AMERICAN AGING ASSOCIATION

18th Annual Meeting

and

AMERICAN COLLEGE OF CLINICAL GERONTOLOGY

3rd Annual Meeting

October 5-8, 1988
Sir Francis Drake Hotel
San Francisco, California

Annual Symposium: "Estimation of Human Physiologic Age"

Dean, W.: **Biological aging measurement in perspective: a concept whose time has come.**

Regelson, W.: **Aspects of quantitation of human aging.**

Elder, W.J.: **Life insurance and biological aging.**

Robbins, L.C.: **Health age assessment.**

Hutchins, E.: **A computer program to measure health risk.**

Short, R.A.: **Measuring the rate of biological aging in pig-tailed macaques: a model for all primates.**

Nakamura, E.: **Statistical approach for the assessment of biological age.**

Heikkinen, E.: **Variations in parameters of aging, their sources, and the best predictors of physiological aging.**

Hochschild, R.: **Ranking of 12 biomarkers of aging for association with ten mortality risk factors.**

Ruiz-Torres, A.: **Estimation of biological age using few parameters to compare aging of human populations.**

Ries, W.: **Determination of human biological age.**

Hershey, D.: **Excess entropy production as a biomarker in longevity analysis.**

Robinson, A.B.: **Quantitative measurement of human physiological age by profiling of body fluids and pattern recognition.**

Monnier, V.M.: **Collagen aging as parameter for estimation of human physiologic age.**

Balin, A.K.: Skin as a biomarker for aging.

Zimmerman, J.: **DHEA with aging.**

Ames, B.: **Measuring oxidative DNA damage in man.**

Cutler, R.: **Oxidative DNA damage in humans as a function of aging.**

Morgan, R.: **Two decades of research and practice with adult growth examination, a brief standardized test of adult aging: the 20th year progress report.**

Earle, R.C.B.: **Unpublished data from a large cohort longitudinal index validation study (morbidity).**

Brant, L.J.: **Age differences in biological markers of mortality.**

Weale, R.: **Light years — and retinal senescence.**

Chodzko-Zajko, W.J.: **Multivariate approach to the quantification of physiologic age.**

Minisymposium: "Gerontology in the New Millenium — The Life Extension Sciences"

1. Packer, L.: **Oxidant and antioxidant hypothesis of aging.**
2. Cole, G.M.: ***In vitro* model systems for Alzheimer's disease.**
3. Vernadakis, A.: **Pharmacological approaches to the aging brain.**
4. Sternberg, H.: **Longevity and nutritional restriction.**
5. Stice*, S.L., and Robl, J.M.: **Cloning of mammals using nuclear transplantation.**
6. Safar, P.: **Resuscitation of the elderly.**
7. Segall*, P.E., and Sternberg, H.: **Breaking the ice: low temperature medicine in gerontology and geriatrics.**
8. Marsh, R.P.: **Ethical implications of life extension.**

Minisymposium: "Molecular Aspects of Aging Research"

Cristofalo, V.: **Growth factor regulation of cellular senescence.**

Bowman, B.: **Expression of the human transferrin gene during aging.**

Johnson, T.: **Genetic analyses of longevity and senescence.**

Bruce, S.: **Differential gene expression during aging in the Serian hamster model.**

Norwood, T.: **Regulation and distribution of DNA polymerase-in senescent human fibroblasts.**

Minisymposium: "The Effect of Age on Neuroplasticity"

1. Flood, D.: **Presence and absence of neuronal plasticity of dendritic trees in different regions of the aging brain.**
2. Bertoni-Freddari, C.: **Synaptic alterations in aging and in senile dementia of the Alzheimer type: an ultrastructural perspective.**
3. Diamond, M. C.: **The influence of the environment on neuronal plasticity in the aging cortex.**
4. Stein, D.: **Behavioral plasticity in development and aging.**
5. Mervis, R. F.: **The effects of various choline-containing diets on dendritic plasticity in the adult and aging rodent neocortex: a potential intervention strategy for the senescent brain?**

Clinical Gerontology

Podolsky, S.: **Pathogenesis, prevention, and treatment of diabetes mellitus, type II.**

Marcus, R.: **Osteoporosis.**

Benedetto, M.: **Aging of the eye.**

Balin, A.K.: **Aging of human skin.**

Luncheon

Luncheon Speaker:
Morton Rothstein, Ph.D.
"Progress in Aging Research"

Annual Awards:
Research Award —
Morton Rothstein, Ph.D.
"Morton Rothstein, Ph.D., is the 1988 recipient of the Research Award. This award is presented to Dr. Rothstein for his significant contributions to biomedical aging research. These contributions include: 1) development of the free-living nematode as a model for aging studies; 2) extensive studies on the effect of age on enzymes; for example, he showed that the "young" and "old" forms of enolase were conformational isomers, and 3) his work contributed to the conclusion that advancing age does not result in "errors" in newly formed protein. In addition, we are indebted to him for his widely read book published in 1982, entitled *Biochemical Approaches to Aging.*"

Walter R. Nicolai Prize in Biomedical Gerontology —
Karen D. Parfitt
"Karen D. Parfitt is the 1988 recipient of the Walter R. Nicolai Prize in Biomedical Gerontology. This prize is presented to Ms. Parfitt for her manuscript, "Age-Related Electrophysiological Changes in Cerebellar Noradrenergic Receptors."

Submitted Papers
Oral Presentations

9. Kleinsek*, D.A.: **Elevation of fibronectin mRNA levels during cellular senescence *in vitro*.**
10. Fleming, J.E., Orr*, P.L., Shibuya, R.B., and Bensch, K.G.: **Hydroxyl radical scavenging in *Drosophila melanogaster*.**
11. Dixon, L. K.: **Strain differences in levels of peroxidase during aging in *Drosophila melanogaster*.**
12. Kalra*, J., Chaudhary, A.K., Cunningham, T.C., and Prasad, K.: **Effect of aging on oxygen free radical producing activity of polymorphonuclear leukocytes.**
13. Recasens*, J.F., and Green, K.: **The effects of age and endotoxin administration on superoxide dismutase activity in the rabbit iris.**
14. Balin*, A.K., Reenstra, W., Mathew, A., and Anzelone, M.J.: **Proliferative lifespan of sequential primary outgrowths of human fibroblasts derived from a single skin biopsy.**
15. Massie*, H., and Sternick, S.: **Calcium and calmodulin changes with aging in Drosophila and mice.**
16. Mesco*, E.R., Sternberg, H., and Timiras, P.S.: **Immunological identification of tau protein in neuroblastoma cells.**
17. Koyal*, S.N, Williams, A.J., Santiago, S, Ellestad, M.H., and Schwartz, D.: **Can biological age be assessed by maximal oxygen uptake?**

Poster Session I

18. Koltover, V. K.: **Stimulation of transcription in mouse liver by nitrogen oxide free radicals.**
19. Tatariunas, A.B.: **Microspectrofluorimetric characteristics of lipofuscin granules in termites *Anacanthotermes Ahngerianus Jacobson.***
20. Gertz*, H.-J., Lowes-Hummel, P., Ferszt, R., and Cervos-Navarro, J.: **The nucleus basalis of Meynert revised: nerve cell number decreases with age.**
21. Nava*, P.B., and Rosario, J.A.: Effects of age and diabetes on murine taste buds.
22. Liu*, C.C., and Howard, G.A.: **Physiological levels of estrogen stimulate bone formation and inhibit bone resorption in ovariectomized (OVX) mature mice.**
23. Fierabracci, V., Novelli, M., Del Roso, A., De Tata, V., Bombara, M., Masiello, P., and Bergamini*, E.: **Effect of age and diet on insulin secretion by rat isolated pancreatic islets.**
24. Slavin*, B.G., and Lerner, S.: **Immunohistochemical studies of A and D cells in pancreatic islets of aged mice.**
25. Wang*, P.S., Wang, W.-C., Liu, J.-Y., Hwang, C.-Y., Hwang, C., Day, C.H., Pan, J.T., and Ho, L.-T.: **Aging effect on the secretion of gastric inhibitory polypeptide.**
26. Riley, P.A.: **Senescence is the consequence of restricted selection in multicellular organisms.**

26a. Busbee, D.L.: **Age-related changes in expression of DNA polymerase α .**

26b. Sell*, D.R., and Monnier, V.M.: **Structure elucidation of a fluorescent crosslink from aging human extracellular matrix.**

26c. Marotta, C.A., Chou, W.-G., Majocha, R.E., Rehman, S., Watkins, R., and Zain*, S.B.: **Cells transfected with the amyloid gene and cloning of amyloid and glial fibrillary acidic protein variants of Alzheimer brain.**

26d. Tate-Ostroff*, B., Majocha, R.E., and Marotta, C.A.: **Distribution of Subdomains of the Amyloid Precursor Protein in Human Brain Tissue.**

Poster Session II

27. Cavallini, G., De Tata, V., Pollera, M., Gori, Z., and Bergamini*, E.: **Age-related changes in specific heart atria granularity in the rat.**
28. Sharma*, B.S., Jolley, W.B., Revankar, G.R., and Robins, R.K.: **Potentiation of human T cell mediated immune responses by a novel nucleoside N10586.**
29. Pickart*, L., Bianne, G., Borel, J.P., Kalis, B., Leutenegger, M., Maquart, F.X., and Salagnac, V.: **Iamin: a human growth factor with multiple skin healing properties.**
30. Engelberg, H.: **Low-dose intermittent heparin therapy decreases plasma fibrinogen levels.**
31. Young, Jr.*, R.C., Rachal, R.E., and Matthew-Thompson, L.: **Pulmonary tuberculosis in the elderly.**
32. Tsai*, T.-P., Chaux, A., Kass, R.M., Blanche, C., Gray, R.J., and Matloff, J.M.: **Coronary artery bypass surgery in the elderly.**
33. Leaf*, D.A., and Parker, D.L.: **Is age a coronary artery disease risk factor?**
34. Galili*, U., Macher, B.A., and Shohet, S.B.: **Evolutionary aspects of the antibody recognition of aging human red cells.**

35. Weiss*, A., Arbell, I., and Silbermann, M.: **Age-related changes in the activities of alkaline and acid phosphatases in femoral condyles of female CW-1 mice and their correlation to changes in bone mass.**

36. Ganguly*, R., Saba, S., Chmel, H., and Pancholy, S.B.: **Age-mediated changes in prostacyclin production by rat lung macrophages.**

Submitted Papers
Oral Presentations

37. Gertz, H.-J.: **The septo-hippocampal pathway in dementia of Alzheimer's type: evidence of neuronal plasticity.**

38. Aloyo, V.J., Vaidya, A.H., and Walker*, R.F.: **Forskolin-modulated serotonin (5HT) release in hypothalamic tissue from young and old male rats.**

39. Bertoni-Freddari*, C., Fattoretti, P., Meire-Ruge, W., and Ulrich, J.: **Increased intranuclear ionic strength in rat hippocampal pyramidal cells during aging.**

40. Mokrasch, L. C.: **Transport of acetylcholine precursors into fibroblasts of Alzheimer's victims and normals is modulatable.**

41. Mervis*, R.F., Bedo-Wierdl, M., and Arendash, G.W.: **Long term nucleus basalis lesions produce an increase in dendritic branching of neocortical pyramidal cells in the aging rat: a quantitative golgi study.**

42. Parfitt*, K.D., and Bickford-Wimer, P.C.: **Age-related electrophysiological changes in cerebellar noradrenergic receptors.**

43. Moss*, M.B., and Rosene, D.L.: **Behavioral and anatomical studies of the limbic system in aged rhesus monkeys.**

44. Kobayashi*, S., Talan, M., Bresnahan, E., Kametani, H., Chachich, M., Gage, F., and Ingram, D.: **Behavioral, physiological, and histological assessment of fetal hypothalamic tissue transplanted to ventral third ventricles of aged mice.**

45. Nandy*, K., Mostofsky, D., and Nandy, L.K.: **Lipofuscin pigment as a marker of neuronal aging.**

ABSTRACTS

ANNUAL SYMPOSIUM: "Estimation of Human Physiologic Age"

1

BIOLOGICAL AGING MEASUREMENT — A CONCEPT WHOSE TIME HAS COME. *W. Dean,* Center for Bio-Gerontology, Los Angeles, CA 91359.

