American Aging Association (AGE)
17th Annual Meeting
and
American College of Clinical Gerontology (ACCG)
2nd Annual Meeting
October 8-10, 1987
Sheraton Centre Hotel
New York, New York

Annual Symposium (AGE): "Aging of the Brain: Recent Advances"

Organized by H. M. Wisniewski

1. Hoyer, S.: **Changes in brain metabolism in aging and dementive disorders.**
2. Barclay, L.: **Differential diagnosis of dementive disease.**
3. Reisberg, B.: **Age-associated cognitive deficiency.**
4. Zemcov, A.: **Cerebral blood flow in dementing disease.**
5. Plum, F.: **Diagnosis and management of brain infarction.**
6. Cahn, J.: **Cognitive deficiency: strategies for treatment and research.**
7. Blass, J.: **Systemic biochemical changes in Alzheimer's disease.**
8. Wisniewski, H .M.: **Pathogenesis of amyloid deposits in aging and Alzheimer's disease.**
9. Bowen, D.: **Neurotransmitters in aging and dementia.**
10. Iqbal, K.: **Biochemical tests for Alzheimer's disease.**
11. Robakis, N.K.: **Molecular approaches to Alzheimer's disease.**
12. Devine-Gage, E.: **Genetics of Alzheimer's disease.**

Annual Symposium (ACCG): "Clinical Gerontology"

1. Vokomos, P.: **Atherogenesis**
2. Upton, A.: **Carcinogenesis**
3. Lindsay, R.: **Osteoporosis**
4. Katz, M.: **Aging of the eye**
5. Kendrick, N.: **Aging of the ear**
6. Balin, A.: **Aging of the skin**
7. Rivilin, R.: **Nutrition and aging**
8. Kendrick, Z.: **Exercise and aging**
9. Franffen, E.: **Aging of the brain**
10. Reynolds, M.: **Dental changes with age**
11. Tideiksaar, R.: **Falls in the elderly**

Minisymposium: "Gene Expression and Aging"

1. Oster-Granite, M.L.: **Cerebrovascular amyloid peptide gene expression in the mouse from very early embryonic stages to aged adults.**
2. Shmookler-Reis, R.: **Altered gene expression during replicative senescence of human diploid fibroblasts.**
3. Busbee, D.L.: **Changes in DNA polymerase expression with age.**
4. Siciliano, M.: **Genetics of human DNA repair.**
5. Papaconstantinou, J.: **Effect of aging on chromatin structure and the DNA modification of the alpha-fetoprotein gene.**
6. Neve, Rachael: **cDNAs for the Alzheimer amyloid beta protein: genetic and neurobiological studies.**

Minisymposium: "Dietary Restriction and Aging"

1. Weindruch, R.: **Dietary restriction and aging: an overview.**
2. Snyder, D.: **Dietary restriction in germ-free and conventional rats.**
3. Hart, R.: **Dietary restriction as a modular of toxicity.**
4. Fernandes, G.: **Modulation of immunologic aging by dietary restriction.**
5. Ingram, D.: **Dietary restriction and biomarkers of aging.**

Luncheon (Friday)

Luncheon Speakers:
Dr. Norman Vincent Peale
Mrs. Norman Vincent Peale
"Always Keep It Going"

Annual Awards:

Walter R. Nicolai Prize in Biomedical Gerontology —
Annette T. Lee

"This prize is presented to Ms. Lee for her manuscript, "The Nonenzymatic Glycosylation of DNA by Reducing Sugars *In Vivo* May Contribute to DNA Damage Associated with Aging."

Research Award —
Charles H. Barrows, Jr., Sc.D.

"This award is presented to Dr. Barrows for his significant studies of biochemical changes produced in animals by dietary restriction and aging. He was one of the first to demonstrate that dietary restriction was effective in increasing the life spans of mature animals.

"For many years Dr. Barrows was chief of the section on Nutritional Biochemistry of the Gerontology Branch of the National Institutes of Health and later of the National Institute on Aging."

Distinguished Achievement Award —
Dr. and Mrs. Norman Vincent Peale

"The Distinguished Achievement Award for 1987 is awarded jointly to Dr. and Mrs. Norman Vincent Peale for their numerous inspirational and religious contributions.

"Dr. Peale, for many years minister of The Marble Collegiate Reform Church in New York City, is author of 34 books. The title of one book, *The Power of Positive Thinking,* has become part of our language. His numerous awards include the Presidential Medal of Freedom presented by President Reagan. An author in her own right, Mrs. Peale has a national radio program called "Yes, You Can!", sponsored by *Plus,* the magazine of positive thinking. Her many awards include "Churchwoman of the Year" by the Religious Heritage of America.

"Dr. and Mrs. Peale are co-editors and publishers of the inspirational monthly magazine, *Guideposts.* Inspirational messages by Dr. Peale are mailed to over a million people monthly from the Foundation for Christian Living, whose chief executive officer is Mrs. Peale.

"Dr. and Mrs. Peale continue to demonstrate, in an outstanding manner, that age is no barrier to achievement."

Submitted Papers

1. Massie, H.R.: **Changes in taurine concentrations during development and aging of *Drosophila melanogaster*.**
2. Baird*, M.B., Hough, J.L., and Decker, R.L.: **Eye color change: a biomarker for aging in *Drosophila*.**
3. Bickford-Wimer*, P.C., Gerhardt, G., and Granholm, A-Ch.: **Age-related changes in cerebellar noradrenergic pre- and postsynaptic mechanisms: intrinsic vs extrinsic determinants evaluated with brain grafts *in oculo*.**
4. Spangler, E.L., Smith*, K., and Ingram*, D.K.: **Systemic injection of DSP-4 impairs complex maze learning in young rats.**
5. Buchweitz-Milton, E.: **Effect of oxotremorine on cerebral blood flow and oxygen consumption in conscious 3 vs 33 month old Fischer-344 rat brain.**
6. Cao, W., Carney, J.M., Duchon, A., Floyd*, R.A., and Chevion, M.: **Direct evidence for the role of oxygen free radicals in ischemia-reperfusion induced brain damage.**
7. Tjioe*, S.A., and Hadjiconstantinou, M.: **MPTP induces lipofuscin accumulation in neuronal tissue.**
8. Wiener*, H.L., Hashim, A., and Sershen, H.: **The effect of deprenyl on brain monoamine oxidase activity in aging mice.**
9. Mokrash, L.C.: **Fibroblasts from Alzheimer victims transport choline and serine slower than do normal fibroblasts.**
10. Weiss*, A., Livne, E., Bernheim, J., and Silbermann, M.: **Relation of PTH to the proliferative activity of chondrocytes from mandibular condyle in aging mice.**
11. Kumar*, V., Giacobini, E., and Elble, R.: **Lumbar CSF choline, acetylcholinesterase and protein in early onset vs late onset Alzheimer's disease patients.**
12. Miller, M.B.: **Concurrent cerebral and peripheral neuropathies in aged diabetics.**
13. Glaser*, J., Brind, J., Eisner, M., Vogelman, J., Dillbeck, M., Chopra, D., and Wallace, K.: **Elevated serum dehydroepiandrosterone sulfate levels in older practitioners of the transcendental meditation and TM-Sidhi programs.**
14. Smith*, D.E., Glaser, J., and Dillbeck, M.: **Zero erythrocyte sedimentation rate (ESR) and the identification of a population with lower than predicted ESR values.**
15. Chavantes*, C., Zamorano, L. Dujovny, M., Malik, G., Shibli, F., and Magilligan, D.: **Lung cancer with brain metastases: surgical approach in elderly patients.**
16. Vance*, D.E., Ehmann, W.D., and Markesbery, W.R.: **Trace element imbalances in hair and nail of Alzheimer's disease patients.**
17. Kumar*, V., Smith, R.C., Giacobini, E., and Elble, R.: **Brain morphology and cholinergic enzymes in Alzheimer's disease.**
18. Arbell*, I., Weiss, A., Steinhagen-Thiessen, E., and Silbermann, M.: **Physical exercise improves the bone structure of femoral head and neck in senescent mice.**
19. Rothschild*, B.M., and Woods, R.J.: **Osteoarthritis in prehistoric native Americans.**
20. Schler, S.H., Vargas, L., and Ganguly*, R.: **Influenza vaccine acceptance in elderly veterans.**
21. Katz*, M.L., and Eldred, G.E.: **Failure of vitamin E to protect against light damage to the retina.**
22. Handelman*, G.J., Dratz, E.A., and van Kuijk, F.J.G.M.: **Age-related accumulation of carotenoids in human retina.**
23. **Kim*, J.-W., and Yu, B.P.: Prevention of age-related loss in hepatic malondialdehyde (MDA) oxidation by food restriction.**
24. Chuknyiska, R.S.: **Antioxidant defense response in endothelial cells subjected to hyperoxia.**
25. Tumer*, N., Fenton, H., Boehm, C., and Roberts, J.: **Effect of age on beta-receptor binding in hearts sympathectomized by 6-hydroxy dopamine.**
26. Sottile, J., and Millis*, A.J.T.: **Enhanced production of collagenase by late passage cultures of human fibroblasts.**
27. Neufeld, D., and Ozer*, H.L.: **Immortalization of human fibroblasts in culture.**
28. Weiss*, A., Livne, E., and Silbermann, M.: **Age-related reduction in the proliferative activity of articular cartilage and its restimulation by various factors *in vitro*.**
29. Ivy, G.O.: **A proteinase inhibitor model of aging.**
30. Vlassara*, H., Harrison, D., Brownlee, M., Pasagian, A., and Cerami, A.: **Function of specific macrophage receptor for glucose-modified proteins decreases with age.**
31. Radoff*, S., Vlassara, H., and Cerami, A.: **Isolation and characterization of a receptor for proteins modified by advanced glycation endproducts (AGE) from a macrophage cell line.**
32. Lee*, A.T., and Cerami, A.: **Elevated glucose 6-phosphate levels are associated with plasmid mutations *in vitro*.**
33. Swenson*, C.D., Siskind, G.W., Thorbecke, G.J., and Coico, R.F.: **IgD-induced effects on Ig production in young and aged mice.**
34. Uitterlinden, A., Slagboom, E., Zurcher, C., Tan, C., de Leeuw, W., and Vijg, J.: **Detection of putative DNA sequence changes in aging inbred rats.**
35. Zebrower, M., Beeber, C., and Kieras, F.J.: **Proteoglycan synthesis in Alzheimer's disease fibroblasts.**
36. Nancy, K., and Nandy, S.: **Effects of dietary manipulations on neuronal lipofuscin pigment in aging mice.**
37. Fudenberg, H.H., and Singh, V.K.: **Immunodiagnosis and immunotherapy of patients with Alzheimer's disease.**

