

ANNUAL REPORT
OF THE OFFICE OF SCIENTIFIC DIRECTOR
NATIONAL INSTITUTE ON AGING
INTRAMURAL RESEARCH

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Annual Report of the Office of Scientific Director National Institute on Aging

The Scientific Director of the NIA is responsible for the overall direction and quality of research conducted by the Intramural Research Program (IRP) which includes nine laboratories and branches in Baltimore and the Laboratory of Neurosciences located in the NIH Clinical Center in Bethesda. The Office of the Scientific Director oversees the central administrative and support activities necessary for the successful operation of the Intra-mural Program. These activities are carried out by the Administrative, Procurement, and Information Offices, and the intramural program Personnel Office.

Fiscal Year 1994 featured a number of changes in key staff. Dr. Gunther L. Eichhorn, chief, Laboratory of Cellular and Molecular Biology retired after a distinguished career spanning 39 years, including serving as NIA acting scientific director in 1988.

Another key player supporting research, Mr. Phillip Thorne, chief of the Research Resources Branch, retired also along with two other important branch staff, These departures have necessitated an evaluation of the resources available and future directions for this important IRP support unit.

Ms. Bertha Voelker, administrative officer, with over 30 years of administrative experience at the Center, retired in December 1993. After an extensive search, Ms. Elise Kreiss was selected to fill this position and joined the IRP in August of 1994. Ms. Kreiss brings impressive NIH administrative and budgetary experience to her new position.

In the research arena, the year proved a productive one for the Intramural Research Program. Selected highlights from the laboratories and branches follow.

Basic Sciences

● GADD153 Activation Pathway

Scientists in the Gene Expression and Aging Section are using the growth arrest and DNA inducible gene, GADD153, to investigate signal transduction pathways operating to regulate gene expression following DNA damage. Evidence has been found in this laboratory indicating that GADD153 is activated through a p53-independent pathway which does not require activation of tyrosine kinases and is independent of c-ras and c-raf activation. However, other recent studies suggest that an alternative pathway dependent on p53 also could contribute to GADD153 induction in response to DNA damage.

● DNA Repair in Cancer and Aging

Different model systems and approaches have been used to explain DNA repair changes that occur in cancer and senescence. One system used by Laboratory of Molecular Genetics (LMG) scientists is that of telomeric DNA. Telomeres are the end regions of chromosomes and are critical for the maintenance of genomic stability. It appears that telomeres decrease with aging. This year a protocol was developed to measure DNA damage and repair in telomeres. There is less repair in old than in young telomeres. Persistence of telomeric DNA could be an alternative explanation for telomeric shortening.

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1000000
1000000

● Cardiovascular Gene Therapy

Gene therapy has great potential for its possible clinical applications and as a tool to learn more about basic biological events. Adenovirus vectors have been used successfully for gene transfer *in vivo* and look promising for treating cardiovascular disorders. Researchers in the Laboratory of Cardiovascular Science have engineered replication-deficient recombinant adenovirus vectors carrying the cDNA for Vascular Endothelial Growth Factor (VEGF), for acidic Fibroblast Growth Factor (aFGF), and for a recombinant form of aFGF modified by the addition of the signal sequence for secretion from FGF-4. These viral vectors cause endothelial cell proliferation, modulate endothelial cell proliferation into capillary-like structures *in vitro*, and induce angiogenesis in a mouse model *in vivo*. The scientists next plan to engineer additional adenovirus vectors which may cause angiogenesis and inhibit restenosis after vascular injury.

● Degenerative Cartilage Disease Therapies

Osteoarthritis (OA) and Alzheimer's disease (AD) are degenerative diseases afflicting millions of older people. Both diseases involve loss of cells required for the maintenance and function of tissues they are associated with, cartilage and the central nervous system, respectively. Cell Biology Unit researchers are testing the hypothesis that this cell loss is due to programmed cell death (apoptosis). This year, they used the TUNEL technique to label nuclei containing fragmented DNA and found that the level of apoptosis in AD brains was elevated in comparison to age-matched control brains. This was most pronounced in the CA-1 region of the hippocampus. Evidence also has been obtained showing that, at least *in vitro*, chondrocytes die by apoptosis in response to conflicting growth signals. Researchers are now testing a rat model of age-associated cartilage degeneration to determine the incidence of apoptosis in this tissue with age and disease.

● Oxidative DNA Damage and Repair

Oxidative DNA damage is very relevant to aging since it is a main product of metabolic processes in cells, and this damage does accumulate with aging. This accumulation is most established for mitochondrial DNA. The DNA damage that may accumulate in regions such as mitochondrial DNA may lead to a rise in mutations gene inactivation, or to deletions commonly found in cancer and aging. Laboratory of Molecular Genetics researchers have developed a technique which allows them to measure the formation of an important oxidative DNA lesion, 8-OH guanosine, at the level of individual genes. Efficient repair of this lesion was found in both nuclear and mitochondrial DNA. It was previously thought no DNA repair took place in mitochondria, but this finding opens a multitude of perspectives. Next these scientists will determine whether the mechanism of repair in mitochondria differs from that in the nucleus, whether mitochondrial DNA repair declines with age, and if local DNA repair defects in mitochondria might lead to DNA deletions.

● Caloric Restriction and Primate Aging

Researchers in the Molecular Physiology and Genetics Section found that both fasting levels and peak glucose levels in a glucose tolerance test are reduced in caloric restricted rhesus monkeys. Thus, with the delayed maturation and reduced body size reported previously, it seems that caloric restriction exerts many effects in primates which parallel those seen in rodents. Other effects seen which agree with those noted in rodents are a decrease in IGF-1 levels and a possible reduction in the normal age-associated increase in the skin collagen pentosidine, an index of glycation. Pentosidine accumulation appears to be proportional to the maximal lifespan of the species examined. It occurs twice as fast in squirrel monkeys as in the rhesus. Other caloric restriction effects include increased activity levels of young rhesus during daylight hours and a possible decrease in fibroblast clonal efficiency.

● Enhancing Memory for Maze Learning

In collaboration with an NIDA scientist, Laboratory of Cellular and Molecular Biology staff found that the polyamine, spermidine, potentiates the antagonistic effects of the NMDA channel blocker, dizocilpine, on the maze learning performance of young rats when both were given systematically. This demonstrates that signal transduction through the NMDA receptor can be augmented by drugs acting at the polyamine site. Studies are now underway to evaluate whether spermidine treatments can improve learning performance in aged rats, but notable side effects require a substantial dosage reduction.

Clinical Studies

● Arecoline Improves Memory in DAT Patients

Nine patients with mild-to-moderate DAT received arecoline by continuous, escalating intravenous infusion for about two weeks in a Laboratory of Neurosciences study. An optimal dose was then given continuously for five days using a placebo-controlled, randomized, double-blind, crossover design. The researchers found that long-term recall increased during dose-finding across subjects, with a U-shaped relation to dose. Measurements were made using the Buschke Selecting Reminding test. Long-term recall improved for five of six initial responders (mean increase six words) during re-infusion at an optimal dose. No side effects were noted. Thus, during reinfusion of arecoline, with individual dose optimization, verbal memory is selectively improved in the majority of patients with mild to moderate Alzheimers disease.

● Brain Aging and Disease

Laboratory of Neurosciences researchers used an extract of normal human entorhinal cortex to generate antibodies which stained axonal/synaptic elements and neurofibrillary tangles in the temporal lobe of Alzheimers brain, and elements of the limbic system in rat brain. They developed a selective method to identify such antibodies (SOFISTIC). This antibody may help determine why the human entorhinal cortex is vulnerable to Alzheimer's pathology.

● Heart Performance and Exercise

Aging is often accompanied by a decrease in physical activity and by a reduction in maximal exercise capacity and cardiac performance. Laboratory of Cardiovascular Science researchers have shown that older endurance trained men have better cardiac pumping capacity during exhaustive bicycle exercise than their untrained age peers. Older athletes (mean age 63 years) achieved 35 percent higher peak work rates and pumped 23 percent more blood at exhaustion than untrained men. In fact, they achieved levels similar to those in younger men. These results suggest that lifestyle factors may account for much of the age-associated decline in maximal cardiovascular performance, and that regular vigorous exercise may help attenuate this decline.

● Exercise Lessens GI Bleeding

A study by investigators in the Epidemiology, Demography and Biometry Branch suggested that older people who exercised at least three times a week, even if only walking or gardening, were 30 percent less likely to suffer severe or fatal gastrointestinal hemorrhages. Regular walking reduced the risk of internal bleeding by about 50 percent. More than 6000 deaths and 542,000 hospitalizations were linked to this problem in 1991. This research involved 8205 people 68 years of age and older who participate in the NIA's Established Populations for Epidemiologic Studies of the Elderly (EPSE).

● Regulation of Insulin Action

Diabetes Unit scientists have shown that incretin hormones, such as the glucagon-like peptide (GLP), which modulates glucose effects on pancreatic beta cells, also modulates insulin effects on insulin sensitive tissues. This was demonstrated in human studies where GLP increased glucose utilization in both insulin-dependent and non-insulin dependent diabetics. It was also shown that GLP stimulates insulin mediated glucose uptake and insulin mediated lipid synthesis in 3T3-L1 adipocytes. The presence of GLP receptor in several insulin sensitive tissues suggests the extrapancreatic effects of GLP on other insulin target tissues.

● Blood Pressure Regulation

Hypoventilation results in a decrease in blood pH. Laboratory of Behavioral Sciences researchers are testing the hypothesis that this increase in acidity could result in an increase in Na^+/H^+ exchange in the kidney and an increase in vascular tone which could lead to an increase in peripheral resistance and high blood pressure. Studies this year have shown (1) human subjects, trained in the laboratory to maintain hypoventilation for 30 minutes, show a significant decrease in urinary sodium excretion and a rise in blood pressure which did not recover to baseline after the task as expected; (2) Among subjects between aged 50 and 80 years, a significant positive correlation exists between resting pCO_2 and blood pressure; (3) In 150 BLSA participants there is a positive relationship between resting levels of blood pressure, plasma ouabain, and a plasma, digoxin-like factor—both ouabain and the digoxin-like factor are known to inhibit sodium pump activity.

● Hip Fractures and Functional Recovery

Hip fractures affect 270,000 people with health costs amounting to \$11 billion annually. A recent study by Behavioral Nursing Research staff, involving 100 community-living persons 65 years of age and older, admitted to two Baltimore hospitals for hip fractures, found that 69 percent were women with an average age of 78 years. Only 35 percent of these patients returned home following hospitalization; 61 percent went nursing homes for rehabilitation or long-term care; and, 4 percent died before discharge. Pre-operatively 74 percent of patients were independent in activities of daily living, but at discharge only a out 20 percent were independent in mobility tasks such as sitting up in bed, transferring out of bed and standing. As a result of these findings a new research program has been designed to see whether nurse- administered exercise and activity can improve the degree of function and discharges to homes.

● Mental and Physical Health Predictors

Psychologists in the Laboratory of Personality and Cognition are investigate interpersonal expression of antagonism, a component of the Type A Behavior Pattern (TABP) that confers risk for coronary heart disease (CHD). Other TABP components such as pressured speech, job involvement, and hurried life pace are unrelated to CHD. A new rating Observed Style, assesses the actual expression of antagonism in an interview setting. Another, Self-Description, rates the degree of antagonism in what respondents say. When these ratings were used this year to rate Structured Interviews from the Multiple Risk Intervention Trial (MRFIT), results confirmed previous findings showing Observed Style conferred risk for CHD. Current research is investigating whether the addition of Self-Description, what respondents say as opposed to how they act in the interview, adds to the predictive value of Observed Style.

● BLSA Prostate Cancer Studies

Longitudinal Study Branch researchers continue to investigate prostate specific antigen (PSA) as a prostate cancer predictor. They found the first documentation that prostate cancer screening criteria based on the rate of change in PSA are affected by the testing interval. Also, when comparing PSA criteria for prostate cancer protection, the average velocity had the highest combined sensitivity and specificity of all compared criteria. In another study component, it was shown that serum testosterone levels are not a strong risk factor for developing prostate cancer.

Information Services

The IRP Information Office completed a successful year which saw preparation of three *NIH Record* stories, including two front pagers— DNA repair (also used for *NIA Research Bulletin*), Meyerhoff Program, Shock Lecture, several articles for *NIH News and Features* on stress induced breathing changes in hypertension, dopamine receptor loss and motor control, and the minority international training program. A story on dental wearers and dry mouth was written for *NIH Healthline*. Wrote items for the NIH Prevention Report. Arranged major interview schedules for Phil Gunby, JAMA; Klaus Mandel, Austrian TV, RAI ITALIAN TV for two days of filming, and interviews for the new CNBC show, “Well Beyond Fifty.” Coordinated and edited 1994 Intramural Annual Report, and the 1993 Scientific Bibliography and 94 Directory. Prepared NIA intramural portion of the International Activity Report, and edited recruitment flyer for the BLSA. Other writing included four IRP NACA Status Reports, a press summary on signal transduction changes for annual GSA meeting, press releases on Dr. Andres’ award and annual Shock lecture, prepared revisions for the intramural portion of the NIH Almanac, and revised the IRP Fact Sheet. In addition, the Office prepared three issues of *Pages of the Ages* for BLSA participants, and 12 issues of the monthly NIA employee newsletter, *Geron News*.

Major media contacts this year dealt with CNN (2), NBC-TV Dateline, CBS-TV Evening News, Brazilian Globo TV, MPT-TV for documentary on Meyerhoff scholars, JAMA American Medical Productions, *U.S. News and World Report*, *Washington Post*, *GEO Magazine*, *Forbes*, *De Volkskrant* (the Netherlands), *Baltimore Sun*, *Medical Tribune* (2), Medical News Network, *Boston Globe*, *Parade*, *McCall's*, *Reader's Digest*, and *Merck Manual of Geriatrics*.

At the request of the NIA assistant to director for special programs, arranged two visits for 74 students attending the National Youth Leadership Forum. Staff participated in GRC briefings for Johns Hopkins graduate students; coordinated the US Savings Bond Drive; CFC; two Blood Drives; helped organize and chaired successful 8th Annual FEB Disability Awareness Training Conference. The Information Office head moderated the NIH 11th Annual Disability Awareness Program; chaired the FEB disability committee; reappointed to NIH and PHS Committees; served on the IRP Human Relations Committee; and as a member of the Bayview Beacham Adult Day Care Advisory Board.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00710-06 GEA

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heat Shock Protein Gene Expression in Response to Stress and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Nikki J. Holbrook, Senior Investigator, GEA, NIA

Others: Timothy Fawcett, Staff Fellow, GEA, NIA; Qingbo Xu, IRTA Fellow, GEA, NIA

COOPERATING UNITS (if any)

Dept. of Surgery, The Johns Hopkins University and Hospital (R. Udelsman)

LAB/BRANCH

SECTION

Gene Expression and Aging

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues X (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Heat shock proteins (HSPs) are induced in response to a variety of cellular stresses, and appear to be critical for maintaining cellular homeostasis. Previously, we demonstrated that restraint or immobilization stress elicits the induction of HSP70 expression selectively in the adrenal gland and vasculature of intact rats. In both tissues this stress-induced HSP70 expression was found to be linked to the activation of the neuroendocrine stress response axes and to be attenuated with age. The adrenal response was found to be dependent on the hypothalamic-pituitary-adrenal axis and require adrenocorticotrophic hormone (ACTH) while the vascular response appeared to be under alpha adrenergic control. Recent studies have focused on the molecular events controlling this response to restraint and the cause for its age-related decline. In the adrenal model we have shown that HSP70 induction is mediated by the heat shock transcription factor HSF1 and have shown that in Wistar rats HSF1 is activated to a DNA binding state by ACTH. Although properties of HSF1 and its activation in response to heat stress have been well studied in cultured cells, little information is available regarding the regulation of its activity *in vivo*. We have noted a number of differences in the properties of HSF1 in our *in vivo* model compared to that observed in heat stressed cells *in vitro*. These include the subcellular localization of HSF1 (which is mostly nuclear in the adrenal gland) and the differential mobility and DNA binding properties of the transcription factor in different rat strains. Despite differences in the nature of DNA binding HSF complexes in two different rat strains, the level DNA binding activity declines with age in both. In vascular tissue we have provided new evidence that in addition to adrenergic hormones, other agents capable of elevating blood pressure likewise induce HSP70 expression in the aorta. This suggests that mechanical stress associated with changes in blood pressure elicit the response. Our findings indicate that the activation of the heat shock response *in vivo* involves greater complexity than is observed in cultured cells in response to heat stress and suggest a broader role for HSPs in the physiologic response to stress than previously appreciated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG00720-03 GEA

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation and Function of the Putative Transcription Factor GADD153

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Nikki J. Holbrook, Senior Investigator, GEA, NIA

Others: Yusen Lui, Visiting Fellow, GEA, NIA; Timothy Fawcett, Staff Fellow, GEA, NIA;

Helen Eastman, IRTA Fellow, GEA, NIA; Myriam Gorospe, IRTA Fellow, GEA, NIA.

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

Gene Expression and Aging

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

GADD153 is a highly conserved mammalian gene whose expression is increased in response to a variety of stresses including growth arrest and DNA damage. It is a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcriptional activators and can dimerize with other C/EBPs through a leucine zipper domain. However, in contrast to other C/EBPs, it lacks the ability to bind to CCAAT DNA sequences and has therefore been proposed to serve as a negative regulator of other members of this family (by virtue of its ability to heterodimerize with them and inhibit their binding to DNA). Studies in this project have focused on the regulation and function of *GADD153* expression in response to stress. The approaches taken have included (1) characterization of *Gadd153* expression (and that of other C/EBPs) in response to diverse growth inhibitory and metabolic stimuli including PGA_2 -mediated growth arrest, glucose deprivation, DNA damage, and inducers of the acute phase response *in vivo* and *in vitro*; (2) use of yeast hybrid protein systems to examine the interaction between the *GADD153* protein and other members of the C/EBP family, and (3) development of transgenic models to address the expression and role of *GADD153* during development and in response to stress *in vivo*. Of particular interest is our finding that the *GADD153* protein provides a functional transactivation domain when fused to a yeast GAL4 transcription factor DNA binding site, suggesting that *GADD153* can act directly (rather than as a dominant negative inhibitor as suggested above) to influence transcription perhaps through as yet unidentified sites. Using the yeast two hybrid system, we have found that in contrast to other C/EBP family members, *GADD153* appears to be unable to form homodimers, suggesting that it must interact with other transcription factors to influence gene expression, or be functional as a monomer.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Activation in Response to DNA Damage and Oxidative Stress in Normal and Aged Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Nikki Holbrook, Senior Investigator, GEA, NIA
 Others: Jennifer D. Luethy-Martindale, Biologist, GEA, NIA; Kate Z. Guyton, IRTA, GEA, NIA;
 Yusen Lui, Visiting Fellow, GEA, NIA; Myriam Gorospe, IRTA, GEA, NIA; Timothy Fawcett,
 Staff Fellow, GEA, NIA.

COOPERATING UNITS (if any)

Pulmonary Division, The Johns Hopkins Hospital (Dr. Augustine Choi)

LAB/BRANCH

SECTION

Gene Expression and Aging

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oxidative stress and DNA damage play a critical role in the development of degenerative diseases, and may underlie the aging process itself. Cells respond to such stresses with the induction of numerous gene products but little is known concerning the signal transduction pathways mediating these effects or the functional significance of the induced gene products. This project encompasses several areas related to these cellular responses. Studies are devoted to understanding basic mechanisms associated with the genetic responses to DNA damage and oxidative stress. We have utilized the growth arrest and DNA damage inducible gene, *GADD153*, as a model to investigate the signal transduction pathways operating to regulate gene expression following DNA damage. Evidence has accumulated from other laboratories to support the existence of at least two distinct pathways operating to enhance gene expression following different types of DNA damaging treatments. One is dependent on the tumor suppressor gene product p53, and appears to be limited to X-ray damage and a small subset of genes. The second, which appears to be a more universal response, is p53 independent, relies on the activation of tyrosine kinases and involves c-ras, c-raf, and ERKs. We have obtained evidence to suggest that *GADD153* is activated through a p53-independent pathway which does not require activation of tyrosine kinases and is independent of c-ras and c-raf activation. However, recent evidence suggests that an alternative pathway dependent on p53 could also contribute to *GADD153* induction in response to DNA damage. A second area of focus in this project is the investigation of the response to DNA damage as a function of aging. These experiments thus far have relied on *in vitro* senescence of fibroblasts as a model of aging. We have obtained preliminary evidence to indicate that a least one subset of genes whose expression is dependent on certain AP-1 transcription factors declines with *in vitro* aging.

* This project is a combination of previous Project #'s: Z01 AG 00723-02 LMG
 and Z01 AG 00728-01 LMG

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00063-26 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Learned Modification of Visceral Functions in Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Bernard T. Engel, Chief LBS, NIA

OTHERS: Mark I. Talan, Medical Officer (Research) LBS, NIA
 David Bush, IPA LBS, NIA
 Robert Abel, Research Volunteer LBS, NIA
 Svetlana Chefer, Visiting Fellow LBS, NIA
 Reginald Quilter, Electronics Technician LBS, NIA

COOPERATING UNITS (if any)

Johns Hopkins Medical School, Bayview Center, Division of Cardiology, Baltimore, MD (D. Bush, R. Abell)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

4.15

PROFESSIONAL:

2.85

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the role of the central nervous system in the regulation of the circulation. Instrumental conditioning is used to directly modify cardiovascular responses. Naturally occurring, adaptive responses are examined by monitoring overnight changes in hemodynamic function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00073-04 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology of Thermoregulation and Aging in Rodents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Mark I. Talan, Medical Officer (Research) LBS, NIA

OTHERS: Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA
 Vladimir Chefer, Visiting Associate LBS, NIA
 Sergey Kirov, Visiting Fellow LBS, NIA
 Lori Clow, Pre-IRTA LBS, NIA

COOPERATING UNITS (if any)

Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (A. Sato, Y. Sato, H. Hatta)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

5.65

PROFESSIONAL:

3.7

OTHER:

.95

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is: (1) to investigate age-related changes in thermoregulation, and (2) to examine the physiological mechanisms underlying these changes.

We have demonstrated that aged mice have diminished cold tolerance and are not able to adapt to repeated cold exposure. The cause of these aged-related aberrations in thermoregulation appears to be, in part, a reduction in metabolic heat production due to change in brown adipose tissue (BAT) and, in part, a reduction in heat conservation.

Efferent sympathetic nervous responses to BAT are enhanced in both cold-acclimated and aged animals and is not changed in animals that "failed" to acclimate. Results indicate that the sympathetic nervous system plays a major role in cold acclimation, but is not responsible for the aged-related decline in thermoregulation. Our result showed that nonshivering thermogenesis is, in fact, enhanced in aged animals.

We have shown that exercise training improves metabolic heat production in aged mice and results in weakening of cold tolerance in adult animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00600-06 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Respiratory Factors in Blood Pressure Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: David E. Anderson, Chief, Behavioral Physiology Section LBS, NIA

OTHERS: Alexei Y. Bagrov, Visiting Associate LBS, NIA
 Olga Fedorova, Visiting Fellow LBS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

2.45

PROFESSIONAL:

2.0

OTHER:

.45

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use stenderd unreduced type. Do not exceed the space provided.)

The development of hypertension is potentiated by synergistic interactions between high sodium intake and behavioral factors, but the mediating physiological mechanisms remain to be clarified. An animal model of hypertension suggests that sodium sensitivity may be influenced by a hypoventilatory breathing pattern evoked by aversive conditioning procedures. Previous studies with an ambulatory respiration monitor have shown that episodes of low frequency/normal tidal volume breathing can be observed in humans in the natural environment. Ongoing laboratory studies with human subjects and laboratory animals are showing that the inhibitory breathing pattern is accompanied by increases in pCO₂, decreases in pH, increases in renal sodium reabsorption, increases in urinary excretion of endogenous digitalis-like factors, decreases in sodium pump activity, and increases in blood pressure, but not heart rate. This physiological pattern provides an alternative to the sympathetic nervous system as a mechanism whereby behavioral factors may interact with high sodium intake to produce sustained hypertension. These studies may lead to nonpharmacological interventions for prevention and reversal of hypertension and associated disorders of aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00603-04 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Implications of Nocturnal Hemodynamic Events

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: David Bush, IPA LBS, NIA

OTHERS: Bernard T. Engel, Chief LBS, NIA
Mark I. Talan, Medical Officer (Research) LBS, NIA
Robert T. Abell, Special Volunteer LBS, NIA

COOPERATING UNITS (if any)

Johns Hopkins Medical School, Bayview Center, Division of Cardiology, Baltimore, MD
(D. Bush); The Johns Hopkins University, Division of Cardiology, Baltimore, MD
(P. Goldschmidt)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.55

PROFESSIONAL:

1.15

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in animals have shown significant nocturnal hemodynamic patterns that differ substantially from daytime patterns in a variety of mammals including man. In patients with heart diseases, these patterns could affect the incidence of morbid events which are known to occur in the morning. This project is designed to evaluate overnight cardiovascular effects in select patient groups, and to test the effectiveness of interventions designed to ameliorate specific adverse effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00607-03 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Effects on Blood Pressure and Circulating Sodium-Pump Inhibitors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: David E. Anderson, Chief, Behavioral Physiology Section LBS, NIA
 OTHERS: Alexei Y. Bagrov, Visiting Associate LBS, NIA
 Joy L. Austin-Lane, Psychologist LBS, NIA

COOPERATING UNITS (if any)

University of Maryland School of Medicine, Department of Physiology, Baltimore MD (J. Hamlyn)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

.65

PROFESSIONAL:

.5

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Epidemiological studies have shown that mean blood pressure and the prevalence of hypertension both increase with age. A possible role for dietary sodium intake in the blood pressure increase in aging individuals has been documented, but little information is available concerning possible age-associated changes in circulating hormones that affect sodium transport across vascular smooth muscle. The present study assesses individual differences in levels of two circulating sodium-pump inhibiting hormones as predictors of age-associated changes in resting blood pressure. Morning supine blood pressure and endogenous ouabain and digoxin-like factor are measured in Baltimore Longitudinal Study on Aging participants who meet the inclusion criteria. The study also investigates possible differences in circulating sodium-pump inhibitors as a function of race and gender. Data supporting the hypothesis of a positive correlation between resting blood pressure and circulating sodium-pump inhibitors could lead to pharmacological and nonpharmacological intervention in hypertension focusing on sodium-pump inhibitors and their determinants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00608-02 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Post-Operative Complications and Mobility Outcomes in Hip Fracture Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Ann H. Myers, Senior Staff Fellow LBS, NIA

OTHERS: Mary H. Palmer, Sr. Staff Fellow CTL, NINR
 Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; Johns Hopkins Medical School, Bayview Center, Baltimore, MD

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Nursing Unit

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.35

PROFESSIONAL:

.6

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hip fractures represent a serious and costly health problem among the elderly. It has been estimated that by the year 2000 there will be 300,000 hospitalizations for hip fractures. Hip fractures account for \$11 billion a year in health care costs. Recovering independence from this serious injury remains a major problem for the elderly. Studies of outcomes suggest that over half of the patients cannot walk independently a year after the injury. Post-operative complications can retard initial rehabilitative efforts. Ambulation status upon discharge is a significant factor associated with post-hospital outcomes. Mobility problems and urinary incontinence often lead to costly institutionalization.

The post-operative complications of pneumonia, decubitus ulcer, urinary retention requiring straight catheterization and urinary incontinence can be reduced by nursing interventions during the acute hospital phase of recovery. Early mobilization probably remains the single most effective method of reducing the incidence of post-operative complications. There were significant changes and deficits in functional status at discharge. Prefracture status and functioning was associated with the development of complications. Prefracture status and functioning and the development of complications have an effect on functional mobility outcomes. The hospital's system of services has an effect on the patient's level of functioning and disposition at discharge.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00609-02 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sodium Pump Inhibitors in Cardiovascular Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Alexei Y. Bagrov, Visiting Associate LBS, NIA
 OTHERS: Olga Fedorova, Visiting Fellow LBS, NIA
 David E. Anderson, Chief, Behavioral Physiology Section LBS, NIA

COOPERATING UNITS (if any)

Dzhanelidze Institute, St. Petersburg, Russia (N. Roukoyatkina)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Medicine Section

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.55

PROFESSIONAL:

1.5

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence has accumulated that digitalis-like factors (EDLF) may play an important role in the pathogenesis of cardiovascular disease (hypertension, congestive heart failure, acute myocardial infarction) via their ability to inhibit sodium pump activity. Much attention has been paid to one of these factors, an endogenous ouabain; however, a digoxin-like immunoreactive factor has also been found previously to contribute to arrhythmogenesis in myocardial ischemia. The objectives of this project are to clarify the role of various sodium pump inhibitors, especially the digoxin-like immunoreactive EDLF, in blood pressure regulation.

During the past year, studies were completed with anesthetized dogs which showed that the increases in endogenous digitalis-like factors, which are evoked by rapid expansion of plasma volume, include a bufodienolide compound in higher concentrations than a concurrently observed digoxin-like factor. Pretreatment of the animals with an antidigitalis antibody prevented the rapidly developing increases in cardiac contractility and more slowly developing natriuresis. A second study showed that voluntary hypoventilatory breathing in human subjects maintained by feedback from a respiratory gas monitor was also associated with increases in urinary excretion of the bufodienolide compound which persisted for 30 minutes following cessation of the breathing task. These studies have implications for understanding of the origins of hypertension and for development of new pharmacological interventions in cardiovascular disorders.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00610-01 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Rehabilitative Nursing Interventions in Elderly Patients with Hip Fracture

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Ann H. Myers, Senior Staff Fellow LBS, NIA

OTHERS: Mary H. Palmer, Sr. Staff Fellow CTL, NINR

Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; Johns Hopkins Medical School, Bayview Center, Baltimore, MD

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Nursing Unit

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.05

PROFESSIONAL:

.55

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The incidence of hip fracture is a major and increasing health problem for the elderly. Over the past 30 years the surgical techniques and prostheses have advanced to benefit a majority of patients. However, recovering independence after a major disabling event remains a serious problem for many older persons. More than half of elderly hip fracture victims remain unable to walk independently, even a year after their fractures; many never recover fully, or to their prefracture status. The mobility/ambulation status of the patient at discharge is a most significant determinant of several important health outcomes in the postoperative period. Therefore, the hospital phase of surgical treatment and rehabilitative care is extremely important and foundational to subsequent patient outcomes. However, there is a paucity of knowledge on interventions designed to effect more optimal outcomes, especially during initial hospitalization.

A prospective experimental study has been designed to evaluate the effects of behavioral nursing interventions preoperatively and postoperatively with community-dwelling elderly admitted to the hospital with a hip fracture. The intervention group and control (standard care) group will be matched on: age, gender, type of fracture, and prefracture functional status.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00611-01 LBS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Nursing Home Residents for Behavioral Nursing Interventions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Ann H. Myers, Senior Staff Fellow LBS, NIA

OTHERS: Mary H. Palmer, Sr. Staff Fellow CTL, NINR
 Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; Johns Hopkins Medical School, Bayview Center, Baltimore, MD; Health Care Financing Administration (B. Cornelius)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Behavioral Nursing Unit

INSTITUTE AND LOCATION

NIA, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

.2

PROFESSIONAL:

.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the 1987 OBRA legislation, Congress mandated a system of assessment and care planning for residents of nursing homes certified by the Health Care Financing Administration (HCFA). The system involves the use of a Minimum Data Set (MDS) to collect data quarterly and annually. HCFA has had a demonstration project in six states with prospective data collected on close to a million residents. The availability of these data provides the unique opportunity to examine many factors over time. The purpose of this project will be to identify populations for specific nursing interventions to promote functional abilities and rehabilitation and reduce dependency, frailty, and complications from chronic morbidity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NR 00004-02 CTL

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Urinary Continence Status and Treatment of Incontinence in Nursing Home Residents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Mary H. Palmer, Sr. Staff Fellow CTL, NINR

OTHERS: Bernard T. Engel, Chief, Laboratory of Behavioral Sciences LBS, NIA

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD

LAB/BRANCH

Laboratory of Behavioral Sciences (Clinical Therapeutics Laboratory, NINR)

SECTION

INSTITUTE AND LOCATION

NIA/NINR, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

.4

PROFESSIONAL:

.35

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Urinary incontinence is prevalent in nursing homes. This project was designed to test the effectiveness of staff performance feedback in conjunction with behavioral treatment of incontinence.

This research project is also being reported by the National Institute of Nursing Research.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 NR 00005-01 CTL
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Urinary Continence Status and Treatment of Incontinence in Nursing Home Residents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)		
PI:	Mary H. Palmer, Sr. Staff Fellow	CTL, NINR
COOPERATING UNITS (if any) National Institute of Nursing Research, NIH, Bethesda, MD		
LAB/BRANCH Laboratory of Behavioral Sciences (Clinical Therapeutics Laboratory, NINR)		
SECTION		
INSTITUTE AND LOCATION NIA/NINR, NIH, Gerontology Research Center, Baltimore, MD 21224		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Organizational characteristics</u> and nursing staff <u>knowledge and attitudes</u> are important in the establishment and maintenance of effective continence programs. This survey was designed to identify antecedents that may effect the implementation of <u>continence programs</u> in the long-term care setting. This research project is also being reported by the National Institute of Nursing Research.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NR 00006-02

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Estrogen on Urinary Incontinence and Symptoms in Post-menopausal Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: Mary H. Palmer, Sr. Staff Fellow

CTL, NINR

OTHERS:

COOPERATING UNITS (if any)

National Institute of Nursing Research, NIH, Bethesda, MD; Johns Hopkins Geriatric Center, Bayview Center, Baltimore, MD (D. Foster); Beacham Ambulatory Care Center, Baltimore, MD (J. Marks).

LAB/BRANCH

Laboratory of Behavioral Sciences (Clinical Therapeutics Laboratory, NINR)

SECTION

INSTITUTE AND LOCATION

NIA/NINR, NIH, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

.3

PROFESSIONAL:

.25

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

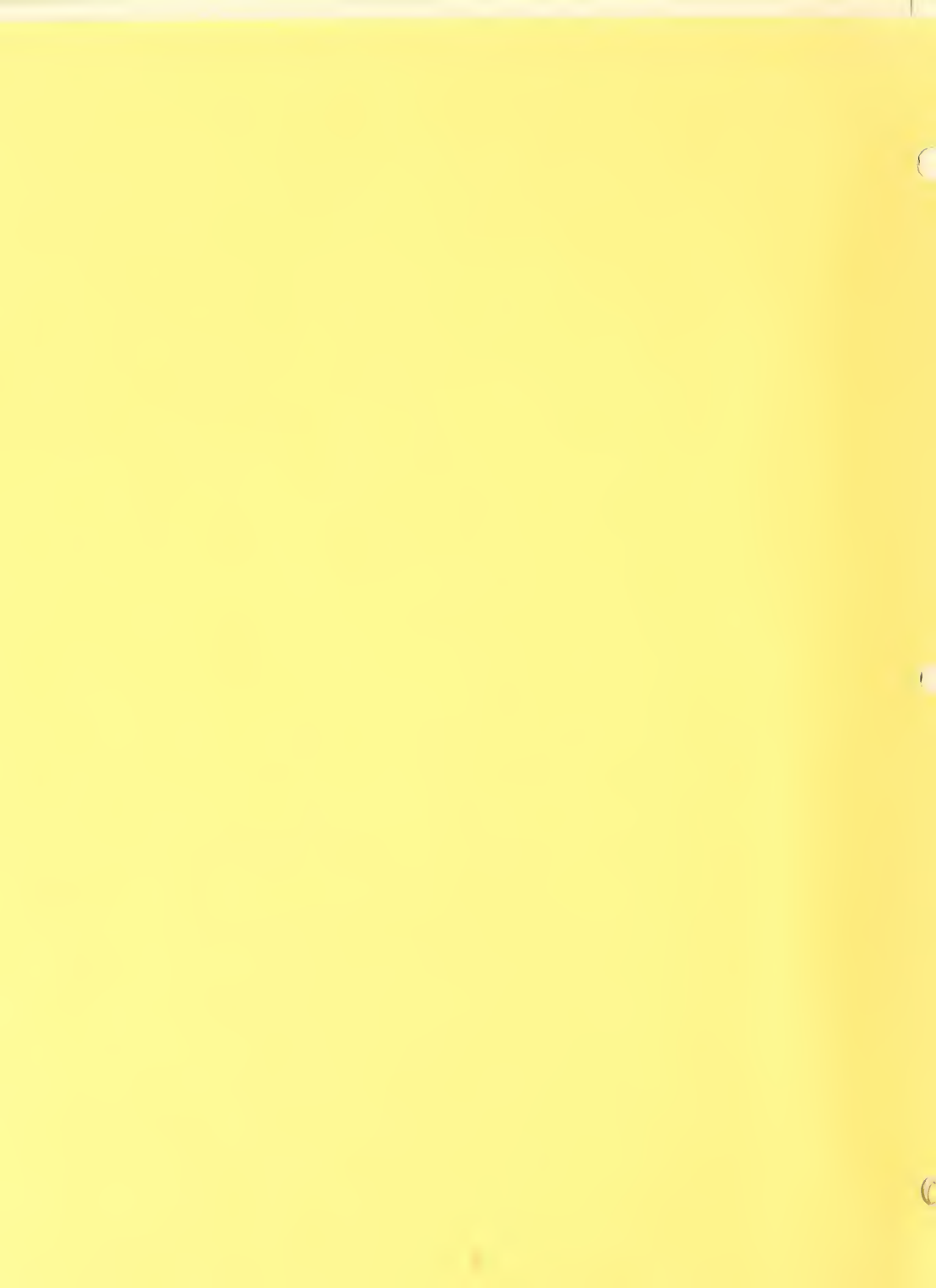
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Incontinence and urinary symptoms of frequency and urgency are prevalent in post-menopausal women. This project is designed to evaluate the effectiveness of topical estrogen in the behavioral treatment of urinary symptoms and incontinence.

This research project is also being reported by the National Institute of Nursing Research.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00057-05 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Cartilage Biology: Mechanisms & Models Related to Aging and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: Walter E. Horton, Jr. Senior Staff Fellow, LBC GRC NIA

Others:

Douglass M. Bradham	Staff Fellow	LBC GRC NIA
Darryl Murray	Biologist	LBC GRC NIA
Richard Balakir	Chemist	LBC GRC NIA
Patricia Precht	Biologist	LBC GRC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

2.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cartilage is a unique tissue that functions to cushion the impact between bones and is a target for degenerative changes that result in osteoarthritis (OA), a disease afflicting millions of elderly individuals. OA involves changes in expression of matrix proteins such as collagen II as well as death of cells that comprise cartilage, the chondrocytes. We previously identified a region in the collagen II gene that functions as a chondrocyte-specific enhancer of transcription and further determined that a decamer sequence serves as binding site for chondrocyte-specific proteins. We have demonstrated that transcription factors belonging to the HLH family interact with the decamer sequence and are involved in regulating enhancer activity. We have now identified partially purified fractions of chondrocyte nuclear extracts that contain the enhancer binding activity. We are also studying the stimulation of chondrogenesis by matrigel and have shown that factors associated with aged mice inhibit this process both *in vivo* and *in vitro*.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00058-05 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Aging, Angiogenesis, and the Growth and Spread of Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: Antonino Passaniti Senior Staff Fellow, LBC GRC NIA

Others:

Joan Chang	Biologist	LBC GRC NIA
Roberto Pili	Visiting Fellow	LBC GRC NIA
Zohreh Naghashfar	Visiting Fellow	LBC GRC NIA
Chunlin Yang	Visiting Fellow	LBC GRC NIA

COOPERATING UNITS (if any)

Hynda Kleinman, Chief, Cell Biology Section, LDB NIDR

Joseph di Paolo, Laboratory of Biology, NCI

Maurizio Capogrossi, Laboratory of Cardiovascular Science, NIA

Richard B. Alexander, Chief of Urology, VA Med. Ctr.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

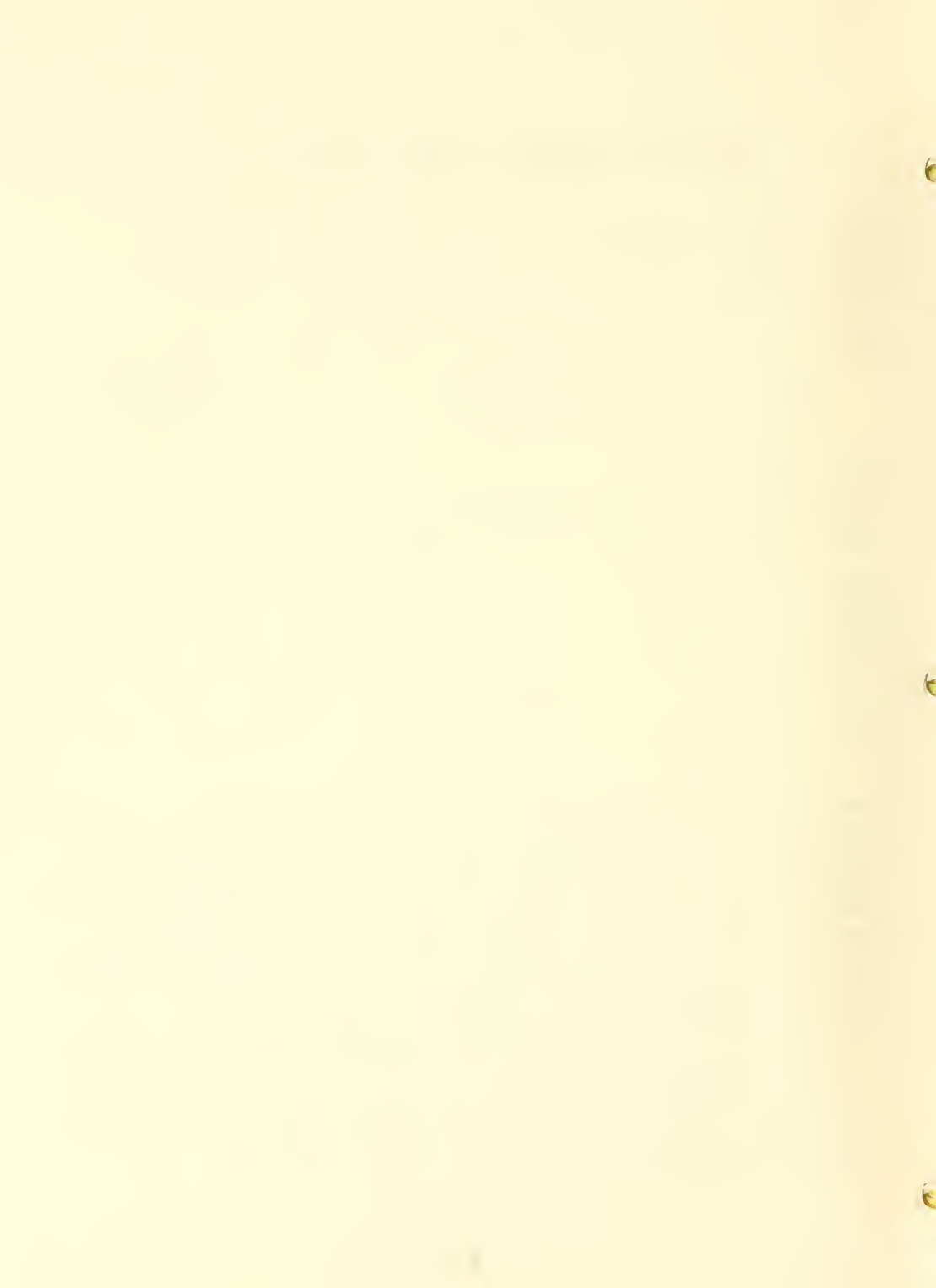
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.25	4.0	1.25

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The incidence of cancer increases strikingly with age while the rate of tumor growth and spread is slowed. To explore the relation between cancer and aging we have adapted spontaneous human prostate and rat breast cancers occurring with age to transplantable lines to allow studies on their origins and properties. Further, we have developed a simple, quantitative assay to measure vascularization and assess angiogenic and potential anti-angiogenic factors. This system has been used to demonstrate that linomide, a useful anti-tumor drug, and castanospermine, a glycosidase inhibitor which we have shown is also a potent anti-tumor drug, exert their action by suppressing the vascularization of tumors. Adenoviral vectors expressing angiogenic factors in this model have been used to promote neovascularization in damaged tissue. We have also compared the growth of tumors in old and young hosts. These studies show a reduced rate of growth of a variety of tumor cells in old hosts. Extracts of tumor tissue grown in old hosts contain a factor(s) that reduces the growth of both normal and transformed cells and which could be a host factor not only slowing the growth of tumors but also impairing tissue repair and regeneration. The administration of the phosphatase inhibitor, orthovanadate, resulted in inhibition of endothelial cell death and enhanced angiogenesis in vivo which may be useful in promoting tissue repair.



PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 AG 00059-04 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mitochondrial DNA Deletions: Role in aging and disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

PI: Charles R. Filburn Research Chemist, LBC GRC NIA

Others:

W. Edris	Biologist	LBC GRC NIA
M. Tamatani	Visiting Fellow	LBC GRC NIA
M. Camp	Biological Science Aide	LBC GRC NIA
A. White	Summer IRTA Fellow	LBC GRC NIA

COOPERATING UNITS (if any)

V. Bohr, Laboratory Chief, LMG GRC NIA

K. Chandrasekaran, Staff Fellow, LNS GRC NIA

A. Richardson, Veterans Administration, San Antonio, Texas

C. Stine, Assistant Professor, Johns Hopkins Univ., School Med.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

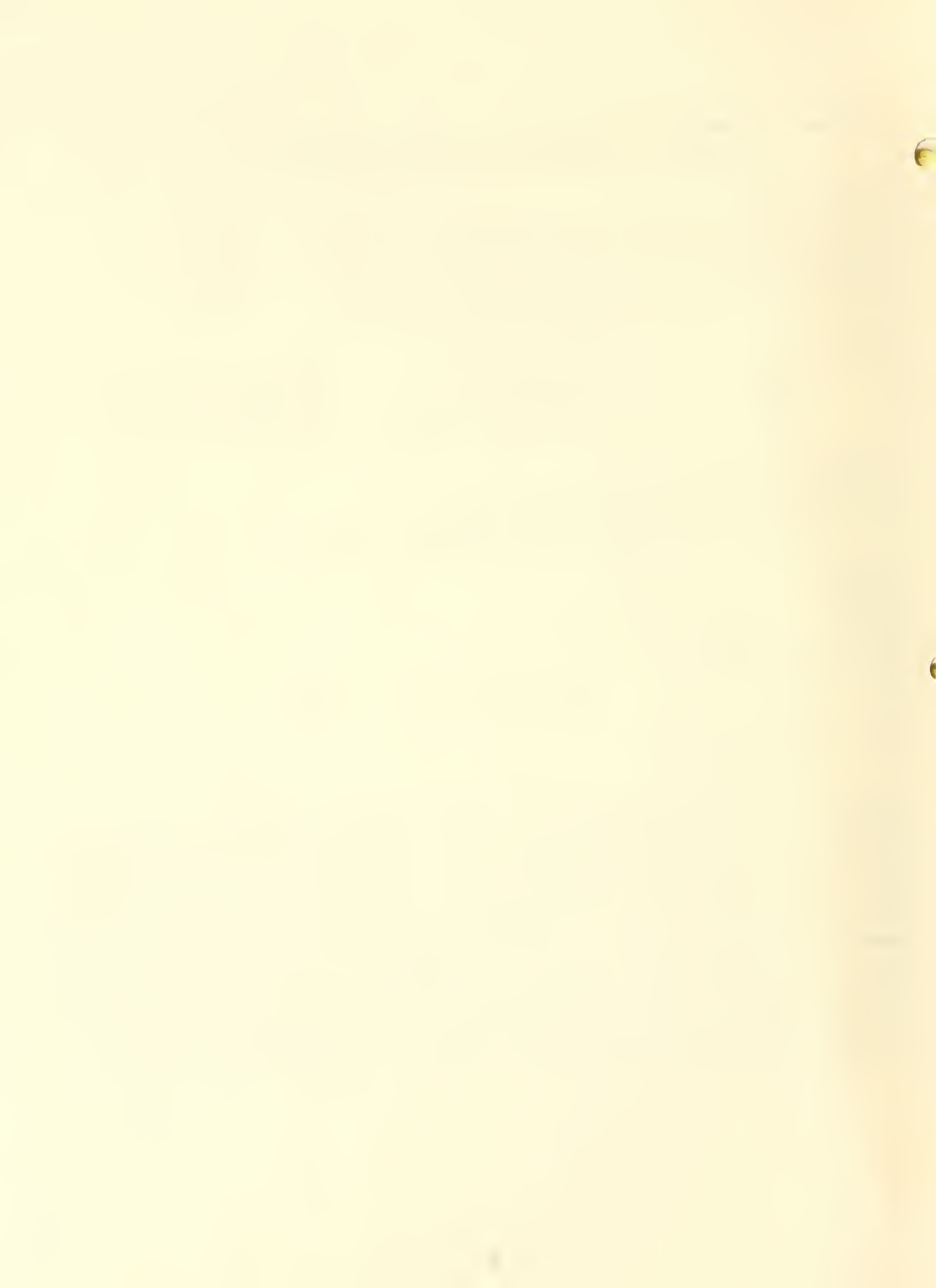
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)

<input type="radio"/> (a) Human subjects	<input checked="" type="radio"/> (b) Human tissues	<input type="radio"/> (c) Neither
<input type="radio"/> (a1) Minors		
<input type="radio"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Damage to mitochondrial DNA (mtDNA) may play an important role in the aging process or in age-associated neurodegenerative diseases. Recent studies indicate that mtDNA mutations cause myopathies, are involved in an increasing number of other diseases, including diabetes, and show marked increases in some human tissues with increasing age. In demonstrating the universality of this age-dependence, we have identified and quantitated a 4.8 kb and a 3.7 kb deletion in rat and mouse, respectively and shown increases with age. Liver mtDNA from diet-restricted rats contained half as much as that from ad libitum fed rats. A correlation was observed between 8-OHdG levels in total DNA of human hearts and the level of a 4.8 kb deletion in mtDNA. Together, these data support the suggestion that oxidative damage, which is reduced in diet-restricted, longer living animals, plays a role in generation of these mutations. MtDNA damage causing these mutations may be important in muscle weakness, neurodegeneration and other age-associated changes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00500-04 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation and Processing of Amyloid Precursor Protein Genes and Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

PI: John W. Kusiak Research Chemist, MNU LBC GRC NIA

Others:

Boyu Zhao	Visiting Fellow	MNU LBC GRC NIA
Michael Prenger	Biologist	MNU LBC GRC NIA

COOPERATING UNITS (if any)

Sangram S. Sisodia, Assistant Professor, Dept. of Pathology, J.H.U

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Molecular Neurobiology

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

3.0	PROFESSIONAL:	OTHER:
	2.0	1.0

CHECK APPROPRIATE BOX(ES)

<input type="radio"/> (a) Human subjects	<input type="radio"/> (b) Human tissues	<input checked="" type="radio"/> (c) Neither
<input type="radio"/> (a1) Minors		
<input type="radio"/> (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alzheimer's Disease is a major neurological disorder of the elderly affecting as many as 50% of the population over 80. No cure for this disease is known and its etiology is obscure. However, a subset of Alzheimer's Disease patients with an early onset form of the disorder have mutations in the gene coding for the amyloid precursor protein (APP). Processing of this protein generates a peptide fragment, termed Ab, which is a major component of the senile plaques in brains of Alzheimer's patients. These observations suggest an important role of APP in the causation of this disorder. Our work has focused on establishing a model system in which mutant APP genes are expressed in several types of cultured cells thought to be important in Alzheimer's Disease. We examined the proteolytic processing of the mutant proteins and the phenotypic consequences to these cells. Both neuronal and endothelial cells expressing three separate mutations in APP contain more carboxyl terminal amyloidogenic fragments. The neuronal cells showed abnormal cell bodies with intact neurites, increased DNA laddering and TUNEL staining, and eventual detachment and cell death. The endothelial cells, cultured on Matrigel, exhibited delayed onset of capillary network formation. Conditioned media from cells expressing mutant APP contained increased amounts of Ab peptide and caused morphological changes in untransfected cells. Electron microscopy, antisense blockade of expression, and FACS analysis will be used to characterize and quantitate the nature of the cell toxicity which, from preliminary results seems to be apoptotic in nature. Other studies will be aimed at determining which cellular systems (e.g. lysosomes, mitochondria) may be affected by the over-expression of mutant APPs and whether other types of insults can result in a similar type of cell death.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00506-01 LBC

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Apoptosis in Degenerative Diseases of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)

(Name, title, laboratory, and institute affiliation.)

PI: Walter E. Horton, Jr. Senior Staff Fellow, LBC GRC NIA

Others:

Georgeann Smale	IRTA Fellow	LBC GRC NIA
Richard Balakir	Chemist	LBC GRC NIA
Patricia Precht	Biologist	LBC GRC NIA
Daniel Brady	Senior Staff Fellow	LN NIA

COOPERATING UNITS (if any)

Laboratory of Neurosciences, NIA

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

Regulatory of Mechanisms Section

INSTITUTE AND LOCATION

Gerontology Research Center, NIA, NIH, Baltimore, MD 21224

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

3.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Degenerative diseases of aging such as Alzheimer's disease (AD) and Osteoarthritis (OA) involve loss of functional cell types. Apoptosis is a form of programmed cell death that is often responsible for elimination of cells during development. The role of apoptosis in age-associated processes is only beginning to be studied. We have utilized an *in situ* method to determine that AD brains show an increased incidence of apoptosis in the hippocampus compared to age-matched controls. Similar studies are underway utilizing rat models of age-associated degenerative cartilage disease. Finally, we have established a cell culture model to study the regulation of apoptosis in chondrocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AG 00044-21 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metal Ions and Information Transfer: mechanism of RNA Synthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gunther L. Eichhorn, Chief, LCMB, NIA

Others: James J. Butzow, Commissioned Officer, IBS, LCMB, NIA; Patricia Clark, Research Chemist, IBS LCMB NIA; Carl Garland, IRTA Fellow, IBS, LCMB, NIA; Thomas Frasier, Predoctoral Fellow, IBS, LCMB, NIA

COOPERATING UNITS (if any)

MDS (LCMB) (J. Rifkind), Centre for Cellular and Molecular Biology; (D. Chatterji); NCI/LMB (S. Adhya, S. Garges), LMG (V. Bohr)

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS

4.5

PROFESSIONAL

3.5

OTHER

1.0

CHECK APPROPRIATE BOXES!

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The effects of metal ions on nucleic acids structure and function have been determined. The primary focus is now on the mechanism of RNA synthesis at the active site of RNA polymerase. The geometry of interaction at the active site of the enzyme is being probed during the transcription process to determine how this geometry changes during transcription and affects its operation. A comprehensive mechanism for assuring fidelity in copying the genetic code has been developed which depends on the sensing by the polymerase of the complementarity between the DNA base and the base of the incoming nucleoside triphosphate. The polymerase shifts its conformation to prevent bond formation when the substrate is not complementary, while the enzyme otherwise remains in the proper conformation for bond formation to allow efficient polymerization with the correct base. The structural model is being applied to understand the effect of DNA damage, promoters, and transcription factors on transcription.

PERIOD COVERED

October 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biomedical Uses of Cyclodextrins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Josef Pitha, Chief, Macromolecular Chemistry Section, NIA/LCMB

Others: Jindrich Jindrich, Ph.D. - MCS/LCMB/NIA; Jiri Horsky, Ph.D. - MCS/LCMB/NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Macromolecular Chemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3

PROFESSIONAL:

3

OTHER:

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hydroxypropyl cyclodextrins, which originated in this section in 1981, are chemical derivatives of enzymatically modified starch and can be used as powerful solubilizers of non-polar, water insoluble substances. Such solubilization makes it possible to prepare highly effective pharmaceutical formulations directly from some hormones and proteins and eliminates the usual need for chemical modification to make them pharmaceutically acceptable. Thus, hydroxypropyl cyclodextrins may be expected to improve therapeutics of chronic diseases of elders, many of which involve hormonal supplementation. The overall success of the project is documented by the progress of the formulations discovered in section toward clinical use; the testosterone formulation is now in USA in Phase III clinical testing, estradiol is in the Phase I in USA and is also being evaluated in Iceland; (these evaluations are performed by parties independent of the section and are not collaborative studies; processes as described by the section nevertheless are used). The present results of the section's work help delineate the conditions of safe therapy by identifying dosages and styles of uses of hydroxypropyl cyclodextrins.

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure-Function Relationships in Hemoglobin and Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Joseph M. Rifkind, Chief, MDS, MDS LCMB NIA

Others: Omoefe Abugo, Visiting Fellow, MDS/LCMB/NIA; Chavali Balagopalakrishna, Visiting Fellow, MDS/LCMB/NIA; Jane Heim, Chemist; P.T. Manoharan; V.S. Sharma; V. MacDonald; D. Danon; D. Ingram; R. Rao; M. Perrella; M. Moss; D. Stern

COOPERATING UNITS (if any)

Indian Inst. of Tech., Madras, India; Walter Reed Army Inst. of Research, Washington, D.C.; Waldenberg Center for Geront. Studies; NIA/LCMB/MPGS; U. of MD, Balt., MD; Univ. of Milan, Italy; Boston Univ.,

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Dynamics Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

3.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses on the mechanism involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. Emphasis is placed on ways in which these functions are impaired and change with age. These studies have focused on the oxidation of hemoglobin, which produces nonfunctional hemoglobin and the simultaneous release of oxyradicals. The enhancement of these oxidative processes under hypoxic conditions is being explored as a possible source of tissue and organ damage, which would be exacerbated during aging. Studies are also included which are directed at the stability of the entire erythrocyte and the erythrocyte membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00301-11 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: I. Hormone Action

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: G.S. Roth, Ph.D., Chief, Molecular Physiology & Genetics Section, LCMB, NIA

Others: S. Kitano, B. Baum, I. Ambudkar, A. Richardson, M.A. Kowatch, G. Kokkonen, T. Reed, V. Bohr, J. Smith

COOPERATING UNITS (if any)

Patient Care Branch National Institute of Dental Research; V.A. G.R.E.C.C., University of Texas, San Antonio, Baylor University, Houston, TX

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

33

PROFESSIONAL:

13

OTHER:

20

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is mainly involved in elucidating those mechanisms by which the ability of hormones and neurotransmitters to regulate physiological functions is altered during aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00302-11 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: III. Behavioral Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and

PI: Donald K. Ingram, Ph.D., Research Psychologist, MGS, LCMB, NIA

Others: D. Danon, R. Crystal, R. Fanelli, N. Greig, H. Ikari, J. Hengemihle, M. Jucker, H. Kametani, J. Kusiak, H. Kleinman, H. Kuo, E. London, J. Rifkind, G. Roth, C. Seltzer, A. Shimada, E. Spangler, A. Mastrangeli.

COOPERATING UNITS (if any)

Cornell Univ. Sch. of Med; Essex Community College; Fukuoka Prefectural University; Nat'l Inst. Drug Abuse; Towson St. Univ.; Miles Pharmaceuticals

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

5.3

PROFESSIONAL:

5.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to assess the effects of aging at a behavioral level of analysis, to identify neurobiological mechanisms associated with these effects, and to test interventions that might alter age-related performance decrements. Rodent models are tested in a battery of sensorimotor and learning/memory tasks. Neurochemical and neurohistological assays are conducted to determine neurobiological correlates of functional losses. Interventions include dietary restriction, exercise, various pharmacologic treatments, neurotrophic factors and gene transfer via adenoviral vectors. Multiple genotypes are examined to determine possible genetic involvement in the pattern of age-related behavioral impairment.



PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genes and Longevity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. G. Cutler, Ph.D., Research Chemist, LCMB, NIA

Others: D.K. Ingram, G.S. Roth, A. Ayala, A.S. Khan, G. Cao, K. Kitani, H. Alessio, I. Zs.-Nagy, J. Joseph.

COOPERATING UNITS (if any)

Miami Univ., Ohio; LMM, NIAID, Bethesda, Tokyo Metrop. Inst. Gerontol.; Verzar Inst. Exp. Geront., Tufts University, Boston, MA

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Dynamics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0.0

CHECK APPROPRIATE BOXES!

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our research program has continued to focus on assessing the possible role oxidative stress state (OSS) may have as a primary cause of aging and that longevity determinant mechanisms may include the numerous different strategies that control OSS. In these studies it is essential to employ sensitive and reliable assays to measure OSS in specific tissues, cells and in the whole organism using both invasive and non-invasive techniques. Towards this objective we have been continuing our development of our Oxygen Radical Absorption Capacity (ORAC) assay and of a PCR technique designed to measure the OSS history of a cell by determining the 8-OHdG per dG content of total DNA as related to 5KB deletions in mitochondrial DNA. We have also continued our studies examining the hypothesis that some aspects of normal aging - particularly in spatial memory and motor control - may involve a deficiency in nitric oxide (NO). In these studies we have used the spin trap N-tert-Butyl alpha Phenylnitron (PBN) plus several other spin traps which have now been found to release NO after reaction with a hydroxyl radical. We have discovered that PBN injected old rats show a remarkable recovery in sensitivity to induction of dopamine release in striatal tissue. PBN addition to the drinking water of 18 month old mice (0.25-1.0 mg/ml) appears to lengthen mean life span by about one month. Breath analysis of ethane and isoprene by GC to measure total body lipid peroxidation and cholesterol synthesis is now being evaluated as another useful assay of OSS.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-AG00304-8 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: V Assessment of Primate

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: G.S. Roth, Ph.D., Chief, Molecular Physiology & Genetics Section, LCMB, NIA
 D.K. Ingram, Ph.D., Research Psychologist, MPGS, LCMB, NIA
 R.G. Cutler, Ph.D., Research Chemist, MDS, LCMB, NIA
 M.A. Lane, Ph.D., IRTA Fellow, MPGS, LCMB, NIA

Others: J. Knapka, D. Barnard, R. Weindruch, W. Ershler, D. Danon, B. Flynn, L. Olsen, N. Wolf, P. Rabinovitch, A. Grossman, C. Harley, M. Reynolds, V. Monnier, S. Ball, D. Cocchi, A. Reznick, H. Kondo, E. London

COOPERATING UNITS (if any)

Department of Medicine, University of Wisconsin, Madison, WI, Department of Pathology, University of Washington, NIDA

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

40

PROFESSIONAL:

20

OTHER:

20

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is attempting to determine whether caloric modification of the diets of Rhesus and squirrel monkeys can affect aging rate as assessed by various physiological, biochemical and behavioral indices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00306-5 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reg. of Physiological Functions During Aging-II Neurotransmitter Responsiveness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

P.I.: G.S. Roth, Chief, MPGS, LCMB, NIA

Others: J. A. Joseph, L. Zhang, D.K. Ingram, P. Mason, J. Kelly, N. Denisova

COOPERATING UNITS (if any)

USDA Human Nutrition Center

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

30

PROFESSIONAL:

25

OTHER:

05

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project attempts to understand those mechanisms involved in age related changes in central nervous system (CNS) responsiveness.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00307-01 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Physiological Functions During Aging: VI. Genes, Neurodegeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Jeffrey M. Chernak, Senior Staff Fellow MPGS, LCMB, GRC, NIA

Others: Peter W. Hoffman, George Roth, Michael Prenger

COOPERATING UNITS (if any)

Genetic Pharmacology Unit, Experimental Therapeutics Branch, Cephalon, Inc., Johns Hopkins University School of Medicine, Mass. General Hospital/Harvard, University of Brescia, Italy

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2

OTHER:

.5

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of our work is on the function and regulation of genes whose expression may change with aging and/or neurodegenerative disease. We have chosen to study regulation of the amyloid precursor protein gene (APP) because of the demonstrated and suspected roles that it plays in the neuropathology and etiology of Alzheimer's Disease (AD), Down's syndrome (DS), and normal brain aging. (This work is a continuation of efforts previously described under project # Z01-G00500-03: Regulation and Processing of Amyloid Precursor Protein Genes and Gene Products.) We have cloned, sequenced and characterized a portion of the rat APP gene promoter (rAPP), and we have identified transcription start points (tsp) and important regulatory elements within this region. We are the first to describe the GAG element, which has a large positive effect in several different cell lines. A second element, designated the SAA element, appears to behave differently in different cell lines. Gel mobility shift experiments suggest that nuclear protein(s) interact directly with the SAA element but not with the GAG element. We are also investigating regulation of the D2 dopamine receptor gene (D2R) because of its demonstrated and suspected roles in the decrease in motor abilities associated with normal aging and neurodegenerative diseases such as Parkinson's Disease (PD), schizophrenia, and tardive dyskinesia. (This project was begun as a collaboration involving MNU and MPGS, and was initially described under project # Z01-AG00500-03. In addition, it is a natural extension of work on the loss of D2 receptors and mRNA with aging, as discussed under project # Z01-AG00306-5: Regulation of Physiological Functions During Aging: II. Central Nervous System Responsiveness.) We have used gel mobility shift experiments to demonstrate that nuclear proteins from human HeLa cell extracts or young and old rat brain tissues will interact with DNA fragments or oligonucleotides (oligos) from this promoter region. In some cases, there appears to be greater binding activity with nuclear extracts from young liver, cerebellum, or striatum than from old liver, cerebellum, or striatum. In addition, we have contributed to the successful demonstration of the use of adenoviral vectors to express functional D2R neurotransmitter receptors in rat brain (project # Z01-AG00302-11 LCMB: Regulation of Physiological Functions III. Behavioral Biology.)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG00308-1 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulations of Physiological Functions During Aging VI: Neuronal Degeneration and Plasticity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PI: Mathias Jucker, Ph.D., Visiting Associate, MPGS, LCMB, NIA

Others: M. Tian, D. Ingram, L. Walker, T. Hagg, H. Hall, P. Bialobok, J. Kusiak, D. Norton, J. Hengemihle, H. Kuo, H. Kleinman, B. Lamb.

COOPERATING UNITS (if any)

J Hopkins U Med Sch, Baltimore; Dalhousie U, Halifax; Swiss Fed Inst Technol; Fisons Corp, Rochester; Natl Inst Dental Res, Bethesda.

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Molecular Physiology and Genetics Section

INSTITUTE AND LOCATION

NIA, Gerontology Research Center, Baltimore, MD 21224

TOTAL STAFF YEARS:

20

PROFESSIONAL:

20

OTHER:

0

CHECK APPROPRIATE BOX(ES)

(a) Human (b) Human (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research project is directed towards understanding molecular mechanisms of age-related neurodegenerative diseases. Emphasis is placed on markers of synaptic loss and neuronal plasticity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AG 00381-04 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Studies of Aging in Cells, Organs, and Animals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard G. S. Spencer, M.D., Ph.D., Chief, In-vivo NMR Unit, LCMB, NIA

Others: Gunther L. Eichhorn, (LCMB); Jerome L. Fleg (LCS); David Spector (FSKMC); Jan Busby (ES); Marc Blackman (ES); George Roth (LCMB); George Weiss (DCRT); Pavel Shkarin (LCMB/NMR); Alena Horska (LCMB/NMR); Chris Tsaio (LCMB/NMR); Kenneth W. Fishbein (LCMB/NMR).

COOPERATING UNITS (if any)

Cardiovascular Section, Clinical Physiology Branch, NIA

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry, In-vivo NMR Unit

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

3

OTHER:

.5

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NMR spectroscopy is currently being used at the NIA to study the phosphorus metabolism of peripheral muscle in BLSA subjects and other volunteers, as well as in animals. Age-related and exercise-related effects are under investigation. Methodologic studies to further develop kinetic and spin-lattice relaxation time measurement methodology are also being actively pursued.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AG 00382-04 LCMB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies by Solid-State NMR

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Richard G. S. Spencer, Unit Chief, In-vivo NMR

Others: Kenneth W. Fishbein, (NMR/LCMB)
Malcolm Levitt (Univ. Stockholm)

COOPERATING UNITS (if any)

University of Stockholm

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry, In-vivo NMR Unit

INSTITUTE AND LOCATION

National Institute on Aging, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ongoing research includes theory and experimental demonstration of solid-state CPMAS NMR experiments.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00013-19 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Hormones, Hormone Receptors, and Aging. III. Aging and Human Endocrine Regulation

PRINCIPAL INVESTIGATOR

S. Mitchell Harman, M.D., Ph.D., Acting Section Chief, ES, LCP, NIA
 Marc R. Blackman, M.D., Guest Scientist, ES, LCP, NIA
 Michele F. Bellantoni, M.D., Guest Scientist, ES, LCP, NIA
 M. Janette Busby-Whitehead, M.D., IPA Fellow, ES, LCP, NIA
 Thomas M. Stevens, M.D., IRTA Fellow, ES, LCP, NIA
 Kieran O'Connor, M.D., IRTA Fellow, ES, LCP, NIA
 Janet Vittone, M.D., Guest Scientist, ES, LCP, NIA
 Robin Roberson, B.S., Chemist, ES, LCP, NIA

COOPERATING UNITS

Reubin Andres, M.D., Chief, Metabolism Section & Branch Chief, LCP, NIA
 William Adler, M.D., Chief, Immunology Section, LCP, NIA
 Jordan Tobin, M.D., Chief, Human Performance Section, LCP, NIA
 Richard Spencer, M.D., Ph.D., Senior Investigator, Laboratory of Molecular and Cellular Biology, NIA

Jesse Roth, M.D., Chief, Diabetes Unit, LCP, NIA & Chief Geriatrics, Johns Hopkins U. School of Medicine
 Alan Shuldiner, M.D., Assoc. Prof., Division of Geriatric Medicine, Johns Hopkins U. School of Medicine
 Richard Bennett, M.D., Asst. Prof., Division of Geriatric Medicine, Johns Hopkins U. School of Medicine
 Kerry Stewart, Ed.D, Division of Cardiology, Johns Hopkins U. School of Medicine
 Edward Shapiro, M.D., Asst. Prof., Division of Cardiology, Johns Hopkins U. School of Medicine
 Ben Caballero, M.D., Chief, Center for Human Nutrition, Johns Hopkins University School of Hygiene
 Ben Hurley, Ph.D., Chief, Dept. Kinesiology, University of Maryland
 Marc Rogers, Ph.D., Asst. Prof., Dept. Kinesiology, University of Maryland

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrine Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore,MD

TOTAL MAN-YEARS

2.1

PROFESSIONAL:

1.6

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human Subjects (b) Human Tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK

In a group of 11 older men, 2 mg of subcutaneous GHRH nightly for 6 weeks produced a relatively small increase in circulating IGF-I but increases in muscle performance and urinary calcium excretion and no effects on body mass index, sleep profiles, or muscle bioenergetics. In a study of GH and sex steroid administration in old men and women, preliminary data show no significant baseline gender differences in GH release, IGF-I, OGTT glucose profiles, or plasma lipids. GH secretion was inversely related to ponderal index, fasting glucose and triglycerides, and positively correlated with levels of HDL. IGF-I was positively correlated only with triglycerides and IGF-BP-3 and not with GH release. Thus, in older men and women GH release may be a better predictor of metabolic and body composition than is IGF-I.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00021-31 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Normal Human Variability and Cross-cultural Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. C. Plato Sr. Research Geneticist LCP, NIA
J. D. Tobin Chief, Applied Physiology Section LCP, NIA

COOPERATING UNITS (if any)

CNS NINDS; CPSPB NCI; University of Maryland; Indiana University;
University of Zagreb, Croatia. The University of Michigan

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.45

PROFESSIONAL:

0.25

OTHER:

0.20

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project represents an ongoing collaborative effort, involving WHO and other national and international laboratories to coordinate the collection, evaluation and interpretation of Normal Genetic Markers in order to study the cross-cultural patterns of genetic and extraneous factors, as they relate to normative aging and to diseases with late onset, including bone loss, osteoarthritis, Alzheimer's disease, breast cancer, amyotrophic lateral sclerosis, and Parkinsonism dementia. Specifically, the objectives of this study are: A) To study the cross cultural patterns of genetic and non-genetic factors in an effort to better understand the process of normative aging. B) To study the distribution of dermatoglyphics, lateral dominance, iris structure and other genetic variables in BLSA participants and other control samples, as well as in patients with late onset diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00022-18 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone Loss with Age: Epidemiological, Familial and Cross-Cultural Considerations

PRINCIPAL INVESTIGATOR (List other professional personnel, below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. C. Plato Sr. Research Geneticist LCP, NIA
 J. D. Tobin Chief, Applied Physiology LCP, NIA

COOPERATING UNITS (if any)

University Zagreb, Croatia; Kyoto University, , Kyoto; University of Maryland; Hopkins Bayview Research Center; Johns Hopkins University; The University of Michigan; CNS, NINDS

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.20

PROFESSIONAL:

1.00

OTHER:

1.20

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the fourth decade of life, the human skeleton begins to lose bone. That is, bone mass decreases in relation to bone volume. Menopause and the associated estrogen deficiency will enhance bone loss in females. It has also been suspected that bone loss is familial, mainly because of the increased prevalence of osteoporosis in relatives, although there are no satisfactory scientific data to support either a familial or a genetic control of bone loss. In long bones, cortical bone is resorbed from the endosteal surface. Because of the thinning of the cortical bone shell, bones lose their mechanical integrity and fracture more readily. The trabecular bone mass of the vertebral column also decreases with age. Vertebral compression fractures and fractures of the femoral neck are the most serious consequences of bone loss.

This project deals with the epidemiological, genetic, cross-sectional, longitudinal, and biomechanical aspects of bone loss (1) among the participants of the Baltimore Longitudinal Study of Aging (BLSA), (2) in genetic isolates of the Adriatic Sea Islands of Croatia and the island of Guam in Micronesia, Japan, Central America, Albania and other parts of the world (cross-cultural), (3) in rats.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00023-18 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Hormones and Aging. Hypothalamic-Pituitary Function in Experimental Animals

PRINCIPAL INVESTIGATOR

S. M. Harman, M.D., Ph.D., Section Chief, ES, LCP, NIA

M. R. Blackman, M.D. Guest Scientist, LCP, NIA

Patricia Ponsalle, Ph.D., IRTA Fellow, LCP, NIA

Marco R. Piñero, M.S., Chemist, LCP, NIA

Robin Roberson, B.S., Chemist, LCP, NIA

Kalonji Collins, Summer Student, LCP, NIA

COOPERATING UNITS

D. Lu, Ph.D, Senior Fellow, Dept. of Geriatrics and Gerontology, University of Texas at Austin

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrine Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD

TOTAL MAN-YEARS

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human Subjects (b) Human Tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK

We are using specific antibodies to distinguish which subspecies of inhibitory GTP binding proteins are altered during aging.

There has been a hiatus in the molecular aspect of this work due to the departure of the principal individual engaged in this project (Dr. Lu). As of January 1994 a new investigator (Dr. P. Ponsalle) has been brought in as an IRTA fellow to reorganize and pursue this work.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00093-22-LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Basis or Regulation of the Humoral Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A. A. Nordin Research Chemist LCP, NIA
 Other: T. K. Kwon Visiting Fellow LCP, NIA
 M. A. Buchholz Biologist LCP, NIA
 F. J. Chrest Biologist LCP, NIA

COOPERATING UNITS (if any)

Dr. J. Shaper and N. Shaper, Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, Dr. E. Gabrielson, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21224

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.3

PROFESSIONAL:

2

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular aspects of T-cell activation and subsequent progression through the cell cycle are investigated using the murine model to establish a basis for the immunodeficiencies associated with advancing age. Progression through and exit from the G1 phase of the cell cycle is limiting in a significant portion of the T-cells derived from old mice. A subfamily of the cyclin-dependent kinases (cdk) represented by cdk4 and cdk6 are thought to play a significant role during G1. Prior to initiating studies with old mice, T-cells derived from young animals were used to characterize cdk4 and cdk6 during the G1 phase of the cell cycle following polyclonal activation with immobilized anti-CD3. These studies have shown that: 1) Specific mRNA for cdk4 and cdk6 are detected 5 hr after activation and maximum levels for cdk4 are attained at 10 hr and 20 hr for cdk6. 2) Protein levels of both kinases were first detected at 5 hr and were significantly increased by 20 hr. 3) D cyclins were consistently found to be associated with cdk4 and cdk6. 4) Although both kinases phosphorylated retinoblastoma protein and E1A binding protein the kinetics of the reaction were different. 5) The availability of cyclin D2 and D3 during the G1 phase of the cell cycle may account for the timing of the kinase reaction. Studies will now be undertaken using T-cells derived from old mice to determine the role of cdk4 and cdk6 and the D cyclins in the failure of activated T-cells to progress through and exit G1.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201-AG-00095-21-LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Cell Membrane Structures on Cellular Recognition

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. H. Adler Medical Officer, PHS LCP, NIA

Other: J. E. Nagel Medical Officer, PHS LCP, NIA
 S. M. Papciak Staff Fellow LCP, NIA
 F. J. Chrest Biologist LCP, NIA
 P. Lal Visiting Fellow LCP, NIA, EOD 10/1/93

COOPERATING UNITS (if any)

Drs. R. Winchurch and D. Kittur, Department of Surgery, FSKMC, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.2

PROFESSIONAL:

1.7

OTHER:

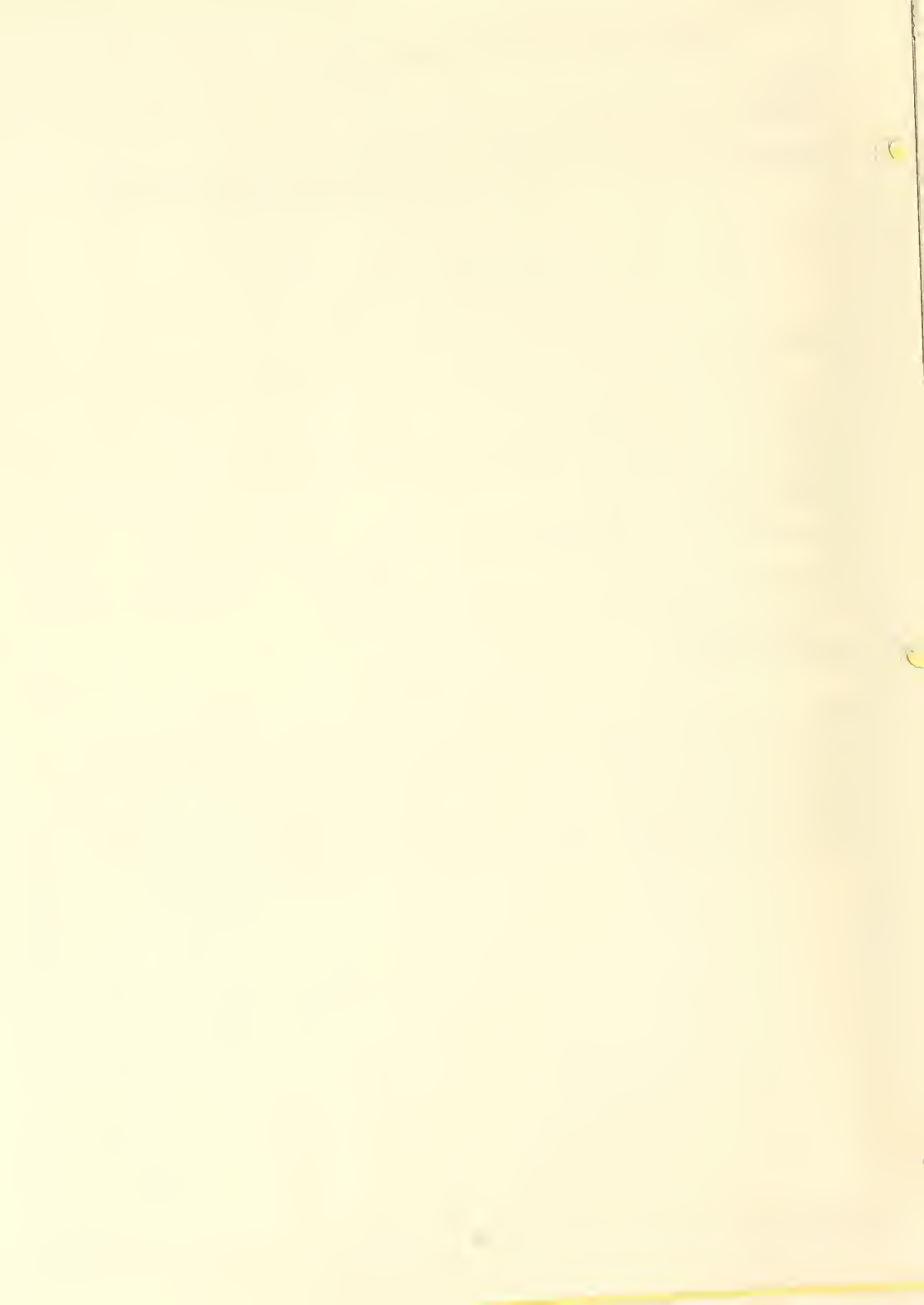
.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There are many cell free factors which are associated with an inflammatory response which are able to be induced in older animals and which are expressed at higher levels than seen in younger mice. The biologic implications of this finding are very important and demonstrate that the immune deficiency of aging is not a simple loss of function or cells but a change in the control of cellular function. The expression of receptors for the factors as well as an analysis of their ability to generate cellular signals in the cells from young and old mice will provide evidence for the biologic role of these factors in the older individuals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00096-21-LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte Activation and Function in Aging Individuals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. A. Brock Research Biologist LCP, NIA
 Other: W. H. Adler Medical Officer, PHS LPS, NIA
 F. J. Chrest Biologist LPS, NIA

COOPERATING UNITS (if any)

Dr. H. J. Hoffman, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1

OTHER:

.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Receptor mediated activation of many cell types is followed by motility related events. In T lymphocytes, lateral redistribution of surface receptors is accompanied by aggregation of actin and myosin in cytoplasmic subcaps. Patching and capping of receptors after activation of lymphocytes from aged animals and humans is impaired, and it was inferred from indirect evidence that age-related changes in cytoskeletal functions are responsible. Concanavalin A activation of resting T lymphocytes resulted in actin polymerization in the cytoskeleton of cells from young but not aged C57BL/6 mice. Bypassing the plasma membrane to activate protein kinase C with PMA induced actin polymerization in resting T lymphocytes and in immunomagnetically isolated CD4 and CD8 positive subpopulations from young and aged mice. A higher percentage of F-actin subcaps in unstimulated CD8 positive cells from aged mice was seen than in all other groups. After activation with PMA, fewer F-actin subcaps formed in both CD4 and CD8 positive cells from aged compared to young animals and their morphology differed from that of cells from young mice. This suggests that although plasma membrane signalling events are bypassed and actin polymerization is initiated in cells from aged mice, the function/s of F-actin change with age. Since T lymphocytes activated with Concanavalin A exhibit circannual rhythms in proliferation and their properties change with age, levels of polymerized actin in resting T lymphocytes and CD4 and CD8 positive cells stimulated with PMA were analyzed for seasonality in responses. Actin polymerized in spring and summer but not during winter months in cells from young mice, and there were no seasonal changes in cells from aged animals. F-actin levels were significantly higher in spring and summer and lower during winter in cells from young compared to aged mice. Therefore, seasonality in lymphocyte functions and obscure age-related defects, and, even though plasma membrane activation events are bypassed, subsequent signal transduction is impaired with age and during the winter season in cells from young animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00104-18-LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Immune Survey of Longitudinal Project Participants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. H. Adler Medical Officer, PHS LCP, NIA
Other: J. E. Nagel Medical Officer, PHS LCP, NIA
L. Song Visiting Associate LCP, NIA
P. Lal Visiting Fellow LCP, NIA, EOD 10/1/93
M. M. Schoonmaker, Biologist, LCP, NIA; F. J. Chrest, Biologist, LCP, NIA;
G. D. Collins, Biologist, LCP, NIA; R. S. Pyle, Biologist, LCP, NIA;
B. A. Dorsey-Cooper, Biologist, LCP, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

5

PROFESSIONAL:

2.4

OTHER:

2.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies use participants in the Baltimore Longitudinal Study of Aging (BLSA) and human cell lines to gain insight into the biochemical and molecular mechanisms underlying age-associated changes in immune function. Recent data indicates that human T lymphocytes activated through different cellular pathways display distinctive patterns of protein phosphorylation, cytokine synthesis and gene expression, only some of which are age-affected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00213-04 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Tris-sulfotyrosyl peptide inhibits protein tyrosine phosphatase activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Bernier Senior Staff Fellow LCP, NIA

Others:

A.S. Liotta Special Expert LCP, NIA
 H.K. Kole Senior Staff Fellow LCP, NIA
 H.M. Fales Senior Investigator LBC, NHLBI

COOPERATING UNITS (if any)

Laboratory of Biophysical Chemistry, NHLBI
 J. Roth, Division of Geriatric Medicine and Gerontology, JHUSM

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS

2.0

PROFESSIONAL

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (aa2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

A synthetic tris-sulfotyrosyl dodecapeptide [TRDIY[S]ETDY[S]Y[S]RK-amide], whose primary sequence is identical to the 1142-1153 sequence of the insulin proreceptor, inhibited insulin receptor dephosphorylation in solubilized membranes, and digitonin-permeabilized cells derived from Chinese hamster ovary (CHO) cells expressing high levels of human insulin receptors (CHO/HIRc). It also inhibited the dephosphorylation of a synthetic tyrosine phosphorylated substrate by recombinant PTP-1B, a protein tyrosine phosphatase (PTPase), indicating that it acted via interaction with PTPase(s). A N-stearyl derivative of the peptide caused an ~ 4.5-fold increase in insulin-stimulated receptor autophosphorylation in intact CHO/HIRc cells. The peptide displayed specificity toward tyrosine-class phosphatases only, as it had no effect on the activities of the serine/threonine phosphatases PP-1 and PP-2A, or alkaline phosphatase. The tyrosine sulfate ester bonds of the peptide were stable when incubated with PTP-1B (1h, 30° C). These data suggest that the sulfotyrosyl peptide functions as a nonhydrolyzable phosphotyrosyl peptide analogue capable of direct interaction with PTPase catalytic domain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01AG00214-04 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Use of an *in vivo* model system to investigate NIDDM

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Egan Senior Staff Fellow LCP, NIA

Others:

R. Perfetti	Visiting Associate	LCP, NIA
T. Henderson	Science Lab Tech	LCP, NIA
A.S. Liotta	Special Expert	LCP, NIA
C. Montrose-Rafizadeh	Senior Staff Fellow	LCP, NIA
Y. Wang	Fogarty Fellow	LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine
 L. Adams, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type Do not exceed the space provided.)

