHILA MELANOGASTER*. *M.R. Rose*, F.J. Ayala, G.S. Spicer, R.H. Tyler, and J.E. Fleming,* Dept. of Ecology and Evolutionary Biol., Univ. of California, Irvine, CA 92717.

Drosophila melanogaster stocks with postponed aging have been created by selection for later reproduction. Mean life span has increased by about 80% in males, among numerous other improvements in aging phenotypes. Five postponed aging stocks exist, along with five controls. These stocks have been electrophoretically analyzed for genetic differentiation associated with postponed aging. In one-dimensional gel electrophoresis studies, the more active superoxide dismutase allele is increased in allele frequency, suggesting that free-radical scavenging may be a factor that controls aging. Additional proteins are differentiated on two-dimensional gels, although these proteins have yet to be identified. The two-dimensional protein data clearly differentiate the selected stocks from the controls in maximum parsimony trees, indicating that the two-dimensional protein electrophoresis technique can reliably reveal proteins involved in postponed aging.

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EXPRESSION OF AN ANTIPROLIFERATIVE PROTEIN, PROHIBITIN. *J.K. McClung*[1], L. Walker[1], V. Friedman[2], M.J. Nuell[2], D.B. Danner[2], and R. Dell'Orco[1],* [1]Biomed. Div., The Noble Fndtn., Ardmore, OK 73402, [2]Lab. of Molec. Genet., Nat. Inst. on Aging, Baltimore, MD 21224.

Recently, the discovery of a number of negative regulators of growth has brought new insight into the development of an immortal phenotype and the maintenance of the normal mortal phenotype which leads to senescence. Prohibitin is a 30,000 dalton protein that has been shown to have antiproliferative activity when the mRNA is microinjected into young human diploid fibroblasts. The fibroblasts are blocked in G_2 and are inhibited from entering S phase of the cell cycle. Expression of prohibitin mRNA and protein is constitutive, but does fluctuate with the cell cycle with peaks of expression occurring in G_1 and G_2. Since the fluctuations in mRNA and protein are only 2 to 4 fold, another control of activity may involve post-translational modifications. The prohibitin amino acid sequence is evolutionarily conserved. Human and rat prohibitins are identical; human and *Drosophila* are about 76% identical; and human and yeast are 51% identical. Therefore, prohibitin appears to play an important role in the regulation of normal cell growth.

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THE SWEDISH ADOPTION/TWIN STUDY ON AGING: A REVIEW. *G. McClearn,* The Pennsylvania State Univ., University Park, PA 16802.

The purpose of the Swedish Adoption/Twin Study on Aging is to assess genetic and environmental variance components in a wide variety of behavioral and health-related phenotypes. In addition to monozygotic (166 pairs) and dizygotic (221 pairs) twins reared conventionally, the study includes a large number of twins separated in early life and reared apart (99 MZ pairs and 238 DZ pairs).

The design is longitudinal with questionnaire (Q) data (personality, self-reported health, environmental circumstances) and, for a subset of twins, in-person testing (IPT) (cognitive functioning, functional competence, pulmonary functioning, etc.) at 3-year intervals. Q data have been collected for 3 occasions; the 2nd IPT measurement is in progress.

This report will review 1st occasion results. For 24 of 25 personality tests, evidence of genetic influences was obtained. Heritability estimates average 0.3, slightly lower but of the general order of those for younger subjects. Almost all environmental variance is non-shared; rearing environment has little effect on twin similarities. Environmental factors reported by the subjects are themselves under substantial genetic influence. Heritabilities of specific cognitive abilities are as high in this older population as are generally reported for younger samples. High heritabilities are found for spirometric measures and other physiological measures.

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MAPPING OF FAMILIAL ALZHEIMER'S DISEASE: ROLE OF THE B-AMYLOID GENE. *P. St. George Hyslop.* Abstract not received.

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THE EFFECTS OF AGING ON DOPAMINE DIFFUSION AND CLEARANCE IN THE STRIATUM OF THE FISCHER 344 RAT. *M.N. Friedemann* and G.A. Gerhardt,* Depts. of Pharmacol. and Psychiat., Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Previous studies have shown that there are age-related changes in dopamine (DA) uptake sites (B_{max} and rates (V_{max}). In order to further investigate the effects of aging on DA uptake processes, the present study combines *in vivo* chronoamperometric recordings and local drug application techniques which allow direct measurement of DA diffusion and clearance from the extracellular space. Male Fischer 344 rats, 6 and 24 months old, were anesthetized with urethane and surgically prepared for *in situ* recording. DA (5-60 pmol) was pressure ejected 240 to 350 µm away from a Nafion-coated carbon fiber electrode at sites within the dorsal or ventral striatum. No significant differences between the age groups for either the amplitude or the clearance time of the DA ejections were found. There were also no significant differences between dorsal and ventral striatum. In addition, the application of the uptake inhibitor, nomifensine (20-200 pmol), just prior (30 sec) to the DA application resulted in significant increases in the amplitude and time course in dorsal striatum. However, in ventral striatum, nomifensine significantly increased the amplitude of the DA signal only in the 6-month-old rats, while the time courses of the signals were significantly increased in both age groups. The inability of nomifensine to modulate the amplitude of the DA signal in the ventral striatum of aged rats suggests that these nerve terminals may be differentially effected by the aging process.

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TISSUE AND REGION SELECTIVITY OF THE EFFECT OF (–)DEPRENYL TO INCREASE SUPEROXIDE DISMUTASE (SOD) ACTIVITIES IN THE RAT. *M.-C. Carrillo*[1], K. Kitani[1], S. Kanai[1], Y. Sato[1], and G.O. Ivy[2].* [1]Dept. of Clin. Physiol., Tokyo Metropol. Inst. of Gerontol., Itabashiku, Tokyo-173, Japan; [2]Div. of Live Sci., Univ. of Toronto, Scarborough, Ontario M1C 1A4, Canada.

A previous study has shown that chronic administration of (–)deprenyl increases activities of SOD and catalase (CA) in rat striatum. The present study attempted to clarify how specific the effect of deprenyl is to certain tissues and brain regions in the rat.

Two mg/kg/day of deprenyl was continuously infused s.c. in young male F-344 rats. On the 22nd day, rats were sacrificed and enzyme activities of SOD and CA were determined in several different brain regions and the liver. Activities of both SOD and CA were significantly increased in striatum and substantia nigra but not in hippocampus, cerebellum or liver. Interestingly, in cerebral cortices of three different regions, activities were also significantly increased, although the increase was not so striking as was observed in striatum and substantia nigra. Both types of SOD (i.e. Cu-Zn SOD and Mn SOD) were significantly increased in striatum, substantia nigra and cerebral cortices but not in liver or other brain regions examined.

The results confirm the previous observation that this drug can increase enzyme activities in striatum and provide further evidence that this effect is specific to certain brain regions and tissues.

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THE OPTIMAL DOSAGE OF (–)DEPRENYL TO INCREASE SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN THE STRIATUM IS SEX AND AGE DEPENDENT IN F-344 RATS. *K. Kitani*[*1], *M.-C. Carrillo*[1], *S. Kanai*[1], *Y. Sato*[1], *and G.O. Ivy*[2], [1]Dept. of Clin. Physiol., Tokyo Metropol. Inst. of Gerontol., Itabashiku, Tokyo-173, Japan; [2]Div. of Life Sci., Univ. of Toronto, Scarborough, Ontario M1C 1A4, Canada.

We have recently reported that chronic treatment with (–)deprenyl, a MAO B inhibitor, significantly increases activities of superoxide dismutase (SOD) and catalase (CA) but not of glutathione peroxidase (GSH Px) in the striatum of *young male* rats (Life Sci. 48:517, 1991). We extended our observations by using different dosages and rat groups. Young (4-7 months) and old (26-28 months) F-344 rats of both sexes were treated with different doses of deprenyl (0.1-2.0 mg/kg/day) for 21 days. On the 22nd day, rats were sacrificed and enzyme activities in striatal tissues were determined. The optimal dosage to increase SOD and CA activities was 10 times lower in young female (0.2 mg/kg/day) than male (2.0 mg/kg/day) rats. Aging decreased and increased the optimal dosage in male and female rats respectively, yielding an optimal dosage of 0.5 mg/kg in old males and of 1.0 mg/kg/day in old females. Doses higher than the optimal dose tended to decrease (rather than increase) SOD activities. Differences in the optimal dose among different groups appear to be due to two different factors 1) metabolism of deprenyl and 2) amount of MAO B. The reported effect of deprenyl in prolonging the life span of rats must be carefully examined in light of optimal drug doses for different ages and sex (and probably species) of animal.

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EFFECTS OF LONG-TERM DEPRENYL ADMINISTRATION ON MOTOR FUNCTION IN F344 RATS. *K. Poth, C. Heron, N. Miniclier, and P. Bickford-Wimer,* Vet. Admin. Med. Ctr. and Dept. of Pharmacol., Univ. of Colorado Health Sciences Ctr., Denver, CO 80262.

Deficits in motor skills in the aged population are well documented. Progressive deterioration in the motor coordination, balance, strength and motor learning occur with senescence. It is likely that changes in neuronal function in the CNS play a role in this decline. Deprenyl (selegiline) is an MAO-B inhibitor which has been shown to be somewhat effective in slowing the progression of Parkinson's disease and improving cognitive function in Alzheimer's disease. In addition, it has been demonstrated that deprenyl increases the maximum life span of aged rats. However, whether deprenyl extends the functional lifespan by improving motor function and learning is less well understood.