1

CHANGES IN TAURINE CONCENTRATIONS DURING DEVELOPMENT AND AGING OF *DROSOPHILA MELANOGASTER.* *H.R. Massie,* Masonic Medical Research Laboratory, Utica, NY 13501.

Taurine (2-aminoethanesulfonic acid) is the most abundant of all the free amino acids in animal tissues but is not incorporated into proteins. At present the biochemical role of taurine is unknown. The whole-body concentration of taurine was found by HPLC to be more than 1000% higher during the adult stage of *D. melanogaster* than during the larval stage. Taurine concentrations continued to increase with aging in adult flies. Adult *Drosophila* failed to produce progeny when fed 0.2M taurine for one week. Life-time feeding of taurine (0.05 to 0.20M) produced no change in life span. Feeding the taurine precursor, hypotaurine, and the taurine mobilizing agent, β-alanine, did not change life span at low concentrations but both decreased life span at higher concentrations. Depletion of taurine in adult flies by feeding β-alanine resulted in early death. These observations suggest that taurine is not merely a metabolic end product of methionine catabolism nor simply an oxidation product of hypotaurine. Although adult flies appear to have a requirement for taurine, the biological role for taurine remains unknown.

2

EYE COLOR CHANGE: A BIOMARKER FOR AGING IN *DROSOPHILA.* *M.B. Baird*, J.L. Hough and R.L. Decker,* Masonic Medical Research Laboratory, Utica, NY 13501-1787.

The most commonly utilized end point for experimental aging studies in most organisms has been death. This end point, although absolute, is of limited value, particularly in attempting to ascribe experimental manipulation of lifespan to a change in the rate of aging. Extensive exploitation of longevity as an end point in experimental gerontology merely reflects the paucity of useful biomarkers for aging.

We have discovered that the normal red eye color of *Drosophila melanogaster* changes to rust brown with advancing age. Eye color change begins well prior to death, undergoes temporal displacement by changing the temperature at which adult flies are maintained, and is observed in flies maintained on food media of varying composition. Paper and thin layer chromatography of eye pigments revealed marked age-related changes in the content of both biopterin and xanthopterin, and disappearance of a yellow pigment tentatively identified as riboflavin. These findings provide a new and convenient biomarker for aging in *Drosophila.* The red eye pigments in fruit flies are pteridines derived from the folic acid metabolic pathway. Analysis of the mechanism for change in this pathway in flies of different ages may prove to be of great value in the study of aging in *Drosophila.*

3

AGE-RELATED CHANGES IN CEREBELLAR NORADRENERGIC PRE- AND POSTSYNAPTIC MECHANISMS: INTRINSIC vs EXTRINSIC DETERMINANTS EVALUATED WITH BRAIN GRAFTS IN OCULO. *P.C. Bickford-Wimer*, G. Gerhardt, and A-Ch. Granholm,* VA Med. Ctr. and Dept. of Pharmacology, UCHSC, Denver, CO 80262.

There is a progressive decline in noradrenergic (NE) function with age in the central nervous system (CNS). The intrinsic vs extrinsic influences on this change were studied using the technique of *in oculo* brain grafting to create age chimeras. Three groups were examined: 1) young cerebellar grafts in young rats (Y/Y), 2) young cerebellar grafts in old rats (Y/O), and 3) old cerebellar grafts in old rats (O/O). The extracellular action potentials from single neurons were recorded from the *in oculo* cerebellar grafts. NE (5-1000 uM) was perfused over the grafts to test for adrenoceptivity. A decreased firing rate was observed with an EC_{50} of 15.9 uM NE in Y/Y grafts and 583 uM NE O/O grafts. The EC_{50} for Y/O grafts was 18.5 uM. The presynaptic aspects of noradrenergic function in the grafts was also examined using the technique of *in vivo* electrochemistry with Nafion coated graphite epoxy capillary electrodes which are highly selective for catecholamines. Illumination of the retina decreases the release of catecholamines. A mean change of 4.2 uM was seen in Y/Y grafts and 2.3 uM in O/O grafts, this difference was not significant. Since the adrenoceptivity in Y/Y and Y/O grafts was not different this suggests that postsynaptic subsensitivity to NE in the cerebellum is intrinsically determined, and that extrinsic circulating hormonal or humoral factors may not determine the changes reported here. In addition, the presynaptic NE elements appear unchanged with age in this system.

4

SYSTEMIC INJECTION OF DSP-4 IMPAIRS COMPLEX MAZE LEARNING IN YOUNG RATS. *E.L. Spangler, K. Smith*, and D.K. Ingram,* Gerontology Research Center, Baltimore, MD 21224.