With growing knowledge of the fundamental mechanisms of aging, an increasing number of scientists and health enthusiasts are adopting and promoting experimental "life extension" regimens to retard aging and extend the human lifespan.

A fundamental problem with this haphazard approach to aging retardation is the lack of a system to rapidly evaluate the efficacy of potential human gerontotherapeutic agents. It is essential to develop a system to accurately measure biological age to perform this evaluation.

A number of international conferences and several books have focused on the subject of aging measurement. However, most of the books and conferences were concerned largely with the philosophy and difficulties of aging measurement. Although a few insect and rodent studies were included, human aging measurements were almost entirely neglected.

Nevertheless, numerous studies have been published over the past 30 years from research teams the world over, demonstrating the ability to measure biological age in man. These papers are briefly reviewed and the significance of particular studies is discussed. Examples of potential protocols for clinically applying aging measurements in the evaluation of experimental gerontotherapeutic regimens are given.

2

ASPECTS OF QUANTITATION OF HUMAN AGING. *W. Regelson,* Med. Coll. of Virginia, Richmond, VA 23298.

We cannot expect the effective entry of physicians or the pharmaceutical industry into the search for anti-aging drugs because of the cost, time and uncertainty involved unless bioquantitation establishes parameters of change that can be reasonably measured over short periods of time.

Currently, aging assessment is primarily concentrated on the elderly, but for it to become an adjunct to clinical intervention, assessment of physiologic age must be made periodically throughout adult life to enable us to act prophylactically to improve the quality of our survival as we age.

Areas of bioquantitation that have relevance to intervention include: forced vital capacity, hand grip strength, cardiac function, bone loss, fingernail growth, wound healing, sensory and neurologic changes, and autonomic function. Endocrine functional changes involving quantitative changes, as in the case of DHEA, or qualitative, as in the case of prolactin; immunologic parameters, biochemical values, which include volatiles in expired air, and enzyme protein/peptide patterns are some of the measures of consequence to intervention procedures that can only be validated if they are seen to produce functional changes.

Modulation of the aging process, with rationale intervention, must be supported by before and after changes to achieve patent office and FDA support.

3

LIFE INSURANCE AND BIOLOGIC AGING. *J. Elder,* Transamerica Life Companies, Los Angeles, CA 90015.

The life insurance industry is always looking for better ways to predict survival. Actuaries have assumed a natural aging process as the explanation for the annual increase in mortality with age. Mathematical models have been constructed for aging to which the effect of accidents and disease can be applied. Little has been done in insurance practice to differentiate between aging and disease. Preferred risk policies have been created on the assumption that physical fitness and absence of risk factors for disease are associated with better than average mortality. The time is ripe for the insurance industry to investigate aging because of the problems posed by an aging population. Approaches can include demographic analysis of insured populations and mortality studies on any piece of information obtained at the time of individual applications for insurance (health history, physical examination, urine or blood test), to which could be added questions or non-invasive tests specific for aging research. A caveat: The nearer it becomes practical to link premiums to the genetic code, the greater will be the pressure from society to prevent it.

4

HEALTH AGE ASSESSMENT. *L.C. Robbins.* Abstract not received.

6

MEASURING THE RATE OF BIOLOGICAL AGING IN PIGTAILED MACAQUES: A MODEL FOR ALL PRIMATES. *R. Short* and D.M. Bowden,* Regional Primate Research Center, Univ. of Washington, Medical Lake, WA 99022.

Interventions thought to extend the maximum human life span must be tested experimentally. In intervention studies using long-lived primates as subjects, the measurement of longevity as an outcome variable is not practical; an index of the rate of biological aging is preferred. We have measured aging rate in a group of 24 female pigtailed macaques of varying ages using a battery of 9 noninvasive tests which include immunological, hematological and serum chemistry variables. Animals were measured at 6 month intervals for up to 5 years. Regression coefficients were computed to represent the rate of change for each animal on each variable. The change variables were intercorrelated, and those having a high loading on the first principal component of the correlation matrix were standardized and summed to form a biological index of aging (BIA). The BIA had an internal consistency reliability of .7, suggesting that the premise of a central aging process was not unreasonable. The next step is to test correlations of the BIA with putative determinants of aging, such as uric acid, ceruloplasmin, vitamin E, and carotenoids, and with known biomarkers of aging, such as joint excursion, creatinine clearance, and reproductive function.

7

STATISTICAL APPROACH FOR THE ASSESSMENT OF BIOLOGICAL AGE. *E. Nakamura,* Dept. of Health and Phys. Education, Kyoto Univ., Sakyoku, Kyoto 606, Japan.

Although aging is a universal biological phenomenon, its various morphological and physical manifestations make it difficult to provide a single process or theory which adequately describes aging. This has led various studies on the prediction of biological age.

The present study was undertaken in an attempt to develop an aging measurement system through application of principal component analysis.

The subjects were 462 healthy Japanese men (ages 30-80). Out of the 30 physiological variables examined in routine check-ups, eleven variables: Hb, albumin, A/G ratio, cholesterol, urea nitrogen, GOT, OGTT (1 hr), vision, pulse, FVC and systolic blood pressure, were selected as suitable for the assessment of biological age based on the results of factor analysis and the physiological meaning of each test.

Principal component analysis of these eleven physiological variables revealed that the first principal component can be used as an overall index related to the aging process of various physiological functions. Moreover, the biological age estimated by this method is practically useful and theoretically valid in contrast with the multiple regression model, because this approach eliminates and overcomes two big problems of multiple regression model: 1) the distortion of individual biological age at the regression edges; 2) a theoretical contradiction in that a perfect model will merely be predicting the subject's chronological age, not his biological age.

8

VARIATION IN PARAMETERS OF AGING, THEIR SOURCES, AND THE BEST PREDICTORS OF PHYSIOLOGICAL AGING. *E. Heikkinen,* Dept. of Health Sciences and Univ. Center on Aging, Univ. of Jyväskylä, 40100 Jyväskylä, Finland.

Since 1971 we have carried out interdisciplinary population studies which aim at describing levels of functional capacity and certain biological properties of people in different age groups. A structural model of functional capacity was adopted for the conceptual framework, and the parameters studied were assumed to change with chronological time, differences in life style and socio-economic status. Large differences were observed both between and within the age groups in all the physical fitness parameters studied, and in sensory, psychomotor and motor functions. When the clustering of better or poorer performance in the functions was studied, it was noticed that long education, a high level of cognitive functions, high occupational status, and a small number of reported occupational hazards were significantly associated with more favorable results in many of these tests. Certain life style factors (e.g. physical exercise) also affected the levels of functional capacity. Differences were observed between the age groups in the LISREL — models which were applied to describe associations between the indicators of physiological functions, cognitive capacity and socio-economic status. This knowledge can be used in developing preventive interventions with the aim of maintaining good functional capacity in different age groups including the elderly.

9

ASSOCIATION BETWEEN BIOLOGICAL AGE AND MORTALITY RISK IN 2,462 OFFICE WORKERS. *R. Hochschild,* Hoch Co., Corona del Mar, CA 92625.

Twelve candidate biomarkers of aging were measured in 2,462 employees of 17 life insurance companies who also answered 33 questions regarding risk factors for mortality and health. A study objective was to explore the feasibility of detecting differences in standardized biological age between sub-groups characterized by differences in mortality risk. Biomarker measurements were made automatically using the H-SCAN, a computerized instrument which repeats procedures identically, reducing variability associated with test operators and technique.

Separately for males and females, significant associations were found between standardized biological age based on all 12 biomarkers and 1) smoking status, 2) amount of red meat in diet, 3) exercise intensity, 4) sum of scores for hours/week + intensity + years of exercise, 5) state life expectancy at birth, 6) state life expectancy at age 45, 7) father's age at death (females only), 8) race (black/white), 9) years of school completed and 10) overall mortality risk percentile based on 11 standardized risk factors combined. As a group, the 12 relatively common biomarkers of aging used in this study appear to be able to distinguish sub-groups characterized by differences in mortality risk based on a number of hereditary, dietary, life style and environmental factors.

10

ESTIMATION OF BIOLOGICAL AGE USING FEW PARAMETERS TO COMPARE AGING OF HUMAN POPULATIONS. *A. Ruiz-Torres.* Abstract not received.

11

DETERMINATION OF HUMAN BIOLOGICAL AGE. *W. Ries.* Abstract not received.

12

EXCESS ENTROPY PRODUCTION AS A BIOMARKER IN LONGEVITY ANALYSIS. *D. Hershey** and *W. Lee,* Dept. of Chem. Engineering, *Univ. of Cincinnati, Cincinnati, OH 45221 and Univ. of South Florida, Tampa, FL 33620.

From the thermodynamics of irreversible processes, the equations for Excess Entropy (EE) and Excess Entropy Production (EEP) are developed. EE is the entropic distance from the final, stationary or equilibrium state, death, while EEP is the rate of approach of EE to this final state. EE and EEP tend towards zero in the vicinity of death. Life span projections can be made, based on these EE and EEP tracks; deviations from the normal track can be used as a diagnostic tool, interpreted as the onset of a significant medical or psychological stress. Data will be shown demonstrating the accuracy of this method, i.e., predicted longevity was within 4 years of the actual death age for about half the individuals in the study. For the group of 39 subjects, the average difference between actual and predicted death age was about 3 years.

13

QUANTITATIVE MEASUREMENT OF HUMAN PHYSIOLOGICAL AGE BY PROFILING OF BODY FLUIDS AND PATTERN RECOGNITION. *A.B. Robinson.* Abstract not received.

14

COLLAGEN AGING AS A PARAMETER FOR ESTIMATION OF HUMAN PHYSIOLOGIC AGE. *V.M. Monnier.* Abstract not received.

15

SKIN AS A BIOMARKER FOR AGING. *A.K. Balin.* Abstract not received.

16

DHEA: MARKER OR MODIFIER OF AGING. *J.A. Zimmerman*+, J.R. Matias*+* and *N.D. Orentreich*.* *Orentreich Foundation for the Advancement of Science, Cold Spring-on-Hudson, NY 10516; and + St. John's University, New York, NY 11439.

concentration fell steadily thereafter in both sexes, reaching 17% of peak by age 70. Longitudinal studies of 15 males over a 25-year period indicated a similar decline in blood DS levels with age. While DHEA administration to humans has been reported to have several beneficial consequences, in our hands feeding 0.4% DHEA to C57Bl/6J male mice (a low tumor strain) failed to modify survival. Thus, while DHEA may be a marker of age and may modify some age-associated disease processes, there is no evidence that this substance is able to modify the underlying process of biological aging.

17

MEASURING OXIDATIVE DNA DAMAGE IN MAN. *B. Ames.* Abstract not received.

18

OXIDATIVE DNA DAMAGE IN HUMANS AS A FUNCTION OF AGING. *R. Cutler.* Abstract not received.

19

TWO DECADES OF RESEARCH AND PRACTICE WITH THE ADULT GROWTH EXAMINATION, A BRIEF STANDARDIZED TEST OF ADULT AGING: THE 20th YEAR PROGRESS REPORT. *R.F. Morgan,* Internat. Assoc. of Applied Psychology's Div. of Geropsychology, Pacific Graduate School of Psychology, Palo Alto, CA 94303.

A review of the history, standarization, reliability, validity, and application of the Adult Growth Examination as a brief measure of adult aging is presented.

Research applications have included biomarker evaluation of stress reduction, hypnosis, race, gender, meditation, vocational choice (nursing specialization), psychological well being, and test development. It allows controls in research for physiological as well as chronological age.

Practice applications have included the effect on measured years of body age from therapeutic interventions, smoking reduction and exercise programs, nutrition programs, cross-generational volunteering (e.g. Foster Grandparents), and self-monitored individual efforts.