The present study investigates the effects of chronic, low dose administration of deprenyl on specific motor behaviors in aged animals. Twenty 12-month-old F344 male rats received deprenyl (.5mg/kg/day) in their drinking water and 20 rats were controls. Seven months after initiation of deprenyl treatment, all deprenyl-treated rats were alive; however, 2 control rats had died. Subjects were then tested for motor coordination, balance and strength using 2 balance beams (2.5cm and 5.0cm diameters), an inclined screen, and a wire hang apparatus. Results indicated no significant differences between the groups on any parameter measured. Further testing of more complex motor skills, i.e., motor learning, is currently in progress.

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DIFFERENTIAL EFFECTS OF AGING UPON HIPPOCAMPAL PYRAMIDAL CELL RESPONSIVENESS TO MUSCARINE AND NICOTINE. *G.M. Rose*, D.A. Engstrom, A.G. Humphreys, and P.C. Bickford-Wimer,* Med. Res. Svc., Vet. Admin. Med. Ctr., and Dept. of Pharmacol., Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80220.

The purpose of this work was to evaluate age-related changes in the responsiveness of hippocampal CA1 pyramidal neurons to cholinergic agonists. Fischer 344 rats at 3-6 (young) or 27-30 (old) months of age were first behaviorally tested in the Morris water maze to evaluate hippocampus-dependent place learning ability. The rats were subsequently anesthetized with sodium pentobarbital and prepared for electrophysiological recording and local drug application. Muscarine (0.1 mM) or nicotine (1.0 mM) was locally applied by pressure microejection onto identified CA1 pyramidal neurons. For each cell, a dose (in p.s.i. X sec) was found which elicited a 300-400% increase in basal firing. These data were used to construct cumulative dose response curves for the populations of neurons tested in the rats of each age group. Young rats displayed rapid place learning, whereas old rats did not learn over a 5-day training period. CA1 neurons recorded from the old rats were significantly *less* sensitive (population ED50's — young: 67±2; old: 103±7) to *locally applied* muscarine, but were significantly *more* sensitive (young: 53±3; old: 10±1) to locally applied nicotine. These results indicate that two types of age-related alterations in hippocampal cholinergic pharmacology are correlated with impaired cognitive ability in aged rats. However, it is not yet clear whether either, or both, of these changes is sufficient to induce the deficits in hippocampus-dependent learning.

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SENESCENCE AS A PROGRAMMED PHENOMENON. *N. Cardarelli,* Univ. of Akron, Akron, OH 44325.

The senescence metaparadigm with its emphasis on the immune theory of aging does not provide insight into the primum movens of immune loss, effector mechanisms or intervention modalities. This review suggests that immune decline is not causative of aging, but rather both are epiphenomenal and under bioclock regulation. A hypothesis is advanced relating to age-dependent homeostatic and homeorhetic alterations to oscillators sited in recognized hypothalamic nuclei. Evidence supporting the existence of a hypothalamus-pineal-lymphon axis controlling the programming of life events is presented. Programming is effected through neuroendocrine transduction with chalones acting as derepressive agents at target genomes. Senescence is related to amplitude losses in rhythmic processes involving key components of the immunoendocrine system.

Bioclocks are entrained and paced by periodic environmental events (zeitgebers). Numerous studies implicate solar associated phenomena, especially photoperiod, as zeitgebers. It is hypothesized that a senescent clock in humans is paced by the periodicity of solar hard electromagnetic radiation. Evidence from hormesis, radiation-induced oversurvival, dermopathology, cell immortalization, human isolation experiments, helioimmunology and other studies support this contention.

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HIGH SERUM COPPER RESULTS IN IMPAIRED IMMUNE RESPONSE. *H. Massie*, W. Ofosu-Appiah, and Valerie Aiello,* Masonic Med. Res. Lab., Utica, NY 13501.

Serum copper concentrations are known to increase in numerous disease states and with aging in both rodents and man. The purpose of this study was to relate the concentration of serum copper in individual mice to immune function. Atomic absorption spectrophotometry was used to determine serum copper concentrations. The response of purified lymphocytes to the mitogens phytohemagglutinin and concanavalin A was determined by cell culture in the presence of tritium labeled thymidine. The mitogen response of *isolated* lymphocytes from the spleens of aging mice was greatly reduced when these cells were taken from animals with naturally occurring serum copper levels in excess of 0.8 ng of copper/mg wet wt. serum. Addition of the copper protein, ceruloplasmin, to lymphocyte cultures *in vitro* reduced the mitogen response of purified splenic lymphocytes. We conclude that excess serum copper and ceruloplasmin are immunosuppressive, especially in older organisms. This may account for the elevated serum copper levels seen in many degenerative disease states associated with aging.

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DIFFERENTIAL INHIBITION OF THE RAT LIVER GLUTAMATE DEHYDROGENASE OF VARIOUS AGES BY METAL IONS. *M. Prabhakaram[1] and S.S. Nath*,* [1]Univ. of Missouri, Columbia, MO 65212; Dept. of Zoology, Banaras Hindu Univ., Varanasi-221 005, India.

Keeping in view the functional and regulatory aspects of glutamate dehydrogenase (L-glutamate NAD (P)+ oxidoreductase, E.C. 1.4.1.2-4, GDH) in mammalian tissues, in the present study, experiments were carried out with purified rat liver GDH (mitochondrial) of different ages of rats. Since the alterations, if any, exist in the enzyme molecule as a function of age can be better understood by using purified enzymes, activity of the purified GDH of the three ages of rats (Immature, 4 weeks; Young, 22 weeks; and Old, 116 weeks) was assayed in presence of different metal ions (anions and cations), and the data were analyzed. The obtained results show that the per activity of GDH of the three ages of rats remained in presence of anions or cations is unaltered with advancing age of the rat. However, the inhibitory effect of cations (silver, cadmium, copper, iron, zinc, calcium, magnesium, etc.) is relatively stronger than anions (sulfite, sulfate and acetate), used in the present study. For better analysis, these data were compared with earlier results obtained with nucleotides and hormones (Prabhakaram and Singh, 1988). Lack of age-related differences among the three ages of GDH demonstrate that GDH is an unaltered enzyme, with advancing age of the rat. Another possibility is the mitochondrial origin of GDH, which may be responsible for the lack of such changes in the enzyme molecule.

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THE EFFECTS OF CHROMIUM PICOLINATE SUPPLEMENTATION ON BODY COMPOSITION IN DIFFERENT AGE GROUPS. *G.R. Kaats*[1], J.A. Ficher[2], and K. Blum[2],* [1]The Living at Goal Weight Ctr., San Antonio, TX 78230 and [2]Dept. of Pharmacol., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78230.

This study examined the effects of supplementation with different amounts of chromium picolinate on the body composition of subjects of two different age groups: 20-46 (Avg = 36) and 47-74 (Avg = 55). All subjects consumed two nutritional drinks a day containing 0, 100, or 200 mcgs of chromium as chromium picolinate. While many of the enrolled subjects were motivated to lose weight, no attempt was made to alter their food intake or exercise levels during the study. We employed a randomized, double-blind, crossover trial with placebo (0 mcg/day), medium (200 mcg/day), and high (400 mcg/day) phases; each phase averaged 72 days. The placebo-control group showed no significant changes in body composition between pre- and post-test measurements, while the chromium-treated group as a whole showed highly significant (P = .001) positive changes in body composition (summing the increase in lean and decrease in body fat). On average, the chromium-treated group lost 4.2 pounds of fat accompanied by a 1.4 pound increase in fat-free mass. (The corresponding non-significant changes in the placebo group were 0.4 pounds and 0.2 pounds, respectively.) In the men alone (22% of the enrolled subjects), fat loss during chromium treatment averaged 7.7 pounds (versus 1.0 pounds during placebo). Although changes in body composition were greater in the 400 mcg (compared to the 200 mcg) and in the older (compared to the younger) groups, in neither case were these differences statistically important.

It was concluded that supplementation with a minimum of 200 mcg of chromium per day as chromium picolinate can lead to significant improvements in body composition irrespective of the age of the user.

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ANTIGLUCOCORTICOID EFFECTS OF DEHYDROEPIANDROSTERONESULFATE (DHEAS): SERUM LEVELS OF CORTISOL AND DHEAS IN NORMAL CONTROLS. *F. Leblhuber*, E. Windhager, J. Weber, F.X. Steinparz, F. Reisecker, and E. Dienstl,* Dept. of Gerontol., Wagner-Jauregg-Krankenhaus, 4020 Linz, Austria.

In 60 normal controls, 34 females (aged 18-81, mean 46, 2±21, 2) and 26 males (aged 21-81, mean 50, 7±15, 6) DHEAS and cortisol plasma levels were determined after an overnight fast, stored at -20°C and tested with the same assay.

Analogous to earlier findings (Sunderland *et al.*: Lancet, 1989) and our own pilot study (Leblhuber *et al.*: Lancet, 1990; Age, 1991) a strong correlation was found between age and DHEAS serum levels (r = 0, 79 for females, r = -0, 73 for males), while there was only mild decrease of cortisol serum levels with increasing age. Sapolsky *et al.* (Endocr Rev, 1986) have reported that chronic glucocorticoid administration leads to hippocampal damage in the rat. On the other hand, Flood and Roberts (Brain Res, 1988) have shown a protective effect of DHEAS on central nervous system. McIntosh and Berdanier (J Nutr, 1988) found that DHEAS antagonizes metabolic effects of glucocorticoids; thus, an appropriate measure of its antiglucocorticoid activity would be the cortisol/DHEAS ratio, by which subjects at risk for the neurotoxic effect of glucocorticoids could be identified.

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THE EFFECT OF AGE ON PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO COGNITIVE STRESS. *W.J. Chodzko-Zajko*, A.J. Caruso, and M.S. Sothmann,* Sch. of PERD, Kent State Univ., Kent, OH 44240.