N-2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4), a noradrenergic-specific neurotoxin, was utilized to assess the involvement of central noradrenergic (NA) systems in the acquisition and retention of a 14-unit T-maze task. Male F-344 rats 3 mo. of age were given preliminary training in 1-way active avoidance (US = 0.8 mA) in a straight runway (1 m long). The criterion was 8/10 successful avoidances in the last of 3 consecutive 10-trial daily sessions. Maze testing began 24 hr later. On Day 1 each rat received 10 trials with a 2-min intertrial interval. On Day 2, 24 hr later, each rat received a similar 10-trial session. Subjects ($n = 47$) were assigned to 1 of 8 groups in which they received an i.p. injection of either DSP-4 (50 mg/kg) or saline as follows: a) 2 weeks prior to acquisition training (AQ), b) immediately after completing the last trial on Day 2 (POST-AQ) or c) 1week after completing the maze training (PRE-RET1 and PRE-RET2). The POST-AQ and PRE-RET1 groups were given an additional 10 trials in the maze two weeks after their initial maze training to assess retention while the PRE-RET2 group was given a similar 10 trial session 3 weeks following the initial training. Immediately following the final maze session each rat was sacrificed by guillotine, and the latency (sec) to the post-decaptitation reflex (PDR) measured. Dependent measures of maze performance included errors, run time, shock received, and number of shocks received. For the PDR, dependent measures included the latency to initial movement and the latency to rapid movement. Separate ANOVAs were computed for each dependent measure of maze performance for acquisition and for retention. For the analysis of acquisition performance, SAL groups were combined with the POST-AQ, PRE-RET1, and PRE-RET2 groups since ANOVA revealed no significant differences ($p < .05$) among groups on any dependent measure. While ANOVAs comparing acquisition performance between the AQ ($n = 9$) group and the combined groups revealed that the AQ group was significantly impaired on all measures of performance ($p < .05$), the impairment appeared to be a moderate one since no significant differences between the groups were evident by the final block of 5 trials ($p > .05$). No significant differences were noted on any dependent measure of retention performance ($p > .05$). The PDR latency to initial movement and to rapid movement increased significantly in DSP-4 treated rats ($p < .05$), indicating decreased central NA activity in all treatment groups. Thus, DSP-4 impaired acquisition performance in young rats but did not impair any aspect of retention. The effects observed in the present study are similar to those reported following scopolamine administration (SC; 1.0 mg/kg) in this task. However, the AQ group in the present study recovered to levels of performance similar to control groups, while in two previous SC (1.0 mg/kg) studies acquisition performance was impaired throughout training. Age-related learning impairments previously observed in rats in this task may be related to declines in central NA systems. DSP-4 treatment of young rats may provide a valid model of these impairments.

5

EFFECT OF OXOTREMORINE ON CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION IN CONSCIOUS 3 vs 33 MONTH OLD FISCHER-344 RAT BRAIN. *E. Buchweitz-Milton,* C.U.N.Y. Medical School, Dept. of Physiology, New York, NY 10031.

This study compared the effects of oxotremorine, a cholinergic muscarinic receptor agonist (OXO), on cerebral blood flow (CBF) and O_2 consumption in specific regions of 3 vs 33 mos old rat brain. The number of muscarinic cholinergic receptors changes during aging. Aged animals should demonstrate an altered cerebral metabolic response to cholinergic muscarinic agonists. Three mos old and 33 mos old rats were divided into

groups of 16 rats each. Eight received 1 ug/kg OXO IV, eight saline IV. CBF was determined with 14C-iodoantipyrine. O_2 consumption was determined microspectrophotometrically. Heart rate was decreased significantly by OXO in both groups. Heart rate was decreased significantly more in the 33 mos old group. CBF averaged 51.9 ± 9.8 ml/min/100g in the 3 mos old group and 61.8 ml/min/100g (mean ± SEM) in the 33 mos old group. Fifteen min after OXO, CBF had increased to 100.3 ± 11.9 ml/min/100g in the 33 mos old group. CBF was not altered significantly in the 3 mos old group. O_2 consumption increased approximately 26.6% in the 33 mos old OXO treated rats, whereas this parameter was not significantly altered in the 3 mos old animals. CBF and O_2 consumption increased similarly and significantly in the 33 mos old brains only. The decrease in the number of muscarinic cholinergic receptors in aged brains may cause an increased sensitivity of the aged brain to cholinergic muscarinic agonists.

6

DIRECT EVIDENCE FOR THE ROLE OF OXYGEN FREE RADICALS IN ISCHEMIA-REPERFUSION INDUCED BRAIN DAMAGE. *W. Cao, J.M. Carney, A. Duchon, R.A. Floyd*, and M. Chevion,* Oklahoma Medical Research Foundation and Dept. of Pharmacology, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73104 and Hebrew Univ. of Jerusalem, Israel.

Stroke causes permanent damage to brain in many cases and is more evident with increasing age. Reperfusion of ischemic brain causes tissue damage. Oxygen free radicals have been implicated as mediating agents in brain damage. Trapping agents which react with oxygen free radicals to yield products that can be quantified extremely sensitively is an approach we have taken to investigate the role of oxygen free radicals in ischemia-reperfusion injury. Salicylate reacts rapidly with hydroxyl free radicals and yields dihydroxybenzoic acid products (DHBAs). These can be quantitated at the nanomolar level by high pressure liquid chromatography with electrochemical detection (LCED). Studies on ischemia reperfusion injury as assessed by behavioral changes were conducted on gerbils using salicylate and LCED methodology. DHBA increased following blood reperfusion. The level of DHBAs reached a maximum if 15 min ischemia was followed by 5 min of reperfusion. The data supports the notion that oxygen free radicals are involved in ischemia-reperfusion induced brain damage.

7

MPTP INDUCES LIPOFUSCIN ACCUMULATION IN NEURONAL TISSUE. *S.A. Tjioe* and M. Hadjiconstantinou,* Dept. of Pharmacology, Ohio State Univ. College of Medicine, Columbus, OH 43210.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) damages dopamine containing neurons in substantia nigra and causes parkinsonism in humans. The compound has been found to be neurotoxic in a number of animals including mice. Since parkinsonism is associated with age, we examined whether MPTP administration would cause other age-related changes in the nervous system. MPTP-treated mice were studied for lipofuscin accumulation in various neuronal tissues. A marked accumulation of lipofuscin fluorescence was found in retinal epithelium and adrenal cortex of the MPTP treated mice. The emission spectra of the lipofuscin fluorophore found in retinas of the MPTP-treated animals were the same as in retinas of old animals. Detailed electron microscope studies of the adrenal cortices of MPTP-treated mice revealed deposition of numerous lipofuscin deposits at the medullocortical junction. Furthermore, membranous whorls and increased number of lysosomes were observed. Preliminary studies have shown an increase in vesicle-packed lysosomes and membrane-packed dense bodies in hippocampus and striatum of the MPTP-treated mice. Our findings indicate that MPTP causes damage in lysosomes and accumulation of lipofuscin in neuronal tissues. Since lipofuscin is associated with aging, our work suggests that MPTP might be a useful tool for studying aging.

8

THE EFFECT OF DEPRENYL ON BRAIN MONOAMINE OXIDASE ACTIVITY IN AGING MICE. *H.L. Wiener*, A. Hashim and H. Sershen,* Center for Neurochemistry, the N.S. Kline Institute, Ward's Island, NY 10035.

Aging mice are more susceptible to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) than younger mice. MPTP is converted to 1-methyl-4-phenylpyridine by monoamine oxidase (MAO) type B. MAO-B inhibitors prevent MPTP induced damage and deprenyl has been suggested for treatment of Parkinson's disease. Since MAO activity increases with age, the effect of deprenyl on both MAO-A and B activities was correlated with changes in enzyme activity in striatum from young (2 months) and old (20 months) female inbred BALB/cBy mice. MAO-B increased 55% ($p<0.02$) between young and old mice, while MAO-A did not. Deprenyl at 0.1, 1, and 5 mg/kg ip decreased MAO-B activity by 54, 78, and 93% respectively in young and by 24, 88, and 94% in old mice. Although deprenyl is classified as a specific MAO-B inhibitor, MAO-A activity was decreased by 17, 37, and 49% in young and by 8, 37, and 45% in old mice. The greater sensitivity to MPTP exhibited by older mice results from its increased oxidation. The results indicate that deprenyl is an effective inhibitor of MAO-B at low doses and as such may serve in a prophylactic manner to slow the progression of Parkinson's disease.

9

FIBROBLASTS FROM ALZHEIMER VICTIMS TRANSPORT CHOLINE AND SERINE SLOWER THAN DO NORMAL FIBROBLASTS. *L.C. Mokrasch,* LSU Medical Ctr., New Orleans, LA 70119.

Disturbances in cholinergic processes are imputed as causes of the neuropathology of Alzheimer's disease. To test whether defects in the transport of acetylcholine precursors exist and whether the transport defects are expressed in fibroblasts from Alzheimer victims, this study was begun. Three lines of Alzheimer cells and 4 lines of age- and sex-matched normals were grown in culture. Choline and serine are transported into normal cells with pseudo-zero order kinetics for about 20 and 200 minutes, respectively; Alzheimer cells transport these compounds linearly with time for slightly shorter periods. Influx kinetic analysis revealed for choline, normals had K_m and V_{max} values averaging 0.54 mM and 0.21 μ mol/hr/mg; the corresponding values for the Alzheimer cells were 0.43 mM and 0.09 μ mol/hr/mg. For the serine K_m's and V_{max}'s, the normals' values were 5.0 mM and 50 nmol/hr/mg and the Alzheimer's values were 3.5 mM and 5 nmol/hr/mg. Hemicholinium-3 inhibited choline influx in both cells with a K_i about 1 μ M. For comparison, in rat C6 astrocytoma cells, the K_m of choline transport is 0.57 mM and the V_{max} is 0.57 μ mol/hr/mg; the sensitivity of choline transport is similar to that of the fibroblasts. The transport of choline into fibroblasts exhibits some of the characteristics of its transport into neural tissue and the influx is slower into Alzheimer cells than into normals.