Age is an independent risk factor for NIDDM. A decline in insulin secretion and a decrease in insulin action occur as people age. This combination makes people more prone to develop NIDDM. A similar phenomenon occurs in Wistar rats. We are using the beta cells from Wistar rats in the aged colony at the GRC to study changes with insulin secretion. We found that mRNA for insulin is preferentially diminished in islets, with glucokinase and glucagon messages unaffected. Therefore, one can envision when a stress is put on the system so more insulin is required, diabetes could result. We are exploring what factors lead to this diminution of insulin message and the possibility that we can prevent or reverse it. We have found the GLP-1 can somewhat restore the number of cells that are again responsive.



NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AG 00281-04 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT

Hormones, Hormone Receptors, and Aging. IV. Hormone Replacement in Menopausal Women

PRINCIPAL INVESTIGATOR

S. Mitchell Harman, M.D., Ph.D., Acting Section Chief, ES, LCP, NIA
 Michele F. Bellantoni, M.D., Guest Scientist, ES, LCP, NIA
 Marc R. Blackman, M.D. Guest Scientist, ES, LCP, NIA
 Janet Vittone, M.D., Guest Scientist, ES, LCP, NIA
 Robin Roberson, B.S., Chemist, ES, LCP, NIA
 Shannon Haszard, Summer Student, ES, LCP, NIA
 Mwango Kashoki, Summer Student, ES, LCP, NIA

COOPERATING UNITS

Dr. Jordan Tobin, Chief Applied Physiology Section, LCP, NIA
 Jay Shapiro, M.D. Div. of Geriatrics, Depts. of Medicine, Hopkins Bayview Medical Ctr and Johns Hopkins University School of Medicine.
 Katherine Bass, M.D., Dept. of Obstetrics & Gynecology, Hopkins Bayview Medical Ctr
 Ann Davidoff, Ph.D., Div. of Geriatrics, Depts. of Medicine, Hopkins Bayview Medical Ctr and Johns Hopkins University School of Medicine.

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Endocrine Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD

TOTAL MAN-YEARS

2.1

PROFESSIONAL:

1.6

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human Subjects (b) Human Tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK

We are studying 30 women 65-75 years of age treated for 2 y with constant oral estrogen/low dose daily progestin (ERT) and 30 age-matched BLSA controls for bone loss (DEXA) and biochemical indices of bone turnover, plasma lipids, anthropometric indices, DEXA measures of body fat, and vaginal maturation indices. Mood, symptoms of estrogen deficiency, and side effects of hormone replacement therapy are assessed. Minor breast tenderness and vaginal bleeding are the only adverse effects observed. Treated women showed a 3.8% decrease in total cholesterol (NS) with a 16% increase in HDL and a 17% decrease in LDL (both $p < 0.001$), and no significant change in plasma triglycerides. DEXA scans revealed a decrease in percent body fat ($p < 0.03$) and increases in lean body mass ($p < 0.03$) and bone mineral density (lumbar spine +7%; femoral trochanter +9%, both $p < 0.01$). Osteocalcin (bone deposition) decreased 35% ($p < 0.01$) and urinary pyridinoline excretion (bone resorption) decreased 30% ($p < 0.01$). Data suggest that ERT in older postmenopausal women improves metabolic function and results in favorable changes in body composition. We find a favorable risk/benefit ratio for ERT in older postmenopausal women.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00290-08 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Osteoarthritis and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. D. Tobin Chief, Applied Physiology Section LCP, NIA
 C. C. Plato Sr. Research Geneticist LCP, NIA

Others: W. Scott Radiologist Johns Hopkins Sch of Med
 M. Hochberg Rheumatologist U. Maryland Medical School
 M. Lethbridge-Cejku Research Associate U. Maryland Medical School

COOPERATING UNITS (if any)

Johns Hopkins University School of Medicine (Scott),
 University of Maryland Medical School (Lethbridge-Cejku and Hochberg)

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Osteoarthritis (OA) is the most common rheumatic disease of the elderly. As part of the ongoing studies of OA in the Baltimore Longitudinal Study of Aging (BLSA) we have evaluated the association of metabolic and physiologic factors with the presence distribution, and progression of hand and knee OA in both sexes. In 234 men and 95 women in the BLSA, aged 60 and above, definite knee OA was present in 65% of those with pain compared to 34% of those without knee pain. Prevalence of self-reported pain is significantly associated with the severity of the grade of OA in the knee. In 130 subjects with longitudinal knee xrays, we found that incidence or progression of OA occurred in 22 (17%) with grade 0-2 osteophyte changes at baseline, over a mean follow-up of 3.8±1.1 yrs; and that both age and BMI at baseline significantly predicted time to incidence or progression. In 465 men and 275 women aged 40 and above, we found that current smoking is associated with a decreased risk of osteophyte formation and knee OA in women independent of age and BMI. We found that subjects aged 40 and above with current knee pain have significantly lower aerobic capacity (AC) than those without knee pain adjusted for radiographic changes of knee OA, confirming the hypothesis that AC is diminished in persons with symptomatic knee OA. In 340 men and 208 women aged 40 and above, mean spine bone mineral density (BMD) was significantly greater in those with knee OA, after adjustment for age and BMI. There was no association between hip BMD and knee OA features. In our evaluation of familial aggregation of hand and generalized OA in the BLSA, we found clinically relevant sibling correlations for OA of the distal interphalangeal joints, after adjustment for age and gender, but no association for other hand sites, for knee OA, or for polyarticular OA (hand and knee).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00293-06 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Parameters of Bone Metabolism: Age and Sex Contrasts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.D. Tobin Chief, Applied Physiology Section LCP, NIA

Others: C.C. Plato Sr. Research Geneticist LCP, NIA

B.F. Hurley Guest Researcher LSB, NIA

R.S. Lindle IRTA Fellow LSB, NIA

COOPERATING UNITS (if any)

University of Maryland, College Park

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Applied Physiology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

.10

OTHER:

.40

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Age-related changes in bone mass have been demonstrated in both men and women. Age and sex related differences hormones, nutritional and physiological variables involved in bone turnover are important in elucidating changes in bone physiology in normal aging and disease. The relationship of muscle strength, as estimated by concentric torque in the lower extremity, to bone mineral density, was evaluated in 80 men and 73 women ranging from 23 to 88 years of age. These normal volunteers from the Baltimore Longitudinal Study of Ageing had highly significant negative correlations of age with muscle strength and bone mineral density. At the same time muscle strength was positively correlated with estimates of bone mineral density. When the effect of age was analyzed by multiple regression techniques there was no age-independent contribution of muscle strength to bone mineral density in women, but there remained a significant relationship of muscle strength to bone mass in men with stronger men having greater bone mass at any age. This different strength-bone relationship in men and women may indicate a role for anabolic sex hormones in the maintenance of bone mass during aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00441-07-LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Host Factors Relating to HIV Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. H. Adler	Medical Officer, PHS	LCP, NIA
Other:	J. E. Nagel	Medical Officer, PHS	LCP, NIA
	G. D. Collins	Biologist	LCP, NIA
	M. Schoonmaker	Biologist	LCP, NIA
	R. S. Pyle	Biologist	LCP, NIA
	B. A. Dorsey-Cooper	Biologist	LCP, NIA
	P. Lal	Visiting Fellow	LCP, NIA, EOD 10/1/93

COOPERATING UNITS (if any)

Dr. J. Bartlett, Dept. Medicine, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.4

PROFESSIONAL:

.8

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immune response to the HIV involves an "autoimmune" component in which the patient makes both anti-HIV antibody as well as anti-HLA antibody. This type of response has also been observed in other viral infections that cause immune deficiencies. During an HIV infection it appears that the older patients have a more extensive loss of T helper cells and reach low levels of CD4+ cells more rapidly than do younger patients. Younger HIV infected patients experience extended periods in which their CD4+ cell level remains in the normal range.



DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00876-03 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Regenerating (REG) gene: A paracrine B-cell growth factor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R. Perfetti Visiting Scientist LCP, NIA

Others:

J.M. Egan Senior Staff Fellow LCP, NIA
 M.E. Zenilman Special Volunteer LCP, NIA
 A.R. Shuldiner Special Volunteer LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Director of Division of Geriatric Medicine & Gerontology, Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS.

1.0

PROFESSIONAL

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pancreatic islets of Langerhans are constituted by cells that rarely undergo through mitosis and that exhibit poor capability of regeneration after injury. Recently, Terazono et al. isolated and characterized a cDNA from regenerating pancreatic derived library, designated reg (for regenerating gene). Reg mRNA levels were shown to be markedly elevated in islets induced to proliferate by pancreatectomy and nicotinamide treatment. The increase in expression of reg gene was temporally correlated with the increase in size of regenerating islets and the decrease of glycosuria. Increase in reg mRNA has also been demonstrated in the hyperplastic islets of the NON mice after treatment with aurothioglucose. In addition, it has been shown that the implantation of a solid insulinoma tumor into rats caused a dramatic reduction of the islets volume as well as of the function of endogenous islet β -cells. This phenomenon was associated with a coordinate suppression of both reg and insulin gene expression. When removal of the tumor was performed, this resulted in a rapid proliferation of the endocrine β -cells and it caused an induction of reg expression and the restoration of the endogenous insulin gene expression. All of those experimental maneuvers show an intriguing correlation between reg gene expression and changes in β -cell mass or β -cell function. We hypothesize that REG may be a crucial autocrine and/or paracrine growth factor during embryogenesis as well as for maintenance of β -cell function in the adult. We believe that alterations of the reg gene expression may be involved in the progressive β -cell dysfunction with aging and diabetes. We plan to use molecular techniques to measure mRNA levels of reg in the pancreas of rodents during the normal aging process, and in rodent strains with genetic forms of type I or type II diabetes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00877-03 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Mechanisms of regulating insulin secretion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Egan Senior Staff Fellow LCP, NIA

Others:

R. Perfetti	Visiting Associate	LCP, NIA
T. Henderson	Science Lab Tech	LCP, NIA
A.S. Liotta	Special Expert	LCP, NIA
C. Montrose	Staff Fellow	LCP, NIA
Y. Wang	Staff Fellow	LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine
L. Adams, Johns Hopkins University

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.5

OTHER

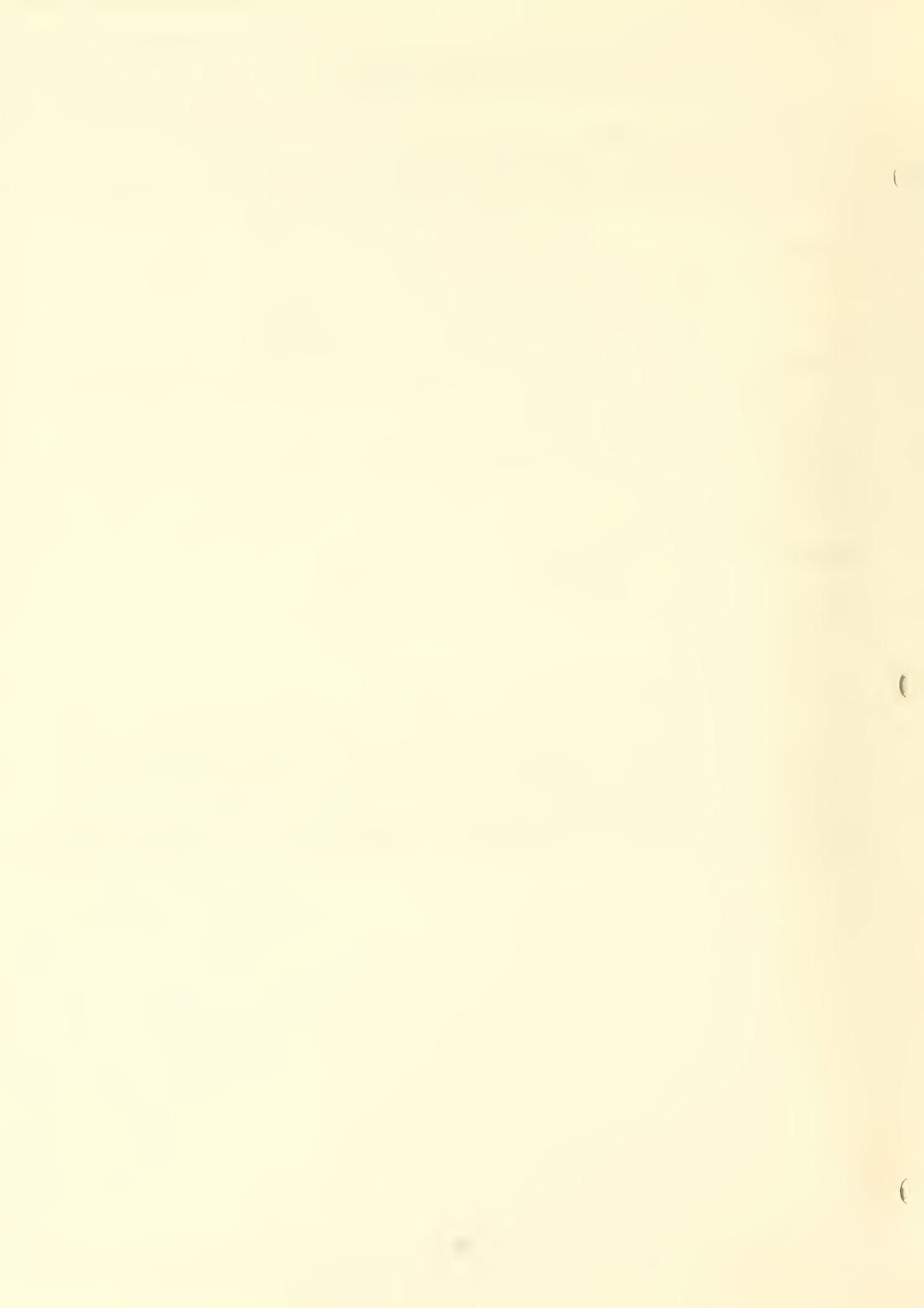
0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Insulin secretion is defective in non-insulin dependent diabetes mellitus (NIDDM). Particularly affected is the glucose signalling of insulin release. Second messengers do not seem to be defective in NIDDM. Rather the signalling of the release of insulin from its granules appears deficient. We are studying these distal events in insulin release as well as mechanisms by which it can be manipulated. We are specially interested in a group of peptide called incretins. These are released from gut in response to food and modulate insulin release in a positive fashion when glucose is present. Moreover, one of the incretins, GLP-1 can normalize the beta cell response to glucose when it has become unresponsive in NIDDM.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00878-03 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Screening for mutations in target genes causing type II diabetes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. S. Celi Visiting Fellow LCP, NIA

Others:

A. R. Shuldiner Special Volunteer LCP, NIA
J. Roth Special Volunteer LCP, NIA

COOPERATING UNITS (if any)

L. Adams, Johns Hopkins University School of Medicine, Division of Geriatric
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LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

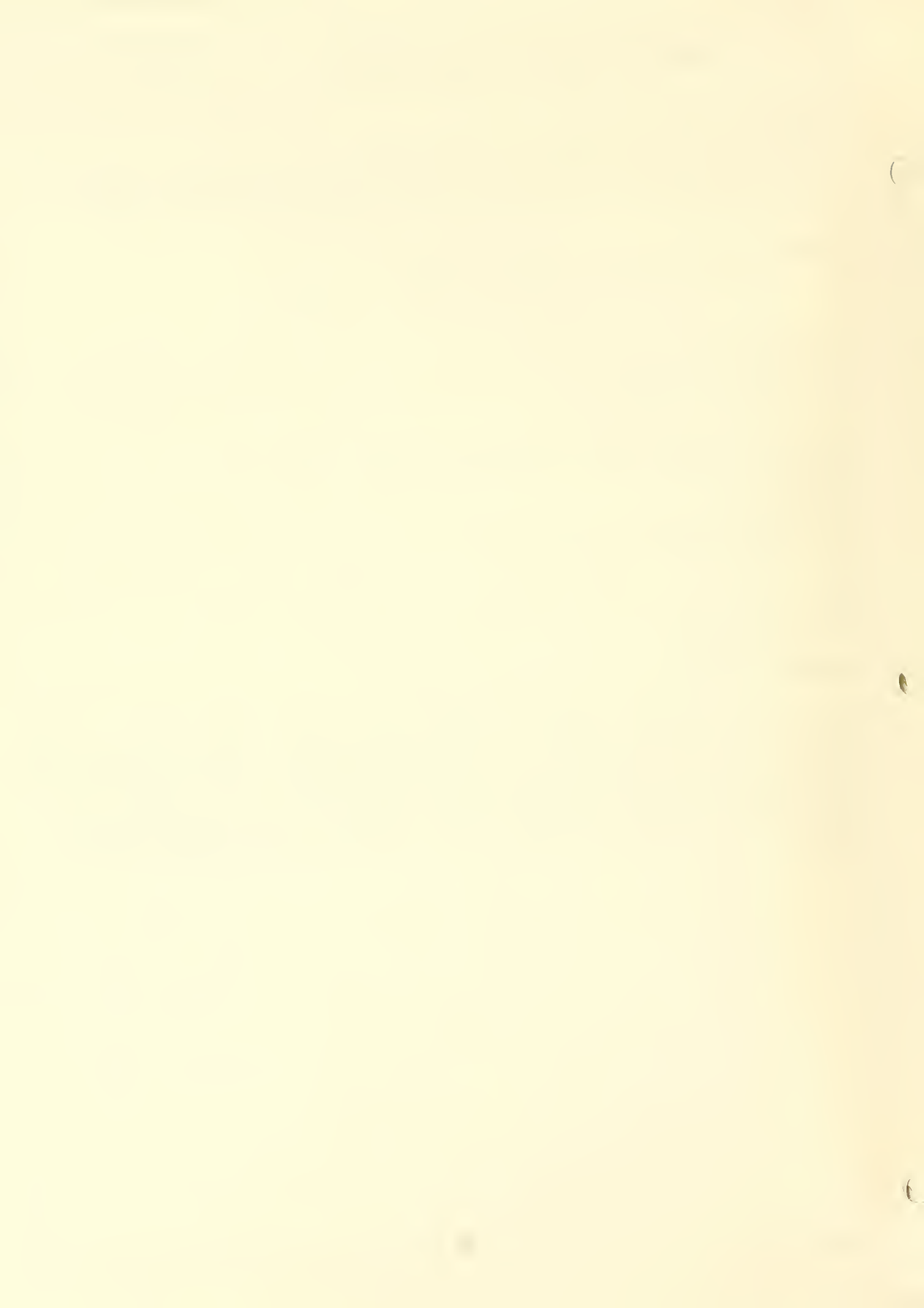
0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type Do not exceed the space provided.)

Mutations in the insulin receptor gene are known to be a cause of some rare forms of extreme insulin resistance. The relevance of mutations in the insulin receptor gene as well as other candidate genes in the pathogenesis of the more common aging-related type II diabetes mellitus are currently unknown. Leading on from our report last year we studied two amino acid substitutions in IRS-1 in Pima indians, a minority population at risk for NIDDM. These substitutions had been associated with NIDDM in a Danish population. Neither, substitution was observed in 242 diabetic or 190 nondiabetic Pima indians. We then looked for other mutations in IRS-1.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00881-02 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Glucagon-like peptide: regulation of insulin action at extrapancreatic sites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Montrose-Rafizadeh Senior Staff Fellow LCP, NIA

Others:

J. Egan	Senior Staff Fellow	LCP, NIA
M. Bernier	Senior Staff Fellow	LCP, NIA
Y. Wang	Fogarty Fellow	LCP, NIA

COOPERATING UNITS (if any)

Dr. Jesse Roth, Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine

LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, GRC, Baltimore, Maryland

TOTAL STAFF YEARS

3.0

PROFESSIONAL

2.5

OTHER

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Non-insulin-dependent diabetes mellitus (NIDDM), type II diabetes, is one of the most common diseases in the elderly population of the USA, and is especially common among minority population. NIDDM is caused by 1) impaired insulin secretion from islets of Langerhans in the pancreas, and 2) impaired sensitivity of peripheral tissues (such as muscle, fat, and liver) to insulin. A promising new approach for treatment of NIDDM is the use of incretin hormones such as glucagon-like peptide. Incretins have many desirable effects which may help NIDDM patients. Incretins stimulate insulin secretion and inhibit glucagon secretion. In addition we have found that GLP stimulates insulin action at insulin target tissues. For example, glucose uptake and lipid synthesis in cultured adipocytes is stimulated by GLP. We have found that GLP receptor mRNA is present in many different tissues from rat, further suggesting extrapancreatic effects of GLP.

We have evidence that the GLP receptor cascade acts differently in pancreas versus extrapancreatic tissues. Our experiments will test whether different GLP receptor isoforms are present in pancreas versus extrapancreatic tissues or whether different signal transduction pathways are coupled to GLP receptor in different tissues. Further molecular cloning of GLP receptor isoforms and the study of signal transduction involved in GLP action will allow the development of new therapeutic agents for NIDDM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00882-01 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Alkylation of Sulfhydryl group(s) on the Insulin Receptor Alter Kinase Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Bernier Senior Staff Fellow LCP, NIA

Others:

O. Nativ Fogarty Fellow LCP, NIA
 H. Kole Senior Staff Fellow LCP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigated the effect of a sulfhydryl-modifying reagent on insulin receptor functions in digitonin-permeabilized Chinese hamster ovary cells that were transfected with human insulin receptors (IR). The reagent used was maleimidobutyryl biocytin (MBB), a thiol-specific biotinylation reagent. Results showed that MBB had an enhancing effect on the insulin receptor tyrosine kinase activity toward an exogenous substrate. In the absence of insulin, MBB caused an increase in basal kinase activity comparable to the level seen with insulin alone. Treatment of permeabilized cells with MBB caused further 2-fold stimulation of receptor kinase activity by insulin. IR β -subunit contains sulfhydryl group(s) that react readily with MBB. The extent of biotinylation of IR β -subunit was identical whether the cells were stimulated or not with insulin. Biotinylation of β -subunit was totally inhibited by L-cysteine but not L-glycine, indicating the specific nature of MBB as a thiol-modifying reagent. Several sulfhydryl reagents were tested as probes for assessing the location of accessible thiol(s) on the receptor as well as determining whether these reactive sulfhydryls are vicinals. It appears that modification of IR sulfhydryl(s) by MBB did not cause reduced accessibility of cellular protein tyrosine phosphatases toward IR phosphorylation sites. We conclude that modification of reactive IR cysteine(s) by MBB results in enhanced IR tyrosine kinase activity, which could suggest an important role played by cellular oxidation of such residues in insulin signalling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AG00883-01 LCP

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical Synthesis of Novel Potent Inhibitors of Protein Tyrosine Phosphatases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. K. Kole Senior Staff Fellow LCP, NIA

Others:

T. R. Burke Senior Investigator LMC, NCI

P. P. Roller Senior Investigator LMC, NCI

COOPERATING UNITS (if any)

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LAB/BRANCH

Gerontology Research Center, Laboratory of Clinical Physiology

SECTION

Diabetes Unit

INSTITUTE AND LOCATION

NIH, NIA, Baltimore, Maryland 21224

TOTAL STAFF YEARS.

2.0

PROFESSIONAL

2.0

OTHER

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Abnormal protein tyrosine phosphatase (PTPase) activity has been reported to be associated with age, diabetes and also with certain cancers. We have been studying the regulation of insulin receptor and epidermal growth factor receptor functions by employing different PTPase inhibitors. Newly synthesized arylphosphonates effectively inhibited dephosphorylation of prelabeled receptors by particulate PTPases and recombinant PTP-1B. Also, peptides containing two variations of the phosphotyrosyl ester moieties have been synthesized and tested for their effect on PTPase activity. These variations were the phosphonomethyl-L-phenylalanine (pmp) and phosphonodifluoromethyl-L-phenylalanine (Fpmp). It has been found that a Fpmp-containing peptide potently inhibited dephosphorylation of insulin receptor by PTP-1B.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00808-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Age and Phenotypic State in Vascular Smooth Muscle Cell Migration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R.R. Pauly Senior Staff Fellow LCS, NIA
 Others: E.G. Lakatta, M.T. Crow, R. Monticone, L. Cheng, S. Sollott, C. Bilato, G.M. Jenkins, J. Fredman, M. Chin

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular disease. The incidence and prevalence of vascular disease increase with age, affecting approximately 50% of men (by age 65) and women (by age 75). We investigated the migratory, proliferative, and differentiative behavior of VSMC derived from young (age 3-6 mo) and old (age 24 mo) rats. Two populations of VSMC (neointimal and medial) were obtained following balloon catheter injury for each age group. Neointimal and medial VSMCs differ in their cellular morphology and pattern of gene expression. We show here that their in vitro migration in response to PDGF gradient differs, as well. Specifically, early passage (P₂-P₅) old medial VSMC exhibit 75% more migratory behavior as compared with (P₂-P₅) young medial VSMC. At later passages (P>10) young medial and old medial VSMC exhibit similar migratory characteristics. In addition, young neointimal (P₂-P₅) cells show 75% greater migration than young medial (P₂-P₅) cells. Both groups showed similar proliferation rates and cell sizes, and both upregulated expression of the immediate-early genes, c-fos and JE (MCP-1) in response to PDGF. The medial cells exhibited a blunted intracellular calcium in response to Ionomycin and a 50% reduction in CamKinaseII activation as compared to neointimal cells. Interestingly, transfection of neointimal cells with Calponin sense DNA (a genetic marker of medial cells) rendered them less migratory while antisense Calponin showed no effect. When VSMC from all groups were growth-arrested, their migratory behavior was less than 20% that of age/phenotype matched proliferating cells. In addition to PDGF, migration of young proliferating cells requires the autocrine production of the growth factor, basic FGF. Old cells, in contrast, do not appear to require this additional factor for migration to occur. We have observed that 72 kD Type IV gelatinase and receptor tyrosine kinase (RTK) activation in response to PDGF is dependent on phenotypic state of the cell as detailed in reports 278-04 and 815-02. Future studies characterizing these differences and further employing genetic markers specific to each group of cells should provide important information in understanding these age-associated behavioral differences in VSMC migration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00809-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dietary Fatty Acid Modulation of Myocardial Function and Influences on Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. Pepe Visiting Fellow LCS, NIA

Others: E. G. Lakatta Chief LCS, NIA
 H. A. Spurgeon Research Physiologist LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cardiovascular Science, Gerontology Research Center

SECTION

Cardiac Function

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS.

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This laboratory has shown that cardiac myocyte contraction time, its action potential and cytosolic Ca⁺⁺ transient are extended with increasing age in rats. In addition, with increased age the threshold for Ca⁺⁺ overload of rat cardiac cells is reduced. Age-related changes in cardiac function and increased vulnerability to arrhythmogenic stimuli may be associated with alterations to membrane composition which may be intervened by dietary lipid modification of myocardial cell membranes. In this project we demonstrated following 8 weeks feeding of a basic reference diet to Wistar rats the proportion of ventricular membrane arachidonic acid (AA; n-6 polyunsaturated fatty acid, PUFA) increased and docosahexaenoic acid (DHA; n-3 PUFA) decreased in 24mo rats compared to 6mo rats. Treatment with a diet rich in saturated fat (SAT) distinctly augmented this age effect whereas FO prevented the age-related decrease in the n-3/n-6 PUFA ratio. An experimental protocol was employed which stresses cell membrane ion homeostasis related to membrane function. When stimulated at 2 Hz contraction amplitudes decreased in all groups, while SAT myocytes had the smallest contraction amplitude, FO myocytes were relatively resistant to this effect. When 0.5 or 2Hz stimulation was ceased no difference in the number of "spontaneous" cell contractions or "waves" (indicative of intracellular Ca⁺⁺ rising above the threshold for Ca⁺⁺ induced Ca⁺⁺ release and causing a spontaneous Ca⁺⁺ release from SR and thus potentially spontaneous contractions). However, the time to the first spontaneous wave was significantly longer for FO myocytes. Isoproterenol or BAYK8644 increased the number of waves and decreased the time to their onset. SAT diet markedly augmented this but FO diet provided protection by attenuating the severity of these effects. Thus results indicate that SAT diet exacerbates, and FO diet provides resistance against, age associated cardiac cell Ca⁺⁺ tolerance and arrhythmogenic triggers. These effects may be due to changes in cell membrane lipid composition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00809-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Dietary Fatty Acid Modulation of Myocardial Function and Influences on Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. Pepe Visiting Fellow LCS, NIA
 Others: E. G. Lakatta Chief LCS, NIA
 H. A. Spurgeon Research Physiologist LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cardiovascular Science, Gerontology Research Center

SECTION

Cardiac Function

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This laboratory has shown that cardiac myocyte contraction time, its action potential and cytosolic Ca⁺⁺ transient are extended with increasing age in rats. In addition, with increased age the threshold for Ca⁺⁺ overload of rat cardiac cells is reduced. Age-related changes in cardiac function and increased vulnerability to arrhythmogenic stimuli may be associated with alterations to membrane composition which may be intervened by dietary lipid modification of myocardial cell membranes. In this project we demonstrated following 8 weeks feeding of a basic reference diet to Wistar rats the proportion of ventricular membrane arachidonic acid (AA; n-6 polyunsaturated fatty acid, PUFA) increased and docosahexaenoic acid (DHA; n-3 PUFA) decreased in 24mo rats compared to 6mo rats. Treatment with a diet rich in saturated fat (SAT) distinctly augmented this age effect whereas FO prevented the age-related decrease in the n-3/n-6 PUFA ratio. An experimental protocol was employed which stresses cell membrane ion homeostasis related to membrane function. When stimulated at 2 Hz contraction amplitudes decreased in all groups, while SAT myocytes had the smallest contraction amplitude, FO myocytes were relatively resistant to this effect. When 0.5 or 2Hz stimulation was ceased no difference in the number of "spontaneous" cell contractions or "waves" (indicative of intracellular Ca²⁺ rising above the threshold for Ca²⁺ induced Ca²⁺ release and causing a spontaneous Ca²⁺ release from SR and thus potentially spontaneous contractions). However, the time to the first spontaneous wave was significantly longer for FO myocytes. Isoproterenol or BAYK8644 increased the number of waves and decreased the time to their onset. SAT diet markedly augmented this but FO diet provided protection by attenuating the severity of these effects. Thus results indicate that SAT diet exacerbates, and FO diet provides resistance against, age associated cardiac cell Ca²⁺ tolerance and arrhythmogenic triggers. These effects may be due to changes in cell membrane lipid composition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00810-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fatty Acid Modulation of Ionic Channel Function and Ca²⁺ Homeostasis in the Heart

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Pepe	Visiting Fellow	LCS, NIA
Others:	K. Bogdanov	Guest Researcher	LCS, NIA
	E. G. Lakatta	Chief	LCS, NIA
	H. A. Spurgeon	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

Dept. of Preventative Med., Harvard Medical School, Mass. General Hospital, Boston

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Laboratory of Cardiovascular Science

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antiarrhythmic effects of polyunsaturated fatty acids following dietary incorporation into cardiac cell membranes have been observed in recent years. The mechanisms of action are yet to be defined. In this project the effect of membrane-free polyunsaturated fatty acids delivered to isolated adult rat cardiac myocytes was investigated (in vivo these can be released from cardiac cell membranes following phospholipase action). The present study investigates the effects of DHA and arachidonic acid (AA;C20:4,n-6) on L-type calcium and K⁺ channel conductance in whole cell voltage-clamp experiments and on cytosolic free calcium fluorescence and contraction in adult rat indo-1 loaded cardiac myocytes. Nitrendipine (10 nM) reduced peak I_{Ca}, measured by whole cell clamp from -40 to -5 mV, twitch contraction amplitude and associated cytosolic indo-1 Ca²⁺ fluorescence measured during electrical stimulation (0.5Hz). DHA (5μM) abolished these effects but AA did not block the nitrendipine effects. Experiments with 10nM BAYK8644 resulted in increased twitch contraction and related cytosolic calcium which could be prevented by DHA but not AA (Fig2). DHA or AA alone had no effect. Neither DHA nor AA altered isoproterenol (1, 0.5, 0.1μM) induced increases in I_{Ca} or twitch amplitude. That DHA abolishes nitrendipine or BAYK8644 effects but has no effect alone, suggests that it binds to Ca²⁺ channels near dihydropyridine binding sites and interferes with I_{Ca} modulation by dihydropyridines. While DHA has no effect on inward rectifier current (I_h) and delayed rectifier current (I_k) it accelerates the inactivation of transient outward K⁺ current (I_w) and decreases its magnitude. These results suggest that selective blockade of I_w and also binding to the dihydropyridine receptor in heart cells by omega-3 PUFA may protect agonist Ca²⁺ overload and ischemia induced arrhythmias. This action may be involved in the antiarrhythmic effects of fish oil diet in vivo both in animal models and in humans with coronary artery disease.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00811-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Coronary Artery Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.C. Capogrossi Medical Officer LCS, NIA
 T. GLoe Visiting Fellow LCS, NIA
 K. Inyaku Visiting Fellow LCS, NIA
 Others: J. Mülhhauser, L. Cheng, C. Cirielli, G.M. Jenkins, A. Passaniti, R. Pili

COOPERATING UNITS (if any)

The New York Hospital Cornell Medical Center, New York (R.G. Crystal); Surgical Neurology Branch, NINDS (J. Merrill); Laboratory of Animal Surgery, NHLBI (M. Jones); Laboratory of Histology, IDI, Rome, Italy (T. Faraggiana)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section, Gene Therapy Unit

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

4.0

OTHER:

.0

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies are aimed at evaluating whether gene therapy with replication-deficient, recombinant adenovirus vectors can be used to prevent and treat restenosis after angioplasty and to induce angiogenesis to restore blood supply to ischemic tissues. For the studies on angiogenesis, we have constructed adenoviral vectors which carry the cDNA for the following angiogenic growth factors: (1) Vascular endothelial growth factor (VEGF), (2) Acidic fibroblast growth factor (aFGF), (3) A recombinant form of aFGF which modified by the addition of the secretory signal sequence from FGF-4 (sp-aFGF), (4) Basic FGF (bFGF), (5) A recombinant form of bFGF which was modified by the addition of the secretory signal sequence form FGF-4, and (6) platelet-derived endothelial cell growth factor (PD-ECGF). For the studies on restenosis after angioplasty, we plan to use adenoviral vectors which carry the cDNA for the following proteins: 1) VEGF and PD-ECGF. These viral vectors may enhance reendothelialization at the site of endovascular injury and decrease the severity of intimal hyperplasia by this mechanism. (2) p53, apoptosis. The following studies have been implemented as part of this study. Studies in vitro have shown that the above adenovirus vectors make functional proteins and modulate cell growth. In experiments in vivo we have shown that the adenovirus vectors which carry the cDNA either for aFGF for the secreted form of aFGF induce angiogenesis in vivo when coinjected with Matrigel, subcutaneously in mice. Since persistent expression of growth factors may have a tumorigenic potential we have examined the biosafety of the adenovirus vectors which carry the cDNA for acidic FGF and for the secreted form of acidic FGF. Our in vivo studies with nude mice show that these vectors do not cause tumor formation. We examined the safety and efficacy of gene transfer into minipig heart either with the intramyocardial (IM) or intracoronary (IC) injection of adCMV.NLS β -gal. IM injection was more effective than IC infusion in targeting cell transduction to a well-defined area of myocardium. Following IM injection exogenous gene expression peaked at 2-4 days and returned to control value within one month. No minipigs died and by epicardial echocardiography there was no evidence of either segmental or global left ventricular dysfunction. Thus, Ad vectors appear safe and effective for gene transfer into the myocardium of large mammals. 5) Experiments on restenosis have been aimed at developing a rat model of intimal hyperplasia after carotid artery injury and minipig model of coronary intimal hyperplasia.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00812-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Free Radicals on Endothelial Cell Ca^{2+} Homeostasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Corda	Visiting Fellow	LCS, NIA
Others:	M. C. Capogrossi	Medical Officer	LCS, NIA
	R. C. Ziegelstein	Guest Researcher	LCS, NIA
	H. A. Spurgeon	Physiologist	LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.8

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been combined with Z01 AG 00272-04 LCS.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00813-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on or > line between the borders.)

Molecular Strategies to Treat Restenosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Cheng Research Chemist LCS, NIA
 M. Jenkins Clinical Associate LCS, NIA

Others: S. Sollott, J. Kinsella, J. Froehlich, C. Bilato, M. Crow, M. Capogrossi, Z Li, C. Nater, P. Heller, E. Lakatta, W.S. Ryu

COOPERATING UNITS (if any)

Department of Biomedical Engineering, Johns Hopkins School of Medicine (Dr. Kam Leong)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although the process of restenosis after percutaneous transluminal coronary angioplasty (PTCA) is complex and not fully understood, vascular smooth muscle cell (VSMC) migration and proliferation are critical underlying events. Recent studies within our lab in rat cultured VSMCs have shown that one important mechanism underlying restenosis involves the expression and activation of matrix metalloproteinases (MMPs). Data from this project shows that in vivo, the production of MMP-2 increases following vascular injury and is preferentially distributed in the newly forming neointimal cells of the restenotic lesion. The functional significance of the MMPs is presently being assessed in an ongoing trial in the pig model in which animals are being treated with a MMP peptide inhibitor following balloon injury of the coronary arteries. Following vascular injury a number of growth factors and cytokines stimulate VSMC migration and proliferation. Studies within our group have focused on understanding signal transduction pathways mediated by these factors involving inositol phospholipid-specific phospholipase C (PLC). Preliminary results suggest the PLC- γ 1 and PLC- δ 1 but not PLC- β 1 are amplified following vascular injury implying that these isoforms of PLC may play a important role in neointima formation following arterial injury. Effective strategies to inhibit PLC activation are being investigated. The application of effective therapeutic strategies to the treatment of restenosis in animals and humans is heavily dependent on the development of intravascular site-specific delivery mechanisms. In collaboration with the gene therapy unit of our laboratory we are using the replication-deficient recombinant adenovirus as a delivery vehicle to assess the functional significance of specific genes on restenosis in the rat and pig. In addition, we have developed sustained-release biodegradable microcapsules via which therapeutic agents can be delivered to the vessel wall. Recently, we have demonstrated that Taxol, a potent anti-cancer drug, administered by this mitral inhibits VSMC migration and proliferation. Additional studies are underway in the pig restenosis model using Taxol impregnated within microcapsules delivered intravascularly. The challenge and hope of our studies are that these therapies developed within the constraints of available animal models will translate into patient benefit in clinical trials of restenosis after PTCA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00814-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Aging and the Hormonal Requirements for Vascular Injury

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Jenkins	Clinical Associate	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
Others:	J. P. Froehlich	Chief, MB	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	C. Nater	Biological Science Lab Tech	LCS, NIA
	D. Tweedy	Biological Science Lab Tech	LCS, NIA
	E. G. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Percutaneous transluminal coronary angioplasty (PTCA) has become an attractive alternative for treating coronary artery disease (CAD) in the elderly because it is associated with less morbidity and mortality than bypass surgery. However it is not known if restenosis following PTCA is more prevalent in the elderly. Therefore, elucidating the effect of advanced age on restenosis will provide important information in advising older individuals on the long term sequelae of PTCA. In order to investigate this issue we used the rat carotid artery injury model of restenosis (i.e., the most extensively studied model of restenosis). In contrast to studies using other model systems, we found that intimal lesion formation following balloon injury was greater in young animals at early time points, and at later time points, there were no age differences in neointimal regrowth.