We examined the effect of age on physiological (heart rate, blood pressure), behavior (response latency) and biochemical (catecholamine) responses to two levels of cognitive stress. Young (n = 20; mean age 23 yrs) and older (n = 20, mean age 68 yrs) adults performed the Stroop Task, a cognitive test in which subjects process stimuli under several different levels of cognitive demand. Two hypotheses were tested: (1) that highly complex (HC) cognitive processes are associated with heightened behavioral and physiological arousal when compared with less complex (LC) control conditions, and (2) that older subjects are more reactive than younger adults especially under HC conditions. Results revealed support for both hypotheses. The old group was significantly slower than the young in HC but not LC conditions. Older subjects exhibited elevated pressor responses when compared to younger subjects, particularly in the HC condition. Preliminary catecholamine analyses revealed markedly greater elevations in plasma epinephrine and norepinephrine in response to the HC task when compared with LC task. These data support the hypothesis that aging is associated with augmented sympathoadrenal reactivity to stressful cognitive tasks.

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TOO OLD FOR A TRANSPLANT? *P.G. Rosario*, S. Greenstein, R.S. Schechner, and V. Tellis,* Dept. of Surg., Bronx Lebanon Hosp., Transplant Prog., Montefiore Med. Ctr., Bronx, NY 10467.

Increased age alone has excluded geriatric patients from renal allograft transplantation. We present three cases in a series of 31 elderly patients to demonstrate that this is inappropriate.

Case 1. A 68-year-old male who had colonic resection, TURP, and appendectomy previously had two successive kidney transplants and is doing well on follow-up three years later.

Case 2. A 68-year-old male with a history of TURP, curative resection of a bladder papilloma, parathyroidectomy, goiter and hiatus hernia tolerated two successive kidney transplants well for over five years.

Case 3. A 64-year-old female with radiation nephritis following radiotherapy for lymphoma had a normal life after transplant, but succumbed to malignancy 10 years later.

Kidney transplant is a gift of life. It adds quality to years, eliminates the need for triweekly hemodialysis and gives more independence to the recipient. Previous major surgery, potentially curable cancer and even serious medical conditions need not be contraindications, and our experience has shown that age alone should not be an eliminating factor for patient selection.

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STABILITY IN ELDERLY MEN IN RELATION TO FOOTWEAR MIDSOLE HARDNESS AND THICKNESS. *J. McClaran* and S. Robbins,* Div. of Geriat. Med., Montreal Gen. Hosp., 1650 Cedar Ave., Montreal, Quebec H3G 1A4 Canada.

Many gerontologists believe that in older individuals stability during locomotion improves when they change from shoes with thin hard soles to footwear that have softer thick lightweight soles made of expanded polymer.

To examine this, we measured balance when walking in 25 healthy young geriatric subjects (mean age 69), when barefoot, and when wearing six pairs of shoes which were identical except for differences in midsole material hardness and thickness, which spanned the respective ranges available in current footwear. We used a balance beam method that requires subjects to walk 0.5 m/sec on an extruded aluminum beam of cross section 7.8 cm x 3.9 cm, and 9 m long, which rested on the floor. Falls from the beam (balance failures; BFS) were quantified.

Balance failure frequency varied significantly ($P<0.05$) in relation to footwear midsole hardness and thickness. Shoes with the softest midsoles (Shore 35; similar to many running shoes) were associated with the poorest stability (8.92 BFS/100m). Shoes with hard soles (Shore 75; similar to leather or hard rubber soled shoes) were among the best (6.51 BFS/100m). Thick soles (6.68 BFS/100m) offered better stability than thin soles (10.16 BFS/100m; $P<0.05$). Stability when barefoot (16.27 BFS/100m) was 17% less than with the shoe offering the least stability, and 271% worse than with the best shoe.

We conclude that physicians should exercise caution when recommending shoes with yielding midsoles to elderly individuals. Also, older men and women with a history of falls, or who are obviously unstable, should avoid barefoot locomotion. Further, these data suggest that footwear could be optimized in terms of stability during locomotion for use by older individuals.

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TESTING OF GEROPROTECTORS AND GEROPROMOTERS WITH THE CELL KINETICS MODEL. *A.N. Khokhlov,* Evolutionary Cytogerontol. Sector, Biolog. Faculty, Moscow State Univ., Moscow, 119899, USSR.

It is known that the "plateau" level (i.e., culture saturation density) of human fibroblast growth curve is lower the more the cell donor's age is, and is very low in progeria. Besides, it was shown that geroprotectors (factors retarding aging) heighten, and geropromoters (ones accelerating aging) lower, the "plateau." Our mathematical model based on the Verhulst-Pearl equation enables precise description of culture growth kinetics for different cell types. With the help of the model, we have found that a classical geropromoter, ionizing radiation, induces the decrease of Chinese hamster cell culture saturation density that is strictly proportional to a dose of treatment. As similar results were obtained for the alkylating agent thiophosphamide and low-frequency electromagnetic field, we related them to geropromoters. On the contrary, geroprotector–antioxidant epigid increased the "plateau" level on the growth curve, not only of human diploid fibroblasts, but also of *Acholeplasma laidlawii* cells. On the basis of these results and other related data, we conclude that cell culture saturation density is the best parameter for gerontological purposes from all ones evaluated with the model.

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LIPID PEROXIDATION AND ANTIOXIDANT DEFENSES AMONG AGED PEOPLE. *K.K. Reddy,* Dept. of Phys. Anthropol., S.V. Univ., Tirupati 517 502 India.

Serum lipid peroxides (SLP), antioxidants, obesity and dietary variables were studied in 340 individuals consisting of males and females of 60+ years. Nonsignificantly higher levels of SLP were observed in females than in males. Higher levels of antioxidants like ascorbic acid (Vit. C) and tocopherol (Vit. E) were observed in males and glutathione peroxidase (GPx) in females. SLP is significantly decreased in males ($P<.05$) and nonsignificantly increased in females with age. However, antioxidants are decreasing with age in both sexes, but the degree of decrease is high in females ($P<.05$). Antioxidants are positively correlated in males and negatively correlated in females with SLP. Mean obesity index and caloric intake is higher in females than males. The higher levels of SLP in the presence of reduced antioxidants may lead to degenerative diseases in the elderly, and the risk is high in females.

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THE INTERACTION BETWEEN HERMAPHRODITE FERTILITY AND LIFE SPAN. *A. Brooks, R. Wold, and T. Johnson,* Dept. of Psychol. and Inst. for Behav. Genet., Univ. of Colorado, Boulder, CO 80309.

An antagonistic relationship between life span and reproductive effort has been observed in several organisms and, as such, has been offered as a mechanism for the evolution of senescence. Here we examined this relationship between hermaphrodite life span and reproductive effort in the nematode *Caenorhabditis elegans.* We compared the life spans of mated and unmated hermaphrodites: Mated hermaphrodites produce significantly more offspring than unmated hermaphrodites. We observed no difference in the life spans of mated and unmated strains which manifest a wild-type life span. However, in strains which carry the *age-1* mutation (a mutation which results in an average 40% increase in life span), we did observe a decrease in the life spans of mated strains. From these data we conclude that an antagonistic relationship between life span and reproductive effort in *C. elegans* is specific to the life extension effects of the *age-1* mutation.

A second question of interest concerns the nature of this negative relationship. Our data suggest that the relationship is not linear, but that any increase in brood size, or perhaps any mating, leads to a detectable decrease in life span ($r = .17$, $p>.05$).

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REGIONAL AGE-RELATED CHANGES IN AMMONIA REGULATION OF PHOSPHATE-ACTIVATED GLUTAMINASE (PAG). *D.R. Wallace*[1] *and R. Dawson*[2], [1]Univ. Colorado Hlth. Sci. Ctr., Dept. Pharmacol., Denver, CO 80262 and [2]Univ. Florida, Dept. Pharmacodynam., Gainesville, FL 32610.

These experiments examined the regulation of PAG by ammonia in the cortex (TCX), striatum (STR) and the hippocampus (HIP) from 8-month-old (N = 9) and 28-month-old (N = 9) Fischer-344 rats. The PAG reaction was started by the addition of 50ul (25-50ug protein) to 950ul of assay buffer containing (in mM): 20 HEPES; 10 phosphate; 0.2 EDTA adjusted to pH 7.4 with 5N NaOH. The reaction proceeded for 15 minutes at 37°C and was terminated by the addition of 1ml of ice-cold 95% ethanol. Samples were stored at -20°C until glutamate production was quantitated using high-performance liquid chromatography. Addition of ammonia (0.5-1mM) to the incubation media resulted in significant reductions in PAG activity from both age groups in all brain regions. This inhibition was significantly attenuated in both the STR and TCX from aged rats. In the STR, attenuation occurred over ammonia concentrations of 01-1.0mM, whereas in TCX, inhibition was attenuated only at 0.5mM and 1.0mM. Ammonia inhibition was unchanged in the HIP from the aged group compared to adult rats. In adult rats, maximal inhibition in the STR was 50% followed by the HIP and TCX at 33% and 31% respectively. These findings may suggest that regional variations, or isozymes, of PAG exist. The Ki values for ammonia inhibition were significantly higher in the TCX (80%) and STR (50%), indicative of the lack of ammonia inhibition seen in these two regions from aged rats. Enzyme kinetics in the presence of set ammonia concentrations did not change the affinity (Km) of PAG for glutamine, but reduced the Vmax (21-42%) in all regions, indicative of noncompetitive inhibition.

These data suggest that ammonia regulation is altered in the STR and TCX of aged F344 rats. The attenuated inhibition seen in these studies may predispose these regions to damage from elevated glutamate and/or ammonia which would result from a lack of inhibition. There was also evidence of regional differences in ammonia inhibition, suggesting the possible existence of regional isozymes.