10

RELATION OF PTH TO THE PROLIFERATIVE ACTIVITY OF CHONDROCYTES FROM MANDIBULAR CONDYLE IN AGING MICE. *A. Weis, E. Livne, J. Bernheim, and M. Silbermann,* Rappaport Institute for Medical Research, Faculty of Medicine, Technion, Haifa 31096, Israel.

Age-related changes of proliferative activity in the mandibular condylar cartilage of CW1 female mice, newborn to 18-mo-old, and its relation to changes in serum levels of iPTH were examined using the following parameters: 1. DNA content, 2. cellular density, 3. quantitative analysis of autoradiograms obtained from condyles of animals that received ip 2 uCi/g body wt. of 3H-thymidine for 24 hrs (*in vivo*) or analysis of autoradiograms of condyles that were cultured for 24 hrs *in vitro* with 2 uCi/ml of 3H-thymidine in BGJb medium supplemented with 10% FCS. The results showed a marked decrease in DNA content of the cartilage by the first three postnatal months followed by a steady state. Similar pattern was noted in cellular density of the tissue. *In vivo* 3H-thymidine autoradiography revealed a rapid decline in cell proliferation during the first 3 mos and virtually no

labeled nuclei could be observed in 6-mo-old specimens or older. On the other hand, even tissues obtained from 18-mo-old animals revealed a certain extent of 3H-thymidine incorporation *in vitro*, although the labeling index was reduced by 90% in comparison to newborns. This age-dependent reduction of all of the above parameters was highly correlated to changes in serum iPTH levels ($r = 0.9$, $p < 0.01$). Moreover, the proliferative activity of chondrocytes from old tissues could be readily restored by including PTH (2 ug/ml) in the culture medium. The findings of the present study clearly indicate toward an involvement of PTH in the regulation of cartilage cells activity.

11

LUMBAR CSF CHOLINE, ACETYLCHOLINESTERASE AND PROTEIN IN EARLY ONSET vs LATE ONSET ALZHEIMER'S DISEASE PATIENTS. *V. Kumar*, E. Giacobini, and R. Elble,* S.I.U. School of Medicine, P.O. Box 3926, Springfield, IL 62708.

The diagnosis of dementia of Alzheimer's type (DAT) includes both presenile and senile forms of the disease. Recent reports (Roth and Wischik, 1985, Seltzer and Sherwin, 1983, Nakamo et al, 1986) have suggested that early onset and late onset forms of Alzheimer's disease differ clinically and biologically. We studied the CSF choline, acetylcholinesterase (AChE) and protein of 50 Alzheimer patients (early onset (EO) (<65 yr.) N = 18, late onset (LO) (>65 yr.) N = 32, and 21 normal age-matched controls (age <65 yr. N = 8, age >65 yr, N = 13). We compared the CSF measures between various groups and found that there was no difference in choline and AChE in EO vs. LO patients, but proteins were non-significantly higher in LO patients than EO ($P = R0.066$). The choline/protein ratio was significantly higher ($P = 0.0067$) in EO cases compared with normal younger controls. Choline was also non-significantly higher in patients than in controls in this sample. In LO cases, choline was significantly higher when compared to age-matched controls. This higher choline in Alzheimer patients reflects changes in brain phospholipid metabolism related to the cholinergic deficit itself. These results suggest that CSF choline may be used as putative markers of the cholinergic disturbance in Alzheimer patients.

12

CONCURRENT CEREBRAL AND PERIPHERAL NEUROPATHIES IN AGED DIABETICS. *M.B. Miller* and N. Miller,* Center-Montefiore Hospital, White Plains, NY 10605.

This study revisits the common syndromata of combined stroke and diabetic neuropathy in the older diabetic. The incidence of peripheral neuropathies in the aged diabetic varies from 20 to 100%. The physical findings are: flaccid paresis, paralysis, atrophy, depressed reflexes, minimal contractures, sensory impairment and autonomic system dysfunction. Unilateral and bilateral cerebral infarcts are also frequent in the aged diabetic. Related disabilities are: dementia, aphasia, dysphagia, dysarthria, hyperreflexia, spastic hemiparesis-hemiplegia, spastic quadriparesis-quadriplegia, contractures and neurogenic bladder and bowel syndrome. The concurrence of both upper and lower neuron disease is inevitable. In the presence of dementia of bilateral cerebral MID, pain and impaired sensory functions are frequently reported. Combined disease is characterized by: (1) residual elements of spasticity but preponderance of lower motor characteristics (atrophy, flaccid paresis, paralysis), (2) contractures less than in MID, (3) characteristic hemiplegic gait absent. Arm reflexes are often hyperactive but depressed or absent in the lowers or vice versa. Sensory changes are manifested by impaired balance and frequency of falls. The presence of dementia and hemianopsias together with peripheral neuropathy of hands and legs characteristic of this disorder. Cases show the above including better rehabilitation prognosis of peripheral neuropathies.

13

ELEVATED SERUM DEHYDROEPIANDROSTERONE SULFATE LEVELS IN OLDER PRACTITIONERS OF THE TRANSCENDENTAL MEDITATION AND TM-SIDHI PROGRAMS. *J. Glaser*, J. Brind, M. Eisner, J. Vogelman, M. Dillbeck, D. Chopra, and K. Wallace,* Maharishi Internat. Univ., Fairfield, IA 52556, and Orentreich Foundation, New York, NY 10021.

Serum dehydroepiandrosterone sulfate (DHEAS) levels were measured in 252 men and 74 women who were experienced practitioners of the Transcendental Meditation (TM) and TM-Sidhi programs. These were compared according to sex and five-year age grouping to levels previously determined in normal, healthy subjects (799 men and 173 women) who were not TM practitioners. The mean DHEAS levels in the TM group were higher in all five of the age groups measured in women ($F = 15.29$, $p = .0001$). There were no systematic differences in younger men, but elevations were observed in TM practitioners in six of seven five-year age groups over 40 years ($F = 7.06$, $p = .0026$). The mean percentage of DHEAS elevation over comparison values was 29% for men over 45 and 48% for women over 45. This effect was independent of diet, obesity, and exercise.

DHEAS decreases systematically with age, and elevated levels have been correlated with reduced incidence of breast cancer, ischemic heart disease and mortality. In conclusion, these findings of elevated DHEAS levels in the TM group, which were comparable to normal non-meditators 10 to 15 years younger, implies that some characteristics of TM practitioners are retarding the usual age- and stress-related deterioration in DHEAS secretion by the adrenal cortex.

14

ZERO ERYTHROCYTE SEDIMENTATION RATE (ESR) AND THE IDENTIFICATION OF A POPULATION WITH LOWER THAN PREDICTED ESR VALUES. *D.E. Smith, J. Glaser, and M. Dillbeck,* Maharishi Internat. Univ., Fairfield, IA 52556.

The finding of an ESR of zero is unusual. Normal values are 1-13 mm in men and 1-20 mm in women. ESR goes up with aging. We have examined 464 volunteer male and female subjects at their usual place of work and found that 12% had zero ESR, when performed using 9 parts whole blood diluted with 1 part buffered citrate in Westergren tubes. Hematocrits in 15 of 50 subjects with zero ESR ranged from 40-50%, ruling out polycythemia as a cause of zero ESR.

The study consisted of 2 cohorts. 252 subjects were practitioners of Transcendental Meditation (TM). The control group consisted of 212 non-practitioners of TM and included Trappist monks and nuns, Seventh Day Adventists, college students, and employees of a machine tool shop. Using discriminate analysis we found that both youth ($F = 11$, $P = .0006$) and maleness ($F = 24$, $P < .0001$) significantly discriminated for zero ESR but that TM was the most powerful discriminator for zero ESR ($F = 35$, $P < .0001$). Using multiple regression analysis we found that ESR tends to increase by .169 mm/yr and that men had lower ESR than women. When the effects of age and sex are factored out, the TM group had ESR values 2.95 mm lower than controls (partial $F = 21.9$, $P < .0001$) or were 17.45 years younger with respect to ESR than controls.