Following specific test procedures, the response of media and the public to the test is explored, as is the growing usage of the test by health practitioners for age norm reference and by educators as laboratory illustration. The Adult Growth Examination continues to be a robust brief standardized measure suited to the field, classroom, or human aging laboratory.

20

BODY AGE INDEX VALIDATION IN CHRONIC FATIGUE SYNDROME PATIENTS. *R.C.B. Earle,* Canadian Inst. of Stress, Fac. of Med., Univ. of Toronto, Toronto, Canada M8X 2X3.

Status validity of Morgan's AGE index was assessed in 324 Chronic Fatigue Syndrome patients. The mean Body Age-Chronological Age (BACA) gap was 7.1 years. Reconstruction of S's past 6 months' morbidity showed strong correlations between size of BACA gap and criterion variables of: physician visits, specialist consultations, disability in activities of daily living, fatigue level, and symptom severity.

S's then underwent a multi-modal intervention (autohypnosis and stress control techniques) designed to support immune function and to moderate catecholamine levels. AGE measures were taken upon entry, and at 3 and 6 months into the intervention. Significant correlations were observed between narrowing of the BACA gap and improvements in morbidity, immunocompetency and catecholamine measures. In summary, retrospective and longitudinal data support the status validity of the AGE as a summary index of impaired/improved physiological functioning, i.e., accelerated or retarded aging.

21

AGE DIFFERENCES IN BIOLOGICAL MARKERS OF MORTALITY. *L.J. Brant.* Abstract not received.

22

LIGHT YEARS — AND RETINAL SENESCENCE. *R. Weale.* Abstract not received.

23

MULTIVARIATE APPROACH TO THE QUANTIFICATION OF PHYSIOLOGIC AGE. *W.J. Chodzko-Zajko.* Abstract not received.

MINISYMPOSIUM: "Gerontology in the New Millenium — The Life Extension Sciences"

1

OXIDANT AND ANTIOXIDANT HYPOTHESIS OF AGING. *L. Packer,* Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Aging hypothesis should include a significant role for oxidants and antioxidants. There is now evidence that free radical reactions accompany aging. Lifespan may critically depend upon antioxygenic enzymes and rates of oxidative DNA damage, consistent with Harman's free radical hypothesis of aging. In our laboratory, we are investigating the increased metabolism due to physical activity with respect to the generation of oxidants and interactions between antioxidants. Relevant information in involvement of vitamin E in metabolism and in the oxidant and antioxidant hypothesis for pathology and aging will be summarized.

2

***IN VITRO* MODELS FOR ALZHEIMER'S DISEASE.** *G.M. Cole,* Dept. of Neuroscience, Univ. of California, San Diego, CA 92093.

Alzheimer's disease (AD) is characterized by the age-related development of brain-specific lesions (plaques and tangles) which contain a human specific paired helical filament (PHF). With no animal model and a protracted time-scale for aging, it would be useful to develop an *in vitro* model using human neuronal cell lines and agents which may accelerate age-related damage. We have shown that several human neurogenic cell lines possess the plaque amyloid precursor protein and tau proteins which generate plaques and tangles, respectively, and that oxidative stresses to the cultured cells can generate modifications to these proteins resembling those found in AD. These neuronal model systems will be discussed in relationship to results from other *in vitro* systems using systemic AD tissues and in relation to potential genetic alterations to these cell lines designed to model selected aspects of AD pathogenesis *in vitro*. We conclude that cultured human neuronal cells will be useful tools for studying the stress related behavior of proteins involved in AD.

3

PHARMACOLOGICAL APPROACHES TO THE AGING BRAIN. *A. Vernadakis** and *N. Sakellaridis,* Depts. of Psychiatry and Pharmacology, Univ. of Colorado School of Medicine, Denver, CO 80262.

Although many physiologic functions are known to deteriorate with advanced age, none are as dramatic as those involving the nervous system. In view of the dominant role that the nervous system plays in homeostasis from the cellular to the organismic level, changes in the nervous system with aging will be reflected in all other systems to varying degrees. In the last decade CNS pharmacology has significantly contributed to our understanding of the CNS function in health and disease and more recently in aging. Using culture techniques we have been investigating the responsiveness of neurons and glial cells to xenobiotics: phenytoin, an anticonvulsant, alcohol and opiates. We have found that neural cells respond differentially to these pharmacologic agents and the response is dependent on the type of cell (neurons or glia), the state of cellular activity (mature vs. differentiating), and the brain area from which cells are derived. In addition, cell-cell interactions and specifically neuron-glial interactions play a key role in drug response. We propose that neurons and glia cells are in a dynamic state throughout the life span and their function and interrelationship can be influenced by epigenetic factors including pharmacologic agents. We propose that further research following this approach may provide clues for future pharmacological intervention in the aging process.

4

LONGEVITY AND NUTRITIONAL RESTRICTION. *H. Sternberg,* Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Diets low in calories but complete in nutrients extend the life span of laboratory rodents. Similarly, animals placed on diets low in tryptophan eat less and also exhibit delayed development and aging. The more restricted the diet was in tryptophan and the earlier in life the diet was initiated, the greater the enhancement of maximum life span. Maximum life span of rats placed on the diet at 3 weeks of age was 1527 days using a diet containing 30% of the normal level of tryptophan (T30%). Those placed on T40% and control diets lived 1347 and 1246 days, respectively. We have focused on delayed reproductive aging to understand more global age changes. Animals placed on the low tryptophan diet at 3 weeks show a more prolonged delay of reproductive aging than those on the diet at 3 months together with a significant younger appearance of several tissues and organs as well as reduced tumor incidence. It is postulated that nutritional restriction extends maximum life span by slowing down the physiologic cascade of nerve cell death, which in turn delays the death of cells within peripheral tissues. Manipulation of the diet represents a successful tool to modify the life span and to understand aging processes.

5

CLONING OF MAMMALS USING NUCLEAR TRANSPLANTATION. *S.L. Stice** and *J.M. Robl,* Univ. of Massachusetts, Amherst, MA 01003.

Nuclear transplantation technology offers investigators the opportunity to determine whether cells can be reprogrammed to undergo an unlimited or extended number of divisions, thus answering important questions about the aging of cells. Recently, nuclear transplantation has been used to clone sheep, cattle and rabbit embryos. Individual cells from eight- to 16-cell stage embryos were fused to enucleated oocytes. The manipulated oocytes were then activated and transferred to foster mothers. These manipulated oocytes then went on to develop into offspring in all the species mentioned above. However, development to offspring by itself does not indicate that cells fused to oocytes have been reprogrammed, since individual cells from eight-cell stage embryos can sometimes develop to offspring without being fused to an oocyte. Experiments in the rabbit were conducted to determine the ability of the oocyte to reprogram cells from early embryos when they were fused to an oocyte. Nuclear swelling, time to blastocyst formation and the number of cells in embryos that formed blastocysts were the parameters used to determine the extent of nuclear reprogramming in the nuclear transplant embryos. The results were then compared with measurements taken from one-cell nonmanipulated embryos and isolated cells from eight-cell stage embryos. Nuclear transfer embryo nuclei swelled to twice their normal size, developed to blastocysts at a similar time and with the same number of cells as nonmanipulated one-cell embryos. The isolated cells did not swell and formed blastocysts earlier and with fewer cells. These results indicate that transplanted nuclei undergo extensive reprogramming, suggesting that the number of divisions an original eight-cell embryo undergoes could be expanded greatly by the repeated transfer of nuclei back to unfertilized oocytes.

6

RESUSCITATION OF THE ELDERLY. *P. Safar*, N. Abramson, K. Detre* and the *BRCT Study Group,* Internat. Resuscitation Research Center, Univ. of Pittsburgh, PA 15260.

Current animal and patient data indicate that, with the use of post-arrest brain-oriented life support by protocol (rather than "usual care"), the longest period of normothermic cardiac arrest that can be reversed to survival with good cerebral function is not 5 min., but 10-20 min. An international multi-center clinical study of brain resuscitation (1979-1989) acquired data on comatose survivors of cardiac arrest, including details of the ischemic insult, resuscitation, protocol-defined intensive care,

survival and quality of life. 800 study patients were followed for 6 months. 74% of patient suffered pre-hospital cardiac arrest. 45% were over 65 years of age. Multi-variate analyses showed that although patients over 65 years of age had a higher incidence of secondary cardiovascular death, survivors had an equal rate of good neurologic recovery compared with younger age groups. Emergency cardiopulmonary-cerebral resuscitation attempts are thus justified, irrespective of age. Accurate early prediction of outcome is now possible using clinical and biochemical methods. These allow physicians to decide on the appropriate level of care, including withdrawal of expensive dehumanizing prolonged life support in cases of persistent vegetative state. Knowledge of the pathophysiologic limits of organ viability and of therapeutic potentials for reversing clinical death has increased greatly. Improved recovery to functional states should soon follow.

7

STATISTICAL APPROACH FOR THE ASSESSMENT OF BIOLOGICAL AGE. *E. Nakamura,* Dept. of Health and Phys. Education, Kyoto Univ., Sakyoku, Kyoto 606, Japan.

Although aging is a universal biological phenomenon, its various morphological and physiological manifestations make it difficult to provide a single process or theory which adequately describes aging. This has led various studies on the prediction of biological age.

The present study was undertaken in an attempt to develop an aging measurement system through the application of principal component analysis.

The subjects were 462 healthy Japanese men (ages 30-80). Out of the 30 physiological variables examined in routine check-ups, eleven variables: Hb, albumin, A/G ratio, cholesterol, urea nitrogen, GOT, OGTT (1 hr), vision, pulse, FVC and systolic blood pressure, were selected as suitable for the assessment of biological age based on the results of factor analysis and the physiological meaning of each test.

Principal component analysis of these eleven physiological variables revealed that the first principal component can be used as an overall index related to the aging process of various physiological functions. Moreover, the biological age estimated by this method is practically useful and theoretically valid in contrast with the multiple regression model, because this approach eliminates and overcomes two big problems of multiple regression model: 1) the distortion of individual biological age at the regression edges; 2) a theoretical contradiction in that a perfect model will merely be predicting the subject's chronological age, not his biological age.

8

ETHICAL IMPLICATIONS OF LIFE EXTENSION. *R.P. Marsh,* San Francisco State Univ., San Francisco, CA 94132.

The purpose of the study was to present a philosophical examination of some of the ethical aspects of the life-extension sciences. No laboratory research was involved.

Ethics is concerned with free choices among values. It is not the same as morality, which is based on socially accepted standards of behavior and which tends to work automatically. No choice is free which is not conscious. The evidence that there can be consciousness without a physical organism to support it is weak at best. Thus, the life-extension researcher, by discovering ways to extend physical existence and strengthen the body, is helping to prolong and clarify consciousness; hence, his/her work is profoundly ethical.

Philosophers and psychologists have said many useful things about the "wisdom" of old age. Their comments usually emphasize the value of "acceptance" of the "inevitable." Thanks to the development of the life-extension sciences, we are beginning to realize that, while old age and death are inevitable now, what is not inevitable is that they will always be inevitable. We are developing "wisdom about wisdom" and moving to a higher level of ethical sophistication.

Further, by showing the physical basis for states of mind once considered exclusively metaphysical, life-extension scientists collectively have made these rewarding and often healing and productive states of mind, available to large numbers of people who previously were excluded from them. Thus, they have enriched the lives of these people and widened the range of possible ethical choices.

Finally, they are developing a counter-argument to the absurd ethic of former Colorado Governor Richard Lamm and others that old people have a "duty to die."

SUBMITTED ABSTRACTS

9

ELEVATION OF FIBRONECTIN mRNA LEVELS DURING CELLULAR SENESCENCE *IN VITRO.* *D.A. Kleinsek,* Dept. of Virology and Epidemiology, Baylor Coll. of Med., Houston, TX 77030.