Hormonal factors have also been shown to play an important role in restenosis. Factors dependent on the pituitary gland are involved in VSMC proliferation and migration after arterial injury as injury-induced neointimal formation is inhibited in hypophysectomized rats. The exact mechanism(s) or factor(s) dependent on the pituitary gland is not known. In an attempt to further clarify this issue and pinpoint specific hormonal factors necessary for intimal thickening, we made rats specifically hypothyroid by dietary manipulation or surgical removal of the thyroid gland. Rats fed thyroid suppressive diets (thiouracil-containing) exhibit a markedly diminished response to neointimal formation 14 days following balloon angioplasty. A similar decrease in neointima formation following balloon injury was also observed in animals following thyroidectomy. This inhibitory effect of hypothyroidism was maintained in animals who were also made hypercholesterolemic. These observations suggest that the anti-proliferative effect of hypophysectomy on neointima formation is in part specifically mediated by a deficiency in thyroid hormone.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00815-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Signal Transduction Pathways Involved in Vascular Smooth Muscle Cell Migration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Crow	Senior Staff Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	R. Pauly	Senior Staff Fellow	LCS, NIA
	L. Smith	Associate	LCS, NIA
	C. Bilato	Visiting Fellow	LCS, NIA
	R. Monticone	Biologist	LCS, NIA
	Y. Gluzband	Chemist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. We have previously shown that VSMC migration in response to PDGF is suppressed in differentiated VSMCs and have sought to identify differences in intracellular signalling between differentiated and proliferating VSMCs that may account for this suppression. Differentiated VSMCs retain their ability to respond to PDGF and upregulate expression of the immediate early response genes, c-fos and MCP-1 (JE) when stimulated by PDGF. Unlike proliferating cells, however, PDGF-stimulated differentiated VSMCs fail to activate calcium/calmodulin-dependent protein kinase (CamKinase) II activity. Blocking CamKinase II activation blocked the migration of proliferating VSMCs by more than 90%. In contrast, inhibitors of protein kinase C had no significant effect on migration. Pretreatment of differentiated cells with ionomycin (1 uM) resulted in an 84 + 6% return to the migration rate of proliferating VSMCs. This return was also blocked by CamKinase inhibitors and was unaffected by inhibitors of PKC. These results suggest that activation of CamKinase plays an important role in VSMC migration and the failure to activate it in differentiated VSMCs may be responsible for the suppression of migration. In addition to PDGF, migration also requires the autocrine production of the growth factor, basic FGF. bFGF blocking antibodies inhibit the PDGF-directed migration of proliferating cells, while the addition of exogenous bFGF enables growth-arrested cells to migrate in response to PDGF. bFGF's effect on migration is likely due its role in enabling PDGF to activate CamKII since 1) bFGF antibodies block activation of CamKII in response to PDGF, while the migration of VSMCs expressing constitutively active CamKII is not affected by these antibodies; and 2) the ability of exogenous bFGF to stimulate migration in growth-arrested cells is blocked by CamKII inhibition. These results demonstrate that, while multiple intracellular signalling pathways triggered by chemoattractant recognition may be required for migration, the regulation VSMCs is controlled by CamKII activation. In addition, these results also demonstrate that to activate VSMC migration requires concurrent action by at least two growth factors/cytokines. This requirement may limit the response of VSMCs to injury to selected group capable of responding to agents and provides a molecular basis for investigating the different abilities of various VSMC populations to migrate.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00816-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Nuclear Transcription and Skeletal and Cardiac Muscle Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Crow Senior Staff Fellow LCS, NIA

Others: E. Lakatta Chief LCS, NIA
 M. Boluyt NRC Fellow LCS, NIA
 X. Long Visiting Fellow LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A significant component of musculoskeletal frailty in aging animals is diminished contractile capacity. The relatively high level of protein accumulation required for the assembly of the contractile apparatus requires specialized systems to selectively stabilize the proteins and mRNA encoding them and a high level of sustained transcriptional activity driven by muscle-specific and ubiquitous nuclear transcription factors. These studies examine three such factors that are present in relatively high levels in both cardiac and skeletal muscle and that are known to drive expression of a number of muscle-specific genes. These are 1) the serum response factor (SRF), 2) the tinman homeobox analog, Csx 3) and the thyroid hormone receptors. The first two factors are involved in the transcription of a number of muscle-specific genes, including sarcomeric actins, the myosin light chains, myoglobin, and the muscle-specific isoforms of creatine kinase. Reagents are being developed to measure their levels of expression in tissues from young and aging animals. The thyroid receptors also act as nuclear factors involved in tissue-specific expression of specific contractile genes in connection with retinoic acid receptors. Their importance to aging lies in the fact that, in terms of gene expression, aging resembles a hypothyroid state. Activation of the genes, such as the b-myosin heavy chain, that are associated with hypothyroidism may have important functional consequences. Since numerous have failed to provide a consensus on whether thyroid hormone levels actually change with age, we have focussed on possible changes in the level of expression or the types of receptors expressed during aging. Our preliminary indication are that there are no changes in thyroid receptor subtypes with aging that could account for the changes seen in gene expression. Since transcriptional activation by thyroid receptors requires a heterodimer complex with retinoic acid receptors, we are currently examining the changes that occur in retinoic acid receptor gene expression with age. A precise understanding of the molecular mechanisms by which cardiac and skeletal muscle-specific gene expression is initiated and sustained provides the background for understanding age-associated changes in gene transcription that may contribute to musculoskeletal frailty in the aging.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 AG 00817-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Smooth Muscle Cell Function and Structure Age-Associated Hypertension

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. P. Froehlich	Chief, MBS	LCS, NIA
	Y. Miyashita	Visiting Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA
	J. L. Kinsella	Research Physiologist	LCS, NIA
	S. J. Sollott	Senior Staff Fellow	LCS, NIA
	L. Cheng	Research Chemist	LCS, NIA
	A. Bagrov	Visiting Associate	LBS, NIA
	Z. Li	Visiting Fellow	LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Behavioral Sciences

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Older individuals demonstrate an increased incidence of hypertension as well as a diminished response to β -adrenergic receptor agonists which mediate vascular smooth muscle relaxation and systemic arterial compliance (vascular stiffness). The etiology of these conditions may involve common factors which affect intracellular ion metabolism and structural components of the vasculature. We have extended our investigations of vascular smooth muscle cell function in a rat model demonstrating age-related systolic hypertension. Freshly-isolated arterial smooth muscle cells (SMC) treated with isoproterenol (ISO) were able to redistribute Ca^{2+} from the sarcoplasmic reticulum (SR) to the extracellular space by a mechanism involving activation of the sarcolemmal Na^+/K^+ pump and Na^+/Ca^{2+} exchanger. Old SMC had a significantly higher SR Ca^{2+} content compared to young cells following ISO exposure, whereas baseline SR and cytoplasmic Ca^{2+} levels were unchanged. Aortic tissue slices from old animals showed reduced $86Rb^+$ uptake in the presence of ISO, indicating a diminished capacity for β -activation of the Na^+/K^+ pump. Western blots demonstrated reduced levels of the $\alpha 1$ isoform of Na^+/K^+ -ATPase in rat tail artery homogenates. These results link the age-related increase in SR Ca^{2+} to a reduced level of stimulation of the sarcolemmal Na^+/K^+ pump by β -agonists. In the presence of vasoconstrictors, the increased SR Ca^{2+} stores following β -stimulation may contribute to diminished smooth muscle relaxation, increased arterial stiffness and systolic hypertension in older animals.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00818-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Signal Transduction of Cardiac Opioid Receptor Stimulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Pepe	Visiting Fellow	LCS, NIA
	R-P. Xiao	Senior Staff Fellow	LCS, NIA
Others:	E. G. Lakatta	Chief	LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cardiovascular Science, Gerontology Research Center

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has been found that cardiac myocytes produce and secrete opioids and have opioid peptide receptors (OPR). We have previously shown that the δ OPR agonist, leucine-enkephalin (LE), specifically reduces twitch, cytosolic Ca^{2+} transient (Ca_i) and L-type Ca^{2+} channel current amplitude in adult rat ventricular myocytes. As it has been shown that opioid peptides are coreleased with catecholamines from autonomic nerve endings within the heart, we propose that OPR stimulation interacts with β -adrenergic receptor (β -AR) stimulation in a negative feedback role. Although it has been reported that OPR stimulation effects involve increased phospholipase C activity and thus IP₃ formation, OPR stimulation also may decrease cardiac cAMP levels. In contrast, β -AR stimulation by norepinephrine (NE) elevates basal cAMP levels. In an isolated heart preparation peak developed pressure was increased to 217% of control by NE ($10^{-6}M$), addition of LE ($10^{-6}M$) resulted in a marked reduction in developed pressure to 66% of control within 15-25min. Further addition of the OPR antagonist naloxone ($10^{-6}M$) to the LE+NE buffer rapidly reversed the LE effect (<1-2min) to 188% of control systolic pressure. LE alone at $10^{-6}M$ had no significant effect on developed pressure but was highly potent following NE. A non-hydrolyzable analog of cAMP, CPTcAMP, at $3 \times 10^{-5}M$ increased peak developed pressure to 176% of control but LE ($10^{-6}M$) +CPTcAMP had no significant effect on peak pressure. Additional experiments were performed in single rat ventricular myocytes where cytosolic Ca^{2+} transient and contraction amplitude were measured during an identical series of experiments. Results from these experiments support those obtained with the isolated heart preparation. These results indicate that there is a distinct interaction of OPR and β -AR stimulation at the postsynaptic level which may alter receptor coupling to adenylate cyclase or inactivate adenylate cyclase itself. Further elucidation of this opioid signal transduction mechanism may shed light on an important negative feedback control β -adrenergic cardiac effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00819-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ca²⁺/Calmodulin-Dependent Protein Kinase II Actions in Heart

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: R-P. Xiao Senior Staff Fellow LCS, NIA

Others: Z. Li Visiting Fellow LCS, NIA

E.G. Lakatta Chief LCS, NIA

COOPERATING UNITS (if any)

Department of Physiology, University of Maryland School of Medicine, Baltimore (H. Cheng, J. Lederer); Department of Biochemistry, Nagoya City University School of Medicine, Nagoya, Japan (T. Suzuki)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ca²⁺ entry through voltage-dependent Ca²⁺ channels is important in cardiac and vascular muscle excitation-contraction coupling. The Ca²⁺ regulated multifunctional protein kinase, Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), has a very important role in signal transduction in nervous system, such as long-term potentiation (LTP) and memory. However, little is known as to whether this protein kinase also modulates the function of cardiac cells. Our studies demonstrate both spatially resolved and temporally distinct novel effects of CaMKII on L-type Ca²⁺ channel current (I_{Ca}) in cardiac cells. Either depolarization alone or calcium influx can increase the amplitude and slow the inactivation of I_{Ca}. The distinct voltage- and Ca²⁺-dependent effects persist with time constants of about 1.7 s and 9 s, respectively. Both effects are completely abolished by a specific peptide inhibitor of CaMKII. This CaMKII inhibitor also suppresses the prolongation of I_{Ca} induced by depolarizing holding potentials. An antibody specific for the autophosphorylated (activated) CaMKII, PY-66, is localized close to sarcolemmal membranes and the profile of CaMKII activation is qualitatively correlated with the changes in I_{Ca} under various conditions. Thus, the action of CaMKII on I_{Ca} is dually regulated by membrane depolarization and by calcium influx: the latter directly activates CaMKII while the former likely promotes the interaction between constitutive CaMKII and the membrane channel proteins. In contrast to the active CaMKII distribution, the intracellular distribution of the total CaMKII enzyme (visualized by using an antibody which specifically reacts with CaMKII δ isoform) but does not sense its activation state is uniform with a higher nuclear distribution. This suggests that CaMKII is translocated to the cell sarcolemma following activation in cardiac myocytes. These findings provide new insights toward understanding the physiological function of the ubiquitous protein kinase, CaMKII in cardiac muscle cells as possibly in other type of cells as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 AG 00820-02 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Novel Effects of Endothelial Cell Derived Substance on the Cardiac Contraction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Lakatta	Chief	LCS, NIA
Others:	A. Mebazza	Guest Researcher	LCS, NIA
	A. Shah	Guest Researcher	LCS, NIA
	J. Sellers		NHLBI
	J. Cuda		NHLBI

COOPERATING UNITS (if any)

Pulmonary Anesthesiology Laboratory, Johns Hopkins Hospital (R.C. Wetzel), Johns Hopkins University (J.L. Robotham), NHLBI Laboratory of Molecular Cardiology (J. Sellers, J. Cudo)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To search for novel paracrine modulators of heart cell function. We studied the effects of effluent of superfused of normoxic and hypoxic endocardial and vascular endothelial cultured cells on contraction and intracellular calcium transients of isolated adult rat cardiac myocytes. We found that EEC synthesize and release endothelin, which accounts for a positive inotropic effect of sheep EEC effluent. Additionally, both endocardial and vascular endothelial cells tonically release a novel substance which rapidly and reversibly decreases the amplitude of myocytes twitch contraction by inducing earlier relaxation, and also increase diastolic cell length. These effects are not associated with any change in the intracellular calcium transient, indicating cardiac myofilament "desensitization". The activity of endothelial cell effluent remained stable at 37°C for several hours or at 4°C for at least 48 hours. The action of this substance did not involve nitric oxide, cyclic GMP or prostanoids, nor changes in intracellular pH. These properties suggest that endothelial cells may rapidly modulate cardiac contraction relaxation coupling and diastolic tonus by altering myofilament properties, as well as exert distant effects because of the unusual stability of this substance. Superfusates of hypoxic endocardial and vascular endothelial cells induced rapid, reversible reduction in myocyte twitch amplitude ($-67.0 \pm 4.9\%$; mean \pm S.E.M.), and decreased diastolic length ($-1.5 \pm 0.3 \mu\text{m}$; both $P < 0.001$; $n=18$). Ca^{2+} transients were however unchanged indicating altered myofilament properties. This effect was not attributable to known endothelial agents, nor to changes in pH in SNARF-loaded myocytes. Superfusate of hypoxic cells (to 1/20 dilution), but not normoxic cells, completely and reversibly inhibited the in vitro sliding of F actin over rat cardiac myosin, and dramatically reduced actin-activated myosin S1 ATPase activity. Neither effect was observed with smooth muscle myosin. These data suggest a novel endothelial cell-mediated feed forward mechanism which senses hypoxia and depresses myocyte contraction, by releasing factor(s) that directly inhibit crossbridge cycling. This may explain the clinical phenomenon of myocardial hibernation, i.e., depressed contraction of viable myocardium during reduced coronary flow.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00821-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chronic Regulation of Mitochondrial Content in Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Moyes	Visiting Fellow (EOD 11/93)	LCS, NIA
	R. G. Hansford	Chief, EMBS	LCS, NIA
Others:	B. Hogue	Chemist	LCS, NIA

COOPERATING UNITS (if any)

Laboratory of Biological Chemistry (Dr. C.F. Filburn)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Energy Metabolism and Bioenergetics Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS.

1.0

PROFESSIONAL

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines the chronic regulation of mitochondrial enzyme activity in muscle, with a view to understanding the decreased capacity of skeletal muscle to respond to endurance training in old age. Our immediate goal is to identify signals which allow the cell to sense increased energy demand in the form of an increased flux through ATP and respond with a coordinated expression of mitochondrial proteins encoded by the nuclear and mitochondrial genomes. This year we have studied the expression of mitochondrial enzymes in an immortal cell line derived from mouse skeletal muscle (C2 cells) as the cells proliferate in media supplemented with 20% fetal calf serum, and differentiate into myocytes and myotubes in response to serum starvation. Further, we have used uncoupling agents, Ca²⁺-ionophores and inhibitors of respiratory chain activity to lower cytosol phosphate potential (ATP/ADP×Pi) in an attempt to stimulate mitochondrial proliferation. During proliferation, the metabolism of these cells resembles that of tumor cells, with 60% of cell ATP needs met by glycolysis. Those mitochondria which are present operate at about 50% of V_{max} capacity. Pyruvate dehydrogenase was found to be only 43% in the active, dephospho form (PDH_a) - indicative of low energy demands. During differentiation the cells become markedly more oxidative. Mitochondrial enzymes (pyruvate dehydrogenase, citrate synthase, NAD-isocitrate dehydrogenase, β-OH acyl CoA dehydrogenase, cytochrome c oxidase) increase 4-fold in activity, and the fraction of ATP flux due to oxidative phosphorylation increases markedly. In contrast, cytosol enzymes (pyruvate kinase, NADP-isocitrate dehydrogenase) remain constant in activity. The fraction of pyruvate dehydrogenase present as PDH_a increases to near 100% after 3 days serum starvation, such that maximum flux through this enzyme is increased 10 fold with differentiation. Several pharmacological interventions are being explored as means to replicate these changes in proliferating cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00822-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Advanced Glycation Endproducts, Their Receptors, and Vascular Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Crow Senior Staff Fellow LCS, NIA

Others: R.R. Pauly Senior Staff Fellow LCS, NIA
 J. Fredman IRTA LCS, NIA
 R. Monticone Biologist LCS, NIA
 L. Cheng Research Chemist LCS, NIA
 G.M. Jenkins Clinical Associate (MSF) LCS, NIA
 E.G. Lakatta Chief LCS, NIA

COOPERATING UNITS (if any)

College of Physicians and Surgeons, Columbia University (D. Stern; A.M. Schmidt)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.7

OTHER:

.3

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The blood vessels of aging animals are characterized by a number of histological changes, including an increase in the number of vascular smooth muscle cells (VSMCs) and monocytes in the intima. These changes are likely to contribute to the increased occurrence and severity of vascular disease that is associated with aging. Advanced glycation endproducts of proteins (AGE) accumulate in the plasma and in tissues with age and at an accelerated rate in diabetes. These products have been shown to stimulate the secretion of chemoattractants and expression of cell adhesion molecules by circulating leukocytes and, in the case of diabetes, have been linked to the development of vascular complications. A receptor for AGE (RAGE) has been identified and cloned. Using an antibody for this receptor, we have shown that RAGE is expressed in the vessel wall by the endothelium and by intimal vascular smooth muscle cells, indicating that they are likely targets of AGEs. Incubation of quiescent VSMCs with AGE-albumin induces the expression of monocyte chemoattractant protein-1 (MCP-1) mRNA over 10-fold, whereas native albumin was without effect. These changes in MCP-1 mRNA expression were inhibited by pre-incubation with the RAGE antibody, which blocks ligand binding, and with the antioxidant, probucol. VSMCs exposed to AGE-albumin also secreted a chemoattractant into conditioned media which stimulated VSMC migration 5-fold (p, 0.01). This activity was blocked by anti-PDGF IgG (78%, p, 0.05), but not by nonimmune IgG. RAGE expression was dramatically upregulated following vessel injury with the increase in expression confined to the developing neointima. This difference in RAGE expression between medial and neointimal cells persisted in culture and is likely responsible for the exaggerated AGE-responsiveness of neointimal cells. These studies demonstrate that VSMCs are targets for AGE and that the consequences of this interaction could result in increased VSMC migration and monocyte infiltration, an early event in atherogenesis. Furthermore, the upregulation of RAGE expression in young, non-diabetic animals in which AGEs are not detectable suggests that receptor activation may also occur through additional unknown ligands and that activation of RAGE may play a role in all forms of vascular injury and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00823-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vascular Smooth Muscle Cell Differentiation Alters PDGF Ca²⁺-Signalling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. J. Sollott	Senior Staff Fellow	LCS, NIA
Others:	D. Rozanski	IRTA Fellow	LCS, NIA
	L. Cheng	Research Chemist, MBS	LCS, NIA
	R. Pauly	Senior Staff Fellow	LCS, NIA
	C. Bilato	Guest Researcher	LCS, NIA
	M. Crow	Senior Staff Fellow	LCS, NIA
	E. G. Lakatta	Chief, LCS	LCS, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Cardiac Function Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOXES)

- (a) Human (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have recently demonstrated that calcium/calmodulin-dependent protein kinase II (CaMK II) activation is required for PDGF-directed Boyden chamber chemotaxis (measured at 4 hrs) by proliferating vascular smooth muscle cells (VSMC) *in vitro*, and that VSMCs induced to differentiate after growth arrest fail to migrate largely due to attenuated CaMK II activation. Since chemotaxis can be restored in growth-arrested VSMCs by Ca²⁺-ionophores, we tested the hypothesis that Ca²⁺-signalling regulates chemotaxis, and that PDGF-stimulated Ca²⁺-signalling differs markedly between these two phenotypes in a cultured VSMC model from rat aorta. PDGF-BB (10 ng/ml) causes a large increase in intracellular Ca²⁺ (Ca_i) in proliferating-VSMCs owing to intracellular release, followed by a tonic elevation, which is accompanied by parallel changes in CaMK II activation via its phosphorylation. In contrast, this Ca_i response and CaMK II activation pattern are both markedly attenuated in growth-arrested VSMCs. Ionomycin (1 μM), which restores migration in growth-arrested VSMCs, induces both a biphasic increase in Ca_i and an immediate activation of CaMK II qualitatively similar to the PDGF response of proliferating-VSMCs. Thus, altered PDGF Ca²⁺-signalling, which accompanies changes in VSMC phenotype, likely regulates both CaMK II activation and chemotaxis in response to PDGF. In addition to differences in chemotaxis between these two differentiation phenotypes, significant heterogeneity in the rate of chemotaxis is also evident among proliferating VSMCs (i.e., only 5-10% chemotax at 4 hrs). Preliminary experiments in a novel microscope-adapted Boyden chamber, enabling simultaneous measurement of VSMC migration and Ca_i in individual cells, suggests that heterogeneous Ca_i activation patterns (i.e., in the magnitude/rate of Ca_i change) could underlie some cellular differences in chemotaxis among proliferating VSMCs. However, that "equalization" of Ca_i-responses with ionomycin in these proliferating VSMCs fails to recruit significant additional chemotaxis (at least at the 4 hr assay time-point) suggests that an additional factor, such as the initial inability to produce and/or utilize activated CaMK II by a large subpopulation (at least 90% of cells), may also be a critical factor in the apparent heterogeneity of chemotaxis. Studies are in progress to measure the coordination of Ca_i and CaMK II activations during chemotaxis in individual cells to resolve these fundamental issues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00824-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Perturbations Affecting Smooth Muscle Cell Function and Blood Pressure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. P. Froehlich	Chief, MBS	LCS, NIA
	Y. Miyashita	Visiting Fellow	LCS, NIA
	S. Pepe	Visiting Fellow	LCS, NIA
Others:	E.G. Lakatta	Chief, LCS	LCS, NIA
	S. J. Sollott	Senior Staff Fellow	LCS, NIA
	J. Kinsella	Research Physiologist	LCS, NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cardiovascular Science, Gerontology Research Center

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.4

OTHER:

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Smooth muscle contractile activity is an important regulator of vascular tone and blood pressure. We are probing the relationship between ion metabolism at the single cell level and blood pressure regulation in vivo using interventions which alter mean systolic blood pressure. 30 mo. old Fisher 344XNB rats showed a significant elevation in systolic blood pressure (155.3+1.64 mm) compared to their 6 mo. old counterparts (131.3+5.96 mm; p<.001). Other studies by us have shown that smooth muscle relaxation induced by β -agonists involves a depletion of SR Ca^{2+} stores which are essential for contraction. Addition of the β -adrenergic receptor (β) agonist isoproterenol (ISO) to freshly-isolated rat arterial smooth muscle cells (SMC) reduced SR Ca^{2+} content to a greater extent in young cells (30.74+7.76% of the pre-ISO level) than in old cells (37.6+2.36%; p<.05). Fish oil diets containing polyunsaturated fatty acids have a blood pressure lowering effect. Dietary supplementation with saturated fatty acids, which increased mean systolic blood pressure in 8 and 12 mo. old rats (p<.05), increased SR Ca^{2+} levels following exposure to ISO. In contrast, supplementation with fish oil, which reduced blood pressure significantly in 12 and 24 mo. old rats (p<.01), decreased SR Ca^{2+} following β -stimulation. Rats given 25% D2O in their drinking water showed an average decline in systolic blood pressure of 35 mm compared to control animals without D2O. Single SMC prepared from D2O-treated rats displayed a lower SR Ca^{2+} content compared to control cells after exposure to ISO in H2O buffer. Thus, interventions that raise blood pressure also raise SR Ca^{2+} while agents lowering blood pressure reduce SR Ca^{2+} . This qualitative correlation is compatible with the view that higher residual SR Ca^{2+} levels following β -stimulation lead to enhanced smooth muscle contractile activity in the presence of vasoconstrictors, thereby contributing to an elevation in blood pressure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00825-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Atherosclerotic Lesion Formation and Progression in the Human Aorta

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Z. Li Visiting Fellow, LCS, NIA
 R-P. Xiao Senior Staff Fellow, LCS, NIA

Others: E.G. Lakatta, R. Pauly, B. Monticone, L. Cheng, M. Crow, W. Steller-stevenson

COOPERATING UNITS (if any)

The Office of Chief Medical Examiner, State of Maryland, University of Maryland; National Cancer Institute, NIH (J.E. Smialek, L. Li)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies in this laboratory and others indicated that 72 kd type IV collagenase (MMP-2) plays a role in migration and proliferation of the arterial SMC of animal. To determine whether this enzyme might be involved in human atherosclerosis, human aortic tissue was collected from autopsies of non-cardiovascular death patients. The MMP-2 was visualized in frozen sections of the aortic wall by an immunofluorescent staining technique with antibody prepared against MMP-2. The quantity of this enzyme was also analyzed by the Western blot technique. Results revealed a greater amounts of MMP-2 in fatty streaks and atherosclerotic lesions than found in normal regions of the aorta. Data suggest that 72 kd type IV collagenase may have a role in promoting early atherosclerotic lesion formation, i.e. from fatty streak to plaque. In addition, the increase in expression of the enzyme in the plaque may be involved in complications of atherosclerosis, such as rupture and ulceration. Since bFGF is a major growth factor involved in SMC proliferation after endothelial injury in animal models, cell biological response of human SMC to this growth factor were undertaken. Immunofluorescent staining was used to investigate bFGF nuclear translocation in normal growing and confluent SMC. Also, the expression of Ca²⁺/calmodulin-dependent protein kinase II (CaM-KII) on the activity of which is necessary for migration in rat SMC and its intracellular distribution in the human SMC were studied following bFGF stimulation. Preliminary results indicate that growing SMC have a quicker response to bFGF, demonstrated as a rapid translocation of bFGF into the nucleus, than do confluent growth arrested cells. Furthermore, bFGF promotes a longer expression of activated nuclear CaM-KII in the growing SMC as compared to confluent SMC. These results provide evidence of specific responses to bFGF stimulation in growing and confluent SMC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00826-01 LCS

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age-dependent Cytoskeletal Alterations in Cultured Smooth Muscle Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Z. Li Visiting Fellow LCS, NIA
 J. Froehlich Chief, MBS LCS, NIA

Others: Y. Miyashita, M. Crow, E.G. Lakatta

COOPERATING UNITS (if any)

Department of Physiology, University of Maryland (H. Cheng, W.J. Lederer)

LAB/BRANCH

Gerontology Research Center, Laboratory of Cardiovascular Science

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.9

OTHER:

0.1

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cytoskeletal fibers are responsible for the maintenance of cell shape, size and phenotype which can significantly affect most cellular functions, especially migration and proliferation. Phenotypic changes in SMC are closely related to pathological behavior in these cells in vascular diseases, such as atherosclerosis and restenosis. Although changes in cytoskeletal proteins have been seen during differentiation of the arterial SMC, how these change with aging and their relationship to arterial disease is not fully understood. To study the cytoskeletal changes with aging, the SMC were cultured from thoracic aortas of young (6 mo.) and old (30 mo.) Fisher 344XNB rats. Cytoskeletal proteins were highlighted in early passage cells (P2) by immunofluorescence techniques with antibodies prepared against muscle myosin, α -smooth muscle actin, vimentin, desmin and tubulin. The immunostaining and fluorescence intensities were analyzed by confocal microscopy. Proliferation of SMC from young and old rats was evaluated from their growth curves in culture. Results indicate that in SMC of old rats, there is a decrease in myosin, vimentin and tubulin, but an increase in desmin. The growth curve shows a higher growth rate in the SMC from old rat aortas. The altered amounts of cytoskeletal proteins in SMC from older animals may influence their growth rate characteristics, affecting aortic wall remodelling during aging and in hypertension associated with aging.

CONTRACT

Name and Number: JOHNS HOPKINS UNIVERSITY (N01-AG-02-2118)

Title: Vascular Stiffness, Arterial Pressure, and Cardiac Mass
With Aging in a Genetically Homogeneous Population
that Differs in Lifestyle

Data Contract Initiated: 1990

Current Annual Level: \$240,358

With advancing age, arterial stiffness increases and is accompanied by increased systolic pressure and mild left ventricular hypertrophy. This study addresses how arterial stiffness affects the myocardium. Does, for example, the increased systolic pressure cause the accompanying increase in heart size, or is it a consequence? A previous study of two Chinese populations published in 1985 identified diet and lifestyle dependent changes in vascular stiffness but did not address the cardiac changes. This study investigates two populations in Taiwan. One population is located in an isolated rural area with little population migration, while the second population comprises an urban setting where diet, exercise habits, and stress are much different. Approximately 2230 subjects in the age range 30-70 plus years were screened for this study, including equal numbers of males and female subjects, and stratified by presence/absence of hypertension. Lifestyle, dietary habits, pulsewave velocities and echocardiographic dimensions have been quantified. The genetic homogeneity of these two populations provides a unique opportunity to assess the causal relationship between cardiac mass and vascular stiffness.

Objectives:

This study seeks to assess whether age associated increase in vascular stiffness is the cause of or results from increased cardiac mass. Because diet, exercise habit, and lifestyle are potent modulators of blood pressure change with advancing age, an increased understanding of these important factors in modulating age-associated cardiac hypertrophy and vascular stiffness will result.

Methods Employed:

Subjects from an isolated rural community (Quemoy), and from a geographically isolated urban population (Puli), both located on the island of Taiwan, are selected using age, sex, and freedom from all clinical cardiovascular disease except hypertension as criteria. Subjects are excluded if historical medical records are inadequate. Lifestyle and dietary habits are assessed by questionnaire, and a comprehensive physical exam performed. Populations for pulsewave study are then stratified into equal sized age and sex matched subjects and into either hypertensive or non-hypertensive groups. These subgroups are then all studied by applanation tonometry and 2D echocardiography to determine pulse wave velocity and ventricular dimensions. Vascular stiffness is a calculated parameter based on pulsewave velocity and blood pressure.

Major Findings:

The field phase of the study is complete. Data were all reverified during the fourth quarter of 1993, and the epidemiological analysis slated to begin in the

CONTRACT

Name and Number: JOHNS HOPKINS UNIVERSITY (N01-AG-4-2109)

Title: Non-Invasive Assessment of Cardiac Structure and Function in Aging Men and Women

Date Contract Initiated: October 1, 1989

Current Annual Level: \$268,327

This is year 5 of a 10 year contract in which rest and exercise thallium and gated blood pool cardiac scans have been performed respectively on nearly 900 and 450 participants in the Baltimore Longitudinal Study on Aging (BLSA). These studies have provided unique insights concerning the prevalence and prognostic significance of exercise-induced myocardial blood flow abnormalities (i.e. ischemia) as well as the effect of age, gender, life-style and disease on cardiac structure and function at rest during aerobic exercise. The present contract is in the sixth year of a 9 year renewal, during which cardiac blood pool and thallium scans are being continued in the groups of individuals listed below.

Although the nuclear cardiac studies are performed at Johns Hopkins Hospital, conceptualization of the specific research questions, the selection of study subjects, and the data analyses are the responsibility of the LCS. Drs. Jerome Fleg and Edward Lakatta direct the overall research effort and review the collected data and plan data analyses with statisticians from our laboratory. The exercise studies are performed under the supervision of Dr. Gary Gerstenblith and the scans are read by Dr. Lewis Becker, both from the Johns Hopkins Cardiology Division. This long-standing collaboration continues to bear fruit as evidenced by the list of derived publications shown.

A. Rest and Exercise Gated Cardiac Blood Pool Scans

1. Longitudinal Study of Cardiac Function. In 100 BLSA men and women who have had blood pool scans at least 5 years previously, the test is repeated, with simultaneous measurement of oxygen consumption (VO_2). This will allow insight into longitudinal changes in cardiac function at rest and during exercise. The VO_2 data will provide information regarding central (cardiac) versus peripheral (arteriovenous oxygen difference) mechanisms for maintaining VO_2 with advancing age. Fifty-seven repeat studies have been performed to date.

2. Cardiac Function in Highly Trained Seniors, Sedentary Subjects Pre- and Post-Training, and Obese Individuals Pre- and Post-Weight Loss. These scans, all performed with simultaneous VO_2 measurements, during maximal upright cycle exercise allow determination of the central and peripheral effects of conditioning status and obesity on aerobic exercise performance, and the interrelations of such lifestyle variables with the aging process. These studies are performed in collaboration with investigators from the Academic Teaching Nursing Home (TNH) project of the University of Maryland. Analysis in 16 senior athletes suggests that central (cardiac) and peripheral factors (arteriovenous oxygen difference) account nearly equally for the age-related decline in peak VO_2 . Nine men have undergone serial scans before and after detraining. These men demonstrate reduction of maximal cardiac index, mediated by reduced end-diastolic and stroke volume indices as well as peak filling rate after 12 weeks of detraining.

3. Cardiac Function in Patients with Latent Coronary Artery Disease (CAD). Data from such individuals with concordant abnormal exercise ECG's and exercise thallium scans, suggest that ischemia and advanced age have additive effects on certain cardiac parameters. Extension of these studies allows more accurate characterization of this interaction between age and silent CAD and help to clarify discrepancies in the cardiovascular aging literature which have resulted from the inclusion of such individuals with silent CAD in some studies but not others. Our findings to date indicate that both age and silent (CAD) cause similar but additive blunting of the ejection fraction response and greater increases in end diastolic volume index (EDVI) during exercise.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00724-02 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Specific DNA Repair

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Vilhelm A Bohr	Chief LMG	LMG, NIA
Others:	Alfred May	Microbiologist	LMG, NIA
	David Orren	Staff Fellow	LMG, NIA
	Florence Larminat	Visiting Fellow	LMG, NIA
	Michele Evans	Senior Investigator	LMG, NIA
	Cynthia Haggerty	Biologist	LMG, NIA
	Edward Beecham	IRTA Fellow	LMG, NIA
	Patricia Kruk	Visiting Fellow	LMG, NIA
	Carleen Cullinane	Visiting Fellow	LMG, NIA

COOPERATING UNITS (if any)

Smithville, Texas (R. Nairn); Galveston Texas (S. Wilson); Rotterdam, The Netherlands (J. Hoeijmakers); Leiden, The Netherlands (L. Mullenders)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

5

PROFESSIONAL:

3

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We are studying the molecular biochemistry of gene specific DNA repair and DNA repair coupling with transcription with a view to clarify which gene products are involved and how these processes are regulated as compared to the DNA repair processes in the general, overall bulk of the genome. There are distinct differences in the efficiency of gene- and strand specific DNA repair dependent upon the type of DNA damage, and it is possible that the local degree of chromosomal distortion is the important element in determining the repair response chosen by the cell. We are suggesting that proficient DNA repair is necessary to secure genomic stability, and we find that certain regions of the genome that undergo translocation or rearrangements in the tumor cells are poorly repaired in cells that are susceptible to cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00725-02 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Specific DNA Repair Throughout the Cell Cycle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Vilhelm A. Bohr	Chief LMG	LMG, NIA
Others:	David Orren	Staff Fellow	LMG, NIA
	Lone Petersen	Guest Worker	LMG, NIA
	Nicholas Rampino	Staff Fellow	LMG, NIA

COOPERATING UNITS (if any)

Cancer Ctr., Columbia Univ. (A. Carothers, D. Grunberger); Oncology Dept., Johns Hopkins Univ. (M. Kastan)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

2

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies are designed to understand the interrelationships between DNA metabolism, specifically DNA damage and its repair, and the cell cycle in mammalian cells. We are particularly interested in characterizing the gene- and strand-specific patterns of repair of various adducts in the different phases of the cell cycle; this will allow us to correlate DNA repair processes with transcription, replication, and mutagenesis. Determination of phase-specific repair processes will also shed light on the possible accumulation and distribution of DNA damage in non-cycling (differentiated and senescent) cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00726-02 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Repair in Cancer and Senescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

CO PI:	Michle Evans	Senior Clinical Investigator	LMG, NIA
CO PI:	Vilhelm A. Bohr	Chief	LMG, NIA
Others:	David K. Webb	IRTA Fellow	LMG, NIA
	Patricia A. Kruk	Visiting Fellow	LMG, NIA
	Cynthia M. Haggerty	Biologist	LMG, NIA

COOPERATING UNITS (if any)

National Cancer Institute, NIH (C.C. Harris, K.H. Kraermer, I. Horak, J.H. Robbins), Dept. of Biochemistry, Johns Hopkins University School of Public Health

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

3

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our interest in understanding the complex interrelationships between DNA repair, cancer and senescence has led us to study the role of DNA repair in several human model systems which are pertinent to both cancer and aging. We and others have identified specific DNA repair phenotypes characteristic of a group of related heritable cancer prone and progeroid human syndromes that have heterogenous clinical manifestations. The role of human DNA repair phenotypes in mutation induction, distribution, and in tumor formation has been explored. By studying gene-specific DNA repair and specifically repair of the p53 tumor suppressor gene in cancer prone disorders, we have been able to further characterize the correlations between DNA repair, mutation distribution, and cancer risk. By investigating DNA damage induction and repair in progeroid syndromes such as Werner's syndrome, we can examine a human mutant which has several clinical manifestations concordant with normal human aging and also associated with an increased cancer incidence. Alzheimer's disease also provides a useful model system in which to study the role of DNA repair in a condition associated with senescence. We have measured gene-specific repair in fibroblasts from patients with familial and sporadically occurring Alzheimer's disease.

Telomeric shortening is one of the age-associated genetic instabilities currently believed to be an important biomarker of aging and cancer. We have developed a novel method to measure DNA damage induction and repair in human telomeres and suspect that repair capacity in telomeres may be related to the genomic instability associated with normal human aging and perhaps with tumorigenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00727-02 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Repair of Oxidative DNA Damage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Vilhelm A. Bohr	Chief, LMG	LMG, NIA
Others:	Bonita Taffe	Staff Fellow	LMG, NIA
	Nicholas Rampino	Staff Fellow	LMG, NIA
	Florence Larminat	Visiting Fellow	LMG, NIA
	Alfred May	Microbiologist	LMG, NIA
	R. Michael Anson	Graduate Student	LMG, NIA
	Deborah Croteau	Graduate Student	LMG, NIA

COOPERATING UNITS (if any)

France (F. Laval); Laboratory of Biological Chemistry, NIA, NIH (C. Filburn), Munchen, Germany (S. Paabo)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIA, NIA, Baltimore, MD 21224

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have been developing assays to detect oxidative lesions in specific genes and thus to quantitate their formation and repair. We generate oxidative DNA damage is generated by several different approaches including hydrogen peroxide, X-irradiation, irradiation with methylene blue, and treatment with 4NQO which forms at least one adduct with oxidative characteristics. Our main approach is to treat cells in culture with acridine orange, which after activation with light forms oxidative lesions in DNA. The main lesion is 8-OH guanosine which can be detected by use of the FaPy glycosylase. This enzyme creates strand breaks in DNA at sites of the lesions, and the single stranded DNA can then be resolved on alkaline gels. We find that 8-OH guanosine is rapidly repaired in active genes in hamster and human cells.

While it has been a general notion that there is no DNA repair in **mitochondria**, we now find that these organelles do have repair capacity. They can not, however, repair all lesions. They are capable of repairing DNA lesions created by monofunctional alkylating agents, but not UV induced pyrimidine dimers. We find fast repair of oxidative damage in mitochondrial DNA, and the mechanism is under investigation. One question is whether the repair in mitochondrial DNA is transcription coupled. We are investigating whether the common deletions in mitochondrial DNA seen in senescence and other conditions could be due to a localized deficiency in DNA repair.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00729-01 LMG

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genomic Instability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Vilhelm A. Bohr Chief LMG, NIA

Others: Jiuping Ji Visiting Fellow LMG, NIA
 Patricia Kruk Visiting Fellow LMG, NIA
 Carleen Cullinane Visiting Fellow LMG, NIA
 Mikael Hjertvik Guest Researcher LMG, NIA

COOPERATING UNITS (if any)

Dept. Oncology, Johns Hopkins University (Bert Vogelstein); Molecular Genetics, The Mayo Clinic, Rochester, Minnesota (Steven Thibodeau); Laboratory of Genetics,

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

3.25

PROFESSIONAL:

3

OTHER:

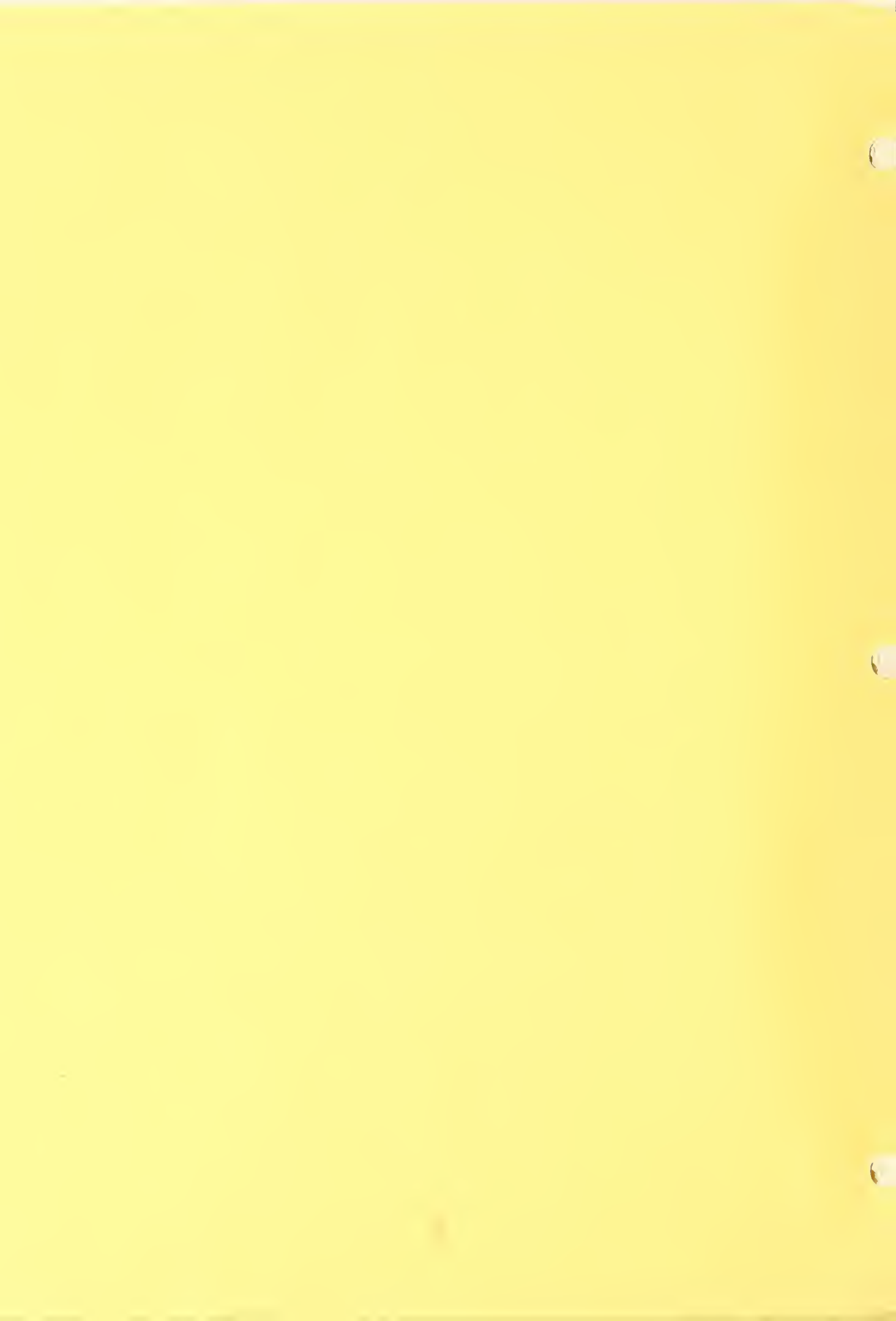
.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genomic instability is a general and characteristic feature of both cancer and aging. We are searching for the underlying mechanisms that are responsible for the development of the genomic instability. Replication errors in DNA, telomeric shortening, increased local DNA damage formation, and localized DNA repair defects, are all possible pathways that can lead to genomic instability. Recently, we have found situations where the genomic instability correlated with a deficiency in DNA repair. We are now examining these pathways in human cells from patients with aging or cancer associated genomic instabilities. A good example is hereditary, non-polyposis colorectal carcinoma (HNPCC), where a mismatch DNA repair defect has been found. We now in preliminary studies find a more generalized DNA repair defect in the pathway that repairs bulky DNA lesions, nucleotide excision repair. This will be further characterized and explored in other HNPCC cell lines. In other studies, we find a distinct shortening of the telomeres in cell lines that have mutations or are deficient in the function of the tumorsuppressor gene, p53, which then leads to genomic instability.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00015-36 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Baltimore Longitudinal Study of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fozard	Assoc. Scientific Director, BLSA	OSD, NIA
Others:	L.J. Brant	Mathematical Statistician	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	B. Hurley	IPA	UM
	C. Morrell	IPA	LC
	J.D. Pearson	Senior Staff Fellow	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA

(Continued next page.)