We conclude that zero ESR is a normal finding in healthy subjects and that TM is highly predictive of zero ESR and is associated with lower ESR levels at all ages in both sexes.

15

LUNG CANCER WITH BRAIN METASTASES: SURGICAL APPROACH IN ELDERLY PATIENTS. *C. Chavantes*, L. Zamorano, M. Dujovny, G. Malik, F. Shibli, and D. Magilligan,* Henry Ford Hospital, Detroit, MI 48202.

In the USA, 40,000 patients/year with bronchogenic carcinoma develop brain metastases. We present a series of 42 patients 65 years old or older who underwent craniotomy for brain metastases. The survival curves were estimated using the Kaplan-Meier product-limit method, and logrank test was used for univariable analysis. Multivariable analysis was performed utilizing Cox regressional analysis to determine the influence of each survival predictor. The median survival from craniotomy time was 228 days, and 494 days from lung treatment. Lung stage (AJCC/TNM) was I in 4, II in 9 and III in 29 cases. The median survival in 20 patients who underwent lung surgery was 953 days. In contrast, 22 patients considered unresectable presented significantly lower median survival of 186 days. Resections were pneumonectomy in 5 and lobectomy in 14 cases, in which the median survival time was 866 and 1185 days, respectively. Karnovsky, lung surgery and resection type were the most important survival determinants. These data did not show any statistical

significance, when compared with our results obtained from 121 patients younger than 65 years old in terms of morbidity, operative mortality, survival and predictor factors. In conclusion, age constituted no impedance for surgical management of brain metastases from pulmonary neoplasm.

16

TRACE ELEMENT IMBALANCES IN HAIR AND NAIL OF ALZHEIMER'S DISEASE PATIENTS. *D.E. Vance[1]*, W.D. Ehmann[1,2], and W.R. Markesbery[2,3],* Depts. of Chemistry[1], Sanders Brown Center on Aging[2], and Pathology and Neurology[3], Univ. of Kentucky, Lexington, KY 40506-0055.

One of the hypotheses regarding the etiology of Alzheimer's disease (AD) implicates trace elements. In this study, 17 elements in 180 nail and 134 hair samples taken from AD and control subjects were determined using the technique of instrumental neutron activation analysis (INAA). In nail, the elements Br, K, Na, and Zn are significantly elevated in AD subjects, while Hg is significantly lower. In hair, Br, Se, and Zn are higher in AD subjects than in matched controls, while Ca and Co are lower. Aluminum was not determined in this work. Imbalances of six of these elements have also been observed in AD brains, although the imbalances are not always in the same direction. These data clearly show that imbalances for elements other than aluminum exist in AD, and that brain imbalances are reflected in non-neural tissues, but they do not provide proof for the direct involvement of trace elements in the etiology of AD.

17

BRAIN MORPHOLOGY AND CHOLINERGIC ENZYMES IN ALZHEIMER'S DISEASE. *V. Kumar*, R.C. Smith, E. Giacobini, and R. Elble,* S.I.U. School of Medicine, P.O. Box 3926, Springfield, IL 62708.

Pearlson and Tune (1986) have recently reported a significant positive correlation between Ventricular Brain Ratio (VBR) and CSF AChE (acetylcholinesterase) levels (AChE/ml), and VBR and mini-mental status scores (MMS), in a small sample of patients with SDAT. They suggested these parameters may, therefore, be used as dual or conjoint markers to help identify patients with this disorder. We studied a similar group of patients with SDAT, and assessed CSF AChE activity (AChE/ml and AChE/mg protein), CSF choline levels, measures of ventricular size (both VBR and global ranking of ventricle size) and cortical atrophy from CT scans, and baseline mental status measures (MMS, Blessed Dementia Rating Scale, CDR). In our sample there was no significant correlation between chemical measures in the CSF of patients with SDAT and measures of ventricular size or cortical atrophy. There was a consistent trend for VBR to be negatively related to mental status test scores or ratings in SDAT patients, but only two of these correlations were statistically significant. There were no correlations between our CSF neurochemical measures and these baseline mental status measures. These results do not support the contention that brain morphology and CSF measures of acetylcholine neurochemistry are closely related in SDAT, or that they can be used as conjoint markers for patients with this disorder.

18

PHYSICAL EXERCISE IMPROVES THE BONE STRUCTURE OF FEMORAL HEAD AND NECK IN SENESCENT MICE. *I. Arbell, A. Weiss, E. Steinhagen-Thiessen,* and M. Silbermann,* The Rappaport Institute for Medical Research, Technion Faculty of Medicine, Haifa, Israel and *The Eppendorf Univ. Hospital, Hamburg, FRG.

Hip joint fractures are common in aged humans and animals. The aim of the present study was to examine the effect of long-term physical exercise on the structure of the bone in the head and the neck of the femur. 30-month-old CW1 female mice were subjected to daily endurance training using a running wheel (30 min/day, 3.5 m/min, 6 day/week) for a period of 18 months. The femurs of trained animals and non-trained controls were processed for light microscopy and morphometry using Image Analysis System (Olympus Cue-2). The results showed a general improvement in the structure of the bone. The thickness of the cortical bone was increased by 62% in trained animals ($p < 0.01$). The cortical bone in the femoral neck was devoid of cracks and cavities characteristic of senescent bone, which was reflected in 53% increase in bone density ($p < 0.01$). Similarly, the density of the trabecular bone of the femoral head was increased by 56% in trained animals. In addition, the articular surface seemed to be better preserved, with a thicker articular cartilage (20%, $p < 0.05$), the cell number per unit area was increased and the signs of osteoarthritis were diminished.

The results of this study indicate clearly that physical exercise, if started early in life, may be beneficial for bone health, and may decrease the incidence of hip joint fractures.

19

OSTEOARTHRITIS IN PREHISTORIC NATIVE AMERICANS. *B.M. Rothschild and R.J. Woods,* St. Elizabeth Hosp. Med. Ctr., NEOUCOM, Kent State and Carnegie Institute, Youngstown, OH 44501.

Samples from the prehistoric records were examined to assess the relationship of body weight and joint use/abuse to development of osteoarthritis. The weight-bearing joints of Woodland Indians (1000-3000 years before present) and dinosaurs (65-200 million years before present) were subjected to gross examination for osteophytes, subchondral sclerosis, cyst formation, and other evidence of remodeling. Radiologic examination of 110 dinosaurs (17 genera representing the 8 major families), including 50 sauropod (Brontosaurus type) dinosaurs weighing up to 60 tons, revealed no evidence of remodeling. Osteoarthritis in the Woodland population was noted 25 years earlier than in contemporary populations examined. Distribution (knee:hip:ankle of 5:2:1) was double contemporary; the frequency of Woodland involvement was much higher than in contemporary; and the patello-femoral component was affected twice as frequently as the tibio-femoral. This reversal of component articulation involvement appears to reflect a mechanically disadvantageous habitual posture (kneeling) and life style. Lack of obvious correlation of osteoarthritis with body weight, and the noted relationship with mechanically disadvantageous use, support the importance of joint protection in its prevention.

20

INFLUENZA VACCINE ACCEPTANCE IN ELDERLY VETERANS. *S.H. Schler, L. Vargas and R. Ganguly*,* James A. Haley Veterans Hosp. and Univ. of South Florida Coll. of Med., Tampa, FL 33612.

Only 10-15% of the high risk groups receive influenza vaccines annually. This study was designed to determine the factors which influence acceptance of annual influenza vaccines in the aged veterans. Three hundred veterans $\geq$ 85 years of age, living at home, were randomly selected. Written questionnaires were developed which were mailed to survey these subjects. They were asked regarding circumstances which influenced their decision for or against the annual vaccine recommendation. Ninety-two persons responded to this survey and data were analyzed six months following the mailing date. Results indicated that the single most important factor for noncompliance was lack of awareness that annual immunization was necessary (36.9%). Fear of shots and side effects was the next most important reason for not taking the vaccine (19.56%). Lack of transportation for going to doctor's office prevented 13.04% of the subjects. Subjects also did not know where or how to get the vaccine (11.96%). Veterans who did not want to bother about taking prophylactic shots were 10.87%.