Normal cells cultured *in vitro* exhibit a finite replicative life span. Previous work indicates that this process is genetically programmed. To understand the molecular events responsible for this cellular senescence, gene expression changes between young, proliferation competent and senescent human diploid fibroblast (HDF) cells is assayed for. This is accomplished by the differential screening of a senescent cDNA library with young and senescent cDNA probes. About 0.05% of the recombinants from the lambda vector show a senescent-specific expression. The senescent-specific cDNA clones characterized hybridize to a mRNA size of 7.8 kilobases. The sequence of these cDNAs is homologous to the 3' terminal portion of human fibronectin. The steady-state mRNA level for this glycoprotein is a function of the growth state of the HDF cells. Young, proliferating cells produce low levels of the mRNA, whereas cells that are senescent or deprived of growth factors have up-regulated levels of 20 or greater fold. This increased message level for fibronectin is not due to an amplification or rearrangement of the fibronectin gene. However, a fibronectin sequence variant produced during senescence may account for the high fibronectin mRNA level.

10

HYDROXYL RADICAL SCAVENGING IN *DROSOPHILA MELANOGASTER.* *J.E. Fleming, P.L. Orr*, R.B. Shibuya* and *K.G. Bensch,* Linus Pauling Inst. of Science and Med., Palo Alto, CA 94306.

Previous work from our laboratory showed that the Swedish C strain of *Drosophila melanogaster* (mean lifespan 44.4 days) is significantly less efficient at scavenging *in vitro* generated •OH than the Samarkand strain (mean life span 68.7 days). In the present study, we report that most of this antioxidative activity is in the water-soluble non-protein fraction of the homogenized tissues. This activity correlates with the concentration of ascorbic acid in these preparations (Sam 0.127 ug/fly; Swed C 0.090 ug/fly). Swed C flies that were fed 1% ascorbic acid showed an increase in •OH scavenging efficiency of 22%. The life span of Swed C, however, is not increased by ascorbate supplementation. Preliminary results suggest that the differences in ascorbate levels observed for these two strains results from different turnover rates. Such data indicate that the Swed C generates ascorbate oxidizing species at a faster rate than the Sam. Thus, we measured the levels of Xanthine Oxidase (X.O.), an O_2-generating enzyme, in these strains. A spectrophotometric assay for X.O. showed 2.8×10^{-4} and 1.8×10^{-4} units/fly for Swed C and Sam respectively. Catalase activity was significantly higher in the long-lived strain (65.4 units/mg protein for Sam and 42.3 units/mg for Swed). Collectively, these data suggest that one of the major •OH scavenging components in *Drosophila* is ascorbate and that the life span of this insect is partially regulated by a balance between synthesis and degradation of O_2 radicals.

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STRAIN DIFFERENCES IN LEVELS OF PEROXIDASE DURING AGING IN *DROSOPHILA MELANOGASTER.* *L.K. Dixon,* Dept. of Biology, Univ. of Colorado at Denver, Denver, CO 80204.

Peroxidase levels were measured in seven strains of *Drosophila* from eclosure to late adulthood. Peroxidase is one of the

three main enzymes responsible for the breakdown of free radicals generated during cellular metabolism. Earlier studies on a random bred strain showed a drastic decrease in peroxidase during aging in *Drosophila*. This study is designed to investigate peroxidase changes in a number of strains and to determine the relationship between these changes and the life span of the strain. Peroxidase was assayed photometrically at 485 nm by measuring the reaction product of p-phenylene-diamine and hydrogen peroxide (2:1). Total protein of each sample was determined using the Bradford assay. Of the three major isozymes of peroxidase: acidic, basic, and neutral, only the latter shows a clear association with age. Thus, fly extract was prepared in a solution buffered at pH 7.4, measuring neutral peroxidase. Initial results show peroxidase decreases with age in all strains, but at different rates among different strains. Moreover, the pattern of decrease differed among the strains. Life span studies are in progress.

12

EFFECT OF AGING ON OXYGEN-FREE RADICALS PRODUCING ACTIVITY OF POLYMORPHONUCLEAR LEUKOCYTES. *J. Kalra*, A.K. Chaudhary, T.C. Cunningham* and *K. Prasad,* Depts. of Pathology and Physiology, Univ. of Saskatchewan and Univ. Hosp., Saskatoon, Saskatchewan, Canada S7N OXO.

Many physiologic and biochemical functions, including antibacterial defenses, have been claimed to be age related. The polymorphonuclear leukocyte (PMN) is a major component of the antibacterial defense system. Reports of age-related functional changes in polymorphonuclear leukocytes have been contradictory. We have measured the luminol-dependent chemiluminescence activity, a sensitive *in vitro* indicator of oxygen-free radical production, to study the response to stimulation of PMN from persons of various ages. Our study included 41 subjects (19 males, 22 females; age range 3-90; mean 42.4) who were grouped for comparison purposes into decades. Blood samples obtained were used as a source of phagocytes to measure luminol-dependent chemiluminescence. The addition of opsonized zymosan-initiated phagocytosis and the resultant chemiluminescence was measured over 60 minutes on an LKB 1251 Luminometer. The luminol-dependent chemiluminescence by polymorphonuclear leukocytes from different age groups did not differ significantly. These results suggest that there were no functional alterations in polymorphonuclear leukocytes with aging, at least in the oxygen-free radical-producing activity as measured by luminol-dependent chemiluminescence.

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THE EFFECTS OF AGE AND ENDOTOXIN ADMINISTRATION ON SUPEROXIDE DISMUTASE ACTIVITY IN THE RABBIT IRIS. *J.F. Recasens* and *K. Green,* Depts. of Ophthalmology and Physiology & Endocrinology, Med. Coll. of Georgia, Augusta, GA 30912.

Superoxide dismutase (SOD) activity was measured by the pyrogallol autoxidation method in the irides of young (4-6 weeks old), adult (6 months old) and aged (over 2 years old) albino rabbits. SOD activity did not significantly change with age equalling 23.44 ± 0.87 units SOD/mg protein in the young, 21.10 ± 1.68 in adult and 24.57 ± 1.38 in aged animals. Normally, a balance exists between antioxidant defenses and oxyradical exposure. However, this balance may be disrupted during inflammatory conditions as cellular infiltrates generate various oxyradicals. Endotoxin is commonly used to produce inflammation of the uveal tract (iris, ciliary body and choroid). Animals in each age group were administered 1 μg of *E. coli* endotoxin in 10 μl H_2O intravitreally 24 hours before enzyme determination. Although endotoxin produced visible signs of ocular inflammation (conjunctival hyperemia, vascular injection and iritis) in each age group, differences in SOD activity were noted independent of the degree of inflammation. In young animals ($n=8$), a 20.8% decrease in SOD activity was determined when compared to age-matched controls ($P<0.001$). In adult animals, endotoxin administration resulted in a 66.9% elevation in SOD activity ($P<0.002$, $n=4$). In aged animals, however, SOD activity was found to decrease by 34.7% after endotoxin ($P<0.001$, $n=8$). The data indicate that a loss in the ability to induce SOD after an inflammatory stimulus such as endotoxin may occur with aging.

14

PROLIFERATIVE LIFE SPAN OF SEQUENTIAL PRIMARY OUTGROWTHS OF HUMAN FIBROBLASTS DERIVED FROM A SINGLE SKIN BIOPSY. *A.K. Balin*, W. Reenstra, A. Mathew,* and *M.J. Anzelone,* The Rockefeller Univ., New York, NY 10021.

We examined the cultural life span of human skin fibroblasts derived from successive outgrowths of primary skin biopsies from four patients. A 1x1 mm fragment of biopsy tissue taken from each patient was used to establish the initial fibroblast outgrowths. Each primary outgrowth was obtained by refeeding the biopsy fragment once each week with Dulbecco's Modified Eagle Medium containing 10% Fetal Bovine Serum until confluent. The cells from these outgrowths were then designated to be at population doubling level (PDL)0. The cells obtained from each primary outgrowth were subcultivated serially at a constant inoculation density of 10,000 cells/cm² until they phased out. The original biopsy fragments were reattached in new flasks to obtain second outgrowths. This procedure was sequentially repeated until no further outgrowths could be obtained from the primary biopsies. Four outgrowths were obtained from each of the four original biopsy fragments; however, it took longer to attain confluence for each consecutive primary outgrowth, and the PDL at phase-out generally decreased for each sequential outgrowth. For example, a biopsy from a 36-year-old female had the following proliferative life span: Crop 1, PDL 42.5; Crop 2, PDL 37.0; Crop 3, PDL 23.7; Crop 4, PDL 12.7. These results indicate that multiple outgrowths can be obtained from a single skin biopsy. This phenomenon indicates that the traditional techniques for establishing cell lines, which utilize the yield only from the first outgrowth, may not have gleaned maximal information regarding differences in the proliferative life span of human fibroblasts as a function of donor age.

15

CALCIUM AND CALMODULIN CHANGES WITH AGING IN *DROSOPHILA* AND MICE. *H. Massie** and *S. Sternick,* Masonic Medical Research Lab., Utica, NY 13501.

Male C57BL/6J mice were used to measure calcium and calmodulin concentrations. The increase in calcium between 0 and 1000 days of age was 260% for kidney, followed by brain (189%), heart (173.5%), lung (106.5%) and liver (78.5%). Calcium in femur declined by 28.2%. The calmodulin content of liver increased with aging. Both liver and kidney calmodulin concentrations declined early in life followed by aging-related increases. Brain, lung and heart calmodulin concentrations did not change significantly with aging.

Total body calcium increased 115% for *Drosophila melanogaster* (Oregon-R) males from 0 to 60 days of adult age at 25°C. The highest values for total calcium were found during the pupal and larval developmental stages. The rate of calcium accumulation was dependent upon the environmental temperature at 11, 20, 25 and 30°. Feeding calcium salts decreased life span. Feeding the calcium channel blockers diltiazem, felodipine, nifedipine and verapamil had little or no influence on life span. Other calcium antagonists, the diphosphonates, also failed to improve survival. Calmodulin concentrations declined during development and increased with aging during the adult stage, but the increase was not significant. We conclude that high dietary intake of calcium salts can increase the rate of aging, but at moderate intakes calcium does not change the rate of aging of *Drosophila*. Calcium antagonists have little or no influence on the rate of aging of *Drosophila*.

16

IMMUNOLOGICAL IDENTIFICATION OF TAU PROTEIN IN NEUROBLASTOMA CELLS. *E.R. Mesco*, H. Sternberg,* and *P.S. Timiras,* Dept. of Physiology/Anatomy, Univ. of California, Berkeley, CA 94720.

We are studying tau protein in a human neuroblastoma cell line (LAN-5). Tau protein is the name for a family of microtubule-associated proteins, predominantly located in neurons, which are implicated in microtubule assembly and are present in neurofibrillary tangles in both Alzheimer's disease and aging. Primary analysis is done by SDS-PAGE and then transfer of proteins to nitrocellulose for immunological identification.

Tau protein, isolated from bovine brain, was used as antigen to generate antisera in rabbits. The purified bovine tau is recognized by the antisera and also by the monoclonal antibody, Tau-1. The tau protein from human LAN-5 neuroblastoma cells runs as lower bands (45-55 kd) on acrylamide gels. Both the antisera and the monoclonal antibody recognize tau from both species, although there is not complete overlap of all the bands recognized by the different antibodies. The antisera also recognizes tau protein isolated from Alzheimer brain samples.

These studies point out the species' variability in tau proteins, but also the homologies which allow for immunological identification. This antisera will enhance the use of our *in vitro* system as a model for the study of tau protein metabolism, and it can also be used to search for alterations of tau which occur in Alzheimer's disease.

17

CAN BIOLOGICAL AGE BE ASSESSED BY MAXIMAL OXYGEN UPTAKE? *S.N. Koyal*, A.J. Williams, S. Santiago, M.H. Ellestad* and *D. Schwartz,* UCLA-VA Med. Ctr., Los Angeles, CA 90073 and Heart Institute of Long Beach, Long Beach, CA 90801.