COOPERATING UNITS (if any)

Johns Hopkins Bayview Medical Center (JHBMC); National Institute of Dental Research (NIDR); University of Maryland, College Park (UM); Loyola College, Baltimore (LC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.7

PROFESSIONAL:

.5

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

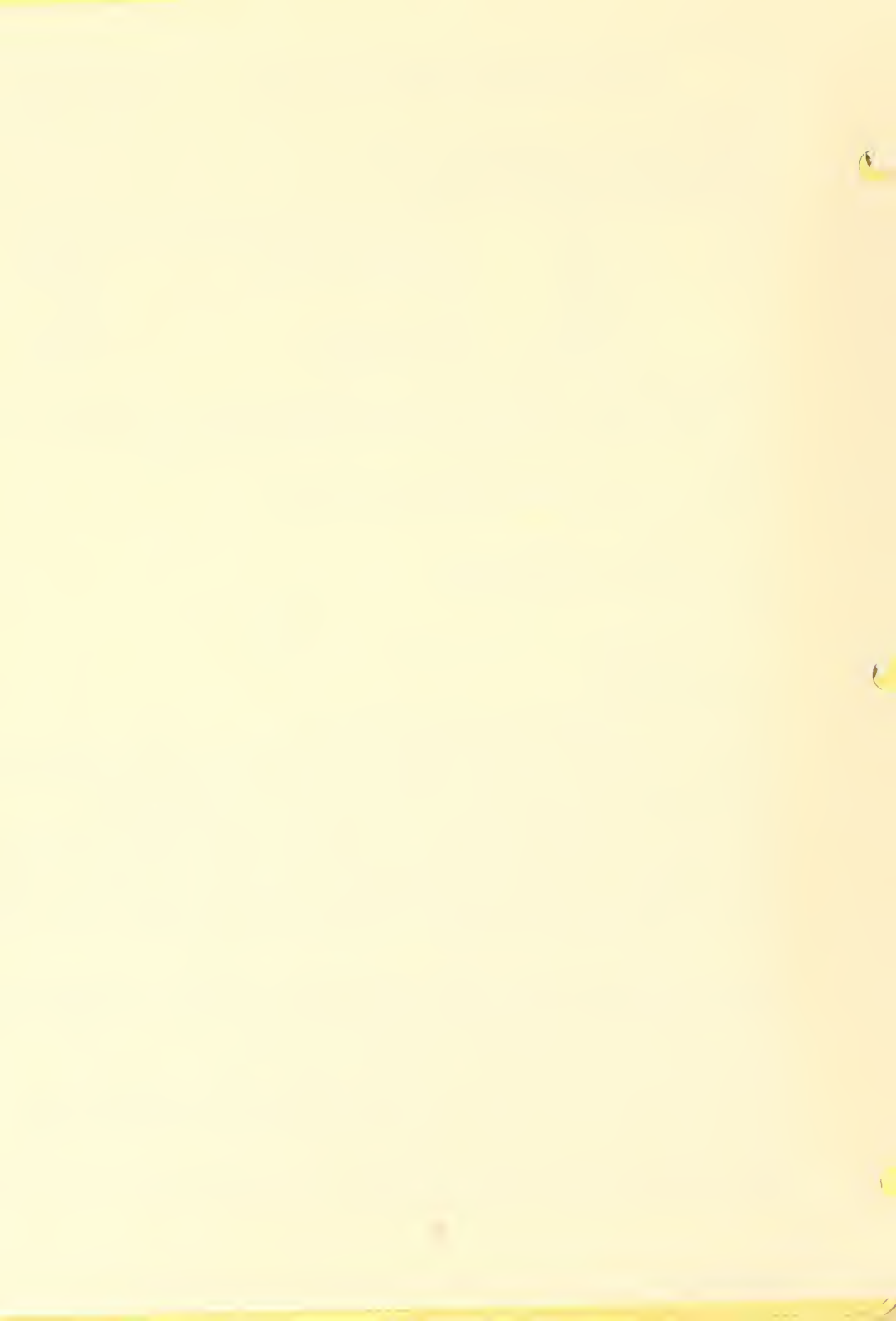
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Baltimore Longitudinal Study of Aging (BLSA), the NIA's major research program on human aging, has been conducted at the Gerontology Research Center since 1958. The study represents a consortium of scientists who work to characterize normal and pathological aging. The BLSA consists of a series of longitudinal and cross-sectional studies of varying degrees of interrelationships oriented toward description, identification of mechanism, prediction and intervention in human aging processes. The scientific goals include identifying age differences among individuals and changes in individuals over time; to characterize transitions from normal to pathological aging; to determine the relative contribution of aging, disease processes, cohort effects and secular effects; to expand scientific understanding about predictors and risk factors for specific diseases and for other end points related to successes and failures of adaptation to aging processes; and where possible to explore mechanisms for normal and/or pathological changes.

Scientists working with BLSA are assigned to 11 sections of 7 laboratories in addition to the LSB. The Chief, LSB, is the Associate Scientific Director, NIA for the BLSA and LSB staff administer and manage the BLSA as well as conduct research with it. During the past year, the Chief, LSB has been on sabbatical at the Eindhoven University of Technology.

The Steering Committee, along with the ASD and Scientific Director are responsible for determining the direction of the study. Three working subcommittees (Scientific Directions, Progress Review, and Resource Management) report to the Steering Committee on routine basis.

Progress has been made in upgrading procedures for data acquisition, storage and retrieval in the BLSA. Procedures have been adopted to increase the work efficiency of the staff, and to be able to manage the increasing number of BLSA participants.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00622-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health Disease Status in the BLSA: Clinical Health Evaluation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
Others:	B.S. Hiscock	Program Analyst	LSB, NIA
	J.L. Fleg	Staff Cardiologist	LCS, NIA
	D. Kramer, D. Binckley	Nurse Practitioners	JHBMC
	D. Lingle, G. Scarinzi	Nurse Practitioners	JHBMC
	C. Bacal, D. Santor	Physician Assistants	JHBMC
	L. Neuman	Nurse	JHBMC

COOPERATING UNITS (if any)

John Hopkins Bayview Medical Center (JHBMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4

PROFESSIONAL:

4

OTHER:

0

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Since 1985, the health evaluation in the BLSA has made major changes. The goal has been to improve the collection of medical information in research participants. Two versions of the health questionnaire have been implemented. The first is used initially to determine previous health complaints and problems. On subsequent visits a second version is used where the participants are queried regarding changes in their health status since their last visit. The interval history questionnaire was started on March 1, 1993. With the new interval questionnaire, we also changed the branching questions for positive responses to include queries about the effects of symptoms and problems on life style and quality of life. Its goal is to identify how a symptom affects the life of the subject. A physical functioning inventory was developed and implemented into the clinical evaluation. It probes for mild to moderate disability. Over the past year, we have added to our quality assurance program to assess the value of the new health questionnaire for BLSA research. The program had included the development by the nurse practitioners/physician assistants of formal guideline for the health questionnaires, and regular QA meetings to discuss issues pertinent to the clinical evaluation. We have begun an analysis of several clinical questions regarding neck, arm, back and leg pain in order to determine whether the question format and the branching questions can be used to understand the role of symptom complaints in aging.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00623-06 LSB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Statistical Methodology for the Analysis of Studies of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L.J. Brant Mathematical Statistician LSB, NIA
 Others: J.D. Pearson Senior Staff Fellow LSB, NIA
 C.H. Morrell Guest Worker LSB, NIA

COOPERATING UNITS (if any)

Department of Mathematical Sciences, Loyola College in Maryland (C.H. Morrell)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Statistical methodology is being applied and developed for longitudinal studies and other studies of aging. The research program focuses on several types of statistical models: 1) longitudinal mixed-effects regression models which consider both within- and between-subject variation in analyzing the repeated measurements for all individuals in the study population, 2) survival analysis for studying risk factors in prospective studies, 3) multiple comparisons for testing group differences in experimental or observational designs, 4) mixture models for describing age changes in distributions of biological markers, and 5) experimental design. Other techniques used include Bayesian, maximum likelihood and numerical computing methods. A major emphasis of the research program is the development of methods which yield cogent yet easily understood results when applied to data.

Several models using mixed-effects regression analyses have been developed to study longitudinal data. A piece-wise non-linear mixed-effects regression model was developed to estimate the time at which individuals with prostate cancer developed their tumors during the follow-up period. The model estimates the time when rapid increases in prostate specific antigen (PSA) were first observed beyond the usual background level of PSA change. The Bayesian nature of the mixed-effects model was also used to obtain estimates of a risk factor response that corrected for measurement error bias due to the random variations in the measurements of a risk factor. These shrinkage estimates were then used to provide a "corrected" value of the risk factor which was used to get a more accurate measure of the strength of the relation between the level of a risk factor and the occurrence of an endpoint such as morbidity or mortality.

The research program has extended earlier methods of longitudinal data analysis, introduced novel methods of describing the natural history of aging, and developed new approaches toward the use of longitudinal data in epidemiological and biomedical studies of aging and associated disease states.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00624-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Baltimore Longitudinal Study of Aging (BLSA): Population Dynamics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fozard	Chief & Assoc. Scientific Director	LSB, NIA
	B.S. Hiscock	Program Analyst	LSB, NIA
Others:	L.J. Brant	Mathematical Statistician	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
	C.L. Dent	Supervisory Biologist	LSB, NIA
	C.B. Willey	Program Coordinator	LSB, NIA

COOPERATING UNITS (if any)

Johns Hopkins Bayview Medical Center (JHBMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

3.8

PROFESSIONAL:

1.2

OTHER:

2.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with optimal management and scientific description of the total BLSA population, which includes, as of 6/30/94, 1167 active participants (543 women; 624 men), 534 inactive (193 women; 341 men), and 615 deceased (61 women; 554 men). Active participants range in age from 20 to 97 years old.

Recruitment efforts are focussed on 45 to 55 year old Caucasian and African American women and African American men and women across the age range to meet the needs of the two new research initiatives, the Perimenopausal and Vascular studies. At present, 9% of all active participants, and 12% of the active women, are African American. Since April, 1992, all new participants have been screened through self report applicant health status questionnaire according to health criteria for either the Vascular or Perimenopausal initiatives.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00625-05 LSB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Baltimore Longitudinal Study of Aging (BLSA) Data Management

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	E. A. Shefrin	Computer Scientist	LSB, NIA
Others:	C. B. Eames	Programmer/Analyst	LSB, NIA
	N. S. Gittings	Programmer/Analyst	LSB, NIA
	G. S. Hammen	Computer Technician	LSB, NIA
	S. M. Pegram	Computer Technician	LSB, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center (GRC), Longitudinal Studies Branch (LSB)

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

4.8

PROFESSIONAL:

2.8

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Data Management work group is responsible for the storage of both paper and computer records generated by the BLSA. They perform the data entry of medical records and manage the data entry of many of the other data collected by the BLSA internal investigators and outside collaborators. Staff members manage the BLSA Computer System and its data base. They support both the administration of the BLSA as well as its scientific activities. Their functions include data extraction, processing, and analysis; consultation; training; hardware and software maintenance; and software development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00626-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Changes in Visual Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
Others:	N.S. Gittings	Computer Programmer	LSB, NIA
	C.L. Dent	Testing Manager	LSB, NIA
	F. Schieber	Guest Researcher	USD
	D.W. Kline, T.S. Kline	Guest Researcher	UC
	E.I. Traboulsi	Guest Researcher	JHU
	H.A. Quigley	Guest Researcher	JHU

COOPERATING UNITS (if any)

University of South Dakota (USD)
 University of Calgary (UC)
 The Wilmer Eye Institute (JHU)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.25

PROFESSIONAL:

0

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Deterioration of vision is a common problem in the elderly and in recent studies has been demonstrated to be an independent and important contributor to physical disability and frailty. Age associated loss of vision can result from aging and from age-associated pathology. The natural history of vision loss has not been well studied in relationship to the development of eye and other pathology and the differentiation between normal visual aging and pathological changes in vision. Furthermore, little attention has been given to what factors may prevent aging changes in vision and what environmental factors can be altered as the changes begin to occur and as they progress.

Research in the BLSA has been designed to address aspects of these age associated changes.

(1) Natural history studies have been done for many years, and several longitudinal studies have been reported. Visual acuity and binocular depth perception is measured on first time participants, and longitudinally on women participants in the BLSA. A laboratory based assessment of visual contrast sensitivity continues to be administered, increasing the number of persons with at least two measures to over a hundred.

(2) A new study of the relationship between intraocular pressure and systemic blood pressure has been developed and has begun in July 1994. The study will identify possible racial and sex differences in blood pressure/intraocular pressure relationships and the effect of the relationship on vision as measured by changes in the visual field.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00627-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Risk Factors for Age-Related Ocular Change

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Sheila West	Associate Professor	Wilmer Institute JHU
		Guest Researcher	LSB NIA
Other:	Neil Bressler	Associate Professor	Wilmer Institute JHU
	Harry Quigley	Professor	Wilmer Institute JHU
	Evan Farmer	Professor, Dermatopathology	Wilmer Institute JHU
	Susan Vitale	Assistant Professor	Wilmer Institute JHU

COOPERATING UNITS (if any)

Wilmer Institute (Johns Hopkins University School of Medicine)
 National Eye Institute

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine risk factors for the leading causes of blindness in the United States, age-related macular degeneration, cataracts, and glaucoma. Specifically, the study is examining the association of dermal elastotic degeneration and antioxidant vitamin status with age-related macular degeneration; the association of vitamin intake with cataract. A total of 719 participants age 40 and older with at least one visit prior to the ocular study were eligible, of whom 96% had macular and lens photographs to assess ocular status.

No new analysis was completed this year. Funding was sought but not received for longitudinal repeat of the measurements, five years after the previous measures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00628-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Aging and Auditory Characteristics

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	James L. Fozard	Chief	LSB, NIA
Others:	Sandra Gordon-Salant	Guest Researcher	LSB, NIA
	Jay D. Pearson	Senior Staff Fellow	LSB, NIA
	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Larry J. Brant	Mathematical Statistician	LSB, NIA
	Christopher H. Morrell	IPA	LSB, NIA

COOPERATING UNITS (if any)

University of Maryland

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.77

PROFESSIONAL:

.27

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project aims to combine assessment of hearing abilities among subjects of different ages over time, together with information from their communication and health histories. Medical and cognitive data collected from subjects in the longitudinal study will be examined with respect to the audiologic and case history data. The two principal objectives of this project are: A) To study the contribution of medical, genetic, dietary and social factors to age-related auditory dysfunction; and B) To determine to what extent age, independent of other etiologic factors, causes a deterioration in hearing abilities. During the past year, approximately 450 subjects from the BLSA have been tested on all of the new measures in the hearing protocol. These measures include assessment of pure-tone hearing sensitivity, sentence understanding in noise, self-perceived hearing handicap, tympanometry, acoustic reflex thresholds, acoustic reflex magnitude, acoustic reflex adaptation, and acoustic reflex latency (the last five measures are part of the acoustic immittance battery of electrophysiologic tests).

We have recently completed a retrospective analysis of gender differences in longitudinal change in hearing sensitivity in a sample screened to exclude otological disorders and evidence of noise-induced hearing loss. We plan to extend these analyses to develop age- and gender-specific nomograms for pure-tone thresholds and to document the natural history of the development of noise-induced hearing loss.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG-00629-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA Men: Distribution of Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
Others:	L.J. Brant	Mathematical Statistician	LSB, NIA
	J.D. Pearson	Senior Staff Fellow	LSB, NIA
	G. Baker	Guest Researcher	LSB, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NO REPORT FOR THIS PROJECT THIS FISCAL YEAR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00630-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA Women: Distribution of Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	D. Kramer	Nurse Practitioner	JHBMC
Others:	D. Binckley, D. Lingle	Nurse Practitioners	JHBMC
	C. Bacal, D. Santor	Physician Assistants	JHBMC
	L. Neuman	Nurse	JHBMC

COOPERATING UNITS (if any)

Johns Hopkins Bayview Medical Center (JHBMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.05

PROFESSIONAL:

.05

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Aging women experience life changes differently than men. Over the past year, continued analysis was made of data from the BLSA to compare gender similarities and differences (1) in drug treatment of hypertension, (2) symptom reporting with the study of chest pain and its association with heart disease, and differences in the reporting of musculoskeletal pains, and (3) the prevalence of urinary stress incontinence in women and its relationship to the aging process. Slow progress has been made in these studies with the greatest advances in the musculoskeletal pain health questions.

Chest pain was found to be a common health complaint for both women and men. The prevalence at different ages was different by sex, with approximately 25% of women reporting chest pain by age throughout the adult life-span, while men showed an increase in reporting with increasing age. Too few cases existed to characterize the nature of the pain or the underlying causes. More women than men complained of chest pain in young adult age range, but the differences were not significant. Women also reported more neck and arm pain, while men reported more back pain. The characterization of the pain did not differ by age group. Both men and women answered questions about time of onset, duration, severity and recency in a similar manner for each of the four body regions, though the frequency of reporting differed. Further analyses are progressing in both of these studies. Women and men complain about pain in different sights at different rates, but the time course and severity of the pains do not differ.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00632-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA Men: Perceived Health Status

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Fozard	Chief	LSB, NIA
Others:	B.S. Hiscock	Program Analyst	LSB, NIA
	J.D. Pearson	Senior Staff Fellow	LSB, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

05

PROFESSIONAL:

05

OTHER:

0

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

A manuscript was completed and submitted for publication.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00633-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health and Disease Status in the BLSA: The Prostate Gland

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.D. Pearson	Senior Staff Fellow	LSB, NIA
	E.J. Metter	Medical Officer	LSB, NIA
Others:	J.L. Fozard	Chief	LSB, NIA
	L.J. Brant	Statistician	LSB, NIA
	R. Andres	Chief	LCP, NIA
	S.M. Harman	Section Chief	LCP, NIA
	H.A. Guess	Guest Researcher	UNC
	H.B. Carter, A.W. Partin	Assistant Professors	JHU
	P.C. Walsh	Professor	JHU

COOPERATING UNITS (if any)

Johns Hopkins University (JHU), Department of Urology
 University of North Carolina (UNC), Department of Epidemiology

LAB/BRANCH

Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, Gerontology Research Center

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new BLSA study of Prostate Growth and Disease was begun in February 1993 to examine anatomic and physiologic correlates of normal prostate growth and the development and progression of benign prostatic hyperplasia and prostate cancer.

In the past year, four studies have been completed. The first BLSA study showed that the epithelial composition of the prostates of men with benign prostatic hyperplasia (BPH) was positively correlated with PSA level and PSA velocity. Thus, PSA could be useful as an inexpensive method of targeting drug treatments at either the epithelial or stromal components of BPH.

The second study demonstrated that the variability in PSA levels was similar in men with BPH whether the samples were drawn at 3 month, 6 month, or 2 year intervals. This documented for the first time that prostate cancer screening criteria based on rate of change in PSA are affected by the testing interval.

The third study compared the sensitivity and specificity of several different candidate PSA criteria for the detection of prostate cancer. Average PSA velocity had the highest combined sensitivity and specificity of all the criteria compared.

The fourth study retrospectively examined serum androgen levels in men with and without prostate cancer. No significant differences were observed in age-adjusted luteinizing hormone, total testosterone, sex-hormone binding globulin, or free testosterone levels. These findings suggest that serum testosterone levels are not a strong risk factor for the development of prostate cancer.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00634-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

Title must fit on one line between the borders.

Age Changes in Pulmonary Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: James L. Fozard, Chief, LSB, NIA
 Jay D. Pearson, Sr. Staff Fellow, LSB, NIA

Other: Jerome L. Fleg, Sr. Staff Cardiologist, LCS, NIA
 Melvyn S. Tockman, Guest Researcher, LSB, NIA
 E. Jeffrey Metter, Medical Officer, LSB, NIA

COOPERATING UNITS (if any)

The Johns Hopkins School of Hygiene
 The Johns Hopkins School of Medicine

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.16

PROFESSIONAL:

.31

OTHER:

.85

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The GRC-BLSA Program in Pulmonary Aging focused upon:

a. Accelerated Decline in Pulmonary Function Predicts Coronary Heart Disease Death

In BLSA men, an elevated risk for cardiac death follows an accelerated decline in FEV₁, independent of the effects of the initial FEV₁, % predicted, cigarette smoking and other common CHD risk factors. There were 79 CHD deaths and 804 survivors over the 1 to 28.5 year follow-up period. After adjusting for common CHD risk factors in a time-dependent Cox proportional hazards analysis, the highest relative risks for cardiac mortality were associated with quintile of subsequent FEV₁ decline, even among never-smokers. The elevated risk associated with decline in FEV₁ was specific to coronary death. Thus, rate of pulmonary function decline appears to be a new risk factor for CHD death.

b. Age-Associated Changes in FEV₁ in Healthy, Non-Smoking Men and Women

Longitudinal analyses of changes in FEV₁ were conducted among 91 men and 82 women who had no history of respiratory problems and had never smoked cigarettes. The FEV₁ data were modeled using a mixed-effects regression model and longitudinal percentile distributions of FEV₁ level were constructed. The findings showed 1) the average longitudinal rate of decline in FEV₁ was approximately 240-340 ml/decade in men and women, 2) none of the participants exhibited a sustained improvement in FEV₁, and 3) between-subjects variability is greater in men than women and increases with age in men, but decreases with age in women.

c. Development of a Mathematical Model of Pulmonary Aging

A new model of normal pulmonary aging is being developed based upon the change in physiologic emptying of the lungs. Digitized spirometry from BLSA healthy, nonsmokers, without evidence of heart disease, is converted into distributions of emptying times by moments analysis. Those healthy nonsmokers with minimal lung function decline will define the standard of optimal pulmonary aging. Aging of the lung will be defined as a significant increase beyond optimal in the proportion of ventilatory emptying described by long time constants. The age-related decline of individual pulmonary function may be described over longitudinal follow-up by a mixed-effects model which includes parameters for Intercept, Time Interval, (Time Interval)², Age and Mean Emptying Time.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00635-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Age Changes in Response Speed and Nerve Conduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.L. Fozard	Chief	LSB, NIA
Others:	E.J. Metter	Medical Officer	LSB, NIA
	J.L. Wood	Psychologist	LSB, NIA
	M. Vercruyssen	Consultant	UM

COOPERATING UNITS (if any)

University of Minnesota (UM)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1

PROFESSIONAL:

0

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three measures of slowing of behavior were analyzed to describe age-related differences as well as age changes: reaction time, movement time, and nerve conduction velocity. Age-related changes in reaction time were not as robust as the cross-sectional age differences suggesting that factors other than age are responsible for part of the age declines observed. Reciprocal manual movement speed declines relatively more rapidly with greater task difficulty in older age. Although cautiously reported, nerve conduction velocity decreased with older age with age difference becoming apparent around age fifty.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00636-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Physical Activities in the BLSA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Linda P. Fried	Guest Researcher	LSB NIA
Other:	Jerome L. Fleg	Cardiologist	LCS NIA
	E. Gundy Zenger	Research Assistant	JHU
	Jordan D. Tobin	Chief, Applied Physiology	LCP NIA
	James L. Fozard	Chief	LSB NIA

COOPERATING UNITS (if any)

Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

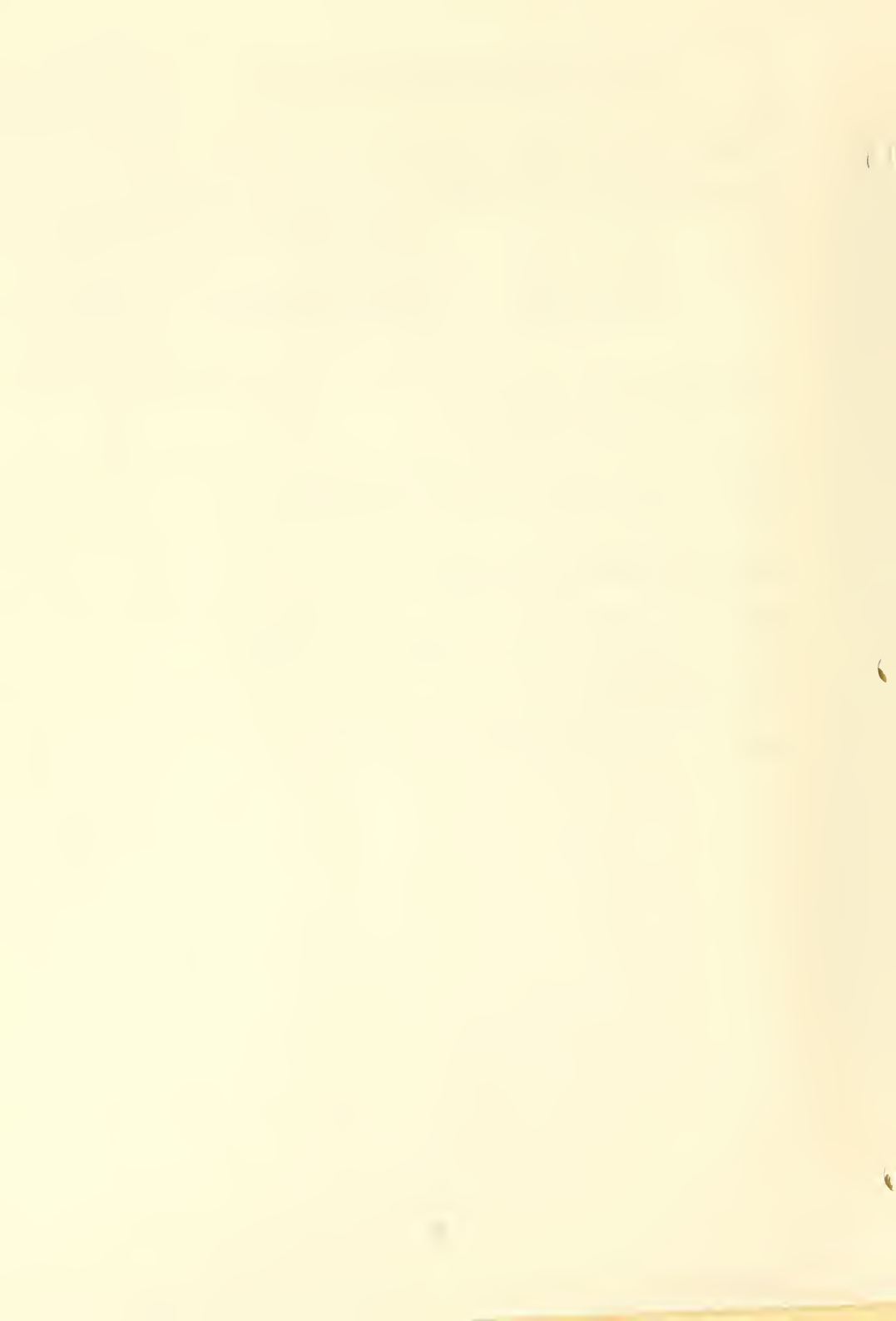
0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NO REPORT FOR THIS PROJECT THIS FISCAL YEAR



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00637-05 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gender Differences and Individual Variability in Human Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.J. Brant	Mathematical Statistician	LSB, NIA
	J.D. Pearson	Senior Staff Fellow	LSB, NIA
	C.H. Morrell	Guest Worker	LSB, NIA
Others:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer, BLSA	LSB, NIA

COOPERATING UNITS (if any)

Laboratory of Cardiovascular Sciences, NIA (J.L. Fleg)
 Speech and Hearing Sciences, University of Maryland (S. Gordon-Salant)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of gender differences and individual variability in age-related phenomena are being carried out to: 1) determine the "normal" range of variability in human aging, 2) identify potential sources of variability which may be responsive to intervention, and 3) determine if there are subgroups of individuals who are more susceptible or resistant to various aspects of aging. The research combines the use of sophisticated statistical methodologies and the unique time depth and multidisciplinary breadth of the existing BLSA data base to examine issues related to the concepts of "normal" and "successful" aging, as well as to increase the power of traditional research designs. The statistical methods used include longitudinal regression models, time dependent proportional hazards analysis, and finite mixture models. Major findings include: 1) the longitudinal rate of hearing loss differs by gender and age (see Project Z01 AG 00628-05 LSB), 2) there is significant variability in longitudinal patterns of hearing loss even in groups carefully screened for otologic disorders and noise induced hearing loss, 3) variability in pulmonary function levels is greater in men than women and increases with age in men but decreases in women, and 4) accelerated decline in pulmonary function is an independent risk factor for coronary heart disease death (see Project Z01 AG 00634-05 LSB). These findings represent significant contributions to the theoretical and methodological development of biomedical risk factor studies, as well as to an increased understanding of the dynamics of the aging process. Research is underway to develop more refined methods of studying variability in aging in order to develop theoretically and methodologically sound approaches to risk factor analysis which account for changes in an individual's covariates over time and the possibility that individuals differ in susceptibility or resistance to aging processes.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00638-05 LSB

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Health Promotion, Modifiable Risk Factors and Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L.J. Brant	Mathematical Statistician	LSB, NIA
Others:	J.L. Fozard	Chief	LSB, NIA
	E.J. Metter	Medical Officer, BLSA	LSB, NIA
	J.D. Pearson	Senior Staff Fellow	LSB, NIA

COOPERATING UNITS (if any)

Department of Urology, Johns Hopkins School of Medicine (HB Carter)
 Speech and Hearing Sciences, University of Maryland (S Gordon-Salant)
 Health Services Research and Development Center, Johns Hopkins Univ. (PS German)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

0.25

PROFESSIONAL:

0.1

OTHER:

0.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Unnecessary morbidity and mortality is an important problem which leads to increased health-care costs and can ultimately result in premature death. It has been estimated that approximately two thirds of mortality is due to potentially preventable causes - 1.2 million deaths (65%) and 8.4 million years of life lost before age 65 (63%). Principal factors associated with unnecessary morbidity and mortality include tobacco use, high blood pressure, improper nutrition, lack of screening and prevention services, alcohol abuse, and injury. This project uses longitudinal data from the Baltimore Longitudinal Study of Aging (BLSA) and other studies to examine the influence of modifiable risk factors on the occurrence of premature deaths and unnecessary morbidity and disability. Identification of risk factors can lead to primary prevention efforts. Examples of BLSA research on the identification of modifiable risk factors include: studies of the relationship between total white blood cell count and coronary heart disease, noise exposure, blood pressure, and smoking in relation to hearing loss; pulmonary change as a risk factor for cardiac events (see Project Z01 AG 00634-05 LSB); and hormonal factors as a risk factor for prostate disease (see Project Z01 AG 00633-05 LSB). One example of BLSA research on secondary prevention practices is the ongoing research to improve the accuracy of the prostate specific antigen test to allow screening for early detection of prostate cancer (see also Project Z01 AG 00633-05 LSB). Information from these studies can have an impact on the development of primary and secondary prevention programs to improve longevity and quality of life for many Americans.



DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00639-03 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Individual Changes in Functioning with Age and Target Conditions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Lois M. Verbrugge	Guest Researcher	LSB, NIA
	Ann L. Gruber-Baldini	Guest Researcher	LSB, NIA
Others:	E. Jeffrey Metter	Medical Officer	LSB, NIA
	Jay D. Pearson	Senior Staff Fellow	LSB, NIA
	Larry Brant	Mathematical Statistician	LSB, NIA
	Cathy Dent	Supervisory Biologist	LSB, NIA
	James L. Fozard	Chief	LSB, NIA

COOPERATING UNITS (if any)

Institute of Gerontology, University of Michigan

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0.0

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal research focus is to analyze longitudinal changes in functioning in 14 domains of activity and to study how these changes vary by sociodemographic and medical factors. The data for these analyses are derived from the "Activity Questionnaire II." It has been filled out by BLSA subjects at each visit since 1966. Subjects estimate the amount of time they spend on numerous specific activities, ranging from personal care to leisure. This data set is unique for its time stretch (up to 25 years for some subjects) and its content (the comprehensive scope of activities). Analyses on this data set include examinations of the cross-sectional, longitudinal, and secular patterns by age and gender. Cross-sectional analyses reveal consistent age and gender differences for participation in and time spent doing various activities, especially work, housework, childcare, and various discretionary activities. Comparisons of longitudinal and cross-sectional results show evidence of secular changes in time spent doing work, housework, and childcare by women.

Examination of the effect of chronic conditions on time spent in activities revealed that the presence of a chronic condition (diabetes, hearing loss, hypertension, ischemic heart disease, musculoskeletal problems, pulmonary dysfunction, and visual acuity problems) increases the time spent in obligatory activities (personal care, sleep) and decreases time in discretionary activities (socializing, public service). The effect of a chronic condition on committed activities (housework, childcare) interacted with gender so that women increased time in these activities while men decreased their time; this may be a function of gender differences in perception of commitment of activities (e.g., women being more committed to housework).



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00640-02 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of Age on Muscle Strength, Body Composition and Health Status

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jamēs L. Fozard Ben Hurley	Chief Guest Researcher	LSB, NIA UM
Others:	E. Jeffrey Metter Rosemary Lindle Jerome L. Fleg Robin Conwit William Brown	Medical Officer IRTA Fellow Sr. Staff Cardiologist Neurologist Neurologist	LSB, NIA LSB, NIA LCS, NIA JHBMC NEMC

COOPERATING UNITS (if any)

Dept. of Kinesiology, College of Health & Human Performance, University of Maryland (UM), College Park; John Hopkins Bayview Medical Center (JHBMC); New England Medical Center (NEMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL STAFF YEARS:

1.67

PROFESSIONAL:

1.67

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study is examining the natural course of age-related changes in muscular strength to determines the relationship of age and strength levels to body composition and health status. The relationship of age to maximal force production (strength) of the upper and lower body musculature during the concentric (shortening) and eccentric (elongation) phases of movement are presently being established. In addition, strength measures are being assessed at slow, fast and zero (isometric) speeds to determine if there is a preferential loss of force at fast speeds with age. Analyses of changes in force production in the prime mover is being compared to changes in the antagonist muscle group to determine if muscle balance is affected with age. In addition, the angle of greatest force production is being assessed to determine if this angle changes with age. Almost 400 male and female subjects from the 20s through the 80s have been tested since implementation.

Age associated declines are found in cross-sectional analysis for both concentric and eccentric testing for all muscle groups and speeds. The rate of decline is greater in the quadriceps femoris than for the biceps brachii. The rates of decline in concentric and eccentric strength tend to be parallel.

LSB has proposed a long term initiative, "BLSA Initiative on Age-associated Changes in Functional Ability (Frailty)." The initiative consist of three projects: 1) to develop a quantitative functional assessment for the BLSA; 2) to investigate how changing vision effects performance; and, 3) a protocol is being developed to estimate the role of age associated changes in the peripheral nerve on the age associated changes in muscle strength. We plan to estimate the number of functional motor units and to follow longitudinal changes in strength and unit count.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00641-02 LSB

PERIOD COVERED

October 1, 1993 through September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Race & Gender Differences in Intracerebral & Carotid Arterial Velocity with Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Metter	Medical Officer	LSB, NIA
	C. Early	Neurologist	JHBMC

COOPERATING UNITS (if any)

Johns Hopkins Bayview Medical Center (JHBMC)

LAB/BRANCH

Gerontology Research Center, Longitudinal Studies Branch

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL STAFF YEARS:

.10

PROFESSIONAL:

.10

OTHER:

0

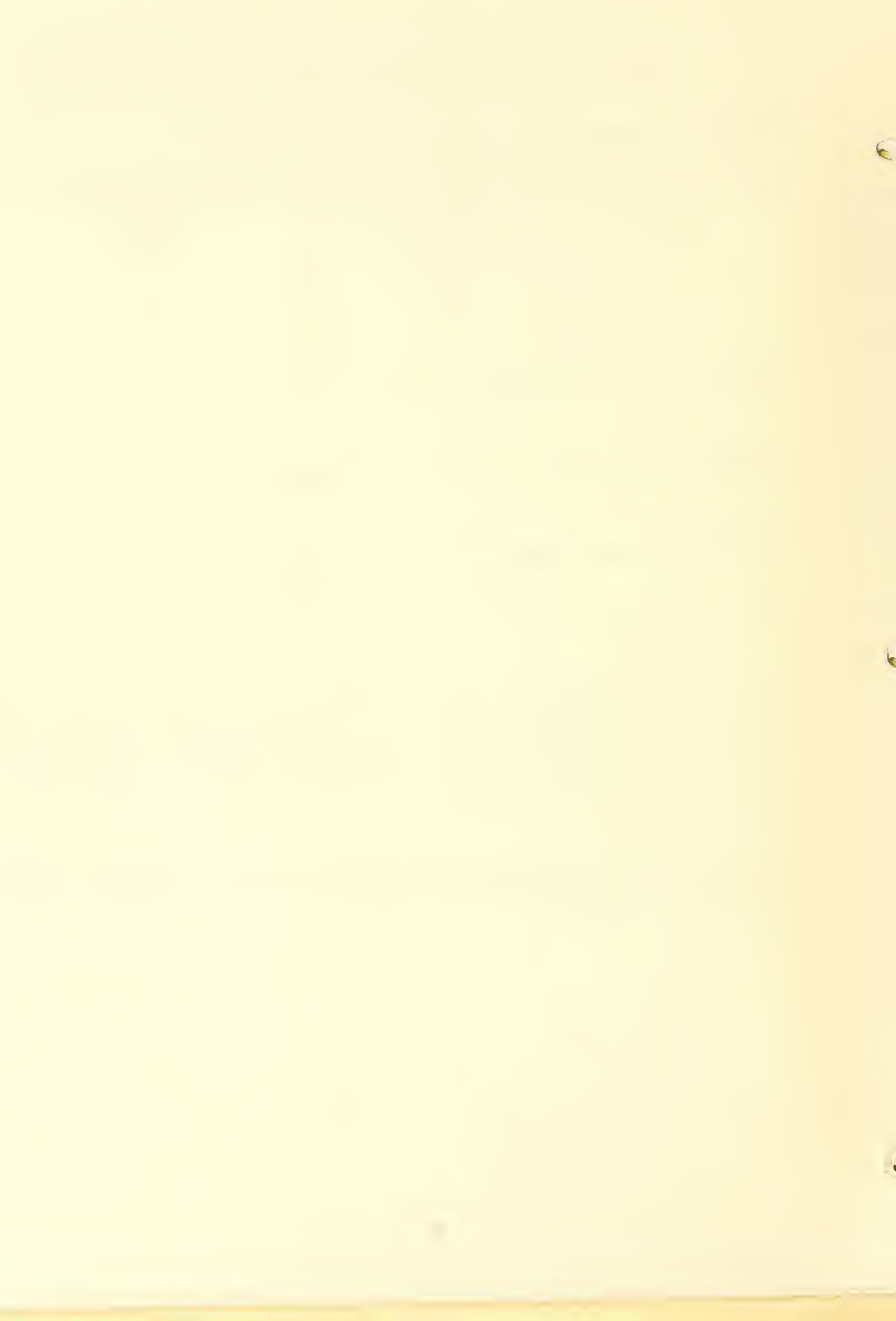
CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data collection was begun on the project in February 1994. By the end of July 1994, 48 subjects were studied. We have identified a foreign visiting scientist with expertise in transcranial doppler to work with us on this program. At present, that person cannot be brought on board because of the current federal hiring freeze. The hiring freeze has resulted in the limited number of studies and the delay in starting this project. With the current limitations, we project to collect data on about 200-250 BLSA participants during the coming year.

The focus of the project over the past year has been to be certain of the accuracy and reliability of the measurements. The current protocol is dependent on the skills of the sonographer. A protocol has been developed for monitoring studies and to monitor the consistency of studies over time, and the accuracy of the equipment.







DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00120-17 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Drug Development and Delivery to the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI N. Greig Visiting Scientist LN, NIA
X-F. Pei Visiting Fellow LN, NIA

Others: T. Soncrant Unit Chief LN, NIA
A. Brossi Professor Georgetown Univ.
D. Ingram Section Chief LCMB, NIA
S. Asthana Asst. Professor U. Washington, Seattle
R. Rothman Chief LCS, NIDA
P. Torrence Section Chief LC, NIDDK

COOPERATING UNITS (if any)

Chemistry Dept., Georgetown Univ., LCMB, GRC/NIA; LCS, NIDA; LC, NIDDK;
Geriatrics Div., U. Washington, Seattle; Drexel U.; NJ; U. Jerusalem, Isreal

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The factors that co-determine drug entry and action in brain (pharmacokinetics and pharmacodynamics) following systemic administration were analyzed to aide in the safer and optimal use of drugs in the elderly and the development of novel neurologic therapeutics. Rationale strategies were developed to design novel, selective, long-duration and centrally active cholinesterase inhibitors for the treatment of Alzheimer's disease and agents also were developed to improve the treatment of (i) cancers of the brain, lymphatics and breast, (ii) AIDS encephalopathy and (iii) drug abuse. Specifically, novel analogues of the alkaloid physostigmine were developed as cognitive enhancers and are being developed towards the clinical arena, and novel lipophilic anticancer alkylating agents, derivatives of dideoxynucleotides and dopamine reuptake inhibitors are being developed. Further strategies are being developed for the treatment of neurologic diseases and to improve the delivery of large compounds to brain.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00121-17 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Function and Structure of Blood-Nerve and Blood-Brain Barriers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI:	K. Wadhvani	Senior Staff Fellow	LN, NIA
	S. Rapoport	Laboratory Chief	LN, NIA
	W. Williams	Guest Worker	LN, NIA
Others:	R. Fukuyama	Visiting Associate	LN, NIA
	K. Chandrasekaran	Visiting Associate	LN, NIA
	A. Balbo	Biologist	LN, NIA
	Q. R. Smith	Section Chief	LN, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Neurochemistry and Brain Transport/Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

(a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Blood-nerve barrier permeabilities to ions and nonelectrolytes is very low, indicating limited exchange between plasma nerve extracellular compartments. In the rat, integrity of the blood-nerve barrier to small nonelectrolytes is maintained with age. The blood-nerve barrier, like the blood-brain barrier, has regulated carriers for the transfer of manganese, neutral amino acids, and basic amino acids. In addition, cationized albumin is taken up into nerve at a greater rate than native albumin, possibly by receptor-mediated transcytosis. Similar properties have been observed at the blood-brain barrier. A modified polymerase chain reaction titration method was developed to quantify glucose transporter mRNA expression at the cerebral microvessels. Lipid composition isolated cerebral capillaries differs with age. There are more unsaturated than saturated fatty acids in capillaries of older rats, a reduced amount of ethanolamine plasmalogen, and reduced phospholipase A2 activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00129-14 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Blood-Brain Barrier and the Regulation of Brain Nutrient and Metal		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal		
PI:	Q. Smith	Chief, SNBT LN, NIA
	Q-S. Deng	Visiting Associate LN, NIA
	M. Hokari	Visiting Fellow LN, NIA
	D. Allen	IRTA LN, NIA
Others:	S. Rapoport	Chief, LNS LN, NIA
	J. Stoll	Senior Staff Fellow LN, NIA
COOPERATING UNITS (if any) National Institute of Standards and Technology; Biomedical Engineering and Instrumentation Branch, NIH; Frederick Cancer Research Center, NCI, Frederick, MD. Unit on Neurotoxicology, INSERM, Paris, France, Food and Drug Administration.		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Neurochemistry and Brain Transport		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A highly sensitive secondary ion mass spectrometry method was developed to image distributions of trace elements in frozen sections of postmortem human brain. Studies were initiated on the distribution of <u>aluminum</u> and other key elements in <u>neurofibrillary tangle-bearing neurons in Alzheimer's disease</u> . The brain transport of manganese, an essential trace metal that produces a Parkinsonian-like syndrome at high concentrations, was studied in rats and found to be mediated by an uptake system that is influenced by plasma protein binding, oxidation state and trace metal competition. The <u>basic amino acid transporter</u> at the <u>blood-brain barrier</u> was cloned and shown to be homologous to System y ⁺ , the classic sodium-independent cationic amino acid carrier. Drugs with high affinity for the <u>large neutral amino acid transporter</u> of the blood-brain barrier were identified and tested for rapid uptake into brain. One drug, <u>D,L-NAM</u> , with high affinity for the neutral amino acid carrier exhibited 20-40 fold greater brain uptake than its clinical analog, L-melphalan, and demonstrated that the saturable nutrient carriers of the blood-brain barrier could be used to improve drug delivery to brain for the treatment of central nervous system diseases.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00130-11 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Neuropsychological Function in Aging and Dementia)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)		
PI:	G. Alexander Unit Chief, Neuropsychology	LN, NIA
	C. Grady Research Psychologist	LN, NIA
	M. Kurkjian Staff Fellow	LN, NIA
Others:	R. Parasuraman Cognitive Psychologist	Catholic University
	P. Greenwood Cognitive Psychologist	Catholic University
	J. Szczepanik Psychologist	LN NIA
	R. Desmond Psychologist	LN NIA
COOPERATING UNITS (if any) Laboratory of Neuropsychology, NIMH Department of Psychology, Catholic University		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Brain Aging and Dementia Section		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 2.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Brain systems that participate in object and spatial vision and in working and long-term visual memory were investigated in healthy young men by measuring regional cerebral blood flow (rCBF) with positron emission tomography (PET) and H2150. The results identified dorsolateral occipital and superior parietal areas activated more by spatial visual processing, and ventral occipital and occipitotemporal areas activated more by object discrimination. Old subjects demonstrated rCBF activations in the same regions as did young subjects, but also demonstrated activation of ventral areas during spatial vision and dorsal areas during object vision, suggesting less functional separation of these visual systems. Patients with dementia of the Alzheimer type (DAT) who could perform an object vision task, demonstrated normal percent baseline activation of rCBF in occipitotemporal cortex during the task, suggesting preserved capacity to recruit this area for perceptual processing. Memory-related modulations of cortical rCBF were found in anterior temporal and prefrontal cortex in DAT patients. Rate of cognitive decline was significantly correlated with rate of brain tissue loss (atrophy) with serial computer-assisted tomography (CT) scans, and with rate of worsening abnormality of resting state regional cerebral metabolic rates for glucose (rCMRglc) as measured by PET and 18F-Fluoro-2-deoxyglucose. Two patterns of cerebral metabolism characteristic of DAT were identified using regional covariance analysis of resting state rCMRglc. These patterns were correlated with specific cognitive deficits. Premorbid intellectual ability was inversely correlated with rCMRglc in several regions of association cortex in DAT patients. Among DAT patients, early age at onset of dementia was related to greater impairment on a measure of visuospatial attention. DAT patients with a family history of dementia showed deficits in rCMRglc of the anterior association regions. A unique neuropsychological profile was observed for a subgroup of DAT patients with early prominent visual disturbances. Older Down syndrome adults perform worse on mental abilities tests than do younger subjects with immediate memory and language less affected by age in Down syndrome than long-term memory and visuospatial function.</u>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00132-10 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Cell Biology of Models for Human Brain Disorders)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: Z. Galdzicki Visiting Associate LN, NIA
L. Acevedo IRTA Fellow LN, NIA
R. Pearce Visiting Fellow LN, NIA
S. Peng IRTA Fellow LN, NIA
J. Stoll Senior Staff Fellow LN, NIA

Others: A. Balbo Biologist LN, NIA

COOPERATING UNITS (if any)

Cellular and Molecular Neuroscience Group, Medical School, University of
Birmingham, UK; Department of Zoology, University of Cambridge, UK

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

4.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

(a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cultured hippocampal neurons from fetal trisomy 16 mouse (Ts16), a model for Down syndrome (DS), showed a significant decrease in the voltage-dependent sodium current. This was due to reduced numbers of voltage-dependent sodium channels in Ts16, relative to diploid neurons, as assessed by binding of radiolabeled saxitoxin. High-voltage-activated calcium currents were significantly larger in trisomic neurons compared to controls. In culture, there was a reduction in the ability of trisomy dorsal rootganglion (DRG) neurons to adhere to laminin-coated dishes in comparison to diploid DRG. This effect was dependent on nerve growth factor (NGF). However, survival in culture, while significantly less in trisomy DRG than diploid neurons, was independent of NGF. In terms of electrical properties, withdrawing NGF from DRG diploid neurons reduced the outward potassium current, whereas for trisomic neurons the outward potassium current increased. Depolarization rate of the action potential and inward sodium current were unaffected by NGF in diploid or trisomic neurons. Septal neurons are NGF-dependent neurons of the central nervous system. In primary culture, both Ts16 and diploid septal neurons had similar electrical properties. However, a subpopulation of highly excitable trisomy 16 septal neurons did evoke faster action potentials and had bigger inward currents than highly excitable diploid septal neurons. These differences were similar to the abnormalities detected for DRG trisomy neurons. The ultrastructural morphology of Ts16 and diploid hippocampus and DRG from gestation day 16 fetuses was the same. Likewise, hippocampal tissue from Ts16 and diploid fetuses, transplanted into the brains of normal mice and allowed to survive for up to 25 months, was ultrastructurally similar. Importantly, the transplanted Ts16 hippocampus grafts did not demonstrate Alzheimer disease-type neuropathology, suggesting that increased expression of genes homologous to those on human chromosome 21 is insufficient to cause Alzheimer-type neurodegeneration in the mouse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00133-11 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Mechanism and Therapeutic Interventions in DAT)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)		
PI:	H-C. Lee	Chief, Unit of LN, NIA Clinical Pharmacology LN, NIA
	U. Freo	Visiting Associate LN, NIA
	M. Kurkjian	Staff Fellow LN, NIA
	W. Hong	Senior Staff Fellow LN, NIA
Others:	G. Alexander	Staff Fellow LN, NIA
	M. Schapiro	Chief, BADS LN, NIA
	S. Rapoport	Chief, LNS LN, NIA
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Brain Aging and Dementia Section		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Two new projects using drugs as pharmacological tools to test the hypothesis of <u>reversible synaptic failure</u> in the early course of dementia of the Alzheimer's type (DAT) were approved by the NIA-IRB. Idazoxan is used to modulate noradrenergic synaptic norepinephrine concentration and physostigmine for cholinergic system. In addition to using pharmacological agents to modulate the neurotransmitter system, we also use cognitive tasks of varying difficulty (parametric stimulation) to potentiate the drug efficacy. A new therapeutic trial using <u>tetrahydrobiopterin</u> to treat DAT was also approved by the IRB. This therapeutic trial is based on our previous work, which showed that biopterine and monoamines are reduced in DAT patients with extrapyramidal signs. We propose that biopterine and monoamine deficiency contributes to the progression of DAT, which can be halted with the supplement of tetrahydrobiopterin. In addition to the three new projects, we have important findings in the pharmacokinetics and pharmacodynamics of <u>arecoline</u> and <u>physostigmine</u> . The verbal memory was improved with arecoline administration in a majority of patients with <u>mild-moderate Alzheimer's disease</u> . The dose response of <u>arecoline</u> on different cognitive modalities showed that verbal ability tended to improve at low doses of arecoline, whereas attention and visuospatial ability tended to improve at higher doses. The mechanism of arecoline-induced memory improvement is not due to induction of stress nor to elevation peripheral corticosteroid levels. Most DAT patients (5/9) responded to chronic intravenous infusion of physostigmine and showed a significant, reproducible but modest improvement in verbal memory. Further, the memory enhancement was significantly correlated with <u>butyrylcholinesterase</u> inhibition but not with plasma <u>physostigmine</u> concentration.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00134-11 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Brain Phospholipid Metabolism Relation to Function Aging and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI:	D. Purdon	Visiting Scientist	LN, NIA
	M. Chang	Senior Staff Fellow	LN, NIA
	C. Jones	NRC Associate	LN, NIA
	E. Grange	Visiting Fellow	LN, NIA
	T. Arai	Visiting Fellow	LN, NIA
	Q. Smith	Chief, SNBT	LN, NIA
	R. Rapoport	Chief, LNS	LN, NIA

COOPERATING UNITS (if any)

Dept. of Nuclear Medicine, Clinical Center NIH; Food and Drug Administration
(CDER/ORR/DRT; CDER/ODEL/DNDP; CDER/ODEL/DOPOP) and Proctor and Gamble

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A mathematical model was used to derive operational equations for examining incorporation and turnover of fatty acids within individual brain phospholipids, under in vivo conditions in animals and humans. In rats, the model was combined with quantitative autoradiography and biochemical analysis to examine these parameters with the saturated [9,10-³H]palmitic acid (³H-PAM) and the unsaturated [1-¹⁴C]arachidonic (¹⁴C-AA) and [1-¹⁴C]docosahexaenoic acid (¹⁴C-DHA). We have refined the model by thorough analysis of phospholipid metabolic precursor pools and defined their rate of turnover and the turnover of fatty acids in membrane phospholipids. We have applied the model to a number of systems in which membrane homeostasis can be either acutely or chronically perturbed. Administration of serotonergic agonists indicated involvement of [¹⁴C]AA but no [³H]PAM in second messenger systems. Incorporation of the three tracers was increased threefold to fivefold compared to normal brain by cerebrally implanted Walker 256 carcinomasarcoma cells in rats. Fractionation of brain membranes after arecoline stimulation indicated rapid turnover of arachidonate in synaptosomes. Chronic treatment of rats with lithium chloride was shown to alter the metabolism of the three tracers in brain phospholipid. Seizures induced in a kindled rat model showed increased incorporation of [³C]PAM in specific brain areas. Rates of fatty acid incorporation were shown by theory and experiment to be independent of cerebral blood flow. An inhibitor of fatty acid oxidation increased the fraction of labeled palmitate that entered brain lipids. We have used the model to define radiolabeled fatty acid imaging of the brain by positron emission tomography (PET). [1-¹¹C]fatty acids were synthesized and gave incorporation coefficients in monkeys comparable to those in rats. PET can be used to image normal and pathological brain in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00135-11 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Molecular Biology of Brain Aging and Disease)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)		
PI:	K. Chandrasekaran	Visiting Scientist LN, NIA
	R. Fukuyama	Visiting Associate LN, NIA
	M. Bennett	NRC Senior Associate LN, NIA
Others:	D. Brady	Senior Staff Fellow LN, NIA
	K. Hatanpaa	Visiting Fellow LN, NIA
	S. Rapoport	Chief LN, NIA
	T. Giordano	Scientist Abbott Labs
COOPERATING UNITS (if any) Abbott labs, Abbott Park, IL; Neuropathology Laboratory, The John Hopkins University School of Medicine, Baltimore, MD		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Purpose of this project is to determine the molecular basis of <u>vulnerability</u> of neurons to <u>Alzheimer's disease</u> (AD). Studies on individual AD patients by <u>positron emission tomography</u> show impaired brain energy metabolism occurring as an early prominent manifestation. We have identified a molecular mechanism involving <u>mitochondrial</u> and <u>nuclear genetic systems</u> of <u>oxidative phosphorylation</u> (OXPHOS) that may account for this impaired <u>energy metabolism</u> in AD. Brains from AD patients showed 40-60% decrease in levels of <u>mRNA</u> of <u>mitochondrial DNA</u> (mtDNA)- <u>encoded cytochrome oxidase</u> (COX) subunits I and III, <u>NADH-dehydrogenase</u> subunit 1 as well as <u>nuclear DNA</u> (nDNA)-encoded COX subunit IV and <u>F₀F₁-ATPase β subunit</u> in an <u>association neocortical region</u> (mid-temporal cortex) known to be affected in AD but not in the unaffected primary <u>motor cortex</u> , as compared to control brains. The level of expression of mitochondrial <u>12S rRNA</u> (mitochondrial transcript) gene, nuclear <u>lactate dehydrogenase</u> subunit B (a marker of <u>glycolytic metabolism</u>) gene, or nuclear <u>β-actin</u> gene was not altered. <u>COX activity</u> measured in brain sections of AD patients also showed a 34% decrease in temporal association neocortex of AD brains as compared to controls. Thus, impairments in mitochondrial <u>oxidative metabolism</u> may contribute to the metabolic failure and to the <u>neuronal degeneration</u> in AD. A <u>rat model</u> of chronic <u>COX inhibition</u> , induced by continuous infusion of the inhibitor <u>sodium azide</u> , has been developed to test the hypothesis that impairment in <u>energy metabolism</u> would develop AD-type <u>neuropathology</u> . Studies on the regulation of <u>amyloid precursor protein</u> (APP) in <u>cell culture</u> systems suggest that <u>cell-cell aggregation</u> and <u>cell-matrix</u> aggregation are not associated with the induction of APP protein and mRNA and that the induction of APP is essentially regulated by <u>Ca²⁺</u> . A modified immunological method - suppression in vivo followed by in vitro stimulation (SOFISTIC) - is developed to identify neuron-specific molecules in the selectively vulnerable AD brain regions.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00401-11 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Cerebral Chemistry in Dementia, Aging and their Treatments)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)		
PI:	H. Shetty Senior Staff Fellow LN, NIA M. Schapiro Chief, SBAD LN, NIA	
OTHERS:	H. Holloway Biologist LN, NIA D. Purdon Visiting Scientist LN, NIA J. Atack Research Scientist Merck	
COOPERATING UNITS (if any) Merck, Sharpe and Dohme, England.		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism/Brain Aging and Dementia		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland, 20892		
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The project entails identification of metabolic defects associated with the pathophysiology of <u>Down syndrome</u> (DS). Subsequently, the implicated metabolic processes would be probed in <u>Alzheimer disease</u> (AD) and in healthy aging. Development of <u>bioanalytical techniques</u> to study brain chemistry is an integral part of this study. Profiles of <u>polyols</u> in DS were examined in view of functional abnormalities that constitute its phenotype. Abnormal levels of polyols lead to neurological disorders and cataract formation in humans (diploids). Since polyols are reduction products of sugars, analysis of polyols may be used to probe glycolytic and pentose phosphate pathways. Several polyol species in <u>cerebrospinal fluid</u> (CSF) and plasma from DS and control subjects were quantitated by a mass spectrometric technique. <u>myo-inositol</u> was found elevated in CSF from DS subjects, 36.32 ± 8.02 ng/ul (mean \pm SD, n =10) versus 24.35 ± 4.20 ng/ul (n = 10) in age-matched control subjects. Other polyols in this fluid and plasma myo-inositol were unaltered. A low degree, positive correlation between the level of CSF myo-inositol and <u>age</u> was observed in controls and not in DS. myo-Inositol plays a central role in signal transduction and osmoregulatory processes in the brain. Thus elevated cerebral myo-inositol in DS may affect the structure and functions of the central nervous system. There is a need to study the relationship between metabolism of myo-inositol and phenotypic abnormalities of DS. A <u>gas chromatographic/mass spectrometric assay</u> for myo-inositol was developed based on generation of a unique fragment ion. Other polyol species in CSF and plasma were analyzed by a similar technique. The technique enabled quantitation of polyol species in microliter volumes of CSF or plasma with satisfactory precision. <u>Physostigmine</u> is a candidate cholinergic drug for treating AD subjects. A <u>liquid chromatographic technique</u> was developed to quantitate this drug in human plasma. Additionally, structure analysis of rat brain <u>phosphatidylcholine</u> (PC) and <u>phosphatidylinositol</u> was carried out. Two <u>polyunsaturated molecular species</u> of PC into which radiolabeled <u>arachidonate</u> incorporated with high specific activity were identified. These molecular species may play an important role in arachidonate turnover in the brain (Project no. Z01 AG 00134-11 LN).		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00403-09 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Genetics and Nongenetic Factors in Alzheimer's Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: M. Schapiro Chief, Section BAD LN, NIA

Others: C. Grady Chief, PET Unit LN, NIA
K. Pettigrew Statistician MHIRP, NIMH
Z. Wu Visiting Associate LN, NIA
B. White Medical Res Officer LCB, NIDDK
K. Sanford Chief LCMB, NCI
R. Parshad Professor Howard Univ

COOPERATING UNITS (if any)

CRND Univ., Toronto; Dept. Pathology, Howard Univ.; Twin Registry, NAS; LCNSS,
NINDS; MHIRP, NIMH; LCMB, NCI; LCB, NIDDK

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Family histories studies showed a familial aggregation of Alzheimer's disease among first-degree relatives of Alzheimer's disease probands compared with controls. A case report shows that dementia in Down syndrome may occur without mental retardation. DNA repair studies show a G2 radiation repair deficiency in Down syndrome. Buffy coats from blood of first-degree relatives with Alzheimer's disease were injected into hamsters to test transmissibility in Alzheimer's disease. In animals surviving up to 301 days after inoculation, no evidence of brain disease was present, arguing against transmissibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 AG 00404-08 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Functional Interactions Among Brain Regions in Aging and Dementia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI:	B. Horwitz	Chief, Unit on Brain Imaging & Computers	LN, NIA
	N. Azari	IRTA Fellow	LN, NIA
	A. R. McIntosh	NSERC Fellow	LN, NIA
Others:	C. L. Grady	Chief, Unit on PET	LN, NIA
	M. B. Schapiro	Chief, BADS	LN, NIA
	T. Soncrant	Senior Staff Fellow	LN, NIA

COOPERATING UNITS (if any)

LN, NIMH; CPB, NIMH; DASR, NIMH, SFBI, NPP, NIMH.

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A correlation method was developed to examine functional interactions between brain regions, by correlating either regional cerebral metabolic rates for glucose or regional cerebral blood flows, as determined by positron emission tomography (PET) in humans. In humans in whom regional cerebral blood flow (rCBF) was measured with PET during a face matching task, correlations among visual brain areas and frontal regions were reduced in patients with mild dementia of the Alzheimer type (DAT) compared to controls. A systems-level neural network-model, fitted to rCBF PET data, permitted determination of the brain regions and their interactions that were involved in two visual processing tasks, and in a working memory task. A multiple regression/discriminant analysis involving PET regional interdependencies distinguished DAT patients from controls. In young adults with Down syndrome, PET values in language areas could be used in a discriminant function to distinguish their PET scans from those of controls. A multiple regression/discriminant analysis applied to PET scans of patients with Obsessive-Compulsive Disorder obtained before and after pharmacotherapy allowed discrimination between patients who responded to drug from those who did not.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00406-04 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Mechanisms for Alzheimer Disease)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)		
PI:	S. Rapoport	Chief, LN LN, NIA
	J. VanMeter	Staff Fellow LN, NIA
OTHERS:	C. Grady	Chief, PET Unit LN, NIA
	J. Maisog	Medical Staff Fellow LN, NIA
	T. Zeffiro	Senior Staff Fellow LN, NIA
	N. Gershfeld	Senior Scientist LPB, NIAMS
	L. Ginsberg	Visiting Associate LPB, NIAMS
COOPERATING UNITS (if any) LPB, NIAMS; LPP, NIMH; Royal Free Hospital, London		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism Section		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Comparative anatomic data suggest that several systems of brain regions underwent selective expansion or differentiation during primate evolution, according to the principle of 'integrated phylogeny'. This involved expansion of the neocortex. Certain human neurodegenerative diseases, including Alzheimer disease, affect such systems, suggesting that they are 'phylogenetic' diseases and that the genetic changes that promoted integrated phylogeny are related to the genetics of these diseases. Measurements of brain blood flow using positron emission tomography, during cognitive stimulation, suggest that reversible synaptic failure underlies early functional deficits in Alzheimer disease. A within-subject method to analyze blood flow responses in Alzheimer patients subjected parametric cognitive stimulation, with and without drugs that modulate synaptic efficacy, has been developed. The critical temperature for maintaining stable lipid monolayers in vitro is reduced from 37°C to less than 30°C, using lipids from temporal association but not cerebellar cortex of Alzheimer brain. Cell membrane instability in vulnerable brain regions likely contributes to progression of Alzheimer disease.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00407-03 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Neuroanatomy and Neuropathology of Aging Primate Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal PI: D. Brady Senior Staff Fellow LN, NIA		
Others: S. Brining IRTA LN, NIA M.C. Bennett NRC Senior Fellow LN, NIA J. Stoll Senior Staff Fellow LN, NIA K. Chandrasekaran Visiting Scientist LN, NIA K. Hatanpaa Visiting Fellow LN, NIA R. Fukuyama Visiting Associate LN, NIA		
COOPERATING UNITS (if any) Lab. of Biological Chemistry, Gerontology Research Center, NIA, Baltimore, MD; Epilepsy Research Branch, NINDS; Division of Intramural Research, NINDS; Division of Neurosciences, Rush-St. Lukes Medical Center, Chicago, IL;		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Cerebral Physiology and Metabolism		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input checked="" type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) There exists a selective vulnerability in the distribution of neuropathology in <u>Alzheimer's disease</u> (AD). We used this neuropathologic characteristic to stage the progression of <u>β-amyloid</u> deposition within <u>neuritic plaques</u> , and its relationship to <u>neuropeptide</u> containing dystrophic neurites and <u>neurofibrillary tangles</u> (NFTs). β-amyloid appears in diffuse plaques prior to the development of dystrophic neurites and the deposition of <u>paired helical filaments</u> . The relationship between <u>neuropathology</u> and <u>apoptosis</u> (programmed cell death) was evaluated in the <u>hippocampus</u> of AD brains, revealing a strong correlation between presence of apoptotic nuclei and AD. However, direct evidence of apoptosis in NFTs was not observed. A more thorough evaluation of other brain regions will elucidate the role of apoptosis in AD. We have developed several approaches to elucidating the molecular basis of selective vulnerability in AD. <u>Monoclonal antibodies</u> were generated against the entorhinal cortex or basolateral amygdala using the SOFISTIC technique. These antibodies label specific subcellular compartments in apparently healthy <u>neurons</u> as well as neurofibrillary tangles. Another approach has been to probe the regional expression of <u>homeobox genes</u> as a means to understand the mechanism of regional differentiation in the cerebral cortex. Analysis of the relationship between <u>oxidative metabolism</u> and AD revealed a deficit the level of COX enzyme activity and MRNA expression in selectively vulnerable brain regions.		
Neuropathologic analysis of AD patients identified clinically with <u>leukoencephalopathy</u> did not support a direct vascular role in the etiology of the disease. Amyloid staining revealed severe cerebral <u>amyloid angiopathy</u> without involvement of white matter vessels. Thus, the increased amyloid burden in the cerebral vasculature does not appear to account for clinically observed leukoencephalopathy in AD patients.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00408-01 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Neuronal Development in Tissue Culture

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI:	Z. Galdzicki	Visiting Associate	LN, NIA
	D. Allen	IRTA Fellow	LN, NIA
	S. Brining	IRTA Fellow	LN, NIA
	R. Fukuyama	Visiting Associate	LN, NIA
	R. Pearce	Visiting Associate	LN, NIA
	K. Wadhvani	Senior Staff Fellow	LN, NIA
	A. Balbo	Biologist	LN, NIA

COOPERATING UNITS (if any)

Department of Zoology, University of Cambridge, UK; CNB, NINDS, NIH.

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.6

PROFESSIONAL:

2.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

β-amyloid peptide increases choline conductance of PC12 cells, which may explain the reduced level of acetylcholine in postmortem brain tissue of Alzheimer's disease patients. The increase in choline leakage out of cholinergic neurons would deplete them of the substrate for acetylcholine synthesis. β-amyloid also increases the activity of endogenous channels, most likely poorly selective chloride channels. Although we did not detect any changes caused by β-amyloid in the calcium conductance an increased calcium-uptake was measured. This increase was sufficient to explain the toxic effect of peptide in culture conditions. The infusion of β-amyloid into rat brain ventricle revealed a small increase in palmitate uptake, which implicates some membrane remodeling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AG 00409-01 LN
PERIOD COVERED October 1, 1993 to September 30, 1994		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the Developmental Models of Human Brain)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal)		
PI:	M. B. Schapiro U. Shetty	Chief, SBAD Visiting Associate LN, NIA LN, NIA
OTHERS:	H. Holloway J. R. Atack I. Hanin M. F. Beal	Biologist Research Scientist Director Neurologist LN, NIA Merck Dept. Pharm, Loyola Univ Mass Gen. Hoap
COOPERATING UNITS (if any) Dept of Pharmacology and Experimental Therapeutics, Loyola Univ.; Dept of Neurology, MA General Hospital, Boston, Ma; Merck, Sharpe and Dohme, England.		
LAB/BRANCH Laboratory of Neurosciences		
SECTION Brain Aging and Dementia/Cerebral Physiology and Metabolism		
INSTITUTE AND LOCATION NIA, NIH, Bethesda, Maryland, 20892		
TOTAL STAFF YEARS: 2.9	PROFESSIONAL: 2.9	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human <input type="checkbox"/> (b) Human <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Correlational analysis</u> and use of <u>multiple regression and discriminant function analysis</u> show that functional associations involving brain <u>language areas</u> are disrupted in young <u>Down syndrome</u> adults. Down syndrome subjects use similar regions to process language as controls, but to a lesser extent. Subjects with <u>Turner syndrome</u> (45,X), including mosaics, had reduced volume of the <u>hippocampus</u> , and <u>impairment of memory and visuospatial abilities</u> . Mosaic Turner syndrome subjects had volumes of left cerebral hemisphere and subcortical nuclei, and metabolism of left middle temporal region and right/left asymmetry ratio in parietal region intermediate between full Turner syndrome subjects and controls. There is a distinctive cognitive profile in <u>fragile X syndrome</u> that is distinct from other forms of mental retardation. An enlarged brain and altered functional interactions among <u>frontal and subcortical brain regions</u> may contribute to the <u>behavioral</u> effects in this disorder; more focal changes in brain structure and metabolism may be related to <u>cognitive</u> changes.		



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00410-01 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Mechanism and Therapeutic Interventions for Vascular Dementia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: H-C. Lee Chief, Unit of LN, NIA
Clinical Pharmacology LN, NIA
A. Dani Visiting Fellow LN, NIA
Others: M. Mentis Senior Staff Fellow LN, NIA
G. Alexander Staff Fellow LN, NIA
C. Grady Research Psychologist LN, NIA
B. Horwitz Res. Mathematician LN, NIA

COOPERATING UNITS (if any)

George Washington University Medical Center, Georgetown University Medical Center

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia Section

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
We found significant cerebral atrophy and abnormal cerebral glucose metabolism in well-treated hypertensive but otherwise healthy people. These findings suggest that longstanding hypertension can result in structural and metabolic brain changes that predate the clinical evidence of vascular dementia or stroke. These findings suggest that good blood pressure control with a conventional antihypertensive regimen fails to prevent hypertension-induced brain damage. White matter abnormalities, including periventricular hyperintensity on magnetic resonance imaging (MRI) or leukoaraiosis on computerized tomography (CT) occur in up to 13% of patients who had first MRI. This change is associated with advancing age and hypertension and has been suggested to be the basis for vascular dementia, but the clinical significance of white matter abnormalities in healthy people and in patients with dementia of the Alzheimer's type (DAT) is unknown. Extensive white matter change can be found in patients with slowly progressive dementia clinically indistinguishable from DAT. These patients differ from DAT patients without white matter change in the pattern of cerebral metabolism of glucose, as measured with positron emission tomography. Three such patients who came to autopsy showed Alzheimer's disease neuropathology and severe cerebral amyloid angiopathy. White matter hyperintensity volumes in older normotensive healthy subjects were significantly predictive of increased lateral ventricular volume, reduced brain volume, and reduced cognitive scores, and lower whole brain and frontal lobe glucose metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH
SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00411-05 LN

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the
Brain Anatomy in Aging and Dementia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal

PI: J. VanMeter Chief, Clinical Magnetic LN, NIA
Resonance Imaging Unit
D. Murphy Visiting Scientist LN, NIA
B. Horwitz Chief, PET Unit LN, NIA
Others: M. Schapiro Chief, BADS LN, NIA
C. DeCarli Senior Staff Fellow ERB, NINDS
S. Rapoport Chief LN, NIA

COOPERATING UNITS (if any)

ERB, NIS, and DIR, NINDS; CC Radiology; General Electric Co., Schnectady, NY

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia Section

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human (b) Human (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Age-related subcortical gray matter atrophy, ventricular dilatation, and expansion of peripheral cerebrospinal fluid (CSF) volume were found in healthy men using magnetic resonance imaging (MRI), employing a threshold technique which allows quantification of volume. MRI showed a significant age related decrease of posterior frontal, but not of temporal lobe volume in healthy men. Men with mild Dementia of the Alzheimers type (DAT) had significantly less brain matter than did healthy age-matched controls. Discriminant analysis of MRI volumes completely separated DAT patients from healthy age- and sex-matched controls. Volumetric analysis of MRI images in Turner's syndrome (TS) revealed smaller hippocampi, basal ganglia and parieto-occipital brain matter than matched controls. Hippocampal volumes and memory test scores were reduced independently of 'X chromosome dosage' in TS subjects, suggesting a role for sex steroids in brain development and death of hippocampal neurons. White matter hyperintensities in healthy controls were found to predict increased ventricular volume, reduced brain volume, and reduced cognitive scores. Using ³¹P MR spectroscopy phosphorus metabolite concentrations were not found to differ between DAT patients and controls despite reduced glucose metabolic rates in the DAT patients. Disturbances in cellular phosphate energy reserves and membrane phosphoester metabolite levels do not appear to play a role in DAT.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Stress, Coping and Personality in Aging Men and Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Robert R. McCrae	Research Psychologist	LPC, NIA
Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA

COOPERATING UNITS (if any)
Longitudinal Studies Branch

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Personality, Stress and Coping

INSTITUTE AND LOCATION
National Institute on Aging, Gerontology Research Center,
Baltimore, MD 21224

TOTAL MAN-YEARS: 2.1 PROFESSIONAL: 1.1 OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
X (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Participants in the Baltimore Longitudinal Study of Aging were asked to indicate which of a set of coping responses they had used in dealing with a loss, threat, or challenge experienced in the previous six months. Responses were correlated with measures of the five major dimensions of personality. An item-level analysis showed predictable associations between enduring dispositions and specific coping responses. Longitudinal research on personality, stress and coping will continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1993 to, September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Basic Research in Personality

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
 (Name, title, laboratory, and institute affiliation.)

Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Robert R. McCrae	Research Psychologist	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Personality and Cognition

SECTION

Personality, Stress and Coping

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224

TOTAL MAN-YEARS: 1.3 PROFESSIONAL: 1.1 OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Personality can be defined in terms of enduring individual differences in emotional, interpersonal, experiential, and motivational styles. The five factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness provide a comprehensive taxonomy of personality traits for the description of personality in aging men and women. Two studies were conducted as part of continuing program of research on these factors. In the first, scales intended to represent an alternative three-factor model of personality were shown to fit better within the five-factor model. In the second, Openness to Experience was related to a measure of the permeability of consciousness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Psychosocial Predictors of Mental and Physical Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Paul T. Costa, Jr.	Chief, LPC	LPC, NIA
Robert R. McCrae	Research Psychologist	LPC, NIA
Alan B. Zonderman	Research Psychologist	LPC, NIA
Stephanie V. Stone	Staff Fellow	LPC, NIA
Chester A. Schmidt	Special Volunteer	FSKMC

COOPERATING UNITS (if any)
Psychiatry Department, FSKMC

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Personality, Stress and Coping

INSTITUTE AND LOCATION
National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224

TOTAL MAN-YEARS: 2.8 PROFESSIONAL: 1.6 OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interpersonal expression of antagonism is the component of the Type A Behavior Pattern (TABP) that confers risk for coronary heart disease (CHD). Other TABP components such as pressured speech, job involvement, and a hurried pace of life are unrelated to CHD. One new rating, Observed Style, assesses the actual expression of antagonism in the interview setting; the other, Self-Description, rates the degree of antagonism in what respondents say. When these ratings were used to rate Structured Interviews from the Multiple Risk Factor Intervention Trial (MRFIT), results confirmed previous research findings: Observed Style conferred risk for CHD. Ongoing research addresses whether the addition of Self-Description, what respondents say as opposed to how they act during the interview, adds to the predictive validity of Observed Style.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Early Markers of Alzheimer's Disease in Longitudinal Participants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Alan B. Zonderman	Research Psychologist	LPC, NIA
Susan Resnick	Senior Staff Fellow	LPC, NIA
Leonard M. Giambra	Research Psychologist	LPC, NIA
Claudia H. Kawas	Staff Neurologist	FSKMC
E. Jeffery Metter	Medical Officer	LSB, NIA
Paul T. Costa, Jr.	Chief, LPC	LPC, NIA

COOPERATING UNITS (if any)

Longitudinal Studies Branch
Department of Neurology, FSKMC

LAB/BRANCH

Laboratory of Personality and Cognition

SECTION

Cognition Section

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224

TOTAL MAN-YEARS: 5.5

PROFESSIONAL: 2.5

OTHER: 3.0

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

(b) Human tissues

(c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Participants in the BLSA aged 60 and older were examined to detect changes in psychological, neurological, and neuropsychological tests related to early signs of Alzheimer's disease (AD). Specific error types on the Benton Visual Retention Test, a test of short-term visual memory, were examined for 2000 participants in the Baltimore Longitudinal Study of Aging. Cross-sectional analyses indicated that all errors increased with age, but differences between age groups in error profiles suggested greater age effects for distortions, omissions, and rotations. Longitudinal analyses of age changes for a subset of 673 participants with three BVRT assessments were consistent with the cross-sectional data and indicated intra-individual increases with age in distortions, omissions and rotations. Although women made more omission errors, men showed steeper increases with age. These findings suggest that aging affects all types of BVRT errors, but has differential effects on particular types of errors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Attentional Processes in Normal and Impaired Elderly

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.)
(Name, title, laboratory, and institute affiliation.)

Leonard M. Giambra Research Psychologist LPC, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Personality and Cognition

SECTION
Cognition

INSTITUTE AND LOCATION National Institute on Aging, Gerontology Research Center, Baltimore, MD
21224

TOTAL MAN-YEARS: 1.0 PROFESSIONAL: 0.50 OTHER: 0.50

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects	(b) Human tissues	(c) Neither
(a1) Minors		
(a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We seek to understand the psychological and biopsychological aspects of normal and pathological aging in terms of attention and attentional processes. We are also concerned with applying that knowledge to develop strategies for improving attentional and cognitive functioning. This year we continue the development of an attention-switching functional test which will be used in our attempts to provide early markers of Alzheimer's Disease. We also begun 6 year longitudinal testing using our sustained attention task and 22 year longitudinal testing using our retrospective self-report questionnaire on spontaneous attention switching to the contents of consciousness.

PROJECT NUMBER Z01 AG 07040 04 EDBP
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Honolulu Aging Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Lon R. White, M.D., M.P.H., Chief, Asia-Pacific Office, EDBP

Richard J. Havlik, M.D., M.P.H., Associate Director, EDBP

James Norman, Ph.D., Field Studies Branch, EBP, NHLBI

G. Webster Ross, M.D., Project Neurologist, DVA

Carolyn Murdaugh, Ph.D., Senior Investigator, NCNR

COOPERATING UNITS (if any)

National Heart Lung and Blood Institute (NHLBI)

National Institute for Nursing Research (NCNR)

Department of Veterans Affairs (DVA)

LAB/BRANCH

Epidemiology, Demography, and Biometry Program

SECTION

Asian-Pacific Office

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

.9

PROFESSIONAL:

.85

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

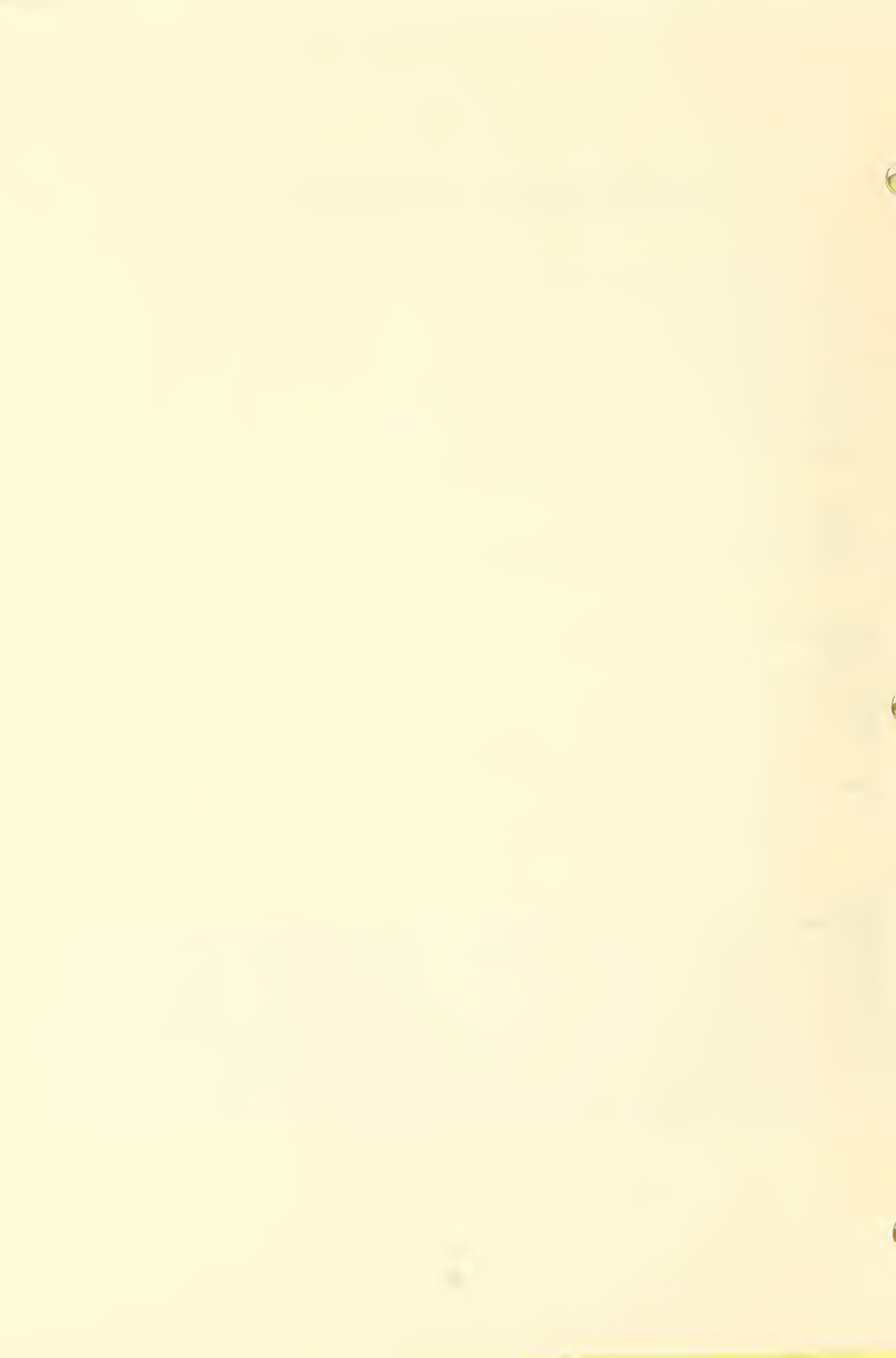
(a1) Minors

(a2) Interviews

X (b) Human tissues

(c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The NIA supplements a research project sponsored by the NHLBI and supported through an NHLBI contract with Kuakini Medical Center in Honolulu, Hawaii, to allow for research on aging and dementia among study participants. The Honolulu Heart Program (HHP) is a prospective study of cardiovascular diseases of American men of Japanese ancestry born from 1900 to 1919 and living on the island of Oahu in 1965. This study focuses on aging, with the emphasis on Alzheimer's disease and multi-infarct dementia. The first wave of examinations was completed in 1993, with call-back dementia evaluations completed in June 1994. A second cycle of examinations to identify new (incident) cases began in March/April 1994. The current plan is to establish a new NIA contract independent of the NHLBI contract, and, when it is in place (target date September 1994), to transfer funding of all NIA research activities to it.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1993 National Mortality Followback Survey

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Dwight B. Brock, Ph.D., Chief, Biometry Office, EDBP, NIA

Eleanor Simonsick, Ph.D., Epidemiology and Demography Office, EDBP, NIA

Paul Placek, Ph.D., Chief, Followback Survey, NCHS

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

Epidemiology, Demography, and Biometry Program

SECTION

Biometry Office

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

.08

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this agreement is to support the collection and analysis of data on cause of death and characteristics of the last year of life in the planning of the 1992 Pretest and 1993 Main Survey of the 1993 National Mortality Followback Survey (NMFS), conducted by NCHS, CDC. This survey will supplement information from death certificates in the vital statistics file with information on characteristics of the decedent. The pretest will examine approximately 800 deaths of individuals aged 15 years and over who died in 1992. The main survey will examine approximately 20,000 deaths of individuals aged 15 years and over who died in 1993. This will include 1,000 deaths to centenarians.

The final version of the questionnaire for this survey was submitted to the Office of Management and Budget (OMB) for clearance during the winter, and final clearance was received recently. The survey has now gone into the field, with Census Bureau interviewers contacting the next of kin of the decedents. Interviewing will continue for approximately one year, and a preliminary version of the data set will be made available to the sponsors of the survey approximately one year thereafter.



Z01 AG 07040 04 EDBP
Honolulu Aging Study
White, L R

The NIA supplements a research project sponsored by the NHLBI and supported through an NHLBI contract with Kuakini Medical Center in Honolulu, Hawaii, to allow for research on aging and dementia among study participants. The Honolulu Heart Program (HHP) is a prospective study of cardiovascular diseases of American men of Japanese ancestry born from 1900 to 1919 and living on the island of Oahu in 1965. This study focuses on aging, with the emphasis on Alzheimer's disease and multi-infarct dementia. The first wave of examinations was completed in 1993, with call-back dementia evaluations completed in June 1994. A second cycle of examinations to identify new (incident) cases began in March/April 1994. The current plan is to establish a new NIA contract independent of the NHLBI contract, and, when it is in place (target date September 1994), to transfer funding of all NIA research activities to it.