These data suggest that lack of awareness, knowledge and information about annual influenza immunication play a major role in its poor acceptance. Health education measures could improve vaccine compliance in this group of elderly population.

21

FAILURE OF VITAMIN E TO PROTECT AGAINST LIGHT DAMAGE TO THE RETINA. *M.L. Katz* and G.E. Eldred,* Univ. of Missouri School of Medicine, Dept. of Ophthalmology, Columbia, MO 65212.

Previous investigations have shown that visible light exposure contributes to the age-related loss of photoreceptor cells from the albino rat retina. Experiments were conducted to determine

whether vitamin E is effective in retarding this light-mediated cell death. Albino Fisher 344 rats were divided into four treatment groups at 21 days of age. Two groups were fed a diet containing 250 mg of dl- α-tocopheryl acetate per kg, and the other two groups were fed the same basal diet lacking vitamin E. One-half of the animals in each dietary group were housed under cyclic light (12 hr on/12 hr off) of 1000 lx, and the remaining animals were maintained on the same lighting cycle, except that the light intensity to which they were exposed was only 15 lx. After 17 weeks the animals were killed and their retinas were examined histologically. Vitamin E deficiency resulted in a modest photoreceptor cell loss in animals exposed to the 15 lx light intensity. All animals maintained under the higher light intensity howed severe loss of photoreceptor cells. In animals fed the vitamin E-supplemented diet, very few photoreceptors remained anywhere in the retina. In most vitamin E-deficient animals, on the other hand, there was a region in the superior retina where a substantial number of photoreceptor cells remained. Therefore, it appears that, contrary to expectations, vitamin E deficiency protects against light damage to the retina.

22

AGE-RELATED ACCUMULATION OF CAROTENOIDS IN HUMAN RETINA. *G.J. Handelman*, E.A. Dratz and F. J.G.M. van Kuijk,* Eye Research Institute, Retina Foundation, Boston, MA 02114, and Dept. of Chemistry, Montana State Univ., Bozeman, MT 59717.

The carotenoid pigments in the whole human retina and in the macular region were measured quantitatively by high-performance liquid chromatography (HPLC) in donor specimens over a broad age range. The dominant carotenoids in the whole retina are lutein and zeaxanthin. Zeaxanthin is concentrated in the macular region, whereas lutein is dispersed throughout the retina. Substantial quantities of both carotenoids are present in the infant retina. Increasing variability is observed in retinal carotenoid levels between individuals of advancing age. In some adults pigment levels were comparable to infant retinas, whereas in other adults levels found were 4-fold or greater than in infants.

Carotenoids have been proposed to be potent antioxidants, protecting membrane lipids from toxic peroxidation reactions. The method presented in this study will allow quantitative investigations of the association between carotenoid levels and age-related degenerations of the retina.

23

PREVENTION OF AGE-RELATED LOSS IN HEPATIC MALONDIALDEHYDE (MDA) OXIDATION BY FOOD RESTRICTION. *J-W. Kim* and B.P. Yu,* Dept. of Physiology, Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7756.

The ubiquitous MDA, a major by-product of lipid peroxidation, has been reported to be mutagenic and cytotoxic by inactivating various cellular components. MDA has also been known to be a major component involved in formation of age pigment in aging tissues. We have explored the effect of anti-lipid peroxidation action of food restriction on *in vitro* MDA oxidation in aged rats. Mitochondria, microsomes and cytosols were prepared from liver isolated from *ad libitum* fed and food restricted rats at various ages. The optimal *in vitro* MDA oxidation requires 1 mM NAD and 10 mM Mg^{++} in the incubation medium (pH 7.4). The results show that the mitochondrial MDA oxidation activity is much higher than cytosol fraction followed by microsomes that show little oxidation activity. It was found that MDA oxidation activity of mitochondria was progressively lost during aging but this age-related loss was slowed by food restriction.

We conclude that food restriction can modulate the age-related declined in MDA elimination process by maintaining high MDA oxidative activity of mitochondria.

24

ANTIOXIDANT DEFENSE RESPONSE IN ENDOTHELIAL CELLS SUBJECTED TO HYPEROXIA. *R.S. Chuknyiska,* Johns Hopkins Medical Inst., Dept. of Surgery, Baltimore, MD 21224.

Hyperoxia can result in the development of a severe form of pulmonary dysfunction. There is an indication that old animals are more sensitive to hyperoxic stress than the younger ones. The reason for higher antioxidant resistance in younger animals is still unknown. Since endothelial cells interface directly with the blood, they are the first ones to be exposed to high oxygen concentrations and its toxic metabolites. We measured the basal endogenous levels of the antioxidant enzymes superoxide dismutase and catalase as well as the inductive capacity of these enzymes in endothelial cells from young and old rat pulmonary artery. Cells were maintained in normal or high oxygen level for various amounts of time. Cells of young rats had improved survival over old cells when exposed to hyperoxia. Basal levels of the antioxidant enzymes in old and young cells were not significantly different, however, young cells underwent a more rapid and statistically greater increase in these enzymes than the old cells under hyperoxic conditions. The protein levels increased in parallel to enzyme activity. This suggests a greater inductive capacity of superoxide dismutase and catalase in younger animals.

25

EFFECT OF AGE ON BETA RECEPTOR BINDING IN HEARTS SYMPATHECTOMIZED BY 6 HYDROXY DOPAMINE. *N. Tumer*, H. Fenton, C. Boehm, and J. Roberts,* Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The effect of age on the capacity of cardiac beta-adrenergic receptors to develop supersensitivity was studied in the hearts of Fischer 344 male rats at three ages (6, 12, and 24 mo). The animals were injected with 6 hydroxy dopamine HBr (6-OHDA), 100 mg/kg, i.v. on day one and on day eight; they were sacrificed on day 15. The binding of the beta-adrenergic antagonist ^{125}I-iodopindolol was used to quantitate and characterize cardiac beta adrenergic receptors. The maximal number of binding sites (B_{max}) in hearts sympathectomized by 6-OHDA were significantly increased at each age. Dissociation constants (K_d) were significantly elevated only in the case of the 12 and 24 month old animals:

	6 month	12 month	24 month
Control			
B_{max}	14.06 ± 2.2	11.55 ± 0.9	7.80 ± 0.04
K_d	0.164 ± 0.04	0.205 ± 0.05	0.132 ± 0.02
6 OHDA			
B_{max}	20.04 ± 0.04*	19.07 ± 1.6*	11.42 ± 0.18*
K_d	0.207 ± 0.04	0.319 ± 0.04†	0.181 ± 0.04†

*P < .0001
†P < .005

These results indicate that beta receptor mechanisms in older hearts can respond to procedures which cause supersensitivity.

26

ENHANCED PRODUCTION OF COLLAGENASE BY LATE PASSAGE CULTURES OF HUMAN FIBROBLASTS. *J. Sottile and A.J.T. Millis*,* Dept. of Biology, SUNY at Albany, Albany, NY 12222.

Comparison of the proteins secreted by early and late passage cultures of human fibroblasts revealed that late passage cell conditioned medium (OCM) contained enhanced levels of three proteins of Mr = 55kDa, 58kDa, and 61kDa. The level of stimulation for 55kDa and 58kDa proteins was from 9-30X. Polyclonal anticollagenase antibodies were used to immunoprecipitate the 55kDa and 58kDa proteins from OCM and to identify them as (latent) collagenases. Other methods for identification included peptide mapping and activity studies (after activation by trypsin). The question of whether the differences observed in the two conditioned media reflected changes in collagenase synthesis or simply changes in levels of secretion was addressed using indirect immunofluorescence. Analysis of intracellular collagenase revealed that 77% of late passage cells stained positively for the enzyme vs 4% of early passage cells. This supports the conclusion that the constitutive level of collagenase synthesis was greater in aged cell cultures. The effects of extracellular matrix, on the regulation of collagenase production, was also addressed. Both early and late passage cultures were seeded onto a matrix produced by human fibroblasts and each responded via enhanced production of collagenase (>12X for early passage cells and >2X for late passage cells). The

response was dependent on protein synthesis as evidenced by its sensitivity to inhibition by cycloheximide. Although cultures of different *in vitro* ages responded positively to matrix-coated substrate, the induced levels were greater in the OCM.