The assessment of biological age has been an objective of gerontological research. Voitenko & Tokar (V&T) published a prediction equation (*J. Expt Aging Research* 1984; 9:4) that derives biological age from chronological age (CA) based on a number of static physiological measurements, e.g., resting H.R., B.P., lung volume. The regression equations of biological aging index (BAI) for males and females were found to be 0.828 *X* CA + 8.586 ($r = 0.937$) and 0.714 × CA + 11.744 ($r = 0.867$) respectively. To test this paradigm, we randomly selected 165 healthy male and female subjects of different ages (30-80) years. Each individual had a series of tests consisting of body anthropometry, blood chemistry, pulmonary function, vascular compliance, muscular strength and endurance, ophthalmological and psychological evaluation. Following these, oxygen uptake and other cardiorespiratory variables were measured at rest and during a maximal treadmill stress test (MTST) at each incremental workload following Ellestad protocol. The biological age (BA) estimated from our measurements compared well with those from the V&T equations up to the age of 40. Above this age our measured BA for both males and females diverged significantly from that of V&T. Based upon statistical correlations of major dynamic physiological variables, we conclude that maximal oxygen uptake is a better indicator for determining the BAI in older subjects, unlike V&T who used only static variable.

18

STIMULATION OF TRANSCRIPTION IN MOUSE LIVER BY NITROGEN OXIDE FREE RADICALS. *V.K. Koltover,* Inst. of Chemical Physics, USSR Academy of Sciences, Chernogolovka, Moscow Region, USSR.

The ESR signal of nitrosyl complexes appears in mouse liver after hydroxylamine injection *in vivo.* This ESR signal testifies to the appearance of NO free radicals. The concomitant accumulation of ^{3}H-uridine in liver RNA evidences for stimulation of transcription. This stimulation is about the same order of magnitude as the stimulation of transcription after gamma-irradiation of mice with lethal doses. It is assumed that NO and, possibly, other oxygen-free radicals can disorder the gene expression machinery in the cells. The data obtained are consistent with the reliability theory model of aging.

19

MICROSPECTROFLUORIMETRIMETRIC CHARACTERISTICS OF LIPOFUSCIN GRANULES IN TERMITES *ANACANTHOTERMES AHNGERIANUS JACOBSON.* *A.B. Tatariunas,* Lab. of Neuron Physiology, Kaunas Med. Inst., str. A. Mickeviciaus 9, Kaunas 233000, Lithuania.

Lipofuscin granules (LG) accumulate in worker termites with increasing age. The fluorescence spectral characteristics of these granules were determined using a microspectrofluorimeter with an interference light filter of variable wavelength ($\lambda_{ex} = 365$nm, emission spot diameter - 7 μm). Since the width of the head capsules (WHC) of termites increases with age, WHC was used to estimate age.

It was determined that LG fluorescence spectra consist of two main maxima in the range of 440-460 and 530-560nm in various tissues of young termites (WHC = 1.4nm). In LG fluorescence spectra of termites (WHC = 2.9mm) the third band is clearly observed in red range (680nm) and in the main range (530-560nm) several sub-maxima — 530, 550, 560nm — can be distinguished. Under continuous excitation of LG by ultraviolet the intensity of the main band 530-560nm increases nearly twofold within 15-20 min (hyperchromic effect). Hemofuscin, on the contrary, is characterized by hypochromic effect of porphyrine compounds.

Thus, LG fluorescence spectra characteristics in various tissues of termites are similar to those of mammals and human beings; this suggests a common pathogenesis.

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THE NUCLEUS BASALIS OF MEYNERT REVISED: NERVE CELL NUMBER DECREASES WITH AGE. *H.-J. Gertz*[1], P. Lowes-Hummel[2], R. Ferszt[2],* and *J. Cervos-Navarro[2],* [1]Dept. of Gerontopsychiatry, Free Univ. of Berlin, Reichsstr. 15, D-1000 Berlin 19, [2]Inst. of Neuropathology, Free Univ. of Berlin, Hindenburgdamm 30, D-1000 Berlin 45.

There is an age-dependent nerve cell loss in some areas of the brain, while other brain regions are stable with aging. The nucleus basalis of Meynert (NbM) is believed to be the source of cholinergic innervation of the cerebral cortex, and loss of its neurons seems to be followed by cognitive deficits. The normal age kinetics of the NbM are therefore of considerable importance. Sixteen autoptic human brains were examined, ages ranging from the 35th week of gestation to 90 years of age. Blocks containing the NbM in its entirety were cut into 20 μ thick serial sections; every 25th section was cresyl-violet stained and underwent morphometric analysis. Nerve cell counts were slightly but significantly higher in the right hemisphere. The total number of neurons in the 9th decade lay 23% below that in newborns. This decrease was statistically significant. We hypothesize that there is a threshold number of nerve cells below which cognitive failure is highly probable.

21

EFFECTS OF AGE AND DIABETES ON MURINE TASTE BUDS. *P.B. Nava** and *J.A. Rosario,* Dept. of Anatomy, L.L.U. School of Medicine, Loma Linda, CA 92350.

Age- and diabetic-related chemosensory gustatory deficits have been reported in humans. However, parallel morphological findings have been few or lacking. Earlier studies by the authors have demonstrated a significant diminution in nerve fibers innervating the fungiform papillae in aged Swiss Webster and diabetic [C57BL/KS(db/db)] mice which, when extrapolated to other animal and human studies, may account for some of the reported gustatory sensitivity losses. This study evaluated the age-related ultrastructural changes in murine vallate taste buds of female diabetic and age-matched nondiabetic littermates (1.25-12 months). Ultrastructural age comparisons were made with 3-24 month old Swiss Webster mice. One of the striking ultrastructural findings in the diabetic animals was the progressive formation, with age and advancement of the disease, of large membrane-bound vesicular structures which occupied as much as 50% of the taste cells, displacing cellular contents, including the nucleus. No such manifest structures were noted in age-matched littermates. Comparison with the vallate taste buds of aged Swiss Webster mice revealed a progressive accumulation of lipofuscin, which is not seen in the diabetic strain or its

age-matched controls. Both the large vesicular structures and lipofuscin may be components of the lysosomal system and products of lipid peroxidation, a result of free radical toxicity which is accelerated by aging and diabetes. These findings suggest that aging and hyperglycemia yield ultrastructural changes that may have an important role in modifying peripheral taste mechanisms.

22

PHYSIOLOGICAL LEVELS OF ESTROGEN STIMULATE BONE FORMATION AND INHIBIT BONE RESORPTION IN OVARIECTOMIZED (OVX) MATURE MICE. *C.C. Liu** and *G.A. Howard*, GRECC and Med. Research, VAMC, American Lake, Tacoma, WA 98493; Dept. of Med., Univ. of Washington, Seattle, WA 98195.

We have previously shown that with age and before senescence there are progressive decreases in osteoblast and osteoclast activities. To determine if estrogen is effective in weanling (as previously reported, Liu and Howard) as well as mature mice, 1 month old Swiss Webster mice were sham-operated or Ovx for 2 months. Ovx mice were injected with 1, 5 or 10 ug 17 β-estradiol benzoate (EB) in oil once/wk for 8 wk (n = 9-10/group). Bone parameters were obtained from cross sections of tibial diaphyses at the fibular junction. Uterine weight was used as an index of serum estrogen levels (not all EB was absorbed). Both the endosteal bone formation rate (EBFR) and endosteal bone apposition rate (EBAR) remained unchanged by Ovx but showed EB dose-dependent stimulation above values in sham-operated mice. The new formed bone was primarily lamellar. The increased medullary area (MA) and endosteal resorbing surface (ERS) caused by Ovx was reversed by estrogen.

	Uterus (mg)	MA (mm^2)	EBFR (10^{-4} xmm^3/d)	EBAR (um/d)	ERS (mm)
Sham	136 ± 12*	.148 ± .013	1.8 ± .6	.3 ± .1	.05 ± .02
Ovx	32 ± 5[a]	.235 ± .014[a]	1.8 ± .5	.3 ± .1	.21 ± .04[a]
Ovx EB 1	41 ± 5	.167 ± .010[b]	14.1 ± 1.8[b]	1.1 ± .1[b]	.05 ± .03[b]
Ovx EB 5	67 ± 10[b]	.140 ± .018[b]	19.6 ± 4.6[b]	1.5 ± .3[b]	.02 ± .01[b]
Ovx EB 10	91 ± 15[b]	.151 ± .017[b]	22.8 ± 2.8[b]	1.8 ± .3[b]	0[b]

*mean ± SE

[a]significantly different compared to sham-operated

[b]significantly different compared to Ovx alone ($P < .05$)

These results suggest: 1) Ovx with attendant low serum estrogen (indicated by uterus weight and preliminary RIA data) caused stimulation of bone formation and inhibition of bone resorption, 2) These effects are independent of high remodeling activity seen in weanling mice, and 3) The mature mouse is a useful model for studying Ovx-induced bone loss.

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EFFECT OF AGE AND DIET ON INSULIN SECRETION BY RAT-ISOLATED PANCREATIC ISLETS. *V. Fierabracci, M. Novelli, A. Del Roso, V. De Tata, M. Bombara, P. Masiello* and *E. Bergamini**, Instituto di Patologia Generale, II cattedra, Universita di Pisa, 56100 Italy.

A decline in glucose- and leucine-stimulated insulin secretion *in vitro* was reported to be an inevitable consequence of the aging process (e.g., E. Reaven et al., *Diabetes* 32: 75, 1983).

In this study we have investigated the effects of glucose and of a number of other insulin secretagogues on islets isolated by the collagenase method from 2-, 6-, 9-, 12- and 24-mo-old rats fed *ad libitum* and from 12-mo-old food-restricted rats (which had had free access to food every other day for 10 months).

With rats on a free feeding, at the lower (2.8mM) glucose concentration the insulin secretory response to the phosphodiesterase inhibitor isobutyl-methyl-xantine (IBMX, 1 mM), to alfaketoisocaproate (KIC, 20 mM) and to KCl (20 mM) always were very low and were not affected by aging, and the response to arginine (20 mM) was significant with the younger rats and slowly declined after 6 mo of age. At the higher (16.7 mM) glucose concentration we observed a significant and progressive decline in the secretory response to glucose, IBMX, KIC and KCl with 6- and 9-mo-old rats. With the 12-mo-old rats, the decrease in insulin-secretary response to glucose and all other secretagogues (including arginine) was remarkable (-68%; -75%; -80%; -75%; -75% respectively as compared with the responses with 2-mo-old rats). Interestingly, the islets isolated from 12-mo-old food-restricted rats always exhibited an insulin secretory capacity like that of the islets from 2-mo-old rats. Hence, an appropriate feeding prevents the decline in the stimulated insulin release and results in youthful-appearing islets in mature rats.

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IMMUNOHISTOCHEMICAL STUDIES OF A AND D CELLS IN PANCREATIC ISLETS OF AGED MICE. *B.G. Slavin** and *S. Lerner*, Dept. of Anat. and Cell Biol., School of Med. and Gerontology Research Inst., U.S.C., Los Angeles, CA 90033.

Cells containing somatostatin and glucagon (two hormones having marked effects on insulin secretion) were analyzed quantitatively in islets of aging C57BL/6J mice. These mice, in contrast to aged rats and other species, are known to secrete more insulin and have improved glucose tolerance.

Pancreata from male mice at 3, 12, and 24 months were divided into a portion consisting of tail, body, and upper head (islets derived from dorsal primordium or DPI) and a smaller portion made of the lower head (islets derived from ventral primordium or VPI). Pancreatic segments were fixed, dehydrated, embedded in Paraplast, and serially sectioned. Sections were exposed to the PAP procedure for localization of glucagon (A cells) and somatostatin (D cells). The cross-sectional area and density (%) were determined using computer-assisted image analysis.

Results showed that the density of glucagon staining was significantly less in VPI of 14 and 24-mo-old mice compared to 3-mo. The density of A cells in 24-mo DPI was less than 3-mo DPI but no different from 14-mo DPI. The cross-sectional area of A cells (only DPI) was significantly less at 24 mo compared to the 3- and 14-mo groups. There were no differences in somatostatin staining when comparing the three age groups.

In conclusion, the major difference between the young and older mice was a deficiency of glucagon-stained cells in older mice. This might be important in explaining the improved glucose tolerance in aged C57BL/6J mice.