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AGE-RELATED ALTERATIONS IN BOTH PHOSPHATE AND CALCIUM REGULATION OF REGIONAL PHOSPHATE-ACTIVATED GLUTAMINASE (PAG). *D.R. Wallace*[1] *and R. Dawson*[2], [1]Univ. Colorado Hlth. Sci. Ctr., Dept. Pharmacol., Denver, CO 80262 and [2]Univ. Florida, Dept. Pharmacodynam., Gainesville, FL 32610.

These experiments examined the regulation of PAG by phosphate and calcium in the cortex (TCX), striatum (STR) and the hippocampus (HIP) from 6-8-month-old and 28-30-month-old Fischer-344 rats. The PAG reaction was started by the addition of 50ul (25-50ug protein)

to 950ul of assay buffer (37°C) and terminated after 15 minutes by the addition of 1ml of ice-cold 95% ethanol. Glutamate production was quantitated using high-performance liquid chromatography. Addition of phosphate significantly increased PAG activity in all three regions and in both age groups. In adult rats, PAG activity in the HIP (100 and 150mM) and in the STR (150mM) was significantly reduced compared to the activity in the TCX. In aged rats phosphate activation was unchanged in the TCX and STR, but significantly reduced in the HIP (50mM and greater). The TCX and STR were unaffected by the aging process, but activity in the HIP was significantly reduced in aged rats at phosphate concentrations of 25mM and greater when compared to adult rats. Additions of calcium resulted in significant increases in PAG activity in both age groups. At 1mM and 2.5mM calcium chloride, activity was significantly reduced in the STR and the HIP when compared to the TCX. A similar trend was seen in aged rats, but at 1mM PAG activity was significantly less than STR PAG activity. Significant reductions in activity were found only in the TCX (2.5mM), and the HIP (0.5 and 1mM) from aged rats compared to adult. Phosphate-independent calcium activation did not occur in either the TCX or the STR, but was apparent in the HIP. Addition of phosphate resulted in a synergistic activation of PAG in the STR and TCX, but this effect was barely additive in the HIP. These findings suggest that PAG undergoes differential regional regulation by both phosphate and calcium, and this regulation is impaired in aged Fischer-344 rats. These data also support the hypothesis that regional isozymes of PAG exist with different regulatory properties.

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MOVEMENT AND PHARYNGEAL PUMPING AS BIOMARKERS OF LONGEVITY IN *CAENORHABDITIS ELEGANS*. *S. Duhon and T. Johnson,* Inst. for Behav. Genet. and Dept. of Psychol., Univ. of Colorado, Boulder, CO 80309.

A biomarker of longevity is a behavioral, physiological, or biochemical measure that displays age-related changes which may predict longevity. These biomarkers could facilitate the work of screening for new Age (long-lived) mutants and establish additional criteria for the analysis of Age mutants. Here we examine two possible biomarkers of longevity in the nematode *C. elegans*.

The first is spontaneous movement. We are examining strains that carry a mutation in the *age-1* gene to determine if they display a linear decline and to determine the rate of decline. We have chosen five strains for these experiments: two have wild-type life span, and three are *age-1* mutants. Spontaneous movement was studied longitudinally on ten nematodes from each of these five strains. Three components of movement were monitored: forward movement, backward movement, and omega turns. We found that movement decreased linearly in all strains, but that an animal's rate of movement on one day could not be used to predict its movement later in life. Age strains have the same rates of movement at all chronological ages as do strains with wild-type life span, but show an extended period of low movement later in life.

The second biomarker is pharyngeal pumping, which is a measure of the amount of food ingested. Pumping was studied longitudinally with twenty animals from each of the five strains above. We found that pharyngeal pumping was only slightly different in the Age strains when compared to the wild-type strains.

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EFFECT OF CHRONIC CALORIC RESTRICTION IN RODENTS: PHYSIOLOGICAL ADAPTATIONS ASSOCIATED WITH A RAPID CHANGE IN THE LEVEL OF FOOD CONSUMPTION. *P.H. Duffy, R.J. Feuers, J.E.A. Leakey, and R.W. Hart,* NCTR, Jefferson, AR 72079.

Physiological variables were continuously monitored in 24-month-old male $B_6C^3F^1$ mice. In one test group, caloric restricted (CR) animals (60% of *ad libitum* [AL] were abruptly switched to an AL diet, and animals in a second test group that had been fed AL were abruptly switched to a CR diet. Physiological performance was monitored before and after the food ration was changed. The experiment was then replicated using the long-lived *Peromyscus leucopus* mouse (AL→CR). The purpose of this study was to compare the effects of acute and chronic CR in species with different longevities.

Generally speaking, physiological parameters changed more rapidly when food intake was switched from CR to AL than from AL to CR. The speed and magnitude of response was greater in the long-lived *Peromyscus leucopus* mice than in $B_6C^3F^1$ mice. The results of this study suggest that the effects of acute CR are similar to those for chronic CR in that both regimens reduce basal and average body temperature and alter metabolic pathways (RQ). However, mice appear to adapt to periodic deficits in metabolic substrates imposed by acute CR by lowering average motor activity and metabolic rate rather than reducing body mass as seen in chronic CR mice. The physiological changes reported here may relate to basic mechanisms by which CR modulates disease and longevity. The fact that CR affects basal metabolic and temperature levels, but not maximum levels, suggests that the rate of aging may be controlled by neuroendocrine systems via hormonal regulation.

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ACUTE AND LONG-TERM EFFECTS OF CALORIC RESTRICTION (CR) ON SERUM HORMONE PROFILES AND RESPONSIVE ENZYMES IN THE FISCHER 344 RAT. *S. Chen, J.E.A. Leakey, J.J. Bazare, Jr., J.R. Harmon, M. Manjgaladze, R.J. Feuers, P.H. Duffy, and R.W. Hart,* Div. of Reproduct. and Develop. Toxicol., Genet. Toxicol., and Office of Dir., NCTR, Jefferson, AR 72079.

Long-term CR in rodents results in increased longevity and a decreased rate of neoplasia. It has been established that CR procedures result in altered circadian rhythms of body temperature and other physiological parameters and in altered feeding behavior. This study was designed to investigate the effects of CR on the circadian profiles of serum hormones in 18-week-old (wk), day-fed rats that had been restricted to 60% of their control's *ad libitum* (AL) calorie consumption for 4 weeks, and in 9-month-old (mo), day-fed rats that had been similarly restricted for 6 months. Serum thyroxine (T4) concentrations were not altered by CR. In both AL and restricted 18-wk rats T4 circadian profiles showed peaks during the early light phase, followed by a sharp decline following feeding. In 9-mo rats, the circadian profiles were shifted so that the restricted rats were higher during the night and the AL rats were higher during the day. Circadian profiles for serum corticosterone (C) were markedly altered by CR. In 9-mo males, a peak occurred during the early light phase prior to feeding. In 18-wk males, a large peak occurred in the late light phase following food allocation. In both cases, the AL rats showed a classic C profile having a broad peak during the dark phase. Serum testosterone concentrations were determined in the 18-wk rats. Although there was a large variation in individual values, the restricted rat profile showed a significant decrease that was coincident with peak levels of C. These data suggest that CR-induced changes in hormonal profiles are apparent as early as 4 weeks after starting CR, but that phase shifting of profiles is not complete at this time.

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ACUTE AND LONG-TERM EFFECTS OF CALORIC RESTRICTION (CR) ON BLOOD GLUCOSE, SERUM INSULIN, AND HEPATIC GLYCOGEN PROFILES IN THE FISCHER 344 RAT. *J.J. Bazare, Jr., J.E.A. Leakey, J.R. Harmon, S. Chen, M. Manjgaladze, R.J. Feuers, P.H. Duffy, and R.W. Hart,* Div. of Reproduct. and Develop. Toxicol., Genet. Toxicol., and Office of Dir., NCTR, Jefferson, AR 72079.

It has been shown that long-term CR procedures result in altered feeding behavior in rats such that restricted rats (R) consume their food rapidly, as soon as it is allocated, whereas *ad libitum*-fed (AL) controls consume their food in a more relaxed manner during the dark. Thus, R rats require a capacity to maintain glucose homeostasis while receiving their caloric intake in short bursts. We have investigated the effects of CR on serum insulin, blood glucose, and hepatic glycogen concentrations and their relationship to hepatic intermediary metabolism. Day-fed, 18-week-old (wk) and 9-month-old (mo) rats were used. R rats received 60% of the AL rats' caloric intake from 14 wks. Blood glucose concentrations ranged from 65 to 90 mg/dl in both AL and R rats at both ages, but were significantly higher in 18-wk and 9-mo AL rats than in R rats during the early dark phase. Serum insulin was not significantly different between AL and R rats at either age

during the light phase, but was significantly higher in AL rats during the dark. Thus, AL rats require higher insulin concentrations to control blood glucose levels than R rats. Significant differences occurred between AL and R rats in hepatic glycogen content. For example, concentrations were 60 ± 3 and 26 ± 3 for AL and R 18-wk male rats, respectively, in the morning, prior to food allocation, and were 48 ± 4 and 76 ± 5 for AL and R rats, respectively, during the night. Thus, R rats exhibited a much greater flux of glycogen content than AL rats. These changes may be related to an increased responsiveness of enzymes, such pyruvate kinase, to activation/deactivation by insulin or glucagon, since such responsiveness is increased during CR.

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ACUTE AND LONG-TERM EFFECTS OF CALORIC RESTRICTION ON HEPATIC CONJUGATING ENZYME EXPRESSION IN THE FISCHER 344 RAT. *M. Manjgaladze, J.E.A. Leakey, J.R. Harmon, J.J. Bazare, Jr, S. Chen, R.J. Feuers, P.H. Duffy, and R.W. Hart,* Div. of Reproduct. and Develop. Toxicol., Genet. Toxicol., and Office of Dir., NCTR, Jefferson, AR 72079.