27

IMMORTALIZATION OF HUMAN FIBROBLASTS IN CULTURE. *D. Neufeld and H.L. Ozer*,* Dept. of Biology, Hunter College, CUNY, New York, NY 10021.

Human fibroblasts (HF) derived from normal tissue undergo a characteristic pattern of growth in cell cultures ending with a non-replicative phase and, finally, cell death ("senescence"). In striking contrast to rodent cells, HF do not spontaneously overcome senescence and become immortal. However, immortalized HF have been reported at low frequency after transformation with SV40-transformed virus. We have isolated a series of SV40-transformed HF using replication-defective viral genomes containing either a wild-type SV40 T antigen (SV/HF) or that of tsA58 encoding a heat-labile T antigen (SVtsA/HF). Such transformants typically show an extended life span in culture and generate immortalized sublines at an increased frequency as compared to those obtained after virus infection. In each system, we have clonally isolated multiple immortalized derivatives for comparative studies on the genetic and biochemical mechanisms of immortalization. Furthermore, preimmortal sublines of SVtsA/HF show temperature-dependent expression of cell growth, permitting synchronous induction of a "senescence-like" growth arrest.

28

AGE-RELATED REDUCTION IN THE PROLIFERATIVE ACTIVITY OF ARTICULAR CARTILAGE AND ITS RESTIMULATION BY VARIOUS FACTORS *IN VITRO*. *A. Weiss, E. Livne, and M. Silbermann,* Rappoport Institute for Medical Research, Technion-Israel Institute of Technology, Haifa 31096, Israel.

The reparative potential of articular cartilage relies to a great extent upon its ability to induce cell division followed by an increase in cellular synthetic activities. Adult chondrocytes have a limited potential for cell replication, although there are evidences for cell divisions in articular cartilage of osteoarthritic joints.

The aim of the present study was to examine the age-related changes in the proliferative activity of chondrocytes and the effect of various growth factors upon this aspect of tissue-healing potential. Mandibular condyles of female CW1 mice aging from 3 to 18 months were cultured for 72 hrs in BGJb medium supplemented with 10% FCS. Test cultures received either PTH (2 ug/ml), MSA (5 ug/ml), PGE1 (20 ug/ml) or pSGF (10 ug/ml). 3H-Thymidine (2uCi/ml) was added for the last 48 hrs in culture, and the specimens were processed for autoradiography and analyzed quantitatively. The results showed a marked, age-dependent decrease in labelling index (-80% in 18 mos old as compared to 3 mos, $p<0.01$). The unique finding in this study was the possibility to resume the proliferative activity of chondrocytes with all of the factors mentioned above even in the old tissues. PTH appeared to be the most effective, as it increased the labeling index in 18 mos old specimens by two folds.

29

A PROTEINASE INHIBITOR MODEL OF AGING. *G.O. Ivy,* Univ. of Toronto, Scarborough Campus, Ontario, Canada M1C 1A4.

The cellular mechanisms underlying the aging process are often obscured by the secondary and tertiary effects of aging. My work is directed toward understanding the causes of aging by developing a model to mimic various morphological and biochemical concomitants of aging in the brains of young rats.

Young rats received either 1) a continuous intraventricular infusion of the thiol proteinase inhibitor, leupeptin, or of artificial CSF for 2 to 10 weeks or 2) intraventricular injections of drug or vehicle for 1-3 days. Some animals were processed for histo- and immunocytochemical analysis while others were used for biochemical isolation of induced ceroid-lipofuscin (CL).

Leupeptin caused the intracellular accumulation of dense bodies which by several morphological and histochemical criteria resemble lipofuscin. There was a 3- to 8-fold increase in the activity of lysosomal enzymes in certain brain regions in drug, but not CSF, treated rats. Treatment with leupeptin also caused other manifestations of brain aging such as neurofillament accumulation, dendritic deterioration, regional cell death and an accumulation of dolichols. Biochemical isolation of the induced CL yielded a five-fold greater amount of protein from brains of drug as compared to CSF treated rats. Analysis of the isolated material demonstrated that two glycoproteins (Mr 84K and 52K) were enriched in the leupeptin-treated brains.

The mechanisms underlying the buildup of lipofuscin may be closely linked to mechanisms underlying cellular aging, and proteinase inhibitors may provide a model for their elucidation.

30

FUNCTION OF SPECIFIC MACROPHAGE RECEPTOR FOR GLUCOSE-MODIFIED PROTEINS DECREASES WITH AGE. *H. Vlassara*, D. Harrison, M. Brownlee, A. Pasagian, and A. Cerami,* The Rockefeller University, New York, NY 10021.

A high affinity macrophage receptor has been shown to mediate the removal of proteins modified by Advanced Non-enzymatic Glycosylation Endproducts (AGE). AGE-proteins are known to accumulate with time in diabetic and aging tissues such as vascular wall, kidney and peripheral nerve causing irreversible damage. We have now hypothesized that during normoglycemic aging macrophages may lose capacity for AGE removal.

In order to evaluate the effect of age on this macrophage receptor-mediated system for the removal of senescent AGE-proteins, we have utilized peritoneal resident macrophages from two groups of normal $C_{57}BL/6$ mice of young (4-10 mos.) and old age (over 20 mos.). Binding and degradation rate of radioiodinated AGE-bovine serum albumin (AGE-BSA) by macrophages from each group were determined from saturation kinetics. Scatchard plot analysis of normal young Balb/c mouse macrophages has previously indicated $1.5X10^5$ receptors per cell, with a binding affinity (Ka) of $1.7X10^7M^{-1}$. A greater than 2-fold decrease in uptake and degradation and a concomitant decrease in receptor number and binding capacity was found in cells from the old group of mice as compared to young ($P<0.005$). These data suggest that aging may adversely affect the AGE-receptor mediated removal of highly crosslinked glycosylated proteins from sites of aging tissue damage.

31

ISOLATION AND CHARACTERIZATION OF A RECEPTOR FOR PROTEINS MODIFIED BY ADVANCED GLYCATION END-PRODUCTS (AGE) FROM A MACROPHAGE CELL LINE. *S. Radoff*, H. Vlassara, and A. Cerami,* The Rockefeller University, New York, NY 10021.

The nonenzymatic reaction of glucose with free protein amino groups results in the formation of a complex group of products capable of cross-linking proteins. These irreversible AGE-protein adducts accumulate with time and have been implicated in tissue damage associated with diabetes and aging. We have previously shown that macrophages bind and degrade AGE-proteins via a specific receptor, thus selectively removing senescent macromolecules.

We have solubilized this receptor from the membranes of the murine macrophage cell line RAW 264.7. We have characterized the nature of receptor ligand interaction by competition studies using modified ligands. Scatchard analysis of solubilized material shows a binding affinity (Ka) of $3.1x10^7M^{-1}$. We have defined the molecular weight of the receptor by chemical cross-linking as 90 Kda, and have isolated a pure protein of this molecular weight which we believe to be the receptor.

These studies have provided further data and material which allows the complete structural analysis of a unique macrophage receptor system for the removal of highly crosslinked glucose modified proteins from aging tissue.

32

ELEVATED GLUCOSE 6-PHOSPHATE LEVELS ARE ASSOCIATED WITH PLASMID MUTATIONS *IN VIVO.* *A.T. Lee* and A. Cerami,* Lab. of Medical Biochemistry, The Rockefeller University, New York, NY 10021.

Previous work has shown that the incubation *in vitro* of plasmid DNA with glucose 6-phosphate has a mutagenic effect when transformed into wild-type *E. coli.* To further investigate the modifications of DNA by the reducing sugar glucose 6-phosphate, we have developed an *in vivo* model to monitor plasmid DNA mutations. *E. coli* strains which are defective in phosphoglucose isomerase alone (DF40) or also defective in glucose 6-phosphate dehydrogenase production (DF2000) were transformed with a plasmid which carries the genes for ampicillin resistance and -galactosidase production. The transformed bacteria were grown in glucose/gluconate minimal medium, then assayed for glucose 6-phosphate levels and plasmid mutation rates. An increase in plasmid mutations (6 and 13 fold) was associated with increased intracellular glucose 6-phosphate levels (20 and 30 fold) present in the DF40 and DF2000 strains, respectively. Growth of the bacteria in gluconate minimal medium does not increase the intracellular levels of glucose 6-phosphate or the rate of plasmid mutations over background. Further characterization of the mutated plasmids showed that insertions, deletions and point mutations were responsible for the loss of -galactosidase production. The increase in plasmid mutations as a function of increased intracellular glucose 6-phosphate levels suggests that the accumulation of adducts formed by glucose 6-phosphate and other reducing sugars over time may contribute to DNA damage associated with aging.