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AGING EFFECT ON THE SECRETION OF GASTRIC INHIBITORY POLYPEPTIDE. *P.S. Wang**, *W.-C. Wang, J.-Y. Liu, C.-Y. Hwang, C. Hwang, C.H. Day, J.T. Pan* and *L.-T. Ho*, Dept. of Physiology, National Yang-Ming Medical College, Taipei, Taiwan 11221, Republic of China.

Effects of aging on the secretion of gastric inhibitory polypeptide (GIP) and insulin in response to oral glucose in female rats were studied. Old (24 months) and young (2½ months) female Sprague-Dawley rats were catheterized through right jugular veins before an overnight fast. Blood samples (0.5-0.6 ml each) were collected at -10, 0, 10, 20, 30, 45 and 60 min after an ingestion of glucose (0.8 g in 2 ml water). Concentrations of GIP and insulin in rat plasma samples were measured by radioimmunoassay. The level of glucose in whole blood was determined by Glucoscan. Concentrations of blood glucose and plasma GIP in young rats increased gradually from 0 to 20 min and 30 min, respectively, after an oral glucose and then dropped. In old rats, the raised levels of blood glucose and plasma GIP were maintained between 30 and 60 min following an oral glucose. The concentrations of blood glucose ($P < 0.05$) and plasma GIP ($P < 0.01$) at 60 min after a glucose load were significantly higher in old than in young rats. The maximal level of plasma insulin in response to an oral glucose was lower ($P < 0.001$) in old than in young rats. The plasma insulin returned to the baseline more slowly in old than in young animals. The basal levels of blood glucose and plasma GIP and insulin were unaffected by aging. These results suggest that the difference in plasma GIP concentration between old and young rats is at least in part due to an impaired glucose tolerance during aging.

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SENESCENCE IS THE CONSEQUENCE OF RESTRICTED SELECTION IN MULTICELLULAR ORGANISMS. *P.A. Riley*, Dept. of Chemical Pathology, UCMSM, London W1P 6DB.

Aging reflects the senescence of the cell populations of which the organism is comprised. Cell population senescence can be viewed as the progressive accumulation of cells rendered defective by mutations. Where there is turnover, defective cells are exposed to competition from unaffected cells with greater proliferative potential. Thus, in homogeneously distributed populations, senescence will be an inverse function of the relative proliferation rates.

A general mathematical model has been developed which illustrates the properties of selection in cell populations. This model demonstrates that restrictions on competition result in senescence of cell populations. Reduced competition results from inhibition of proliferation and is strongly dependent on spatial factors. Some of the features of the model are that it explains phenomena such as the Hayflick limit and the action of growth stimulators in "immortalizing" cell populations *in vitro.*

The effect of the total constraint on selection imposed by nonproliferating cell populations, such as those of the central nervous system, may explain the primary role of these populations in determining organismal aging and the correlation between longevity and brain size of a species.

26a

AGE-RELATED CHANGES IN EXPRESSION OF DNA POLYMERASE α . *D. Busbee*, G. Curtin, J. Norman,* and *R. Tilley,* Dept. of Anatomy and Cell Biology, Coll. of Veterinary Med., Texas A&M Univ., College Station, TX 77843.

DNA polymerase α isolated from human fibroblasts exhibited at least two enzyme forms, one of which had low affinity of binding to DNA and low specific activity (A_1). Cell lines from older donors, vs fetal cell lines, exhibited increased expression of polymerase A_1 and decreased expression of a fetal enzyme form (A_2) with high specific activity and high affinity of binding to DNA. DNA polymerase α A_1, but not A_2, was significantly activated by treatment with phosphatidylinositol-4-monophosphate or its phospholipase C hydrolysis product, inositol-1,4-biphosphate. None of the other precursors, intermediates, or products of the phosphatidylinositol cascade interacted with the enzyme. Activated Pol A_1 reverted to the low activity enzyme form with loss of the IP2 activator as a function of time. Activation or increased expression of the adult isozyme appeared to correlate with EGF-stimulated increased mitosis in cells from aged donors.

26b

STRUCTURE ELUCIDATION OF A FLUORESCENT CROSSLINK FROM AGING HUMAN EXTRACELLULAR MATRIX. *D.R. Sell** and *V.M. Monnier,* Inst. of Pathology, Case Western Reserve Univ., Cleveland, OH 44106.

Collagen-linked fluorescence was found to increase in aging human skin and to correlate with overall severity of diabetic complications. In order to elucidate the structure of the fluorophore insoluble human dura mater was digested with trypsin and fractionated by Sephadex G-50 chromatography. The high molecular weight fraction was further digested with collagenase, aminopeptidase M and carboxypeptidase Y, and chromatographed on Biogel P2. The low molecular weight fraction was purified by paper chromatography and HPLC. Two highly fluorescent chromophores, P & M, were obtained. Fluorophore P had excit./fluor. max at 335/385 nm; UV max at 325 nm was resistant to acid hydrolysis and borohydride reduction. Fluorophore M had excit/fluor. max at 360/460 nm, and was labile to acid hydrolysis and was borohydride reducible. The acid resistant fluorophore P was purified from human collagen using a combination of gel, paper and HPL chromatography. Structure elucidation was carried out using ^{1}H-NMR, ^{13}C-NMR and MS/MS fast atom bombardment spectroscopy. The results indicate the presence of a lysine-arginine crosslink formed by nonenzymatic glycosylation. The fluorescent crosslink was found to increase exponentially with age in human skin. Its level was dramatically increased in Type I diabetic subjects with severe renal disease. This report is the first demonstration of a specific advanced glycosylation end product crosslink in biological tissues.

26c

CELLS TRANSFECTED WITH THE AMYLOID GENE AND CLONING OF AMYLOID AND GLIAL FIBRILLARY ACIDIC PROTEIN VARIANTS OF ALZHEIMER BRAIN. *C.A. Marotta, W.-G. Chou, R.E. Majocha, S. Rehman, R. Watkins,* and *S.B. Zain**, Cancer Center and Dept. of Biochem., Univ. of Rochester Med. School, Rochester, NY 15642; Dept. of Psychiat. and Neurosci. Prog., Harvard Med. School, and Massachusetts General Hosp., Boston, MA 02114 and McLean Hosp., Belmont, MA 02178.

We are examining the effects of amyloid overproduction in genetically engineered cell lines as a means to elucidate aspects of amyloid accumulation in the Alzheimer's Disease (AD) brain. Cells were transfected with cloned A4 amyloid ((40)) cDNA linked to tumor virus vectors. After transfection, a variety of cell types overexpressed A4 immunoreactivity that was detected by highly specific monoclonal antibodies to A4 amyloid. Overproduction of cellular A4 amyloid was possible in the absence of the endogenous Kunitz-type protease inhibitor found in amyloid protein variants. The transformed cells were readily propagated in culture and may provide an experimental medium to elucidate aspects of the molecular pathogenesis of AD. The cellular models may also serve as tools for deriving potentially useful therapeutic agents. Other postmortem molecular cloning experiments revealed that the AD brain contains variants of both the amyloid mRNA and glial fibrillary acidic protein mRNA. These findings may be related to the molecular pathogenesis of the disease.

26d

DISTRIBUTION OF SUBDOMAINS OF THE AMYLOID PRECURSOR PROTEIN IN HUMAN BRAIN TISSUE. B *Tate-Ostroff*, R.E. Majocha* and *C.A. Marotta,* Harvard Med. School, Mass. General Hosp., Boston, MA 02114, McLean Hosp., Belmont, MA 02178.

Information concerning the distribution of various subdomains of the amyloid precursor protein (APP) in brain may illuminate aspects of the normal metabolism of this membrane-associated protein, as well as putative abnormal processing that may occur in Alzheimer's Disease (AD). We prepared affinity-purified antibody (P2) against an extracytoplasmic APP site and applied it along with monoclonal antibodies to the β-peptide, or A4 region, to control and AD brain sections. In contrast to A4 epitopes, which are easily demonstrable only in extracellular senile plaques of AD brain, the extracytoplasmic P2 antigen was found in association with neurons, glia and blood vessels in both normal and AD cortices and hippocampi. A subset of senile plaques contained both A4 and P2 antigen, and in some cases the P2 antigen was predominant. In addition, the P2 antigen, but not the A4 antigen, was associated with corpora amylacea. The results support the view that the extracytoplasmic domain of the APP, rather than the A4 region exclusively, undergoes processing and deposition at extracellular sites. However, the unique characteristics of the two domains suggest that they may have different degradative pathways.

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AGE-RELATED CHANGES IN SPECIFIC HEART ATRIA GRANULARITY IN THE RAT: A MORPHOMETRIC STUDY. *G. Cavallini, V. DeTata, M. Pollera, Z. Gori* and *E. Bergamini**, Instituto de Patologia Generale, II Cattedra, Univ. of Pisa, Italy.

The atrial cardiocytes harbor a large Golgi complex and secretory granules containing potent peptide(s) (atrial natriuretic factor) with varied biological effects: induction of diuresis and natriuresis, vasodilation, inhibition of hypertension and of the secretion of adrenal hormones. Age-related alteration in the bioactivity of the atrial extracts has been reported (E.W. Insho et al., *Endocrinology* 121: 1662, 1987).

In this study we explored age-related changes in atrial granularity. Hearts were taken from 1-, 2-, 6- and 12-mo-old male Sprague-Dawley rats fed *ad libitum,* and samples from the tip of the right auricle were processed by the standard techniques and sections examined at the electron microscope and data analyzed by the morphometric techniques described by Weibel (*Int. Rev. Cytol.,* 26: 235, 1969).

Results indicate that the appearance of the granules changes between 1 and 2 mo of age, with a significant increase in their number, volume and surface densities. Thereafter, in the 6-mo-old rats granules decrease in number and become larger (number density decreases and volume density increases further) and in 12-mo old rats granules become smaller and with an altered shape (both number and volume densities are decreased, whereas surface density stays high).

It is concluded that the morphometric properties of the heart secretory granules change through adult life (with a decrease in the total mass of granules per unit body weight). These factors may help understanding of age-related alteration in the bioactivity of crude atrial extracts.

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POTENTIATION OF HUMAN T CELL MEDIATED IMMUNE RESPONSES BY A NOVEL NUCLEOSIDE, N10586. *B.S. Sharma*, W.B. Jolley, G.R. Revankar* and *R.K. Robins*, Nucleic Acid Research Inst., an ICN Pharmaceuticals, Inc. & Eastman Kodak Co. Partnership, 3300 Hyland Ave., Costa Mesa, CA 92626.

The aging process is associated with a decline in cell-mediated immunity and an increase in incidence of infections and malignancy. We evaluated the effect of a unique guanosine nucleoside (N10586) on alloantigen or mitogen induced human lymphocyte proliferation. Peripheral blood mononuclear cells (PBMNC) were isolated over Ficoll-Hypaque and resuspended in RPMI-1640 with human AB serum. The cells ($1x10^5$/0.2ml) were incubated with mitogens or irradiated allogeneic PBMNC in the presence and absence of N10586 for 3-5 days. Cultures were pulsed with ^{3}H-thymidine and its incorporation was determined. The results demonstrate that N10586 is not mitogenic to T or B cells. At concentrations 0.01-1mM, N10586 mediated a marked increase (up to 1171%) in both PHA and Con A induced proliferation of T cells. Together these findings suggest that mitogenic activity is not essential for N10586 to exert its immunoadjuvant effect. N10586 also potentiated T cell proliferation in response to alloantigens in a dose and antigen dependent manner, but had no potentiating effect on B cells activated either with *S. aureus cowan* or PWM, suggesting that N10586 is mainly a T cell function immunopotentiator. Interleukin-2 (IL-2) could restore depressed T cell functions. We examined whether N10586, like IL-2, would also overcome cyclosporin (CsA) induced immunosuppression. In the presence of CsA, N10586 was able to override CsA-caused inhibition of T cell response. These findings indicate that N10586 may have potential to enhance T cell functions that are involved in defense against viruses and tumors.