Conjugation with glucuronic acid, sulfate, and glutathione are the primary pathways by which lipophilic molecules are made polar and more readily excretable in bile or urine. The hepatic enzymes responsible for such conjugation, the UDP-glucuronyltransferases (UDPGT), the sulfotransferases (ST) and the glutathione-*S*-transferases (GSHT) exist as families of isoforms which exhibit distinct substrate specificities and independent regulation. The expression of several UDPGT, ST, and GSHT activities that are selective for individual isoforms were compared in *ad libitum*-fed and 40% calorically restricted rats. The effects of caloric restriction on the conjugating enzymes were dependent on age, sex, and isoform. For example, long-term caloric restriction in 9- or 22-month-old rats resulted in increased UDPGT activity towards bilirubin, but not towards 5-hydroxytryptamine or testosterone. GSHT activity towards dichloronitrobenzene was increased by caloric restriction in 22-month-old male rats, as was γ-glutamyltranspeptidase activity, but activity towards androstenedione was not. ST activity towards corticosterone was not significantly affected by caloric restriction in 22-month-old male or female rats, but was increased by caloric restriction in 9-month-old and 18-week-old male rats that had been restricted from 14 weeks of age. Conversely, ST activity towards corticosterone was decreased by caloric restriction in 18-week-old female rats. These data suggest that the effect of caloric restriction on the elimination of lipophilic drugs and other conjugating enzyme substrates will be dependent upon the age and sex of the organism used and the specific isoform that conjugates the compound.

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FOOD RESTRICTION PREVENTS AGING-INDUCED DECREMENTS IN BRAIN PHOSPHOINOSITIDE METABOLISM. *A.S. Undie* and E. Friedman,* Neurochem. Div., Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

Receptor-activated hydrolysis of [^{3}H]inositol-labeled phosphatidylinositide (PI) was evaluated in brain slices of 6-month-old (6M) and 24-month-old (24M) Fischer-344 rats. The dopamine D_1 receptor agonist, SKF38393, and the muscarinic cholinergic agonist, carbachol, significantly stimulated the accumulation of [^{3}H]inositol phosphates in hippocampal, striatal, and frontal cortical slices of 6M and 24M rats. However, the responses to either agonist were significantly lower in each of the 3 brain areas of the 24M rats, which suggests a decreased capacity of the aged brain to respond to receptor-activated PI hydrolysis. Examination of the labeling of PI with [^{3}H]inositol showed significantly lower maximal labeling of the phospholipids in the 24M animals. Hence, the decreased formation of inositol phosphates in the aged brain preparations may result from changes inherent in the inositol cycle as well as from possible aging-related alterations in the investigated receptors.

The effects of food restriction (40% calorie restriction) were studied by comparing agonist effects in 6M rats fed *ad lib.* (AL) or restricted diet (RD), 24M AL and 24M RD groups. Accumulation of inositol phosphates in slices from the 6M diet groups was not different. However, striatal or cortical slices of the 24M RD rats gave significantly higher responses to carbachol or SKF38393. In fact the responses in the 24M RD animals were not significantly different from corresponding responses in the 6M AL rats, implying complete prevention of the aging effect in the diet-restricted animals up to at least 24 months of age. These results indicate that aging is associated with marked decrements in the generation of inositide second messengers, and that the aging effect may be abated by diet restriction.

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EFFECT OF CALORIC RESTRICTION ON CYTOCHROME P450 (CYT P450) ISOFORM EXPRESSION IN FISCHER 344 RATS. *J.R. Harmon, J.E.A. Leakey, J.J. Bazare, Jr., S. Chen, M. Manjgaladze, R.J. Feuers, P.H. Duffy, and R.W. Hart,* Div. of Reproduct. and Develop. Toxicol, Genet Toxicol., and Office of Dir., NCTR, Jefferson, AR 72079.

The rat cyt P450s are a family of at least 20 isoforms that catalyze the oxidation of a wide range of xenobiotics and endogenous compounds. They are responsible for both detoxication and metabolic activation of many drugs and carcinogens and for the microsomal production of oxygen radicals. Altered expression of cyt P450 isoforms can, therefore, result in altered rates of drug pharmacokinetics, carcinogenic initiation, and free-radical induced tissue damage. Expression of cyt P450 isoforms was investigated in *ad libitum*-fed and 40% calorically restricted Fischer 344 rats at 18 weeks, 9 months, and 22 months of age, using isozyme-specific activities and immunoblotting techniques. Although caloric restriction resulted in only minor changes in total cyt P450 concentrations, large changes occurred in the expression of individual isoforms. For example, expression of cyt P450 2C11-dependent testosterone 2α- and 16α-hydroxylase activities was decreased to 40-60% of control activities in 18-week- and 9-month-old rats; however, caloric restriction increased these activities in 22-month-old rats, which normally express only low levels of cyt P450 2C11-dependent activity. Cyt P450 3A-dependent testosterone 6β-hydroxylase activity was also increased by caloric restriction in 22-month-old male rats. Cyt P450 2E1-dependent 4-nitrophenol hydroxylase activity was increased in both the 9-month-old and 22-month-old rats, but the increase was dependent upon circadian time-point. These alterations in cyt P450 isoform expression may play a major role in mediating the observed changes in genotoxic damage that occur in calorically restricted rats following exposure to chemical carcinogens.

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THE EFFECT OF CALORIC RESTRICTION ON REDUCTION POTENTIAL FOR MAINTENANCE OF FREE RADICAL DETOXIFICATION. *F. Chen* and R.J. Feuers,* Natl. Ctr. for Toxicol. Res., Jefferson, AR 72079.

It has been suggested that caloric restriction (CR) may produce some of its life span increasing effects by improving free radical detoxification. To investigate this possibility, we have evaluated the activities of three enzymes: glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and glutathione reductase (GR), in young *ad libitum* (AL) and CR Fischer 344 male and female rats at various times of day. In CR rats, both G6PDH and ME had lower activities when compared with AL rats. The Km of NADP for G6PDH was significantly lower in male and female CR rats. Even though the apparent G6PDH concentration was higher in AL rats, the actual level of activity at NADP concentrations similar to endogenous levels was significantly lower. The result for ME was similar to that seen in old (24M) rats. We also found that the activity of GR in both CR and AL rats did not show statistical differences, but the activity was always higher in CR rats. There were similar circadian rhythms in the activities of these enzymes, and maximums were several h after feeding. We also investigated the NADPH and NADH levels in the liver cytosol in these rats. The result displayed that both NADPH and NADH levels were higher in female rats, their maximum levels were correlated with food intake and there was a tendency toward increased levels in CR rats. All these important responses to CR may contribute to the mechanism through which CR enhances free radical detoxification, even at young age with short-term caloric restriction.

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EFFECTS OF DIET ON HYDROGEN PEROXIDE DETOXIFICATION IN FISCHER 344 RATS. *E.T. Oriaku* and R.J. Feuers,* Natl. Ctr. for Toxicolog. Res., Jefferson, AR 72079.

Caloric restriction (CR) is the only effective means known to increase maximum achievable life span and offset age-related degenerative diseases. Effect of CR on the activity of liver cytosol catalase (CAT) and glutathione peroxidase (GP), involved in hydrogen peroxide detoxification, was assessed in both male and female Fischer 344 rats. From 12 wks post partum, CR animals received 60% of the food (NIH-31 diet) consumed by the control group (AL) for 6 wks. CR animals received their food at 11:00 CST. Six animals/group were sacrificed every 4 h at 6 time points beginning at 0200 h. There were circadian patterns in the activities of both enzymes in both sexes and maximal activities were highest during the period of food intake. Effective activity (U/L x time to substrate inhibition) of CAT was significantly higher ($p<0.01$) from male and female CR groups at 1000 h and again at 1800 h for females in comparison to AL. This is consistent with our observations in mid- and old-age rats, and we suggest that CR slows the rate of accumulation of compound II (oxidized, inactive CAT), and maintains higher levels of this radical scavenger with age. We have also found that the reduction potential (G6PDH and NADPH for maintaining active CAT is much higher in male and female CR animals at this age. Even though no statistical difference due to diet was observed at other times, CR rats always had higher levels. Thus, we have shown that detoxification of H_2O_2 appears to be enhanced by CR at a very young age and after a very short period of restriction.

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INFLUENCE OF EXERCISE AND AGE ON MYOCARDIAL β-ADRENERGIC RECEPTOR PROPERTIES. *N. Turner*, D.T. Lowenthal, and P.J. Scarpace,* Geriat. Res., Educ. and Clin. Ctr., VAMC and Dept. of Pharmacol. and Therapeutics, Univ. of Florida, Gainesville, FL 32610.

The bradycardia following physical training may be mediated by an alteration in β-adrenergic receptor number or agonist affinity. We characterized the interaction between age and exercise on myocardial β-adrenergic receptor number and agonist affinity in 4- and 24-month-old female F-344 rats to test the hypothesis that the effects of training should be blunted in older rats. β-adrenergic receptor density was unchanged with age (16.2 ± 0.8 fmol/mg protein, 4 mo; and 16.1 ± 1.0, 24 mo) or training (16.1 ± 1.4, 4 mo; and 15.5 ± 0.9, 24 mo). The total number of receptors per heart increased by 56% with age due to increased ventricle weight. With training in the senescent rats the total number of receptors decreased by 32% due to a reduced amount of homogenate protein recovered from the ventricle, the significance of which is unknown. The receptor agonist dissociation constant for isoproterenol was determined in both the absence and presence of 1.5 mM β, γ-imidoguanosine 5'-triphosphate [Gpp(NH)p] and did not change with age or training (i.e., K_d = 0.243 ± 0.015 mM without Gpp(NH)p for young control). Approximately 60 percent of the receptors displayed high affinity binding for isoproterenol and this amount was unchanged with age or training. Neither training nor age influenced β-adrenergic receptor characteristics, suggesting that training bradycardia is not mediated by an alteration in β-adrenergic receptors.