Z01 AG 07050 02 EDBP
1993 National Mortality Followback Survey
Brock, D B

The purpose of this agreement is to support the collection and analysis of data on cause of death and characteristics of the last year of life in the planning of the 1992 Pretest and 1993 Main Survey of the 1993 National Mortality Followback Survey (NMFS), conducted by NCHS, CDC. This survey will supplement information from death certificates in the vital statistics file with information on characteristics of the decedent. The pretest will examine approximately 800 deaths of individuals aged 15 years and over who died in 1992. The main survey will examine approximately 20,000 deaths of individuals aged 15 years and over who died in 1993. This will include 1,000 deaths to centenarians.

The final version of the questionnaire for this survey was submitted to the Office of Management and Budget (OMB) for clearance during the winter, and final clearance was received recently. The survey has now gone into the field, with Census Bureau interviewers contacting the next of kin of the decedents. Interviewing will continue for approximately one year, and a preliminary version of the data set will be made available to the sponsors of the survey approximately one year thereafter.

Z01 AG 07060 02 EDBP
Biological Mediators of the Disease-Weight Loss Relation Among Older Persons in the Framingham Heart Study
Harris, T

The purpose of this agreement is to fund the drawing, transporting, processing and analysis of blood specimens from the Framingham Heart Study cohort in Examination Cycle 22 and the collection of bioelectric impedance data from the members of the Framingham Heart Study cohort in Examination Cycle 22.

Data collection in Framingham is complete and final files are being prepared. The measurement of cytokine levels in mononuclear cells at Tufts University, levels of inflammatory proteins and associated other biochemistries have been successful. Methodologic work over the year suggests that the delay of an hour in laboratory processing while the specimens are being transported from Framingham to Tufts has some effect on increasing the measurements; however, this effect is present in health younger persons as well as elderly and therefore should not affect the analysis of correlates of levels. Preliminary data on both stimulated and unstimulated levels of IL-1, TNF and IL-6 show fairly normal distributions of secretors and non-secretors for each cytokine.

Z01 AG 07080 01 EDBP

Effect of Weight on Change in Body Composition and Function in the Framingham Heart Study
Harris, T

In the Framingham Heart Study, about 22 percent of men aged 65 and older and 15 percent of women lost 5 percent or more of body weight. To investigate the relationship of weight change to change in body composition in this older cohort, we entered into an agreement to obtain body composition measurements with dual energy x-ray absorptiometry (DEXA) for muscle, bone and body fat as well as to obtain reasons for weight change. This study began in December of 1993.

Z01 AG 07090 01 EDBP

Body Composition Measurement in the Cardiovascular Health Study
Harris, T

All members of two Cardiovascular Health Study (CHS) Field Center cohorts will be invited to participate in a study using dual energy x-ray absorptiometry (DEXA) for whole body composition and bone mineral density of the hip: Bowman-Gray School of Medicine in Winston-Salem, North Carolina and the University of Pittsburgh. OMB approval was obtained and the agreement was signed in July 1994 with NHLBI and measurements were begun at that time in the Pittsburgh, Pennsylvania CHS Field Center. Data collection at the CHS Field Center sites is estimated to require one year. Receipt of data and subsequent analysis of data by the CHS Coordinating Center is estimated to require one additional year for a total of 2 years.

The study will require approximately 15 minutes of scan time for each participant. A short set of questions relevant to the body composition measure will be asked as well. Estimates of bone mineral density from the hip, total body segmental bone mineral content, fat and lean mass, will be forwarded to the CHS Coordinating Center. Quality control and clean-up of the DEXA data will be carried out through the CHS Coordinating Center. Analyses of the data will be carried out by the CHS Coordinating Center.

Z01 AG 07100 01 EDBP

Measuring Exercise Tolerance in Frail Older Adults

Simonsick, E M

The purpose of this agreement is to develop and test a set of measures and protocols to measure exercise tolerance and aerobic capacity for use in population-based studies of older adults; and to develop a method for scaling performance across tests.

Hard copy records of examination results and raw data files will be delivered within 30 days of the completion of the last examination. Description and documentation of testing procedures, including reliability data, in a form suitable for scientific use will be delivered within 3 months of the last examination. This project is a 10-month commitment.



DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Mediators of the Disease-Weight Loss Relation Among Older Persons in the Framingham Heart Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Tamara Harris, M.D., M.S. Chief, Geriatric Epidemiology Office, EDBP, NIA

Ronald Prior, Ph.D., Scientific Program Officer, Human Nutrition Research Center on Aging

Irwin Rosenberg, M.D., Director, Human Nutrition Research Center on Aging at Tufts University

COOPERATING UNITS (if any)

USDA-Agricultural Research Service

LAB/BRANCH

Epidemiology, Demography, and Biometry Program

SECTION

Geriatric, Demography, Office

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this agreement is to fund the drawing, transporting, processing and analysis of blood specimens from the Framingham Heart Study cohort in Examination Cycle 22 and the collection of bioelectric impedance data from the members of the Framingham Heart Study cohort in Examination Cycle 22.

Data collection in Framingham is complete and final files are being prepared. The measurement of cytokine levels in mononuclear cells at Tufts University, levels of inflammatory proteins and associated other biochemistries have been successful. Methodologic work over the year suggests that the delay of an hour in laboratory processing while the specimens are being transported from Framingham to Tufts has some effect on increasing the measurements; however, this effect is present in health younger persons as well as elderly and therefore should not affect the analysis of correlates of levels. Preliminary data on both stimulated and unstimulated levels of IL-1, TNF and IL-6 show fairly normal distributions of secretors and non-secretors for each cytokine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Weight on Change in Body Composition and Function in the Framingham Heart Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Tamara Harris, M.D., M.S. Chief, Geriatric Epidemiology Office, EDBP, NIA

Ronald Prior, Ph.D., Scientific Program Officer, Human Nutrition Research Center on Aging

Irwin Rosenberg, M.D., Director, Human Nutrition Research Center on Aging at Tufts University

COOPERATING UNITS (if any)

USDA-Agricultural Research Service

LAB/BRANCH

Epidemiology, Demography, and Biometry Program

SECTION

Geriatric Epidemiology Office

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

(a) Human subjects

(b) Human tissues

(c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the Framingham Heart Study, about 22 percent of men aged 65 and older and 15 percent of women lost 5 percent or more of body weight. To investigate the relationship of weight change to change in body composition in this older cohort, we entered into an agreement to obtain body composition measurements with dual energy x-ray absorptiometry (DEXA) for muscle, bone and body fat as well as to obtain reasons for weight change. This study began in December of 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Body Composition Measurement in the Cardiovascular Health Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Tamara Harris, M.D., M.S., Chief, Geriatric Epidemiology Office, EDBP, NIA

Teri Manolio, M.S., M.H.S., Medical Officer, Division of Epidemiology and Clinical Applications, NHLBI

COOPERATING UNITS (if any)

NHLBI Division of Epidemiology and Clinical Applications

LAB/BRANCH

Epidemiology, Demography, and Biometry Program

SECTION

Geriatric Epidemiology Office

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects

X (b) Human tissues

O (c) Neither

O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

All members of two Cardiovascular Health Study (CHS) Field Center cohorts will be invited to participate in a study using dual energy x-ray absorptiometry (DEXA) for whole body composition and bone mineral density of the hip: Bowman-Gray School of Medicine in Winston-Salem, North Carolina and the University of Pittsburgh. OMB approval was obtained and the agreement was signed in July 1994 with NHLBI and measurements were begun at that time in the Pittsburgh, Pennsylvania CHS Field Center. Data collection at the CHS Field Center sites is estimated to require one year. Receipt of data and subsequent analysis of data by the CHS Coordinating Center is estimated to require one additional year for a total of 2 years.

The study will require approximately 15 minutes of scan time for each participant. A short set of questions relevant to the body composition measure will be asked as well. Estimates of bone mineral density from the hip, total body segmental bone mineral content, fat and lean mass, will be forwarded to the CHS Coordinating Center. Quality control and clean-up of the DEXA data will be carried out through the CHS Coordinating Center. Analyses of the data will be carried out by the CHS Coordinating Center.

PROJECT NUMBER Z01 AG 07100 01 EDBP
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1993 to September 30, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Measuring Exercise Tolerance in Frail Older Adults

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Eleanor M. Simonsick, Ph.D., Epidemiologist, Epidemiology and Demography Office, EDBP,
Eric T. Poehlman, M.D., Geriatrics Service, Baltimore Veterans' Administration Medical Center

COOPERATING UNITS (if any)

Baltimore Veterans' Administration Medical Center

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NIA, NIH, Bethesda, MD 20892

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PROFESSIONAL:

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.05

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(a) Human subjects

(b) Human tissues

(c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this agreement is to develop and test a set of measures and protocols to measure exercise tolerance and aerobic capacity for use in population-based studies of older adults; and to develop a method for scaling performance across tests.

Hard copy records of examination results and raw data files will be delivered within 30 days of the completion of the last examination. Description and documentation of testing procedures, including reliability data, in a form suitable for scientific use will be delivered within 3 months of the last examination. This project is a 10-month commitment.

CONTRACT

Name and Number: The Johns Hopkins University School of Medicine (N01-AG-1-2112)

Title: Women's Health and Aging Study

Date Contract Initiated: July 1, 1991

Current Annual Level: -0- (Forward funded (\$1,0127,654) in FY93)

Objectives: The overall goal of the study is to understand the causes and course of physical disability (defined as a deviation or alteration in normal functional performance) in older women living in the community. This will be accomplished by (1) screening a representative population of community-dwelling older women to recruit a study cohort of women with moderate to severe dependence in physical functioning, (2) characterizing prevalent diseases and conditions in members of this cohort and assessing the impact of these conditions on physical function, and (3) following the cohort prospectively for a period of 3 years to evaluate change in functional status.

Methods Employed: Participants in the study are selected at random from among women aged 65 and over living in community residences in 12 zip code areas of Baltimore City and County. The initial sample will include 5,500 women, stratified by age so as to ensure sufficient numbers in the oldest age groups. A brief screening interview will identify approximately 1,270 women who are moderately to severely disabled, defined as being disabled in 2 or more of 4 specified domains of functioning. Of these, about 1,000 are expected to agree to participate in the remainder of the study. These women will complete a baseline interview and an examination conducted in their homes by a nurse practitioner that includes performance-based measures of disability. Follow-up interviews will be conducted with participants every 6 months for the next 3 years.

Significance to Biomedical Research: This study has a number of aspects that make it unique as an epidemiologic study and add substantially to previous work done on disability in older populations. Epidemiologic studies in general have tended to study factors leading to the onset of incident disease, with little study of subjects once disease occurs. This study will examine women who already have disease and disability, attempt to understand the diseases underlying that disability and then prospectively evaluate the course of disability and how the underlying diseases as well as health habits, psychological, cognitive, social and other factors affect that course. Unlike previous epidemiologic research on disability, this study will intensively evaluate diseases and physiologic dysfunction that are associated with disability.

CONTRACT

Name and Number: Duke University Medical Center (N01-AG-1-2102)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: January 1, 1991

Current Annual Level: -0- (forward funded (\$507,736) in FY93)

Objectives: The purpose of this project is to conduct epidemiologic investigations in a biracial elderly population, 65 years of age and older, selected from both urban and rural locations in North Carolina.

Methods Employed: Descriptive and analytical epidemiologic studies of existing problems and surveillance of newly developing problems all with an emphasis upon future intervention and prevention have been conducted. Investigators conducted cross-sectional and prospective studies as well as more detailed problem-related studies in a carefully defined and accessible population using standard field and analytical techniques.

Significance to Biomedical Research: The NIA began funding three population studies of the elderly to determine the influences of social, environmental, behavioral, and economic forces on the mortality, morbidity, and utilization of health services in the elderly. These studies, however, were not fully representative of the U.S. elderly; specifically, they did not include a significant proportion of blacks. It is well known that distributions of certain risk factors and diseases differ between U.S. blacks and other racial groups. Therefore, the purpose of the 1984 contract was to conduct epidemiologic investigations in an elderly population of which at least 50 percent is black in order to develop new knowledge concerning the medical and social factors in health and diseases of the aging black population. In addition, both black and white subgroups in the study exhibit an excellent distribution on indicators of socioeconomic status, and participants have been selected from both urban and rural locations.

Proposed Course: A new 7-year contract was awarded to Duke University Medical Center January 1, 1991, to conduct a third in-person survey wave and continue surveillance for major endpoints. In this wave, begun in May 1992, Duke gathered information comparable to that obtained by the other EPESE sites as well as items which are important for the study of the health of older black persons and racial and urban/rural differences. Physical performance measures taken include tests of balance, a timed walk, chair stands, a test of shoulder range of motion, and functional reach. Waist/hip ratio, height and weight, and vision were also ascertained. Blood assays include complete blood count, automated serum chemistries, and HDL cholesterol.

Z01 AG 07100 02 EDBP

Measuring Exercise Tolerance in Frail Older Adults

Simonsick, EM

The objective of this project is to develop a package of brief, safe and reliable measures of exercise tolerance that, in total, is broadly applicable and highly discriminating in population studies of older persons. For the purposes of comparison across studies and assessment of change over time, the inter-relationship of measures of exercise tolerance to one another will be examined. Testing and evaluation is ongoing at the Baltimore Veteran's Administration Medical Center. The following tests of exercise tolerance are being administered sequentially on two occasions 7 to 10 days apart to a volunteer sample of 50 men and women age 70-79 years, 35 of whom have no limitations in lower extremity function and 15 of whom have peripheral arterial disease: (1) self-report physical function; (2) timed measured walks (usual and fast pace 4-meter and 20-meter walks); (3) a 6-minute corridor walk; (4) seated step-test; and (5) treadmill walk-test. Heart rate at work, recovery heart rate, blood pressure, and oxygen consumption were measured. In addition, two questionnaires are being piloted. One is a further modification and refinement of Taylor's Leisure Time Physical Activity questionnaire and the other is a self-report measure of physical function that assesses ease of performance and level of fatigue as well as difficulty in performing higher order functional tasks. At the end of July, 28 of the 50 subjects have completed testing.

Z01 AG 07080 02 EDBP

Effect of Weight on Change in Body Composition and Function in the Framingham Heart Study

Harris, T

To investigate the relationship of weight change to change in body composition in the Framingham Heart Study cohort, we entered into an agreement with the USDA Human Nutrition Research Center on Aging and researchers at Harvard School of Medicine and Boston University School of Medicine to obtain body composition measurements with dual energy x-ray absorptiometry (DEXA) for muscle, bone and body fat as well as to obtain reasons for weight change. Data collection of repeat body composition measurements is almost complete and we anticipate beginning data analysis in January 1996. NIA has had a major interest in frailty, both in terms of epidemiology and in terms of intervention studies. In addition, there is currently interest in trophic factors which may promote conservation of muscle in particular and aid in rehabilitation of frail older persons. This study allowed examination of body composition and mediators of weight loss in a major longitudinal study of now very old persons in which there has been assessment of weight, weight related risk factors, and many of the chronic diseases of importance in old age including heart disease, stroke, congestive heart failure, osteoarthritis, osteoporosis, pulmonary disease, and cancer, as well as indicators of inflammation. From this viewpoint, it would allow broadening of the perspective not only on frailty, but the interaction of frailty with comorbidity.

PROJECT NUMBER Z01 AG 07100 02 EDBP
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Measuring Exercise Tolerance in Frail Older Adults

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Eleanor M. Simonsick, Ph.D., Epidemiologist, Epidemiology and Demography Office, EDBP,
Eric T. Poehلمان, M.D., Geriatrics Service, Baltimore Veterans' Administration Medical Center

COOPERATING UNITS (if any)

Baltimore Veterans' Administration Medical Center

LAB/BRANCH

Epidemiology, Demography, and Biometry Program

SECTION

Epidemiology and Demography Office

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS: PROFESSIONAL: OTHER:

.05

CHECK APPROPRIATE BOX(ES)

X (a) Human subjects (b) Human tissues (c) Neither

 ○ (a1) Minors

 ○ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to develop a package of brief, safe and reliable measures of exercise tolerance that, in total, is broadly applicable and highly discriminating in population studies of older persons. For the purposes of comparison across studies and assessment of change over time, the inter-relationship of measures of exercise tolerance to one another will be examined. Testing and evaluation is ongoing at the Baltimore Veteran's Administration Medical Center. The following tests of exercise tolerance are being administered sequentially on two occasions 7 to 10 days apart to a volunteer sample of 50 men and women age 70-79 years, 35 of whom have no limitations in lower extremity function and 15 of whom have peripheral arterial disease: (1) self-report physical function; (2) timed measured walks (usual and fast pace 4-meter and 20-meter walks); (3) a 6-minute corridor walk; (4) seated step-test; and (5) treadmill walk-test. Heart rate at work, recovery heart rate, blood pressure, and oxygen consumption were measured. In addition, two questionnaires are being piloted. One is a further modification and refinement of Taylor's Leisure Time Physical Activity questionnaire and the other is a self-report measure of physical function that assesses ease of performance and level of fatigue as well as difficulty in performing higher order functional tasks. At the end of July, 28 of the 50 subjects have completed testing.



PROJECT NUMBER Z01 AG 07080 02 EDBP
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of Weight on Change in Body Composition and Function in the Framingham Heart Study

PRINCIPLE INVESTIGATOR (List other professional personnel below the Principle Investigator.)

(Name, title, laboratory, and institute affiliation.)

Tamara Harris, M.D., M.S. Chief, Geriatric Epidemiology Office, EDBP, NIA
Ronald Prior, Ph.D., Scientific Program Officer, Human Nutrition Research Center on Aging
Irwin Rosenberg, M.D., Director, Human Nutrition Research Center on Aging at Tufts University

COOPERATING UNITS (if any)

USDA-Agricultural Research Service

LAB/BRANCH

Geriatric Epidemiology Office

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O (a1) Minors

O (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To investigate the relationship of weight change to change in body composition in the Framingham Heart Study cohort, we entered into an agreement with the USDA Human Nutrition Research Center on Aging and researchers at Harvard School of Medicine and Boston University School of Medicine to obtain body composition measurements with dual energy x-ray absorptiometry (DEXA) for muscle, bone and body fat as well as to obtain reasons for weight change. Data collection of repeat body composition measurements is almost complete and we anticipate beginning data analysis in January 1996. NIA has had a major interest in frailty, both in terms of epidemiology and in terms of intervention studies. In addition, there is currently interest in trophic factors which may promote conservation of muscle in particular and aid in rehabilitation of frail older persons. This study allowed examination of body composition and mediators of weight loss in a major longitudinal study of now very old persons in which there has been assessment of weight, weight related risk factors, and many of the chronic diseases of importance in old age including heart disease, stroke, congestive heart failure, osteoarthritis, osteoporosis, pulmonary disease, and cancer, as well as indicators of inflammation. From this viewpoint, it would allow broadening of the perspective not only on frailty, but the interaction of frailty with comorbidity.



Z01AG00013-20 LCP

Hormones, Hormone Receptors, and Aging. III. Aging and Human Endocrine Regulation.
S. Mitchell Harman, M.D., Ph.D.,

In 11 older men, subcutaneous GHRH nightly for 6 weeks produced a small increase in muscle strength and reduced the need for anaerobic metabolism during stressful exercise. In a study of GH and sex steroid administration for 6 months, baseline data from 41 healthy women and men >65y suggest that: (1) Serum IGF-I does not predict integrated GH secretion; (2) GH secretion is unrelated to lean body mass or strength, but inversely correlated with total body fat and abdominal fat mass; (3) abdominal fat is a predictor of triglyceride and HDL cholesterol, whereas total and LDL cholesterol are more closely related to GH secretion; (4) There are no effects of basal GH or IGF-I, on VO_2 max. Thus, age-related reductions in GH alter cardiometabolic risk factors both directly and via effects on abdominal fat. A study of 2800 banked sera from 443 BLSA men aged 25-92 y at entry shows both cross-sectional and longitudinal decreases in testosterone (T) and free T with aging with a constant rate of change after age 30, and independent effects of smoking on sex hormone binding globulin and free T index.

Z01AG00021-32 LCP

Study of Normal Human Variability and Cross-Cultural Aging
Plato, CC

This is an ongoing collaborative effort involving several national and international laboratories to coordinate the collection, evaluation and interpretation of data on normal genetic markers in order to assess the cross-cultural patterns of genetic and extraneous factors as they relate to normative aging and to diseases of late onset, including osteoporosis, osteoarthritis, Alzheimer's disease, breast cancer, amyotrophic lateral sclerosis and parkinsonism dementia. The specific objectives of the study are: 1) To better understand the contributions of genetic and non-genetic factors contributing to the process of normative aging, and 2) To study the cross-cultural variability in the occurrence of dermatoglyphics, lateral dominance, iris structure and other genetic markers in the BLSA participants and other control samples and compare them with those of patients with diseases with late onset.

Z01AG00022-19 LCP

Bone Loss with Age: Epidemiological, Families and Cross-cultural Considerations
Plato, CC

After the third decade of life the human skeleton begins to lose bone. That is, bone mass decreases in relation to bone volume. Endosteal cortical and trabecular bone is lost from both the axial and the peripheral sites of the skeleton. Menopause and the associated estrogen deficiency will enhance bone loss in females. It has also been suspected that bone loss is familial, mainly because of the increased prevalence of osteoporosis in relatives, although there are no satisfactory scientific data to support a genetic control of bone loss. While age-related bone loss does not usually interfere with the normal function of the individual, occasionally it will produce considerable thinning of the cortical bone shell, causing bones to lose their integrity and fracture more readily. Decreases in the trabecular bone would result to vertebral compression of fractures



and fractures of the femoral neck. This project investigates the epidemiological, genetic, cross-sectional, longitudinal and cross-cultural aspects of bone loss among participants of the BLSA, and among genetic isolates from Europe, Japan, Australasia, Central America and Africa.

Z01AG00023-19 LCP

TITLE: Hormones and Aging. Hypothalamic-Pituitary Function in Experimental Animals.
S. M. Harman, M.D., Ph.D., Section Chief, ES, LCP, NIA

Many hormone secretion and tissue responses to hormones that depend on cell membrane receptor transduction are blunted in old animals when compared to young animals. Such changes in hormonal responses are frequently due to alterations in the levels or ratios of stimulatory GTP-binding proteins (G_s) and inhibitory GTP binding proteins (G_i) which mediate many membrane-bound receptor responses intracellularly. We have been conducting investigations of the effects of aging on the G-protein components of cyclase mediated catecholamines receptors in fat cell and hepatic cell membranes, and, more recently, have initiated studies of the effects of aging on responses of skeletal muscle and other tissues to GH and testosterone concentrating on EF-1 α , a G protein which is a key mediator of protein synthesis at the ribosome. Activities in the past year have been devoted mainly to methods development and optimization of new assays for cyclase mediating G-protein complexes, for EF-1 α mRNA to detect gene activation, and for EF-1 α protein in tissue.

Z01AG00093-23 LCP

Cellular Basis of Regulation of the Humoral Immune Response
Nordin, AA

The molecular aspects of T-cell activation and subsequent progression through the cell cycle are investigated using the murine model to establish a basis for the immunodeficiencies associated with advancing age. Comparative studies of polyclonally activated G0 T-cells from young and old C57Bl/6 mice showed that a significantly higher portion of cells derived from the old mice underwent apoptotic cell death which was restricted to the G1 phase of the cell cycle. Irrespective of age apoptosis involved both CD4+ and CD8+ cells. There were significantly higher numbers of CD4+ memory cells within the G0 T-cells from the old mice and was identified as the major apoptotic cell population. In addition to an increase in apoptosis in the activated T-cells from the old mice, a significantly higher number of viable activated cells failed to complete progression through the G1 phase of the cell cycle. Initial evidence suggest that the expression of the genes encoding the G1 cyclin-dependent kinases and the partner cyclins is not significantly different from that observed in T-cells from young mice. The kinetics of the kinase expression in the T-cells from the old mice differs from that observed in young animals suggesting that the family of cyclin-dependent kinase inhibitors may be involved in regulating the kinase activity in the cells from the old mice. Northern analysis of the kinetic expression of specific mRNA for two of these inhibitors i.e. p21 and p27, showed prolonged synthesis in the T-cells from the old mice.

Z01AG00095-22 LCP

The Role of Cell Membrane Structures on Cellular Recognition

Adler, WH

Cell free factors mediate immune function as well as the expression of an inflammatory response. Since the expression of many of these factors is increased in the cells from elderly donors the biologic implication of this finding are very important. Resistance to and severity of infectious diseases in the elderly may be due to a change in the control of cellular function. The mechanism for the increase in the cells from old donors appears to be due to an increase in a subset of memory T cells which synthesize and release these factors. The expression of receptors for the factors as well as an analysis of their ability to generate cellular signals in the cells from young and old mice will provide evidence for the biologic role of these factors in the older individuals.

Z01AG00096-22 LCP

Lymphocyte Activation and Function in Aging Individuals
Brock, MA

Receptor mediated activation of many cell types is followed by motility related events. In T lymphocytes, lateral redistribution of surface receptors is accompanied by aggregation of actin and myosin in cytoplasmic subcaps. Patching and capping of receptors after activation of lymphocytes from aged animals and humans is impaired, and it was inferred from indirect evidence that age-related changes in cytoskeletal functions are responsible. Concanavalin A activation of resting T lymphocytes resulted in actin polymerization in the cytoskeleton of cells from young but not aged C57BL/6 mice. Bypassing the plasma membrane to activate protein kinase C with PMA induced actin polymerization in resting T lymphocytes and in immunomagnetically isolated CD4 and CD8 positive subpopulations from young and aged mice, but the values were lower in cells from aged animals. Light microscopy visualization of the individual cells showed that following activation with PMA, the actin cytoskeleton reorganized and subcaps formed in CD4 and CD8 positive lymphocytes from both young and aged mice. However, in aged animals a significantly smaller percentage (30.5) of the cells exhibited cytoskeletal rearrangement compared to the percentage of cells from young mice (53.5), and the subcellular actin filament morphology differed from that of cells from young animals. The kinetics of the response to PMA did not differ in the two age groups. This suggests that although plasma membrane signalling events are bypassed and actin polymerization is initiated in cells from aged mice, actin filament function/s change with age and may depend on differences in actin binding protein and phosphatidylinositol metabolism. This project will be terminated as the Principal Investigator has retired on June 30, 1995.

Z01AG00104-19 LCP

Clinical Immune Survey of Longitudinal Project Participants
Adler, WH

This project uses participants in the Baltimore Longitudinal Study of Aging (BLSA) to gain insight into the biochemical and molecular mechanisms underlying age-associated changes in human immune function. Recent data suggests that human T lymphocytes activated through different cellular pathways display distinctive patterns of protein phosphorylation, cytokine



synthesis and gene expression, only some of which are age-affected. With increasing age, an increasing large proportion of T-cells undergo cytokine-mediated apoptosis.

Z01AG00213-05 LCP

Tris-sulfotyrosyl dedecapeptide specifically enhanced insulin receptor function *in situ*
Kole, HK

We have recently shown that a synthetic tris-sulfotyrosyl dodecapeptide (3S-peptide-I) that corresponds to the major autophosphorylation domain within the insulin receptor β -subunit potently inhibited protein tyrosine phosphatase (PTPase) activity *in vitro* (Liotta et al. J Biol Chem 1994, 269: 22996-23001). In the present study, we found that the introduction of a N-stearyl derivative of 3S-peptide-I in cells caused a significant increase in the insulin-stimulated phosphorylation of the insulin receptor, with a maximum effect at 25 μ M. In contrast, ligand-stimulated phosphorylation of epidermal growth factor (EGF) receptor was not affected by the presence of N-stearyl 3S-peptide-I. Furthermore, stearyl-3S-peptide-I at a concentration of 50 μ M was capable of stimulating the activity of downstream key signal regulators of insulin action such as phosphatidylinositol 3-kinase and mitogen activated protein kinase by 2-2.5-fold and 1.8-fold, respectively. These data suggest that by inhibiting dephosphorylation of the insulin receptor in intact cells, 3S-peptide-I specifically enhanced insulin signalling.

Z01AG000214-04 LCP

Aging and the pancreas
Egan, JM

Aging is an etiologic factor in non-insulin-dependent diabetes mellitus. In order to characterize the β -cell abnormalities that occur with age, we investigated glucose-stimulated insulin release, pancreatic insulin content and mRNA levels for islet-specific genes in aging Wistar rates. Ten minutes after glucose stimulation, 6-month-old islets had approximately 40% more cells secreting insulin than 24-month old islets ($p < 0.0001$); after 1 hour 67 \pm 1.0% islet cells from 6-month old rat secreted insulin, compared to 51 \pm 3.5% from 24-month-old rat ($p < 0.0001$). The amount of insulin secreted by each β -cell was also less in the older animals ($p < 0.0001$). Despite increases in islet size ($p < 0.0001$) and in β -cell number ($p < 0.0001$) with age, whole pancreas insulin content of 24-month-old pancreas had less insulin than 6-month-old pancreas (0.61 \pm 0.06 vs. 0.84 \pm 0.08 μ g/mg pancreatic protein; $p < 0.05$). Finally, insulin mRNA levels declined to 50% of the newborn value in 24-month-old islets ($p < 0.0001$), while glucagon mRNA levels showed a much smaller decline with age. Somatostatin mRNA levels did not vary significantly. Of great interest is the new information that these changes can be reversed. We treated 5 young (6 months) and 5 old (23 months) Wistar rates with an infusion of human recombinant GLP-1 (1.5 pmol per kg body weight per minute) using an ALZET osmotic pump (1003D) implanted subcutaneously in the neck for 48 hours. Insulin mRNA was increased 3 fold in the GLP-1 infused animals. Even the old rats which have a lower amount of mRNA (50% lower) for insulin than young rats, had a 3 fold increase in insulin mRNA. The abnormality in the insulin secretion with aging was also normalized by this treatment.

Z01AG00216-05 LCP

Glucose and Insulin Metabolism in Normal Aging and Disease

Reubin Andres, M.D.

A comprehensive examination of basal insulin levels and insulin responses to oral glucose loading has been carried out in 472 men and 299 women aged 20 to 96 years. Men had significantly lower body fat content than women, but higher plasma glucose levels, and a much higher waist/hip ratio. Both fasting and past-glucose insulin levels were higher in men, but when adjusted for the sex differences in obesity and glucose levels, this sex difference disappeared. Insulin levels fell progressively with age even when "corrected" for differences in body composition and glucose variables, from 323 pM to 267, 253, and 228 in young (20-39 yr) middle-aged, old, and very old (80-96 yr) individuals. Thus the decline in insulin response cannot be attributed to other age-associated changes. In essence, this demonstrates that a defect in insulin secretion occurs in addition to the previously demonstrated defect in insulin sensitivity with aging.

Data from the 30 year experience of the BLSA were used to provide the evidence needed to remove the upper age limit from the collaborative clinical trial on prevention of diabetes (conversion of subjects from Impaired Glucose Tolerance (IGT) to WHO-classifiable Diabetes Mellitus. The BLSA data set provides the most extensive experience on Caucasian subjects across the adult span of years. Data provided include: (1) conversion rates as modified by sex, BMI, fasting glucose, glucose tolerance, and family history of diabetes; (2) incidence rates of development of coronary heart disease in IGT; (3) conversion formulae of plasma creatinine levels to 24-hr creatinine clearance for potential dry studies; (4) mortality rates in IGT subjects compared to those with normal glucose tolerance.

Data from BLSA participants on incidence of development of diabetes are included in a publication under the auspices of the Diabetes Prevention Program which examines this incidence rate as influenced by age, sex, and race in a summary of the several studies that provide reliable incidence data from various ethnic groups world-wide.

Z01AG00220-03 LCP

Body Composition and Fat Distribution in Aging and Disease

Reubin Andres, M.D.

The goal of this long-term study is to quantify changes in body composition that occur with aging and to determine the significance of these changes to health, morbidity, and mortality. As a spin-off of these analyses normative standards are being developed which are age-specific. A comprehensive summary of all studies reporting longitudinal changes in height has been performed and to these studies has been added the results of analyses of height change with aging in men and women from the Baltimore Longitudinal Study of Aging. From these analyses a clear picture emerges of the nature of the height changes with age; results from several confusing reports whose results are clearly far outside the consensus of these studies can be disregarded. The data show surprisingly, that height continues to increase into the 20s, does not change in the 30s and 40s, then begins to fall at an ever increasing rate into old age with height loss being much more rapid in women than in men. Of importance is the fact that the impact of height loss on computation of the Body Mass Index (a commonly used surrogate for obesity) is quite small and does not explain the finding that the BMI associated with lowest mortality increases progressively with age.



We have reported a remarkable set of longitudinal observations on 24-hr urinary creatinine excretion, an index of total body muscle mass, obtained over a period of more than two decades. Simple height and weight measurements to compute the Body Mass Index is flawed as an estimate of obesity mainly because body weight obviously includes not only adipose tissue but also the markedly variable inter-individual mass of muscle. The question of a possible contributory role of muscle to the correlative role of BMI to health variables has not been comprehensively examined. Data from the BLSA have provided unexpected results: (1) Muscle mass has no predictive value for mortality at any age and (2) Muscle mass is unrelated to the common coronary disease risk factors (glucose tolerance, plasma lipids, or blood pressure).

Z01AG00281-05 LCP

Hormones, Hormone Receptors, and Aging. IV. Hormone Replacement in Menopausal Women. S. Mitchell Harman, M.D., Ph.D.,

In a study of 30 healthy women 65-75 years of age treated for 2 y with constant combined daily oral estrogen and low dose progestin compared with 30 age-matched female BLSA participants who do not receive ERT, 18 treated subjects completing one year showed significant decreases in depression, anger, and tension as measured by the Profile of Mood Scale (n=18), and significant decreases in vasomotor symptoms. In 16 completing the second year, there were significant increases in total body bone mineral density ($3 \pm 7\%$), and lumbar spine density ($7 \pm 1\%$) which correlated with decreases in biomarkers of bone resorption including urinary pyridinoline ($30 \pm 6\%$) and serum osteocalcin ($35 \pm 6\%$). There were also significant decreases in total cholesterol ($6 \pm 2\%$) and LDL cholesterol ($14 \pm 3\%$) and increases in HDL cholesterol ($9 \pm 4\%$). Adverse effects included breast swelling and tenderness and vaginal spotting which decreased from a mean of 2.3 ± 1.3 days/mo at 3 months to $0.3 \pm .2$ days/mo by 1 year. The positive effects on bone, lipids, and affect and minimal adverse effects, suggest that older women should be included in future randomized clinical trials of estrogen use in postmenopausal women. In a longitudinal study of changes in hormone regulation, bone and mineral metabolism, and glucose and lipid metabolism in healthy women during the menopausal transition, 87 women have completed a total of 297 GCRC outpatient visits ranging from 1 to 11 visits per individual. Eight women have completed 12 GCRC inpatient visits for 12 hour nocturnal blood sampling. Cross-sectional data analyses comparing the endocrine profiles to the bone biochemistries, bone density measurements, and body composition assessments were begun in August 1995.

Z01AG000290-09 LCP

Osteoarthritis and Aging
Tobin, JD

Osteoarthritis (OA) is the most common form of arthritis in the elderly, and is a major cause of activity limitation, physical disability and health services utilization in the elderly. As part of the ongoing studies of OA in the Baltimore Longitudinal Study of Aging (BLSA) we examined the association of metabolic and physiologic factors with the presence and progression of knee OA in both sexes. The relationship between cardiovascular disease risk factors [blood pressure, fasting lipids, glucose tolerance and obesity] and knee OA was examined in 464 men and 275

women aged 40 and above. In univariate analysis, women with hypertension had significantly higher odds of knee OA; after adjustment for age and obesity, this association was no longer significant. No association was found for fasting lipids or glucose and knee OA in either sex. The relationship between reproductive and gynecologic factors and knee OA was examined in 229 postmenopausal women aged 40 and above. In univariate analysis, women with knee OA had later mean age at menopause, more years of fertility, and were less likely to have used oral contraceptives than women without knee OA. After adjustment for age, obesity and smoking status, none of these factors were related to the presence of knee OA. There was no evidence of a protective effect of current use of estrogen replacement therapy on knee OA. In 146 subjects with longitudinal xrays, we studied the progression of knee OA using the technique of chondrometry. In 110 subjects without knee OA on baseline films, joint space did not significantly decrease over a mean follow-up of 4 years. In contrast, in 36 subjects with signs of knee OA on baseline films, joint space narrowing occurred at a rate of 0.09 mm/year (P.0.01). Neither age, gender nor body mass index at first radiograph predicted amount of loss of joint space. In addition the above completed work, molecular genetic analysis of fibroblast samples is underway to examine restriction fragment length polymorphisms of the Type II procollagen gene in BLSA subjects with hand and knee radiographs, in order to test the hypothesis that genetic markers may be implicated in the expression of OA, especially generalized (polyarticular) OA. We have also re-initiated the protocol for collecting more knee radiographs on BLSA subjects aged 40 and over, in order to increase the numbers of individual with longitudinal data.

Z01AG000293-06 LCP

Biochemical Parameters of Bone Metabolism: Age and Sex Contrasts

Tobin, JD

Age-related changes in bone mass have been demonstrated in both men and women. Age and sex related differences hormones, nutritional and physiological variables involved in bone turnover are important in elucidating changes in bone physiology in normal aging and disease. Specifically, the relationship age and sex to changes in body composition, both as a marker of obesity and as a measure of the weight bearing load on bone are important factors in bone status and rates of change. The most dramatic rates of change in bone mass occur at the menopause, and changes of body composition, bone mass, and bone markers and hormones are being investigated in a cohort of peri-menopausal women recruited to the Baltimore Longitudinal Study of Aging, who will be followed at 3 month intervals as they traverse the menopause. These studies will allow for the interpretation of the changes that occur prior to and immediately around the time of the cessation of menses, changes that can only be appreciated in a prospective longitudinal study.

Z01AG00441-08 LCP

Host Factors Relating to HIV Infections

Adler, WH

During an HIV-infection there is a loss of T cells with an increase in production and repopulation of the lymphoid system with new T cells. With age there is a decline in the ability to replace the T cells lost in the disease process. Younger HIV- infected patients can experience extended



periods where their CD4+ cell level remains in the normal range and they are free of opportunistic infections. The mechanisms accounting for the T cell loss are still unclear. They are thought to be linked to an autoimmune process and/or an apoptotic event during cellular activation. Both mechanisms play a role. The immune response to HIV involves both anti-HIV antibody as well as anti-HLA antibody. This suggests the possibility that individuals with some HLA haplotypes may be more resistant to the effects of an HIV infection especially since genetics plays a role in resistance to infectious illness as well as to the immune response. Furthermore, genetic manipulation of cell lines allows the cells to resist the detrimental effects of the HIV as well as to resist infection.

Z01AG000876-04 LCP

Regenerating (REG) gene: A paracrine B-cell growth factor
Perfetti, R

The *reg* gene was cloned from a regenerating pancreas following surgical subtotal pancreatectomy and treatment with nicotinamide. Interventions that cause a reduction in β -cell mass or suppression of β -cell function (i.e., implantation of an insulinoma) result in a decrease in *reg* mRNA levels, while interventions that stimulate islet proliferation (i.e., removal of an insulinoma or surgical wrapping/partial occlusion of the pancreatic duct) are associated with marked increases in *reg* mRNA levels. Watanabe has recently shown that administration of recombinant *reg* to 90% depancreatized rats induces β -cell proliferation. Furthermore, *reg* protein has been shown to induce DNA synthesis in isolated islets in culture as well as in islet-derived cell lines. These findings raised the possibility that *reg* may be an important growth and maintenance factor for pancreatic β -cells.

We hypothesize that REG may be a crucial autocrine and/or paracrine growth factor during embryogenesis as well as for maintenance of β -cell function in the adult. The aim of this study was to further explore these hypotheses. The project has been developed in 6 different interrelated studies. 1) Cloning of the mouse pancreatic *reg* gene. 2) Expression study of the mouse *reg* gene during embryogenesis. 3) Characterization of the pattern of gene expression of the two nonallelic mouse *reg* genes during normal aging. 4) *Reg* expression study in an *in vivo* model of islet regeneration. 5) Effect of *reg* protein on pancreatic derived cell lines. 6) Gene mapping and subchromosomal localization of the human *reg* gene.

Z01AG000877-03 LCP

Mechanisms of insulin release
Egan, JM

Glucagon-like peptide (7-36) amide (GLP-1) is a hormone produced in the L cells of the small bowel and proximal colon, and released into the blood stream in response to food ingestion. It belongs to a class of peptides, known as incretins, which enhance insulin release from beta cells in the presence of glucose. It is the most potent of all the known incretins. Under physiological conditions it is required to maintain normal glucose tolerance in humans. Acute studies of GLP-1 have shown that it can synergize with glucose in stimulating insulin secretion both *in vivo* and



in vitro. Here, we studied the effects of long-term exposure of RIN 1046-38 cells, an insulin secreting cell line, to GLP-1 and the mechanisms by which GLP-1 synergizes with glucose in stimulating insulin secretion. Incubation of cells with 100 nM GLP-1 for 12 or 24 hours significantly increased release, intracellular insulin content, and insulin mRNA when compared to cells cultured with glucose alone. The insulinotropic effects of GLP-1 on RIN 1046-38 cells were accompanied by an up-regulation of GLUT-1 and hexokinase I mRNA compared to control cells. We did further studies to investigate the beneficial effects of GLP-1 on beta cells.

Z01AG000881-03 LCP

Glucagon-like peptide: regulation of insulin action at extrapancreatic sites
Montrose-Rafizadeh, C

Non-insulin-dependent diabetes mellitus (NIDDM), type II diabetes) is one of the most common diseases in the elderly population of the USA, and is especially common among minority population. NIDDM is caused by 1) impaired insulin secretion from islets of Langerhans in the pancreas, and 2) impaired sensitivity of peripheral tissues (such as muscle, fat, and liver) to insulin. A promising new approach for treatment of NIDDM is the use of incretin hormones such as glucagon-like peptide (GLP). Incretins have many desirable effects which may help NIDDM patients. Incretins stimulate insulin secretion and inhibit glucagon secretion. In addition we have found that GLP stimulates insulin action at insulin target tissues. For example, glucose uptake and lipid synthesis are stimulated by GLP in cultured adipocytes, and GLP stimulates glucose oxidation and glycogen synthesis in rat myotubes in culture. We have found that GLP receptor mRNA is present in many different tissues from rat, further suggesting extrapancreatic effects of GLP.