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IAMIN: A HUMAN GROWTH FACTOR WITH MULTIPLE SKIN HEALING PROPERTIES. *L. Pickart*[1], G. Bianne[2], J.P. Borel[2], B. Kalis[2], M. Leutenegger[2], F.X. Maquart[2]* and *V. Salagnac[2]*, [1]ProCyte Corporation, Redmond, WA 98052 and [2]Univ. of Reims, Reims, France 51095.

The human growth factor, Iamin[Gly-His-Lys:Cu (II)] possesses multiple healing-related properties. Iamin is angiogenic, induces nerve outgrowth and stimulates collagen secretion by fibroblasts. Iamin's healing action may be secondary to its specific chemoattractant properties for cells critical for healing (macrophage, monocyte, mast cell) which in turn may secrete growth factors such as EFG, FGF and PDGF.

Initial clinical tests of Iamin involved 30 patients of age 46-100 with non-healing venous stasis and diabetic skin ulcers. Iamin-containing creams were applied topically on days 1-5, then every other day. All patients initially responded positively to the treatment with the development of granulation tissue within 3-10 days. This was followed by re-epithelialization with better than 50% of lesions progressing to full coverage of the lesion within 6 to 8 weeks. Venous stasis ulcers responded more rapidly to treatment than diabetic ulcers. Age was less a factor in patient response than was overall patient condition. Some very elderly patients (82-100 years) responded with rapid healing and re-epithelialization. Controlled studies are currently in progress.

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LOW-DOSE INTERMITTENT HEPARIN THERAPY DECREASES PLASMA FIBRINOGEN LEVELS. *H. Engelberg**, California Arteriosclerosis Research Foundation, Beverly Hills, CA 90210.

Elevated plasma fibrinogen levels have been shown to be an important predictor of myocardial infarctions and strokes. In the past year 12 patients with coronary heart disease were started on intermittent subcutaneous heparin therapy. They were given 10,000 units every other day, a non-anticoagulant dose. Plasma fibrinogen decreased in every patient. Omitting one exceptional instance, where fibrinogen dropped from 535 mg.% to 199 mg%, the average decline was 26% (367-272). The decrease was continuous and gradual. Thus subcutaneous intermittent low-dose heparin therapy significantly lowers fibrinogen levels.

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PULMONARY TUBERCULOSIS IN THE ELDERLY. *R.C. Young, Jr.,* R.E. Rachal,* and *L. Matthew-Thompson*, Howard Univ. Hosp., Washington, DC 20060.

Tuberculosis in the elderly has a new significance: at home and in long-term care centers. This division is similar to the infectious pneumonias (community acquired vs nosocomial). The case of an 87-year-old female nursing home resident is presented. She was admitted to the hospital with atypical pulmonary manifestations and right pleural effusion. Tuberculin skin tests were negative. The patient deteriorated and succumbed to her illness. Disease due to M. tuberculosis was discovered by culture after death. Most geriatric patients are not immunodeficient. The prevalence of tuberculosis in the aged is increasing as the population ages. At home it occurs as sporadic disease and as recrudescence of infection acquired earlier in life. Long-term care residents, infected early in life, outlive their tubercle bacilli. They are at great risk of being infected again. Since the aged lack immunity, it may cause disease and atypical chest radiographic findings, making diagnosis prolonged and difficult. Diagnosis may be first discovered at death. Thus a threat is posed to both residents and staff. Continuous clinical awareness in the geriatric segment of the population is essential if tuberculosis control is to be achieved. Tuberculin skin testing is important: chemoprophylaxis for converters and treatment for active disease.

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CORONARY ARTERY BYPASS SURGERY IN THE ELDERLY. *T.-P. Tsai*, A. Chaux, R.M. Kass, C. Blanche, R.J. Gray* and *J.M. Matloff*, Cedars-Sinai Med. Ctr., Los Angeles, CA 90048.

Consecutive 629 patients over age 70 (Group I) and 64 patients over age 80 (Group II) underwent isolated coronary artery bypass surgery with cardiopulmonary bypass. Hypothermia (mean 22°C) and hyperkalemic cardioplegia were used in each. There were 468 men and 161 women in Group I (mean age 73) and 41 men and 23 women in Group II (mean age 82). Most patients were in NYHA Functional Class III (Group I, 25%; Group II, 23%) and in Class IV (Group I, 39%; Group II, 72%) preoperatively. There were 41 early mortalities in Group I (6.5%) and two early mortalities in Group II (3.1%) (average of 3.9 grafts/patient). The cardiac-related mortalities were 6.4% and 6.3% respectively.

Over 30-day survivors were 588 patients in Group I and 62 patients in Group II. There were 17.5% in Group I and 35.9% in Group II who developed major complications, including bleeding, pericardial tamponade, sternal dehiscence, myocardial infarction, arrhythmia and pump failure. Mean hospital stays were 14.8 and 19 days respectively. At follow-up (mean 38.2 months) these patients showed significant functional improvement by one or more classes in 80% (Group I) and 60% (Group II).

This experience proves that if adequate trial of medical treatment of the elderly with angina fails, coronary artery bypass surgery becomes a successful alternative.

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IS AGE A CORONARY ARTERY DISEASE RISK FACTOR? *D.A. Leaf* and *D.L. Parker,* Dept. of Med., Univ. of California at Irvine, Irvine, CA 92600.

Coronary artery disease (CAD) represents a leading cause of death and disability for the elderly. Although increased age is associated with increased CAD risk, the aging process is associated with deleterious changes in other CAD risk factors. One hundred twenty-eight healthy nonsmoking men, mean age 42.5 ± 9.9 years with no family history of CAD, evidenced a significant positive correlation ($p < 0.05$) between age and percent body fat, total plasma cholesterol, plasma low-density lipoprotein cholesterol, plasma triglycerides, and blood glucose. Analysis also showed an inverse correlation between age and plasma high-density lipoprotein cholesterol and VO_2MAX. A trend toward decreasing leisure-time physical activity with increasing age suggests that changes in CAD risk factors attributed to increasing age may be behaviorally related. This emphasizes that preventive cardiology in the elderly should target the modification of CAD risk factors associated with aging-related behaviors such as decreased physical activity and attributes such as increased body weight.

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EVOLUTIONARY ASPECTS OF THE ANTIBODY RECOGNITION OF AGING HUMAN RED CELLS. *U. Galili, B.A. Macher* and *S.B. Shohet,* Cancer Research Inst., Univ. of California Med. Ctr., San Francisco, CA 94143.

Our previous studies have suggested that, as the human red cell ages *in vivo,* a cryptic carbohydrate epitope with Galα1➡3Gal epitope is exposed. The binding of a few hundred molecules of anti-Gal to the aging red cells labels them for phagocytosis by macrophages of the reticuloendothelial system. In order to understand the nature of this antigen, a comparative study was carried out to determine the expression of Galα1➡3Gal epitopes on red cells and nucleated cells of various mammalian species. This epitope was found to be abundantly expressed on red cells and nucleated cells ($1 \times 10^6 - 35 \times 10^6$/cell) of non-primate mammals, prosimians, and New World monkeys. In contrast, it was not found in Old World monkeys, apes, and humans. The latter species, however, produce large amounts of anti-Gal IgG in their serum (50-100 μg/ml). The difference between the various species in regard to the expression of the Galα1➡3Gal epitope was found to be a consequence of the activity of the enzyme α1➡3 galactosyltransferase, which catalyzes the following reaction:

Galβ1-4GlcNAc-R + UDP-Gal ➡ Galα1-3Galβ1-4GlcN Ac-R + UDP

This enzyme displays high activity in cells of non-primate mammals, prosimians, and New World Monkeys, whereas in Old World monkeys, apes, and humans, it is largely suppressed. Our studies suggest that low activity of this enzyme limits the synthesis of Galα1➡Gal epitopes on human red cells to such an extent that it is present only in a cryptic form. In aged red cells, this epitope becomes exposed and binds the anti-Gal antibody.

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AGE-RELATED CHANGES IN THE ACTIVITIES OF ALKALINE AND ACID PHOSPHATASES IN FEMORAL CONDYLES OF FEMALE CW-1 MICE AND THEIR CORRELATION TO CHANGES IN BONE MASS. *A. Weiss*, I. Arbell* and *M. Silbermann,* Rappaport Family Institute for Med. Research and Faculty of Med., Technion, P.O.B. 9697, Haifa 31096, Israel.

The study examined changes in the activities of alkaline (ALP) and acid (ACP) phosphatases in the heads and necks of the femur of CW-1 female mice aged from 9 to 24 months. Specimens were homogenized in 0.05M Tris-HCl buffer (pH 7.4) containing 0.1M Triton X-100, and 24,000xg supernatants were used for the determination of enzyme activities. The assays for ALP and ACP were carried out at pH 10.5 and 5.5 respectively, and p-nitrophenyl phosphate served as a substrate. Additional specimens were processed for histological examination and were used for bone morphometry.

The results showed that ALP activity (expressed in units/g wet weight) both in the head and neck of femur reached a peak at the age of 12 months and was followed by a gradual decrease, so that by the age of 24 months the activity of ALP was decreased by 26.4% in the head and 22.2% in the neck ($p < 0.05$). The activity of ACP at the same age was decreased by 21% and 37.5% respectively ($p < 0.05$). Similarly, peak bone mass was also reached at the age of 12 months and declined thereafter. A high degree of correlation between the changes in ALP activity and the changes in bone mass was observed: ALP activity in the head was highly correlated with Trabecular Bone Volume ($r = 0.91$, $p < 0.05$), and in the neck with Cortical Bone Volume ($r = 0.79$, $p < 0.05$). No significant correlation between ACP activity and bone mass was observed.

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AGE-MEDIATED CHANGES IN PROSTACYCLIN PRODUCTION BY RAT LUNG MACROPHAGES. *R. Ganguly*, S. Saba, H. Chmel,* and *S.B. Pancholy,* James A. Haley VA Hosp. and Univ. of South Florida College of Med., Tampa, FL 33612.

This study examined the effect of age on prostacyclin (PGI_2) synthesis by rat alveolar macrophages. Lung-derived macrophages were obtained by bronchoalveolar lavage from young adult and aged Fischer-344 rats at 12 and 30 months of age respectively. PGI_2 was measured by 6-Keto-Prostaglandin $F_{1\alpha}$ radioimmunoassay in the lung lavage fluids (basal levels) and in the cell supernatants following *in vitro* incubation of macrophages with arachidonic acid. Data indicate that aged animals had low basal levels of PGI_2. Aged lung macrophages synthesized significantly greater amounts of PGI_2 than macrophages from young adult animals upon exposure to arachidonic acid. Furthermore, in presence of arachidonic acid the increase in PGI_2 production with time in old animals was significantly higher than in the young adult animals. Low basal levels of PGI_2 in the aged animals could lead to upregulation of PGI_2 receptors. This coupled with excessive PGI_2 synthesis might result in enhanced inflammatory response and compromise of the already weakened immune functions of the respiratory tract.

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THE SEPTO-HIPPOCAMPAL PATHWAY IN DEMENTIA OF ALZHEIMER'S TYPE: EVIDENCE OF NEURONAL PLASTICITY. *H.-J. Gertz**, Dept. of Gerontopsychiatry, Free Univ. of Berlin, Reichsstr. 15, D-1000 Berlin 19.

The influence of the loss of subcortical cholinergic neurons in senile dementia of Alzheimer's type (SDAT) on postsynaptic morphology of the cholinergic innervated cortical nerve cells has been investigated. In seven autoptic cases of patients suffering from SDAT and in seven age-matched controls, nerve cells of the medial septal nuclei (area CH 1), and of the vertical limb of the diagonal band of Broca (area CH 2) of the right hemisphere were counted in cresyl-fast-violet-stained serial sections. The granular cells of the ipsilateral fascia dentata (FD) were Golgi-stained and dentritic spine density was quantified in 10 m segments. There was a significant loss of neurons in areas CH 1 and CH 2 in SDAT cases compared to controls. The spine density of the granular cell dendrites was significantly reduced in the distal parts of the dendrites. In the most proximal part, where cholinergic septal fibers form synapses, the spine density was not significantly different between the two groups. We assumed that collateral sprouting of undamaged inputs occurs maintaining a constant number of spines in the proximal segments despite the loss of source neurons within CH 1 and CH 2.