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AGING AND ENZYME INDUCTION: EFFECT ON CHEMICALLY-INDUCED DNA REPAIR IN NON-PROLIFERATING RAT HEPATOCYTES. *J.G. Shaddock*, R.J. Feuers, and D.A. Casciano,* Natl. Ctr. for Toxicolog. Res., Jefferson, AR 72079.

Although it is unclear whether DNA repair changes with age, the accumulation of DNA damage in somatic cells has been proposed as one of the basic mechanisms underlying the aging process. Several studies have shown strong correlations between UV-induced DNA repair and species life span. The purpose of this study has been to measure the effect of aging and enzyme induction on the genotoxicity of four carcinogens, representing four different classes of chemicals, in the *in vitro* rat hepatocyte/DNA repair assay. Hepatocyte cultures were isolated from untreated young male Fischer (F344) rats and old male F344 rats which were either untreated or Aroclor-induced (ARO). Cultures from the untreated old rats, treated with 2-acetylaminofluorene (2AAF), aflatoxin B_1 (AFB_1), 7,12-dimethylbenz[a]anthracene (DMBA) and dimethylnitrosamine (DMN), exhibited age-dependent decreases in DNA repair of 75%, 49%, 40% and 48% respectively, when compared to untreated young rats. By contrast, cultures from ARO-induced old rats exhibited significant increases in DNA repair of 78%, 43%, 54% and 44% with 2AAF, AFB_1, DMBA and DMN, respectively, when compared to results from untreated old F344 rats. These data indicate that the observed age-dependent decrease in DNA repair in the untreated old F344 rats may not be directly related to the DNA repair process, but to the levels of hepatic metabolic activation enzymes responsible for the metabolism of potentially toxic chemicals.

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THE AGE-RELATED ABERRATIONS IN LIPID METABOLISM OF RAT ADIPOCYTES. *L.-T. Ho*, C.-Y. Cheng, and M.-S. Shiao,* Dept. of Med., Vet. Gen. Hosp., Taipei, Taiwan, R.O.C.

Adipocytes isolated from male Sprague-Dawley rats of old (O ~ 80 wk) and young group (Y ~ 8 wk) were used to study the impact of aging upon lipid metabolism. Lipogenesis (LG) was measured as the incorporation ratio of radioactivity of either [U-14C] glucose or [2-3H] acetate into triglycerides (TG). The released free fatty acids (FFA) and glycerol (G) were measured as an index of lipolysis (L), either at adenin deaminase primed basal (BL) or isopreterenol (ISO) or insulin (I) treated conditions. Adipocyte's I bindings, plasma glucose, FFA and IRI (immunoreactive I) were also measured. The results showed that the O group had (1) decreased basal as well as I stimulated LG (-I:O/Y=2.4%/6.6%; +I:3.6%/12.6%), (2) decreased I's anti-L (O/Y=13%/82% inhibition at 0.5 nM of I) (3) decreased ISO-stimulated L (O/Y: $IC50=10^{-7}$ M/10^{-8}M; Vmax=410 nM/640nM/ul cell), (4) higher plasma IRI (24±10/18±9 uU/ml) & lower FFA (394±141/517±155uM), & (5) lower adipocyte I binding (<10% of Y). It is concluded that aging is associated with aberrant lipid metabolism in adipocytes as shown by (1) I resistance in both LG & anti-L, probably via reduction of I receptor number, (2) decreased sensitivity and responsiveness to the L effect of ISO, and (3) drastic decrease of LG. The net effect may be reflected in the lower plasma FFA and higher IRI in the aged rats.

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IMMUNIZATION OF THE INSTITUTIONALIZED ELDERLY AGAINST PNEUMOCOCCAL INFECTION. *R. Ganguly*, T.B. Webster, J. Sinnott, and H. Chmel,* The Univ. of So. Florida, James A. Haley Vet. Hosp., Tampa FL 33612 and Bay Pines VA Med. Ctr., St. Petersburg, FL 33504.

Elderly residents (201, mostly male, ≥65 years old) from the VA nursing home care unit (NHCU) in Florida were surveyed by a questionnaire as to whether they were voluntarily immunized with pneumococcal vaccine in the past and what factors had affected their decision. These veterans had an average length of stay at the NHCU of 12 months and >70% suffered from chronic diseases with a past history of smoking. Approximately one-fifth of the residents (42 subjects, 20.9%) were immunized against pneumonia. The remaining 159 subjects were either not immunized (42.8%), uncertain of their immunization status (31.8%) or did not reply (4.5%). Lack of knowledge regarding the vaccine need and availability emerged as the major obstacle to vaccine compliance (55.2% of all important reasons given for nonimmunization). Health education intervention measures may be necessary to rectify the low vaccine acceptance rate among the institutionalized elderly who suffer from high death rates and complications from pneumonia.

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CYTOCHROME OXIDASE INHIBITION BY SODIUM AZIDE INFUSION IMPAIRS LEARNING IN THE MORRIS WATER MAZE. *M.C. Bennett and G.M. Rose,* Dept. of Pharmacol., UCHSC and VAMC, Denver, CO.

Cytochrome oxidase activity is significantly reduced in blood platelet mitochondria from Alzheimer's disease (AD) patients [Parker *et al., Neurol.,* 40, 1301 (1990)]. Previously, we reported that chronic and selective inhibition of cytochrome oxidase by sodium azide impaired

learning on two behavioral tasks and impaired hippocampal plasticity [Benet *et al., Neurosci. Abst., 16,* 1346 (1990)]. We now report that sodium azide treatment impairs learning in the Morris water maze.

Adult male Sprague-Dawley rats were implanted with Alzet osmotic minipumps (2ML4) containing 0.9% saline (CONTROL) or 160 mg/ml (400 µg/hr) of sodium azide (AZIDE) 7-21 days prior to training. Rats were given 4 daily trials for 7 days in a tank filled with opaque water maintained 24-25°C. Swim time to a hidden platform, which occupied a fixed position relative to extramaze cues, was the performance measure. The AZIDE rats exhibited overall poorer performance across trials [MANOVA main effect: $F(1,23) = 16.0$; $p<0.001$]. AZIDE rats were also impaired in performance on a probe trial retention test conducted on the 8th day of training ($t = 2.47$, $p<0.05$). First day performances did not differ significantly between groups ($t = 0.78$, NS), indicating that the deficit was not due to a motor impairment.

These results extend our previous findings and indicate that chronic cytochrome oxidase inhibition produces a generalized learning deficit. The present finding is consistent with the hypothesis that tonic infusion azide in rats models some characteristics of AD.

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THE *IN VITRO* EFFECTS OF VARIOUS HORMONES ON THE SYNTHESIS OF COLLAGEN AND NONCOLLAGEN PROTEINS IN THE ARTICULR CARTILAGE OF AGING MICE. *A. Weiss*, E. Livne, and M. Silbermann,* Rappaport Inst. for Med. Res., Fac. of Med., Technion, Haifa, Israel.

Age-related changes in the rate of synthesis of collagen (CP) and noncollagen proteins (NCP) in articular cartilage were studied. Mandibular condyles of male ICR mice, aged 1 to 12 months, were cultured for 48h in BGJb medium containing 10% ECS, ^{3}H-proline (5 uCi/ml) and 1-84 PTH (1 ug/ml), PGE_1 (10 ug/ml), PGE_2 (10 ug/ml) or dexamethasone (10 M). Purified bacterial collagenase was used for determination of the incorporation of ^{3}H-proline into CP and NCP; some specimens were used for autoradiography. A marked age-dependent decrease in the synthesis of collagen from 1 to 6 months (-64%, $p<0.01$), followed by an additional decrease of -77% at 12 months, $p<0.01$, was observed. The synthesis of NCP declined at a slower rate (-54% at 12 months). As a result, the relative synthesis of collagen declined from 28% at 1 month to 18% at 12 months of age. PTH caused a significant increase in both CP and NCP synthesis in young specimens; however, it had no effect on 6 months and older. PGE_1 caused specific increase in collagen synthesis at all ages, while dexamethasone caused a slight decrease of collagen synthesis only. The findings were substantiated by autoradiography. Hence, it is concluded that synthesis of collagen in chondrocytes declines at a higher rate than that of noncollagen proteins with aging, yet it can be stimulated by various hormones such as PTH and PGE_1.

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DIFFERENTIAL GENE EXPRESSION DURING THE ADULT LIFE SPAN OF *C. ELEGANS.* *T.J. Fabian* and T.E. Johnson,* Inst. for Behav. Genet. and Dept. of MCD Biol., Univ. of Colorado, Boulder, CO 80309-0447.

We are exploring age-dependent changes in gene expression during the adult life span of the nematode *Caenorhabditis elegans.* We have developed methods for culturing large numbers of synchronous adults to enable the recovery of age-specific RNA. These age-specific RNAs are being used to determine whether there are consistent age-dependent patterns of transcript abundance for a number of cloned genes. In addition, to directly identify any abundantly-expressed genes which exhibit altered expression during the adult life span, we differentially screened a cDNA library with cDNA probes from young adult and aged adult nematodes. Of the 67 clones recovered from this screen, 33 gave a stronger signal with the aged adult cDNA probe, ad 34 gave a stronger signal with the young adult cDNA probe.

We are currently analyzing the clones recovered in the differential screen to determine a) if their age-dependent expression is reproducible in independently-grown cultures, and 6) if this pattern is altered in strains with genetically-extended life spans. We will begin sequencing and mapping any clones passing these criteria. We will also attempt to clone, via subtractive hybridization, cDNAs for less-abundant transcripts which are differentially expressed with age, or which are differentially expressed between strains with wild type life spans and strains with genetically-extended life spans.