33

IgD-INDUCED EFFECTS ON Ig PRODUCTION IN YOUNG AND AGED MICE. *C.D. Swenson*, G.W. Siskind, G.J. Thorbecke and R.F. Coico,* NYU Medical Ctr., New York, NY 10016, Cornell Medical College, New York, NY 10021, and CUNY Medical School, New York, NY 10031.

IgD induces Fc δ receptors on T cells of the L3T4+, $Ly2^-$ phenotype in young adult BALB/c mice after exposure to IgD *in vitro* or after injections of IgD *in vivo.* Such "T δ cells" have immune augmenting properties *in vivo.* Aged BALB/c mice, 20 mo. old, do not respond to IgD with increased expression of Fc δ receptors. Therefore, in the present study the functional effects of IgD on the immune response in young and old mice were compared. Six to eight week old adult mice receiving $\geq$ weekly injections of IgD-containing ascites showed increased production of all Ig isotypes tested (but not of IgD itself) as enumerated by the reverse plaque forming cell (PFC) assay. Treatment with IgD augments the production of IgM, IgG_2, IgG_1, and IgA, 8, 80, 25 and 7 fold, respectively. This effect on Ig secretion is absent in aged ($\geq$20 months) mice.

Although neither IgD nor T δ cells induce proliferation of B cells *in vitro,* there was an effect of IgD on B cell proliferation in young adult mice *in vivo* as examined histologically. Weekly injections, $\geq$ 2, of IgD-containing ascites caused a significant increase in the incidence of lymphoid follicles containing germinal centers. Approximately 60% of the follicles in IgD-treated mice contained germinal centers as compared to 23% in saline-treated controls. Again, this effect of IgD was absent in aged mice: the percentage of lymphoid follicles containing germinal centers in IgD-treated aged mice was 37%, which was not significantly different from the 21% observed in age-matched controls. In addition, while injections of IgD given prior to a primary injection of a T-dependent antigen enhanced both primary and secondary responses to that antigen in young adult mice, such an effect of IgD was not observed in aged mice. Attempts to overcome the resistance of aged mice to IgD-mediated augmentation of the immune response by injection of lymphokines are under way.

34

DETECTION OF PUTATIVE DNA SEQUENCE CHANGES IN AGING INBRED RATS. *A. Uitterlinden*, E. Slagboom, C. Zurcher, C. Tan, W. de Leeuw and J. Vijg,* TNO Institute for Exp. Gerontology, PO Box 5815, 2280 HV Rijswijk, The Netherlands.

The continuous exposure of aging organisms to DNA damaging agents might ultimately lead to changes in the DNA sequence organization. Whether these changes occur randomly throughout the genome or in certain "hot spots" is unknown. To address this problem we are presently analyzing various types of genomic sequences with respect to putative age-related changes in their organization and expression in different organs and tissues. As a model system for both longitudinal and cross-sectional studies, inbred rats are used, the pathology of which has been well characterized. So far, Southern blot analysis of several single- and multi-gene systems in normal tissues has revealed no large-scale DNA rearrangements in cross-sectional studies. In order to improve the sensitivity of our analysis and to include repetitive DNA sequences in our studies, we are now applying a novel DNA separation technique which we have further developed: two-dimensional "DNA fingerprinting". Using this technique we have successfully resolved highly repetitive model sequences. Currently, we are using several other repeat-probes derived from families with a dispersed organization, to be able to analyze a large number of different regions in the genome. Application of these probes in the two-dimensional DNA fingerprinting analysis will enable us to establish whether high frequency DNA sequence changes occur during development and aging.

35

PROTEOGLYCAN SYNTHESIS IN ALZHEIMER'S DISEASE FIBROBLASTS. *M. Zebrower, C. Beeber, and F.J. Kieras,* Office of Mental Retardation and Developmental Disabilities, New York State Inst. for Basic Research in Dev. Disabil., 1050 Forest Hill Rd., Staten Island, NY 10314.

Skin fibroblast lines established from patients with Alzheimer's disease and from old and young normal individuals were labelled for three days with ^{35}S-sulfate and ^{3}H-glucosamine. Proteoglycans were then extracted, using guanidine hydrochloride-containing protease inhibitors and purified by Sephadex G-50 and DEAE-Sephacel chromatography. Sulfate incorporation into proteoglycans was increased three-fold while glucosamine incorporation was the same in Alzheimer's disease relative to normal controls. Thus, the sulfate-to-hexosamine ratio was three-fold greater in Alzheimer's disease. Enzymatic digestion of these proteoglycans showed that sulfate incorporation into chondroitin sulfate proteoglycans and heparan sulfate proteoglycans was increased two-fold and four-fold, respectively, in Alzheimer's disease relative to normal controls. In addition, a higher proportion of both chondroitin sulfate proteoglycans and heparan sulfate proteoglycans remained cell-associated in Alzheimer's disease relative to normal controls.

36

EFFECTS OF DIETARY MANIPULATIONS ON NEURONAL LIPOFUSCIN PIGMENT IN AGING MICE. *K. Nandy and S. Nandy,* Depts. of Anatomy and Neurology, Boston Univ. Med. Sch. and Boston City Hosp., Boston, Massachusetts 02118.

The effects of dietary (caloric) restriction on the neuronal lipofuscin pigment in mice of different ages have been studied. Young (3 mos.) and old (24 mos.) female C57BL/6 mice were subjected to restricted diet by feedings of 2.0 gm/day of Purina mouse chow for 24 and 6 months respectively. Control animals consisted of mice of the same age and sex fed *ad libitum* on the same diet (average daily intake of about 5gm). Animals were kept in a temperature- and humidity-controlled environmental chamber and were weighed weekly. Young mice were sacrificed after 3, 6 and 24 months, and older animals were sacrificed after 3 and 6 months. After sacrifice, the brains and other organs were dissected out, weighed and stored at −70C until use. In this study, the frozen sections of the brains were cut in a cryostat for examination of lipofuscin pigment in the areas of the hippocampus and frontal cortex. Lipofuscin was visualized by its characteristic autofluorescence and was measured by the use of an ocular grid in the fluorescence microscope. There was a significant decrease of the pigment in the dietary group of young mice compared to the controls. On the other hand, the old dietary animals did not show any significant change in the pigment. It was apparent that the dietary restriction was most effective in the young animals, and less and less so as the animals grew

older. If lipofuscin is considered a reliable marker of neuronal aging, the dietary restriction at an early age might be considered as an effective tool which may be used to modify neuronal aging.

37

IMMUNODIAGNOSIS AND IMMUNOTHERAPY OF PATIENTS WITH ALZHEIMER'S SYNDROME. *H.H. Fudenberg and V.K. Singh,* Med. Univ. of South Carolina, Dept. of Microbiology and Immunology, Charleston, SC 29425.

Based upon the unique structural and functional similaries of central nervous system (CNS) cells and peripheral blood immune cells, we utilized peripheral blood immune cells for studies of patients with CNS disorders, especially of the effects of therapy. Using this approach in Alzheimer's Disease (AD), we found aberrations of both cellular immunity and humoral immunity. Depending upon the nature of the immune deficits and patients' responsiveness to appropriate immunomodulant therapy, we have thus far distinguished 4 subsets of AD patients: one subset with defect (membrane fluidity) of a specific T cell and response to pyrrolidone therapy; a second subset with serum antibodies to neuron-axon filament proteins; these patients have clinical improvement after therapy with Dialyzable Leucocyte Extract (DLE); a third subset with antibodies to brain antigens (autoimmune) for which therapy is not yet developed; and a fourth subset with none of the abnormalities mentioned above, probably heterogeneous due to multiple biochemical deficiencies. We believe that different therapeutic modalities will be necessary for different subsets, much like the situation with other "diseases" such as anemia and diabetes. These results provided additional evidence that AD is a syndrome, not a single disease. Additionally, the clinical improvement demonstrated that the defective function in AD is not because the relevant neuronal cells are dead, but they are either atrophied or functionally suppressed.