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FORSKOLIN-MODULATED SEROTONIN (5HT) RELEASE IN HYPOTHALAMIC TISSUE FROM YOUNG AND OLD MALE RATS. *V.J. Aloyo, A.H. Vaidya* and *R.F. Walker**, Med. Coll. of Pennsylvania, Dept. of Pharmacology, Philadelphia, PA 19129.

The purpose of this study was to evaluate the effect of age on cAMP-modulated hypothalamic 5HT release. The cell-permeable adenylate cyclase activator, forskolin, was used to modulate cAMP levels. Hypothalamic slices from 6 to 24 month old Fischer

344 rats were preloaded with ^{3}H-5HT, placed in perifusion chambers, and perifused with oxygenated buffer. After achieving basal efflux of ^{3}H-5HT, the slices were subjected to two consecutive 30 mM K+ – depolarizations, each of 2 min duration separated by 70 min. Forskolin (3 to 30 mM) was added to the perifusion buffer 30 min before and during the second K+ stimulation. The modulatory effects of forskolin on K+-mediated 5HT were expressed as a ratio of the second to the first depolarization. Forskolin increased 5HT release from young tissue in a dose-related fashion. Although comparable increases occurred in tissue from some old rats, forskolin was ineffective in others. The extreme variability of responses in old tissue obliterated any group effect of forskolin. The data suggest an age-related but non-uniform decay of the hypothalamic cyclic nucleotide system(s) that influence 5HT efflux.

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INCREASED INTRANUCLEAR IONIC STRENGTH IN RAT HIPPOCAMPAL PYRAMIDAL CELLS DURING AGING. *C. Bertoni-Freddari*, P. Fattoretti, W. Meire-Ruge* and *J. Ulrich,* Ctr. for Surgical Res. and Biochemistry, INRCA Res. Dept., Via Birarelli 8, 60121 Ancona, Italy and Div. of Neuropathology, Univ. Basel, Switzerland.

Na^+, *Cl- and* K^+ intranuclear contents were measured in CA1 hippocampal pyramidal cells of 3, 12 and 24 month old male Wistar rats by means of X-ray microanalysis. Quickly frozen, fractured and dried hippocampal samples were analyzed according to a method elaborated in our laboratory (I. Zs.-Nagy et al., *J. Ultrastruct. Res.* 58: 22-33, 1977). 100 nuclei were measured per group of age. The data, expressed as percent of nuclear dry mass, showed no difference in Na^+, Cl- and K^+ contents between 3 and 12 months of age. In the old animals, Na^+ significantly decreased and K^+ increased, respectively, when compared to adult and young rats. No difference was found between old and adult groups in Cl- contents, whereas a significant decrease of this ion was evident comparing old and young animals. Considering the known age-dependent decrease of the intracellular water content, the present data: (a) demonstrate an increase of the intranuclear ionic strength in Ca1 hippocampal pyramidal cells of old rats due to K^+ ions and (b) support an age-related impairment of nuclear membrane permeability.

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TRANSPORT OF ACETYLCHOLINE PRECURSORS INTO FIBROBLASTS OF ALZHEIMER'S VICTIMS AND NORMALS IS MODULATABLE. *L.C. Mokrasch**, Dept. of Biochemistry, LSU Med. Ctr., New Orleans, LA 70119.

Disturbances in cholinergic processes are imputed as causes of the neuropathoses of Alzheimer's disease and of aging. This work tested whether the defects of transport of acetylcholine precursors into fibroblasts of Alzheimer's victims were reversible. Six lines of Alzheimer's cells and 5 lines of age- and sex-matched normals were grownin culture. Choline and serine are transported into the cells with pseudo-zero order kinetics up to 20 and 200 minutes, respectively. Kinetic constants for choline: normals had Km and Vmax values averaging 0.65 mM and 222 nMol/hr/mg protein; for Alzheimer's 0.22 mM and 41 nMol/hr/mg protein. Constants for serine: normals, 5.0 mM and 65 nMol/hr/mg protein; Alzheimer's, 3.2 mM and 17 nMol/hr/mg protein. Insulin and glutathione, which cause large increases of amino acids influx into cultured cells, have no effect on choline transport, and an increase in serine transport. Among other compounds tested, caffeine and dexamethazone increase the Vmax for choline transport up to 4-fold. Both express a maximal effect after 5 minutes' preincubation, suggesting a site of action on the plasmalemma. The effect appears to be dependent on the residual concentrations of other factors in the old culture medium. Hemicholinium-3 inhibits choline transport with a Ki about 1 uM. The transport of choline into fibroblasts exhibits some characteristics of its transport into neural cells; the unmodulated transport is slower in cells from Alzheimer's victims.

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LONG-TERM NUCLEUS LESIONS PRODUCE AN INCREASE IN DENDRITIC BRANCHING OF NEOCORTICAL PYRAMIDAL CELLS IN THE AGING RAT: A QUANTITATIVE GOLGI STUDY. *R.F. Mervis*, M. Bedo-Wierdl,* and *G.W. Arendash*†, Div. of Neuropathology, The Ohio State Univ. Med. Ctr., Columbus, OH 43210, and †Dept. of Biology, Univ. of South Florida, Tampa, FL 33620.

The effects of long-term excitotoxic lesions of the nucleus basalis magnocellularis (nBM) on dendritic morphology of cortical pyramidal cells was evaluated in the aging rat. 21- month-old Sprague-Dawley rats were infused unilaterally with ibotenic acid and sacrificed 5 months later. Histologically, the side ipsilateral to the lesion revealed a significant decrease in neuronal density and the appearance of senile plaque- and neurofibrillary tangle-like structures. Neurochemically, cholinergic markers in the cortex were also reduced on the lesioned side. Sholl "concentric circle" morphometric analysis of Golgi-impregnated layer III pyramidal cells in the frontal cortex (from the region specific for extrinsic cholinergic innervation by the nBM) showed that there was a significant increase in dendritic branching on the lesioned side relative to the contralateral hemisphere. Due to the long-time interval between lesion and sacrifice, the results suggest that the increase in dendritic material in these remaining cells may be an expression of compensatory dendritic hypertrophy accompanying loss of cortical neurons.

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AGE-RELATED ELECTROPHYSIOLOGICAL CHANGES IN CEREBELLAR NORADRENERGIC RECEPTORS. *K.D. Parfitt* and *P.C. Bickford-Wimer,* Univ. of Colorado Health Sciences Ctr. and VA Medical Ctr., Denver, CO 80262.

Noradrenergic transmission in the central nervous system declines early in the aging process. This decline can be demonstrated as subsensitivity to the depressant effects of norepinephrine on Purkinje neurons of aged rats. In young rats $alpha_1$, $alpha_2$ and beta adrenergic receptors are present and functional in the cerebellar cortex. The purpose of this study was to determine which of these receptor subtypes alters their response with age. Inhibition of the spontaneous activity of Purkinje neurons by selective noradrenergic agonists was compared in young (3-month-old) and aged (18- and 26-28-month-old) Fischer 344 rats. These agonists were applied to Purkinje neurons by pressure micro-ejection from multibarreled micropipettes and the change in neuronal action potential discharge rate was recorded. Purkinje cells of both groups of aged rats were significantly less sensitive to locally-applied isoproterenol, a beta-adrenergic agonist, than Purkinje cells of young rats. Subsensitivity to the alpha, agonist phenylephrine and the $alpha_2$ agonist clonidine was not observed in the aged rats. These results suggest an age-related functional decline in an adenylate cyclase-linked receptor system with no concomitant functional change in receptor systems linked to other second messengers.

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BEHAVIORAL AND ANATOMICAL STUDIES OF THE LIMBIC SYSTEM IN AGED RHESUS MONKEYS. *M.B. Moss** and *D.L. Rosene,* Dept. of Anatomy, Boston Univ. School of Med., Boston, MA and Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA.

The goal of this study is to determine the extent to which the rhesus monkey serves as a suitable experimental model of age-related cognitive dysfunction in human aging. Performance by six rhesus monkeys 26 to 27 years of age was compared with that of six young adult monkeys (five years of age) on a delayed recognition span task. This task, originally developed to assess spatial memory in monkeys with selected temporal lobe damage, is a test of short-term memory that has been shown to be sensitive to age-related memory decline in normal humans and in patients in the early stages of Alzheimer's disease. The task requires the subject to identify, trial by trial, a new stimulus among an increasing set of previously presented familiar stimuli. The task was administered using two stimulus parameters: spatial position and colors. The number of correct responses

made before the first error was committed constituted the "recognition span" for each stimulus parameter. Following behavioral testing, the animals were sacrificed and perfusion-fixed. Their brains were analyzed to determine the density of acetylcholinesterase (AChE) and distribution of neuritic plaques in the hippocampal formation, an area known to be critical for normal memory function in monkeys and humans. On the recognition task, aged monkeys were significantly impaired relative to the young adult group on both stimulus parameters. The impairment paralleled closely that seen in studies of normal human aging. Initial histochemical findings revealed an age-related decline of AChE in the hippocampal formation of the same monkeys that evidenced a recognition memory impairment. The relationship between memory impairment and loss of cholinergic activity in the hippocampal formation may help our understanding of the neurobiological basis of memory dysfunction in normal aging and in certain age-related dementias.

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BEHAVIORAL, PHYSIOLOGICAL, AND HISTOLOGICAL ASSESSMENT OF FETAL HYPOTHALAMIC TISSUE TRANSPLANTED TO VENTRAL THIRD VENTRICLES OF AGED MICE.

S. Kobayashi, M. Talan, E. Bresnahan, H. Kametani, M. Chachich, F. Gage* and *D. Ingram,* Gerontology Res. Ctr., NIA, NIH, Baltimore, MD 21224 and Dept. of Neuroscience, School of Med., UCSD, San Diego, CA 92093.

We are investigating whether fetal (E-15) hypothalamic (HTH) grafts transplanted into the brains of 26-mo old C57BL/6J male mice would improve or prevent further age-related functional decline 4 mo after surgery, since impaired HTH function is hypothesized as a mechanism of mammalian aging. Previously, we reported that HTH grafts transplanted to the dorsal third ventricle (D3V) had little effect on performance in an age-sensitive battery, including open-field activity, tightrope test, rotorod test, water consumption, body temperature, oxygen consumption, cold tolerance, runwheel activity and running speed test, although the grafts were histologically viable in D3V and well-developed neuroanatomical contact between host brain and grafts was observed. In this study, the effect of HTH grafts transplanted to the ventral third ventricle (V3V) on performance in the same battery was examined to ascertain that grafts more proximal to host HTH would have beneficial effects on test performance. Grafts appeared to develop in V3V as well as grafts in D3V in these 30-mo old mice. Although transplantation to V3V was associated with significant differences in several tests compared to aged unoperated controls, the difference showed a trend of accelerated deterioration in these age-associated parameters. These results indicate that fetal HTH tissue transplantation to aged mouse brain, whose function may have already deteriorated, do not appear to improve function in the tests examined.

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LIPOFUSCIN PIGMENT AS A MARKER OF NEURONAL AGING.

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One of the most consistent neuronal changes associated with mammalian aging is the cytoplasmic deposition of lipofuscin age pigment. The pigment is generally visualized by its characteristic autofluorescence, histochemical and ultrastructural properties. Lipofuscin has been quantitated in tissue sections morphometrically using a fluorescence microscope. The pigment formation was markedly increased in the neurons by vitamin E deficient diet in the animals. On the other hand, pigment was reduced by vitamin E excess diet or dietary or caloric restriction to the mice. Although it is generally agreed that antioxidants work by influencing lipid peroxidation in the tissues, the mechanism of action of dietary restriction is not clearly understood. It was further noted that the alterations of the pigment in the neurons of CNS were associated with changes in the learning and memory of the animals. *In vitro* studies using neuroblastoma cells indicated a similar age-relationship of the pigment. Therefore, it appears that lipofuscin pigment is a reliable marker of neuronal aging and this property might be useful in studying the effects of experimental manipulations of the aging process.