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PYRRALINE AND PENTOSIDINE AS BIOMARKERS OF AGING. *V.M. Monnier*, S. Miyata, D.R. Sell, S. Grandhee, and R. Nagaraj,* Inst. of Pathol., Case Western Reserve Univ., Cleveland, OH 44106.

Work from this laboratory led to the identification of two advanced Maillard Reaction products which form during normal aging and at an accelerated rate in diabetes.

Pyrraline is formed from glycated residues through a reaction involving 3-deoxy-glucosone. Poly- and monoclonal antibodies were used to localize pyrraline in histological tissue sections. Intense immunoreactivity was observed in the extracellular matrix (EC) of renal glomeruli and arteries that are sclerosed because of the diabetic and aging procces. Brain microvessels, lung bronchus wall and EC matrix collagen in general were positive for pyrraline which was also present in normal and diabetic plasma.

Pentosidine, a lysyl/arginine crosslink mediated by a pentose increases linearly in human dura mater, exponentially in skin and plateau-shaped in glomerular basement membrane to reach levels of 250,100, and 50 pmol/mg protein respectively. Tail tendon pentosidine levels increase with age in F344 rats and are decreased 30-40% in food-restricted animals (Collab. with Dr. E. Masoro).

Whereas only glucose can serve as pyrraline precursor, the origin of pentosidine *in vivo* is less clear. Recent studies revealed that not only pentoses, but also hexoses, ascorbate and other carbohydrates can form pentosidine. A high correlation between pentosidine and the degree of lens pigmentation and crosslinking was uncovered (Collab. with Dr. B.J. Ortwerth) which is highly suggestive for a role of ascorbate in the aging of the human lens. These data emphasize the growing role of the Maillard reaction in the aging process. The notion that "glycoxidation" or "auto-oxidation" modulates the Maillard reaction provide important new insight into the free radical theory of aging.

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STRUCTURAL/FUNCTIONAL CHANGES OF MATRIX PROTEINS BY GLYCATION. *A. Charonis*, N. Kouzi-Koliakos, C. Haitoglou, S. Anderson, and E. Tsilibary,* Dept. of Lab. Med. and Pathol., Univ. of Minnesota Med. Sch., Minneapolis, MN 55455.

Laminin and type IV collagen are two major basement membrane glycoproteins. We have performed *in vitro* nonenzymatic glucosylation of these macromolecules and examined their structural and functional alterations. Nonenzymatic glucosylation of laminin created major shape alterations and impaired its ability to polymerize at two levels: the formation of long-to-long arm dimers (first step) and the formation of large aggregates (second step). Aminoguanidine prevented these structural and functional changes. Nonenzymatic glucosylation of type IV collagen reduced its ability to laterally associate and polymerize; also, domaine NC1 of type IV collagen lost its ability to interact with the triple helical domain of type IV collagen. Both macromolecules promote the adhesion of endothelial and mesangial cells. Nonenzymatic glucosylation resulted in impairment of this function, even at very low levels of glucose incorporation. Isolated kidney basement membranes were examined after incubation in the presence of high glucose. It was observed that glucose generated crosslinks (observed by gel electrophoresis) and local openings in the structure (observed by low voltage scanning electron microscopy). It is concluded that matrix macromolecules undergo important structural and functional alterations due to nonenzymatic glucosylation.

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DNA GLYCATION AND AGING. *A.T. Lee* and A. Cerami,* The Rockefeller Univ., New York, NY 10021.

The nonenzymatic reaction of reducing sugars such as glucose and glucose-6-phosphate with the amino groups of proteins occurs both *in vitro* and *in vivo* to form permanent modifications. The extent of glycation is dependent on sugar concentration and period of exposure to the protein. Early glycation products progressively rearrange

to form a series of crosslinking moieties referred to as Advanced Glycation Endproducts (AGEs). It has been shown that reducing sugars are capable of reacting with the amino groups of DNA bases in an analogous manner. The *in vitro* modification of DNA by glucose or glucose-6-phosphate results in significant alterations of both physical and biological properties of DNA. In a bacterial model system, it was shown that an increase in mutations of a target plasmid occurred as the result of *in vivo* exposure to elevated glucose 6-phosphate levels in glycolytic mutants of *E. coli*. An intermediate formed in the reaction of glucose-6-phosphate and lysine was found to react with DNA to form stable DNA adducts *in vitro* which also resulted in plasmid DNA mutations. Analysis of the mutated plasmids observed from both *in vivo* and *in vitro* reactions showed them to be the result of gross DNA rearrangements. The glycation of DNA by reducing sugars directly or through an intermediate can result in DNA damage and mutation and their accumulation may contribute to a number of genetic abnormalities associated with aging.

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ADVANCED GLYCATION AND HYPERTENSION IN AGING: ROLE OF NITRIC OXIDE. *R. Bucala and A. Cerami,* The Rockefeller Univ., New York, NY 10021.

The prevalence of hypertension increases with age and high blood pressure is the single greatest risk factor for cardiovascular disease. The precise etiology of the increased risk of hypertension with age is unclear. Important insight into the pathophysiology of hypertension has been achieved recently with the identification of nitric oxide (NO) as endothelium-derived relaxing factor (EDRF). Nitric oxide is released by endothelial cells and signals smooth muscle cells to relax, thus maintaining normal vascular tone. Recent studies indicate that the pathway of NO action is specifically inhibited by the accumulation of advanced glycation products in the vascular wall. These products, called AGEs (advanced glycosylation endproducts), are the terminal adducts of the glycation reaction between glucose and long-lived proteins. AGEs accumulate with age on vascular wall collagen and form at an accelerated rate in patients with diabetes. Advanced glycosylation endproducts inactivate NO via a direct chemical reaction and this inactivation contributes to the impaired vasodilatory responses which occur in diabetes. Impaired vasodilatation occurs in normal aging as well, and may contribute to the pathogenesis of age-related hypertension. Ongoing investigations are addressing the hypothesis that NO inactivation by vascular wall AGE plays a role in age-related hypertension. Nitric oxide also has been found to be an intracellular messenger in a variety of other organs such as the immune and the central nervous systems. The inactivation of NO by AGEs may lead to a generalized, age-associated decline in cell communication and contribute to aging on a multicellular level.

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***IN VIVO* ADVANCED GLYCOSYLATION-INDUCED VASCULAR PERMEABILITY AND MONOCYTIC MIGRATION IS INHIBITED BY AMINOGUANIDINE.** *H. Vlassara*[*1], *H. Fuh*[1], *Z. Makita*[1], *and S. Morgello*[2], [1]Lab. of Med. Biochem., The Rockefeller Univ., New York, NY 10021 and [2]Dept. of Pathol., Mount Sinai Sch. of Med., New York, NY.

Irreversible crosslinking Advanced Glycosylation Endproducts (AGE) are implicated in aging and diabetic vascular disease. In both conditions atherosclerotic changes are preceded by vascular permeability and monocytic migration. Monocyte/macrophages contain AGE-specific receptors which allow a) selective transendothelial migration toward matrix AGE *in vitro*, b) endocytosis and removal of AGE proteins, and c) macrophage activation and elaboration of proliferative cytokines/growth factors, which may contribute to tissue damage. Using an AGE-specific radioreceptor assay, we confirmed that rat arterial AGE levels increase as a function of age, consistent with an age-dependent increase of AGE in human coronary arteries. We now provide *in vivo* evidence indicating that AGEs are capable of inducing both increased vascular permeability and monocytic infiltration in tissues of healthy young rats. Following the injection of AGE-rat serum aslbumin (RSA) for 14 days (100 mg/day, i.v.), increased vascular permeability was observed in most tissues. In addition, marked perivascular mononuclear infiltration of hepatic vessels, heart, and brain was noted, compared to native RSA-treated, and negative control animals. Simultaneous treatment with the AGE-crosslinking inhibitor Aminoguanidine (60 mg/kg/day, i.p.) abolished these indings. In conclusion, several pathologic changes consistent with early atherosclerosis can be duplicated *in vivo* by Advanced Glycation Endproducts, independently of biological aging, or lipid/glucose abnormalities.

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PHARMACOLOGIC INTERVENTION OF NONENZYMATIC GLYCATION. *M. Brownlee.* Abstract appears on page 146.

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ACCUMULATION OF GLYCOXIDATION PRODUCTS IN SKIN COLLAGEN IN AGING AND DIABETES. *D.G. Dyer, J.A. Dunn, J.A. Blackledge, T.J. Lyons, D.R. McCance, S. Thorpe, and J. Baynes**, Univ. So. Carolina, Columbia, SC 29208; Med. Univ. So. Carolina, Charleston, SC 29425; Royal Victoria Hosp., Belfast, Northern Ireland BT12 6BA.

Glycation is the first step in the Maillard or browning reaction between sugars and proteins. In addition to fructoselysine (FL), other products of this reaction identified in tissue proteins include N^{ϵ}-(carboxymethyl) lysine (CML) and pentosidine (P). CML and P are termed glycoxidation products because they are formed in protein by sequential glycation and oxidation reactions. To assess the role of glycation and glycoxidation reactions in chemical modification of proteins in aging and diabetes, FL, CML and P were measured in skin collagen from control and diabetic subjects. FL increased 33% between ages 20 and 80 in control subjects. CML, P and Maillard-type fluorescence (Ex/Em = 325/375 nm) increased ~ 5-fold (500%) and correlated strongly with age. FL (~ 5 mmol FL/mol Lys in control collagen) was increased ~ 3-fold in diabetes. CML, P and fluorescence also increased up to 2-fold in diabetes, reaching 2 mmol CML/mol Lys and 0.1 mmol P/mol Lys in collagen from aged diabetic patients. There were strong correlations between CML, P and fluorescence in both control and diabetic groups, providing evidence of age-dependent glycoxidative damage to skin collagen via the Maillard reaction and acceleration of this process in diabetes. We conclude that glycoxidation products should be useful as biomarkers of age-dependent and diabetes-related glycative and oxidative damage to tissue proteins.

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TREATING HYPERTENSION IN THE ELDERLY. *M. Moser,* Yale Univ. Sch. of Med., New Haven, CT 06510.

A review of all of the clinical trials indicates that pharmacologic treatment of systolic/diastolic hypertension in patients 60 years of age or older improves outcome and reduces cardiovascular complications. An initial three to six months of non-drug therapy should be tried in most cases, but if this fails, antihypertensive drug therapy should be instituted. Diuretic monotherapy in small doses will lower blood pressure to goal levels in a high percentage of elderly patients. Combination therapy, using small doses of a beta-adrenergic inhibitor, calcium blocker, or angiotensin-converting enzyme inhibitor with a diuretic, may increase the number of responders. THe recently reported findings of the SHEP study also indicate that therapy of isolated systolic hypertension is beneficial. Stroke deaths were reduced by 36% with a decrease of 27% in total fatal and non-fatal CHD events in treated compared to placebo subjects. In general, the elderly subject tolerates therapy well without a major effect on quality of care. Dosages of medications should be kept as low as possible.

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ANTIHYPERTENSIVE THERAPY: VA TRIALS ON HYPERTENSION IN THE ELDERLY. *W.C. Cushman.* VA Coop. Study Group on Antihypertensive Agents, VA Med. Ctr., Memphis, TN 38104.

The VA Cooperative Study Group on Antihypertensive Agents has completed clinical trials demonstrating that diastolic and isolated systolic hypertension (HTN) in older individuals can be effectively and safely treated with various antihypertensive agents. In 690 patients with diastolic HTN, 50% achieved goal blood pressure on 25-50 mg of hydrochlorothiazide (HCTZ) and 58% on 50-100 mg (p=0.1). The addition of a second agent controlled over 70% of the nonresponders, so that over 85% achieved goal blood pressure on a one or two drug diuretic-based regimen. Side effects were minimal with HCTZ, reserpine, metoprolol or hydralazine, but were frequent with methyldopa. Cognitive–behavioral function was not adversely affected with any of these agents. Of particular note, reserpine was associated with no increase in adverse complaints nor with any deterioration in mean depression scores. Low doses of HCTZ were nearly as effective as standard doses, but caused less biochemical changes. The addition of hydralazine reduced glucose, cholesterol, and triglycerides; reserpine and methyldopa also reduced cholesterol, and metoprolol increased potassium and triglycerides. In isolated systolic HTN (n=51), goal blood pressure was achieved without any significant incidence of adverse effects with low (78%) or high dose HCTZ (p=0.4), but the lower dose had less hypokalemia. These data demonstrate that a substantial proportion of older patients with HTN may have their blood pressure controlled with an inexpensive, well tolerated, and convenient regimen.

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ANTIHYPERTENSIVE MEDICATIONS. *J. Tuckman,* Bronx VA Med. Ctr., Bronx, NY 10468; Dept of Geriat., Mount Sinai Sch. of Med., New York, NY 10029.

Large long-term clinical trials of antihypertensive treatment in the elderly have shown important reductions in stroke, smaller decreases of coronary artery disease complications and few side effects. These trials used diuretics, beta blockers and methyldopa, However, many workers now suggest that alpha blockers, ACE inhibitors or calcium channel antagonists are the preferred drugs as they cause fewer metabolic and electrolytic disturbances, less CNS effects and theoretically might have a more beneficial effect on coronary artery morbidity and mortality. Within this context, the use of the six major groups of antihypertensive medications will be discussed. It will be concluded that all of the drugs have a significant place in the treatment of hypertension in the elderly, and importantly, that low dose diuretic therapy should play a major role in initial and combined therapy.

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RESEARCH DIRECTIONS FOR ANTIOXIDANT VITAMINS AND AGING. *J. Blumberg*,* USDA Human Nutrition Research Center on Aging, Tufts Univ., Boston, MA 02111.

The principal value of the antioxidant vitamins (AV) appears based in their ability to restrict the damage of reactive free radicals on cellular components thereby affecting the rate of aging processes and the initiation/promotion of a variety of chronic diseases. AV status may partially determine the rate of aging as suggested by the positive correlations of carotenoid and α-tocopherol tissue concentrations with life span in mammals. Similarly, resistance of tissues to spontaneous autooxidation and oxidative damage to DNA correlate inversely with life span. AV differentially display antimutagenic, chemopreventive and immunoenhancing properties which may underlie a significant component of the reduced risk of cancer in those with high dietary intakes. The reduced rate of oxidative modification of LDL-cholesterol by AV may partially explain their protective value against atherosclerosis and ischemic heart disease. The cataractogenic effect of oxyradicals also appears to be thwarted by AV. Emerging evidence suggests a beneficial action of AV in arthritis, hypoxic reperfusion injury, Type II diabetes and Parkinson's disease. Future AV research must focus both on their differential mechanisms and their application as prophylactic/therapeutic agents in slowing the rate of aging and the onset of associated chronic diseases.

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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 the decline in cognitive function, ranging from "benign senile forgetfullness" to full-blown dementing illnesses like Alzheimer's or Parkinson's diseases. Mechanisms of memory and normal cognitive function are briefly reviewed, as well as the most likely causes of age-related cognitive impairment (to include alterations in neurotransmitter levels, decrements in cerebral blood flow and neuronal metabolism, and accumulation of neurotoxic substances).

Many cognitive-enhancing agents are now approved by pharmaceutical regulatory agencies around the world, and over 100 additional substances in this class are in various stages of development and evaluation. Experimental and clinical studies for many of these agents are reviewed, including Hydergine, Cognex (THA), the nootropics (piracetam, oxiracetam, aniracetam, and primaracetam), vinpocetine, acetyl-L-carnitine, vasopressin, idebinone, nimodipine, pyritinol, sabeluzole, and others. Indications for use, side effects, dosages, and availability are outlined.

The growing phenomenon of the unapproved use of these agents by "normal" individuals for cognitive enhancement and prevention of age-related cognitive impairment, as well as the legal and ethical implications of such use, are discussed.

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NUTRITIONAL MODULATION OF AGING PROCESSES: HAVE WE TURNED THE CORNER FOR INTERVENTION? *D.K. Ingram,* Nathan W. Shock Labs., Gerontol. Res. Ctr., Natl. Inst. on Aging, Baltimore, MD 21224.

Epidemiological research linking specific nutrients to risk for developing age-related disease, such as heart disease and cancer, has been well established now at the end of the 20th century. In turn, experimental research in laboratory animals continues to gain momentum in demonstrating that a wide range of age-related physiological changes can be modulated by caloric intake in addition to the delay or prevention of age-related diseases. Many questions can be raised in regard to whether these two lines of research are beginning to converge in considering that caloric intake may be a primary environmental factor governing aging processes? Books have been written proposing that caloric restriction can retard aging in humans. Do we have enough data to reach this conclusion? What additional studies are needed? Is the food industry responding to this possibility as they responded to the need for nutritional supplementation in the early part of this century? How might this area of research impact upon our dietary habits in the next century? Have we turned the corner for intervention? These and other questions are to be addressed in this overview of the nutritional modulation of aging processes.

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EXERCISE AND AGING. *R.A. Fielding,* Human Physiol. Lab., USDA Human Nutr. Res. Ctr., Tufts Univ., Boston, MA 02111

Human aging is associated with an increased incidence of several chronic diseases including coronary artery disease, non-insulin dependent diabetes mellitus and osteoporosis. Relating to this increased disease risk are age-related changes in body composition that include an increased body fat mass and a progressive decline in skeletal muscle mass. Our laboratory has been investigating the effects of physical activity and exercise training on the body composition and functional capacity of older men and women for the past eight years. Results from our studies indicate that aerobic exercise training can increase maximal oxygen consumption approximately 25% in men and women aged 60 to 75 yrs following 12 wks of cycle ergometer training (75% VO2 max). In addition, our studies have shown that older men and women up to age 99 yrs have the capacity to improve dynamic muscle strength and muscle size (hypertrophy) in response to heavy resistance training (80% of the one repetition maximum). The results of our work suggest that older individuals are responsive to exercise training interventions. In addition, exercise training programs may be a suitable strategy for reducing the prevalence of age-related chronic disease.

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PHARMACOLOGIC INHIBITION OF THE MAILLARD REACTION. *M. Brownlee, D. Edelstein, H.-P. Hammes, I. Giardano, and E. Mullukondov,* Diabetes Res. Ctr., Albert Einstein Coll. of Med., New York, NY 10021.

The accumulation of advanced glycosylation products (AGPs) in diabetic tissues has been used as a model of glucose-induced aging. The prototype inhibitor, aminoguanidine HCl, effectively inhibits AGP formation. Aminoguanidine's mechanism of action involves reaction with soluble sugar-derived compounds. *In vitro,* aminoguanidine effectively inhibits AGP-induced matrix abnormalities and AGP–matrix induced defects in cell adhesion and proliferation. *In vivo,* aminoguanidine treatment prevents diabetes-induced increases in retinal vascular permeability, AGP formation, the pathologic development of retinal microaneurysms and an 18-fold increase in acellular capillaries. In diabetic kidney, aminoguanidine treatment decreases glomerular basement membrane AGP content, reduces basement membrane thickening and mesangial expansion, and reduces urinary albumin excretion to near-normal values. In diabetic peripheral nerve, axonal atrophy, motor and sensory nerve conduction velocities, and vasa nervorum blood flow are also normalized in animals treated with aminoguanidine.