Our data also suggest the presence of different GLP receptor isoforms in pancreas versus extrapancreatic tissues. We have evidence that GLP receptor signaling cascade acts differently in pancreas versus extrapancreatic tissues. Further, different agonist specificity for GLP analogues are noted in pancreas versus extrapancreatic tissues. This suggests the exciting possibility that it may be possible to selectively activate GLP receptor isoforms in different tissues in NIDDM therapy. Our goal is to devote effort to molecular cloning of GLP receptor isoforms and the study of signal transduction involved in GLP action to help develop new therapeutic agents for NIDDM.

Z01AG000882-02 LCP

Thiol-Biotinylation of the Insulin Receptor
Bernier, M

We examined the reactivity of insulin receptor sulfhydryls to biotinylation in Chinese Hamster Ovary cells that express high levels of human insulin receptors (CHO/HIRc cells). Following the biotinylation reaction, the insulin receptor was purified by immunoprecipitation, and resolved by SDS-polyacrylamide gel electrophoresis before electrotransfer to membranes. The use of enzyme-linked streptavidin in conjunction with a chemiluminescent technique allowed the detection of thiol-biotinylated receptor β -subunit, with no modification of the alpha-subunit. In



cells expressing large numbers of IGF-1 receptors, the same technique enabled the detection of thiol-biotinylated IGF-1 receptors as well. Thiol-alkylation of intact CHO/HIRc cells with an impermeant reagent did not impair the ability of maleimidobiotinyl biocytin (MBB) to biotinylate sulfhydryls on the receptor β -subunit after cell permeabilization with digitonin. In contrast, thiol-alkylation of digitonin-permeabilized cells prevented MBB-induced receptor biotinylation. The basal and insulin-activated insulin receptors exhibited insulin receptors exhibited a comparable reactivity to MBB. Furthermore, the use of affinity-purification on monomeric avidin-agarose enabled us to learn that the biotinylation reaction was near quantitative. MBB had no effect on insulin binding nor on receptor autophosphorylation and insulin-dependent receptor kinase activity. However, basal levels of receptor kinase activity were significantly elevated by thiol biotinylation. Further, in the presence of vanadate, MBB retained the ability to enhance receptor kinase activity in permeabilized cells, consistent with the notion that this increased exogenous substrate phosphorylation was not accounted for by inactivation of protein tyrosine phosphatases. The dephosphorylation of thiol-biotinylated, ^{32}P -labeled insulin receptors by particulate protein tyrosine phosphatases was not affected. These results suggest that modification of reactive sulfhydryls located in the cytoplasmic domain of the insulin receptor β -subunit may play a critical role in insulin receptor function.

Z01AG00883-02 LCP

Chemical Synthesis of Novel Potent Inhibitors of Protein Tyrosine Phosphatases

Kole, HK

Peptides containing phosphonate based, non-hydrolyzable phosphotyrosyl (pTyr) mimetics and also arylphosphonate-containing small molecules have been previously shown to be competitive inhibitors of protein-tyrosine phosphatases (PTPases). These agents suffer from low cellular penetration which is partially attributable to ionization of the phosphonate group at physiological pH. We have developed the non-phosphorous containing pTyr mimetic, L-O-malonyltyrosine (L-OMT) and its fluoro-derivative (FOMT) and incorporated them into a hexamer peptide Ac-D-A-D-E-X-L-amide (X = L-OMT or FOMT). Both peptide derivatives potently inhibited dephosphorylation of insulin receptor by a recombinant PTPase, PTP-1B. FOMT-containing peptide showed 10-fold higher activity compared to its L-OMT analog. Prodrug protection of L-OMT or FOMT moieties as carboxylic acid diester could potentially increase cellular penetration, thereby making them valuable reagents for cellular studies.

Z01AG000884-01 LCP

Arginine and Insulin Response in Adipocytes

Bernier, M

The present study was undertaken to define the role of L-arginine (L-Arg) in glucose metabolism in differentiated 3T3-L1 adipocytes in culture. L-Arg alone had no effect on 2-deoxyglucose uptake or basal glycogen synthesis, but this amino acid increased by 153±10% ($p < 0.01$) the incorporation of glucose into glycogen in insulin-treated cells. L-Glutamate (L-Glu), a major metabolite of L-Arg, also enhanced insulin-stimulated glycogen synthesis. The response to insulin was not altered by L-lysine (L-Lys), but the effect of L-Arg was markedly attenuated by L-Lys. Cell incubation with L-Arg markedly enhanced arginase-mediated urea synthesis while

L-Lys abolished this response. The stimulatory effect of L-Arg on insulin-stimulated glycogen synthesis did not appear to be accounted for by the generation of polyamines or the production of nitric oxide, both potentially derived from the enzymatic conversion of L-Arg. In the presence of insulin, cellular ATP levels were significantly increased by L-Arg, L-Glu and L-Lys as well. These data suggest that metabolic degradation of L-Arg not related to citric acid cycle activity is important in the mechanism by which L-Arg enhances insulin-stimulated glycogen synthesis.

Z01AG000885-01 LCP

Receptor Tyrosine Kinase and DNA Repair

Bernier, M

We report the characterization of a member of the DNA excision repair gene family that is enhanced in cells expressing high levels of insulin receptors or various growth factor receptor tyrosine kinases. The partial hamster ERCC-1 cDNA was cloned by reverse-transcription polymerase-chain reaction of total cellular RNA extracted from Chinese hamster ovary cell lines expressing human insulin receptors (CHO/HIRc). It encodes a protein whose overall homology at the amino acid level is 91.3% and 96% with human and murine ERCC-1, respectively. CHO/HIRc cells and cells overexpressing functionally active IGF-1 receptors and EGF receptors induced an increase of mRNA levels for ERCC-1 gene versus control CHO/neo cells. The enhanced expression of ERCC-1 mRNA in CHO/HIRc cells compared to control CHO/neo cells cannot be ascribed to cell cycle-dependent expression of the ERCC-1 gene. Moreover, this activation of ERCC-1 gene was absent in cells expressing kinase-deficient insulin receptors or a mutant form of the receptor with a substitution of tyrosine for phenylalanine at position 960 in the juxtamembrane region of the protein. There was a correlation between the level of ERCC-1 gene expression and cell survival after UV irradiation. Finally, the enhanced ERCC-1 gene expression was independent of glucose utilization since the rate of glucose consumption was identical in all cell lines. Taken together, these data support an association between receptor tyrosine kinase activity and enhancement of ERCC-1 expression and function.

Z-1AG000886-01 LCP

Modulation of Insulin receptor Function

Bernier, M

The role of the insulin receptor C-terminal domain in the regulation of insulin signal transduction was studied with a synthetic peptide (peptide HC) whose structure corresponds to residues 1293-1307 of the insulin proreceptor sequence. Peptide HC enhanced insulin-stimulated autophosphorylation of the insulin receptor in cell-free systems and in digitonin-permeabilized cells without any detectable effect on basal autophosphorylation levels on receptor dephosphorylation. The peptide was modified by addition of a stearyl moieties at its N-terminus, and introduced in intact Chinese hamster ovary (CHO) cells transfected with an expression plasmid encoding the human insulin receptor. We found that stearyl-peptide HC enhanced several fold insulin-stimulated insulin receptor autophosphorylation while having no effect on ligand-stimulated receptor phosphorylation activity in CHO cells overexpressing either the IGF-1 receptor or EGF receptor. To explore the effect of stearyl-peptide HC in regulating insulin signaling, we evaluated the association of phosphatidylinositol 3'-kinase (PI 3'-K) to tyrosine

phosphorylated insulin receptor substrate-1 (IRS-1), and the levels of phosphorylation of mitogen-activated protein (MAP) kinase by hyperphosphorylation gel shift assay. Loading of cells with stearyl-peptide HC resulted in a 1.7-fold increase in the amount of insulin-stimulated PI 3'-K activity detected in anti-IRS-1 immunoprecipitates, and a 1.8-fold increase in MAP kinase hyperphosphorylated species. Taken together our data provide evidence for the important role of the sequence 1293-1307 in the C-terminus of the insulin receptor in the transmission of biological effects and could account, as least in part, for receptor specificity.

Z01AG000887-01 LCP

Studies on the regulation of the insulin -sensitive glucose transporter, GLUT4
Egan, J

Insulin is necessary for glucose uptake in the periphery. It induces the translocation of GLUT4, a specific insulin-sensitive glucose transporter, to the plasma membrane of fat cells and muscle cells. Chronic elevation of insulin has been known to cause downregulation of this transporter, leading to reduced insulin-mediated glucose uptake. The fact that glucagon-like peptide-1 (GLP-1) an incretin hormone, modulated insulin signalling in the 3T3-L1 adipocytes (Endocrinology 135:2070-2075, 1994) prompted us to investigate the effects of GLP-1 on the GLUT4 transporter in these cells. It appeared that GLP-1 could reverse the downregulation induced by high concentration of insulin on GLUT4 mRNA levels. GLP-1 also caused an increase in the levels of GLUT1 mRNA and protein, GLUT1 being widely distributed among all tissues. In conjunction with these findings, we also showed that the downregulation of GLUT4 mRNA induced by insulin in cells incubated with 22mM glucose could be prevented by reducing the glucose concentration in the medium. The C/EBP family of transcription factors is coexpressed with adipocyte genes (e.g. GLUT4) during the differentiation of 3T3-L1 adipocytes. We looked at the relationship between *gadd 153* (which encodes a C/EBP-related protein that lacks a functional DNA-binding domain), C/EBP alpha and GLUT4, and found an association between the mRNA levels for C/EBP and GLUT4 in a variety of culture conditions. It appeared, however, that the levels of *gadd 153* mRNA differed from that of C/EBP alpha depending on the glucose levels in the culture medium.

Z01AG00901-03 LCP

Dietary and nutritional factors in aging, health, and disease
Denis C. Muller, M.S.

Nutritional evaluation in the male and female participants of the Baltimore Longitudinal Study of Aging (BLSA) has been carried out by periodic collection of 7-day dietary diaries and by measurement of vitamin levels in plasma. The diary technique has provided data over a 35 year period in men and a 15 year period in women. The ages of the participants range from 20 to 95 years. Since the BLSA is a multi-disciplinary study, it is possible to carry out correlations between nutritional intakes and levels and other potentially related variables and outcomes.

The results of longitudinal changes in diet (aging and secular effects) over three decades (1960s, 1970s, and 1980s) have been reported. We have re-initiated dietary diary collection for the 1990s by establishing collaborations with USDA and HNRCA scientists (see above).

There are no data sets elsewhere on nutritional variables in human beings of comparable duration, reliability, number of subjects, and association with important physiologic and outcome variables.

Plasma samples collected under the conditions for assay of trace minerals in the BLSA population are currently being analyzed by Dr. Hallfrisch. This will permit tests of the various hypothesized roles of trace minerals in aging and disease.

A study of two groups of older men who differ markedly in their activity patterns but who are otherwise carefully matched for body mass index, for age, and for health status has been published. The study is unique in having conducted detailed dietary evaluation in these free-living 58 to 75 year old men. Sixteen endurance-trained senior athletes were matched with 24 inactive but healthy participants in the Baltimore Longitudinal Study of Aging. The aerobic capacity of the older athletes was 50% higher on average than that of the sedentary group. Despite comparable body weights, the sedentary group had 20% higher body fat. The fat distribution pattern was also significantly more favorable in the athletes (waist to hip ratio of 0.87 vs 0.92). Although the weight for height ratios were comparable, the athletes had a significantly different dietary pattern in that the caloric intake was higher and the distribution of calories showed a higher intake of protein and carbohydrate and a lower intake of fat.

Z01AG00903-01 LCP

Establishment and Maintenance of Banked BLSA Blood Samples

Reubin Andres, M.D.

The Baltimore Longitudinal Study of Aging was initiated in 1958 (men) and 1978 (women). Storage of samples of blood or blood fractions began in 1963 and has been systematically continued since that time. Storage of multiple individual samples of serum, plasma, and, later of lyophilized plasma of whole blood (including leucocytes), and of erythrocytes is continuing. In addition, aliquots of 24-hr urinary collections have been stored. Samples have been removed for various approved protocols over the years. Most recently a longitudinal study of prostate-specific antigen was retrospectively performed. It showed that the longitudinal trajectory of the PSA concentration could detect prostatic carcinoma many years prior to usual clinical measures. Analyses of sex hormones were simultaneously measured on frozen samples.

A comprehensive inventory of the "bank holdings" is now being conducted. An ancillary creation of a DNA bank is in the planning phase.

Z01NR00004-03 CTL

Urinary Continence Status and Treatment of Incontinence in Nursing Home Residents
Palmer, MH

Urinary incontinence is highly prevalent in nursing home residents. A prompted voiding intervention has been documented as an effective method to reduce incontinent episodes in nursing home residents. However, the literature indicates that nursing staff often do not use this intervention consistently. The present study investigates the effects of the intervention delivered by the nursing staff with verbal feedback from the immediate supervisor on the number of incontinent episodes and the compliance of the staff to the intervention over a six-month period.

Z01NR00005-02 CTL

A Survey Regarding Continence Programs
Palmer, MH

Research has shown that staff compliance to behavioral interventions in nursing homes is critical to the success of the program. Awareness of antecedent factors that can hinder or promote the success of continence programs, such as staff knowledge and beliefs about incontinence and its treatment is important prior to implementation. A questionnaire was administered to nurses, who attended a workshop on urinary incontinence in nursing homes. Information about potential staff resistance and deficits in knowledge about incontinence and its care can assist nurses and administrators in development of strategies to reduce resistance, increase knowledge, and change staff behavior that leads to improved continence status of nursing home residents.

Z01NR00006-03 CTL

Role of Estrogen on Urinary Incontinence and Symptoms in Post-menopausal Women
Palmer, MH

Estrogen deficiency has been implicated in stress and urge incontinence in post-menopausal women. Studies have shown that estrogen may increase the response of alpha-adrenergic receptors located along the urethra. Its role in the treatment of urge incontinence is less clear, but there is evidence that urge symptoms can be alleviated with estrogen therapy. The effect of localized estrogen therapy and pelvic muscle exercise with biofeedback on stress and urge incontinence and urinary symptoms is being investigated in the current study. Findings from this study could lead to the development of combined pharmacological and non-surgical interventions in the treatment of prevalent urinary conditions in post-menopausal women.

Z01AG00063-27 LBS

Learned Modification of Visceral Functions in Animals
Talan, MI

The purpose of this project is: (1) to investigate the role and functional organization of different brain sites in the integration of the cardiovascular adjustment to exercise and learned modification of cardiovascular functions; (2) to understand the mechanisms of naturally occurring nocturnal hemodynamic patterns and adaptive responses related to modification of those patterns. Monkeys

are operantly conditioned to exercise while attenuating the exercise related increase in heart rate. Activation of different sites of the brain related to cardiovascular control can interact with learned attenuation of the tachycardia of exercise.

Z01AG00073-05 LBS

Physiology of Thermoregulation and Aging in Rodents
Talan, MI

The purpose of this project is to (1) investigate age-related changes in thermo- regulation and (2) examine physiological mechanisms underlying these changes. Aged mice have diminished cold tolerance and are not able to adapt to repeated cold exposure. Recent research has shown that the central (sympathetic nervous system) component of responses to cold was intact or even enhanced in aged mice. This project investigates the extent to which deficits in mechanisms of heat production in brown adipose tissue are responsible for age-related deterioration in thermoregulation.

Z01AG00600-07 LBS

Respiratory Factors in Blood Pressure Regulation
Anderson, DE

Experimental studies with laboratory animals have shown that behaviorally-induced hypoventilatory breathing potentiates the hypertensinogenic effects of high sodium intake. Current studies in this project focus on the interactions of pCO₂, sodium intake and blood pressure in humans. One experimental study investigates the correlation that exists between resting pCO₂ and (a) higher ambulatory blood pressure in the natural environment, and (b) the magnitude of pressor response during laboratory role playing of social conflict. A study in progress also investigates blood pressure sensitivity to high sodium intake as a function of resting pCO₂.

Z01AG00603-05 LBS

Clinical Implications of Nocturnal Hemodynamic Events
Bush, D

Nocturnal hemodynamic patterns differ substantially from daytime patterns in a variety of mammals including man. In patients with heart disease, these patterns could affect the incidence of morbid events which are known to occur in the morning. This project is designed to test the effectiveness of interventions which may ameliorate adverse effects of nocturnal hemodynamics. A Doppler echocardiographic study on patients with mitral regurgitation investigates morning rehydration effects on viscosity and cardiac functions.

Z01AG00607-04 LBS

Age Effects on Blood Pressure and Circulating Sodium-Pump Inhibitors
Anderson, DE

Previous research has shown that endogenous digitalis-like factors (EDLF) are increased in humans and laboratory animals with clinical and experimental hypertension. However, the extent to which EDLF increases with age is not known. The chemical structures of various EDLF remain to be

clarified, but human plasma and urine appears to contain both a ouabain-like substance and a digoxin-like substance which may be a bufadienolide. The Baltimore Longitudinal Study on Aging provides the opportunity to determine whether the plasma and/or urinary ouabain-like and bufagenin-like factors are associated with age.

Z01AG00608-03 LBS

Post-Operative Complications and Mobility Outcomes in Hip Fracture Patients
Myers, AH

Hip fractures represent a serious and costly health problem among the elderly. A study of 100 patients admitted with hip fractures to two hospitals investigates the relationships of prefracture functional status to development of postoperative complications and mobility outcomes and disposition at discharge. The findings of this study may assist clinicians in identifying patients on admission who may benefit from specific rehabilitative protocols.

Z01AG00609-03 LBS

Sodium Pump Inhibitors in Blood Pressure Regulation
Anderson, DE

Increased concentrations of endogenous digitalis-like sodium pump inhibitors have been observed in chronic hypertension in humans and laboratory animals, but whether they are a cause or effect of the hypertension remains to be clarified. Digitalis-like factors might constitute a previously unrecognized group of stress hormones that could contribute to the development of human hypertension via interaction with dietary sodium intake. The present research tests the hypothesis that the blood pressure elevations occurring in micropigs during behavioral stress are mediated by increases in circulating levels of endogenous digitalis-like factors and inhibition of erythrocyte sodium pump activity.

Z01AG00610-02 LBS

Rehabilitative Nursing Interventions in Elderly Patients with Hip Fractures
Myers, AH

Over the past 30 years, advances in surgical techniques and prostheses have benefitted patients with hip fractures. However, recovering independence after a major disabling event remains a serious problem for many older persons. A prospective experimental study has been designed to evaluate the effects of behavioral nursing interventions preoperatively and post-operatively with community-dwelling elderly patients admitted to the hospital with hip fracture.

TERMINATED.

Z01AG00611-02 LBS

Identification of Nursing Home Residents for Behavioral Nursing Interventions
Myers, AH

The Health Care Financing Administration (HCFA) has a large longitudinal data set on nursing

home residents which is available for investigation of changes in various health parameters after admission to nursing homes. Changes in functional status of up to 350,000 nursing home residents in six states will be examined in order to design and evaluate behavioral nursing interventions to reduce morbidity and facilitate independence in older residents.

INACTIVE.



Z01AG00044-22 LCMB

Metal Ions and Information Transfer: Mechanism of RNA Synthesis

Eichhorn, GL

The effects of metal ions on nucleic acids structure and function have been determined. The primary focus is now on the mechanism of RNA synthesis at the active site of RNA polymerase. The geometry of interaction at the active site of the enzyme is being probed during the transcription process to determine how this geometry changes during transcription and affects its operation. A comprehensive mechanism for assuring fidelity in copying the genetic code has been developed which depends on the sensing by the polymerase of the complementarity between the DNA base and the base of the incoming nucleoside triphosphate. The polymerase shifts its conformation to prevent bond formation when the substrate is not complementary, while the enzyme otherwise remains in the proper conformation for bond formation to allow efficient polymerization with the correct base. The structural model is being applied to understand the effect of DNA damage, promoters, and transcription factors on transcription.

Z01AG00047-25 LCMB

Structure-Function Relationships in Hemoglobin and Erythrocytes

Rifkind, JM

This project focuses on the mechanism involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. Emphasis is placed on ways in which these functions are impaired and change with age. These studies have focused on the oxidation of hemoglobin, which produces nonfunctional hemoglobin and the simultaneous release of oxyradicals. The enhancement of these oxidative processes under hypoxic conditions is being explored as a possible source of tissue and organ damage, which would be exacerbated during aging. Studies are also included which are directed at the stability of the entire erythrocyte and the erythrocyte membrane.

Z01AG00301-12 LCMB

Regulation of Physiological Functions During Aging: I. Hormone Action

Roth, GS

This project is mainly involved in elucidating those mechanisms by which the ability of hormones and neurotransmitters to regulate physiological functions is altered during aging. We have established changes in a variety of signal transduction components/events ranging from receptors to gene expression, during aging. Current studies are focussing on basic molecular mechanisms of aging which are responsible for these alterations, as well as interventions at the same levels to ameliorate such dysfunctions.

Z01AG00302-12 LCMB

Regulation of Physiological Functions During Aging: III. Behavioral Biology

Ingram, DK



The purpose of this project is to assess the effects of aging at a behavioral level of analysis, to identify neurobiological mechanisms associated with these effects, and to test interventions that might alter age-related performance decrements. Rodent models are tested in a battery of sensorimotor and learning/memory tasks. Neurochemical and neurohistological assays are conducted to determine neurobiological correlates of functional losses. Interventions include dietary restriction, exercise, various pharmacologic treatments, neurotrophic factors and gene transfer via adenoviral vectors. Multiple genotypes are examined to determine possible genetic involvement in the pattern of age-related behavioral impairment.

Z01AG00304-09 LCMB

Regulation of Physiological Function During Aging: V. Assessment of Primate
Roth, GSR

This project is attempting to determine whether caloric modification of the diets of Rhesus and squirrel monkeys can affect aging rate as assessed by various physiological, biochemical and behavioral indices. Although it has been known for 60 years that reduced feeding extends lifespan, delays and reduces age-related diseases and slows overall aging rate in rodents and lower animals, relevance to primates (especially humans) has never been established. We are examining a battery of indices of aging, as well as acute metabolic effects, of caloric restriction in order to finally answer this question.

Z01AG00306-06 LCMB

Regulation of Physiological Functions During Aging:II. Neurotransmitter Responsiveness
Roth, GS

This project attempts to understand those mechanisms involved in age related changes in central nervous system (CNS) responsiveness. Studies are focussed at the level of neuronal degeneration and death as well as molecular changes in signal transduction components/events. In addition the relationship between these processes is under examination. Information obtained from these investigators is being used to devise interventions to overcome age related dysfunctions in the CNS.

Z01AG00307-2 LCMB

Regulation of Physiological Function During Aging: VI. Genes, Neurodegeneration
Chernak, JM

The focus of our work is on the function and regulation of genes whose expression changes with aging and/or neurodegenerative disease. We have chosen to study regulation of the D2 dopamine receptor (D2R) gene because of its demonstrated and suspected roles in the decrease in motor abilities associated with normal aging as well as neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), and tardive dyskinesia. This project is a natural extension and refinement of work on the loss of D2 receptors and mRNA with aging (see project # Z01-AG00306-6 LCMB: Regulation of Physiological Functions During Aging: II. Neurotransmitter Responsiveness). In addition, we are continuing to contribute to studies



on the transfer of functional D2R to rat brain and any resulting cellular and behavioral effects (see project # Z01-AG00302-12 LCMB: Regulation of Physiological Functions: III. Behavioral Biology). We are also investigating regulation of the amyloid precursor protein (APP) gene because of the demonstrated and suspected roles that it plays in the neuropathology and etiology of Alzheimer's disease (AD), Down's syndrome (DS), and normal brain aging. Finally, as a result of an internal competition to identify new and important collaborative projects within the GRC, we have received support from the Acting Scientific Director to proceed with our plans to use adenoviral vectors to deliver wildtype and mutant APP genes to localized areas of rat (and/or monkey) brain in order to study expression, processing and normal function of the APP gene products, as well as to possibly reproduce the pathology and/or behavioral changes associated with AD in an animal model.

Z01AG00308-02 LCMB

Regulations of Physiological Functions During Aging VI: Neuronal degeneration and plasticity
Jucker, M

This research project is directed towards the understanding of molecular mechanisms of age-related neurodegenerative diseases. Emphasis is placed on markers of synaptic loss and neuronal plasticity.

Z01AG00309-01 LCMB

Neuronal Aging and Regeneration
Wallace, WC

This project aims to understand the molecular mechanisms that underlie the response of the mature and aged neuron to various stresses. The unsuccessful response to these stresses leads to neurodegeneration. We are examining (1) The biological role of secreted amyloid precursor protein (APP) in response to subcortical lesions of the rat cortex and (2) The role of heat shock proteins as neuroprotective agents.

Z01AG00310-01 LCMB

Interrelationships Between Stress, Differentiation, Growth Arrest and Programmed Cell Death
Holbrook, NJ

Cell cycle progression is subject to major points of restriction, or checkpoints, which serve to arrest growth if certain processes are incomplete, or damage is incurred. The tumor suppressor gene product p53 has elucidated important connections between cell cycle progression and tumorigenesis. p53 is required for the activation of the G1 checkpoint after certain forms of genotoxic stress and appears to initiate growth arrest, at least in part, through transactivation of effector genes including the cdk inhibitor p21 (Waf1, Cip a and Sdi1). In certain cell types, the same treatments result in apoptosis rather than G1 arrest. The mechanisms involved in inducing apoptosis under such conditions are poorly understood, but p53 also appears to contribute to this response. In addition, the factors responsible for determining whether a cell will undergo apoptosis or growth arrest have yet to be clarified. Better understanding of the critical mediators acting downstream of p53 could provide answers



to these questions. Our studies thus far have focused primarily on the induction of p21 in response to treatment with the prostaglandin PGA_2 , a potent suppressor of growth *in vitro* which also exhibits antitumor activity *in vivo*. We have shown that treatment of breast carcinoma MCF-7 cells leads to G1 arrest associated with a dramatic increase in p21 expression that is independent of p53 status, and have provided evidence that p21 arrests cell cycle progression by inhibiting cdk2 activity. Other studies have focused on the transcriptional activation of p21 in response to growth factors and genotoxic stress. We have provided evidence to indicate that p21 is induced in response to both treatments through a Ras, Raf and MEKK dependent pathway, implicating ERK as a key intermediate in these responses.

Z01AG00381-05 LCMB

NMR Studies of Aging in Cells, Organs and Animals

Spencer, RJS

NMR spectroscopy is currently being used at the NIA to study the phosphorus metabolism of peripheral muscle. Age-related, hormone related and exercise-related effects are under investigation. Methodologic studies to further develop kinetic and spin-lattice relaxation time measurement methodology are also being actively pursued. Connective tissue work has been initiated.

Z01AG00710-07 LCMB

Heat Shock Protein Gene Expression in Response to Stress and Aging

Holbrook, NJ

Heat shock proteins (HSPs) are induced in response to a variety of cellular stresses, and appear to be critical for maintaining cellular homeostasis. Previously, we demonstrated that restraint or immobilization stress elicits the induction of HSP70 expression selectively in the adrenal gland and vasculature of intact rats. In both tissues this stress-induced HSP70 expression was found to be linked to the activation of the neuroendocrine stress response axes and to be attenuated with age. The adrenal response was found to be dependent on the hypothalamic-pituitary-adrenal axis and require adrenocorticotrophic hormone (ACTH) while the vascular response appears to be under alpha adrenergic control. Studies in this project are focused on defining the physiologic and molecular events involved in controlling this response to restraint and the determining the cause and significance of its age-related decline.

The past year's studies have concentrated most heavily on the vascular response where we have further established a link between restraint-induced hypertension and the induction of the HSP70 in the aorta. As previously shown for the adrenal response, we have provided evidence that restraint-induced HSP70 expression in the aorta is mediated via activation of the heat shock transcription factor HSF1.

We have identified two additional conditions associated with tissue selective HSP70 expression in response to stress *in vivo*: HSP70 induction in rat renal tubular cells following administration of vasopressin and HSP0 induction in brown fat of rodents exposed to hypothermia.



Z01AG00720-04 LCMB

Regulation and Function of the Putative Transcription Factor GADD 153

Holbrook, NJ

GADD153 is a highly conserved mammalian gene whose expression is increased in response to a variety of stresses including growth arrest and DNA damage. It is a member of the CCAAT/enhancer-binding protein (C/EBP) family of transcriptional activators and can dimerize with other C/EBPs through a leucine zipper domain. However, in contrast to other C/EBPs, it lacks the ability to bind to CCAAT DNA sequences and has therefore been proposed to serve as a negative regulator of other members of this family (by virtue of its ability to heterodimerize with them and inhibit their binding to DNA). Members of the C/EBP family are thought to play important roles during adipocyte and liver specific gene expression. Studies in our laboratory suggest that C/EBPs are also likely to serve a more ubiquitous role in regulating gene expression during the cellular response to stress. Studies in this project have focused on the regulation and function of *GADD153*. The approaches taken have included (1) characterization of *Gadd153* expression (and that of other C/EBPs) in response to diverse growth inhibitory and metabolic stimuli including PGA_2 -mediated growth arrest, glucose deprivation, genotoxic stress, and inducers of the acute phase response *in vivo* and *in vitro*; (2) definition of critical regulatory elements in the *GADD153* promoter which control its activity in response to stress, and (3) examination of interactions between GADD153 and other related transcription factors during various conditions of stress.

Z01AG00902-02 LCMB

Gene Activation in Response to DNA Damage and Oxidative Stress in Normal Aged Cells

Holbrook, NJ

Oxidative stress and DNA damage play a critical role in the development of degenerative diseases, and may underlie the aging process itself. Cells respond to such stresses with the induction of numerous gene products but little is known concerning the signal transduction pathways mediating these effects or the functional significance of the induced gene products. This project encompasses several areas related to these cellular responses. Much effort is being devoted to understanding basic mechanisms associated with the activation of genetic responses to different forms of genotoxic stress and the consequences of the response. Specific topics we are addressing include (1) the role of various MAP kinases (ERK, JNK/SAPK and p38) and/or the p53 tumor suppressor protein in mediating the cellular response to different agents; (2) the role of oxidant injury versus DNA damage in sensing damage and triggering the response; (3) mechanisms involved in attenuating the response; and (4) the relationships between acute cellular responses and subsequent effects including growth inhibition and cytotoxicity. A second area of focus in this project area is the investigation of the response to DNA damage as a function of aging. We have proposed that the cellular response to stress declines with aging and using two different models of aging have obtained evidence to support this hypothesis. These include hepatocytes obtained from young versus old rats and *in vitro* aged human diploid fibroblasts.



Z01 AG 00015-37 LSB

The Baltimore Longitudinal Study of Aging

Fozard, JL

The Baltimore Longitudinal Study of Aging (BLSA), the NIA's major research program on human aging, has been conducted at the Gerontology Research Center since 1958. The study represents a consortium of scientists who work to characterize normal and pathological aging. The BLSA consists of a series of longitudinal and cross-sectional studies of varying degrees of interrelationships oriented toward description, identification of mechanism, prediction and intervention in human aging processes. The scientific goals include identifying age differences among individuals and changes in individuals over time; to characterize transitions from normal to pathological aging; to determine the relative contribution of aging, disease processes, cohort effects and secular effects; to expand scientific understanding about predictors and risk factors for specific diseases and for other end points related to successes and failures of adaptation to aging processes; and where possible to explore mechanisms for normal and/or pathological changes.

Scientists working with BLSA are assigned to 11 sections of 7 laboratories in addition to the LSB. The Chief, LSB, is the Associate Scientific Director, NIA for the BLSA and LSB staff administrator and manage the BLSA as well as conduct research with it.

The BLSA Steering Committee, an internally comprised committee of GRC scientists and the ASD, is responsible for determining the direction of the study. Three working subcommittees (Scientific Directions, Progress Review, and Resource Management) report to the Steering Committee on routine basis.

Progress has been made in upgrading procedures for data acquisition, storage and retrieval in the BLSA. Procedures have been adopted to increase the work efficiency of the staff, and to be able to manage the increasing number of BLSA participants.

Z01 AG 00622-06 LSB

Health Disease Status in the BLSA: Clinical Health Evaluation

Metter, EJ; Fozard, JL

Starting in 1985, the BLSA health evaluation has undergone major changes to improve the medical information about research participants. Two versions of the health questionnaire have been implemented. The initial questionnaire, introduced March 1, 1991, is used on participant's first visit to determine previous health complaints and problems. The interval history questionnaire, instituted March 1, 1993, is used for all subsequent visits to document changes in health complaints and status since their previous visit and to identify the effects of symptoms on daily living. The interval history provides participant's previous responses to each items and allows the participant to indicate changes. The interval history questionnaire also included an expansion of branching questions used for positive health complaints with questions assessing effects on daily activities and emotional well being. The health evaluation also provides health screening for a number of research protocols. The unit has increased formalized handling of screening and research protocols; provided technical support to investigators in the development of research studies; and worked to minimize and/or prevent potential adverse outcomes or other problems. The staff worked with investigators on a blood pressure protocol, improved



assessment of hormonal status and clean up of the historical dataset, and the collection of biopsy material. To meet research needs for studying the elderly who at times are frail, the unit is developing procedures to identify the frail individuals and a special nursing protocol with appropriate changes in visit routine. Other changes include the implementation of a physical functioning inventory to probe for mild to moderate disability in physical activity. Over the past year, we have continued as part of our quality assurance program to assess the value of the new health questionnaire for BLSA research. We continue to develop formal guidelines as needed.

Z01 AG 00623-07 LSB

Development of Statistical Methodology for the Analysis of Studies of Aging
Brant, LJ

Statistical methodology is being applied and developed for longitudinal studies and other studies of aging. The research program focuses on several types of statistical models: 1) longitudinal mixed-effects regression models which consider both within- and between-subject variation in analyzing the repeated measurements for all individuals in the study population, 2) survival analysis for studying risk factors in prospective studies, 3) multiple comparisons for testing group differences in experimental or observational designs, 4) mixture models for describing age changes in distributions of biological markers, and 5) experimental design. Other techniques used include Bayesian, maximum likelihood and numerical computing methods. A major emphasis of the research program is the development of methods which yield cogent yet easily understood results when applied to data.

The effect of measurement error bias on risk factor analyses has been investigated using a Monte Carlo simulation study. Various methods of representing the baseline value of a risk factor have been examined, including the usual single baseline measurement, means of multiple baseline measurements, and predicted values from repeated measurements using mixed-effects regression analyses. Results show that individual predicted values from the mixed-effects model provide a more accurate measure of the strength of the relation between the level of a risk factor and the occurrence of an endpoint such as morbidity or mortality. In a second area, a nonlinear mixed-effects model was used to describe longitudinal data on changes in PSA level in men with prostate cancer. The model is piecewise with a linear phase and an exponential phase of PSA increases. This allows for the estimation of the time before diagnosis at which the transition between the slow linear phase and the rapid exponential phase occurs.

The research program has extended earlier methods of longitudinal data analysis, introduced novel methods of describing the natural history of aging, and developed new approaches toward the use of longitudinal data in epidemiological and biomedical studies of aging and associated disease states.

Z01 AG 00624-06 LSB

Baltimore Longitudinal Study of Aging (BLSA): Population Dynamics
Fozard, JL; Hiscock, BS

This project is concerned with optimal management and scientific description of the total BLSA population, which includes, as of 7/10/95, 1165 active participants (558 women; 607 men), 542 inactive (199 women; 343 men), and 646 deceased (71 women; 575 men). At present, 10% of all



active men, and 13% of the active women, are African American.

Active Participants. Between 7/1/94 and 6/30/95, 533 participants visited the GRC for the regular 2- to 2 1/2-day visit. This includes 42 new participants (27 white; 15 black), and 35 formerly inactive participants.

Inactive Participants. The most recent telephone follow-up of inactive BLSA participants was completed in 1992 to collect and update demographic information, health, cognitive, and functional status, depressive state, reasons for not returning to the GRC, and plans for continued participation. Data collection from inactive participants is expected to resume in 1996 using a half-time employee to be hired through the Johns Hopkins Bayview contract early next year.

Deceased Participants. Currently on file are death certificates for 96% of the deceased, physician and/or hospital reports for 38%, and autopsy reports for 15%. Cause of death information is kept current by LSB staff with the cooperation of two staff physicians from LCP and LCS who assist with cause-of-death coding according to a system developed at the GRC and using all available death information. In addition, cause of death is coded according to the standardized NCHS coding system using only death certificate information.

Z01 AG 00625-06 LSB

Baltimore Longitudinal Study of Aging (BLSA): Data Management
Shefrin, EA

The Data Management work group is responsible for the storage of both paper and computer records generated by the BLSA. They perform the data entry of medical records and manage the data entry of many of the other data collected by the BLSA internal investigators and outside collaborators. Staff members manage the BLSA Computer System and its data base. They support both the administration of the BLSA as well as its scientific activities. Their functions include data extraction, processing, and analysis; consultation; training; hardware and software maintenance; and software development.

Z01 AG 00626-06 LSB

Age Changes in Visual Function
Fozard, JL; Metter, EJ

Deterioration of vision is a common problem in the elderly and in recent studies has been demonstrated to be an independent and important contributor to physical disability and frailty. Age associated loss of vision can result from aging and from age-associated pathology. The natural history of vision loss has not been well studied in relationship to the development of eye and other pathology and the differentiation between normal visual aging and pathological changes in vision. Furthermore, little attention has been given to what factors may prevent aging changes in vision and what environmental factors can be altered as the changes begin to occur and as they progress. Research in the BLSA has been designed to address aspects of these age associated changes. (1) Natural history studies have been done for many years, and several longitudinal studies have been reported. A laboratory based assessment of visual contrast sensitivity continues to be administered, increasing the number of persons with at least two measures to over two hundred. (2) A new study of the relationship between intraocular pressure and systemic blood pressure was begun in July 1994. The study will identify possible racial and



sex differences in blood pressure/intraocular pressure relationships and the effect of the relationship on vision as measured by changes in the visual field.

Z01 AG 00627-06 LSB

Risk Factors for Age-Related Ocular Change
West, S

No report for this project this fiscal year. Publication only:

West S, Vitale S, Hallfrisch J, Muñoz B, Muller D, Bressler S, Bressler, N. Are antioxidants or supplements protective for age-related macular degeneration. Arch Ophthalmol 1994;112:222-7.

Z01 AG 000628-06 LSB

Aging and Auditory Characteristics
Fozard JL, Metter, EJ

This project aims to combine assessment of hearing abilities among subjects of different ages over time, together with information from their communication and health histories. Medical and cognitive data collected from subjects in the longitudinal study will be examined with respect to the audiologic and case history data. The two principal objectives of this project are:

A) To study the contribution of medical, genetic, dietary and social factors to age-related auditory dysfunction; and B) To determine to what extent age, independent of other etiologic factors, causes a deterioration in hearing abilities. During the past year, approximately 450 subjects from the BLSA have been tested on all of the new measures in the hearing protocol. These measures include assessment of pure-tone hearing sensitivity, sentence understanding in noise, self-perceived hearing handicap, tympanometry, acoustic reflex thresholds, acoustic reflex magnitude, acoustic reflex adaptation, and acoustic reflex latency (the last five measures are part of the acoustic immittance battery of electrophysiologic tests).

We have recently published a retrospective analysis of gender differences in longitudinal change in hearing sensitivity in a sample screened to exclude otological disorders and evidence of noise-induced hearing loss, and published a risk factor analysis showing that high blood pressure is associated with hearing loss in the speech frequencies (see Project Z01 AG 00638-06 LSB). We are now extending these analyses to develop age- and gender-specific nomograms for pure-tone thresholds and to identify modifiable risk factors for age-associated high-frequency hearing loss. Long range plans are to implement a collaboration with the National Temporal Bone Registry to study the relationship between inner ear pathology and longitudinal changes in audiological function, and to implement additional electrophysiological measures to provide corroborative evidence of the site of auditory system degeneration.

Z01 AG 00629-06 LSB

Health and Disease Status in the BLSA Men: Distribution of Diseases
Metter, EJ; Fozard, JL

No report for this project for this fiscal year.



Z01 AG 00630-06 LSB

Health and Disease Status in the BLSA Women: Distribution of Diseases

Metter EJ; Kramer DK

No report for this project this fiscal year.

Z01 AG 00632-06 LSB

Health and Disease Status in the BLSA Men: Perceived Status

Metter, EJ; Fozard JL

No report for this project this fiscal year.

Z01 AG 00633-06 LSB

Health and Disease Status in the BLSA: The Prostate Gland

Pearson, JD; Metter, EJ

A new BLSA study of Prostate Growth and Disease was begun in February 1993 to examine anatomic and physiologic correlates of normal prostate growth and the development and progression of benign prostatic hyperplasia (BPH) and prostate cancer. In the past year, three studies have been completed. The first BLSA study developed age-specific reference ranges for prostate-specific antigen velocity (rate of change) and compared the sensitivity and specificity of different prostate-specific antigen (PSA) criteria for the detection of prostate cancer.

In order to determine the optimal PSA testing regimen, the second study examined the variability in PSA levels in men with BPH and prostate cancer when measured at different time intervals. This study showed that PSA measurements obtained at 3 month or 6 month intervals are unlikely to be clinically useful.

The third study developed age-specific norms for serum androgen levels in men (see Project Z01 AG 0013-20 LCP). These norms will be used to examine the relationship between age-adjusted androgen levels and risk of BPH or prostate cancer.

Z01 AG 00634-06 LSB

Age Changes in Pulmonary Function

Fozard, JL; Pearson JD

The BLSA Program in Pulmonary Aging has focused on describing longitudinal changes in pulmonary function and demonstrating the importance of pulmonary aging in determining the health of individuals as they age. The BLSA recently published the first study to demonstrate that an accelerated decline in forced expiratory volume at one second predicts coronary heart disease death.

Current research is focusing on establishing longitudinal norms for age-associated changes in pulmonary FEV_1 in healthy, non-smoking men and women. Longitudinal analyses of changes in FEV_1 were conducted among 91 men and 82 women who had no history of respiratory problems and had never smoked cigarettes. The FEV_1 data were modeled using a mixed-effects regression model and longitudinal percentile distributions of FEV_1 level were constructed. The findings showed 1) the average longitudinal rate of decline in FEV_1 was



approximately 240-340 ml/decade in men and women, 2) none of the participants exhibited a sustained improvement in FEV₁, and 3) between-subjects variability is greater in men than women and increases with age in men, but decreases with age in women. The age- and gender-specific percentile distributions are the first nomograms which reflect age differences in the variability in pulmonary function.

Future research will expand the work on developing age- and gender-specific nomograms for other pulmonary measures and will examine the effect of smoking cessation on pulmonary function. The pulmonary testing protocol is currently under review to develop new research directions.

Z01 AG 00635-06 LSB

Age Changes in Response Speed and Nerve Conduction

Fozard, JL; Metter, EJ; Wood J; Verduyssen M

Age is associated with a slowing of movement and loss of strength that affects the accuracy of arm and leg use. At a critical level this can be an important factor leading to functional disability in the elderly. The natural history of how these changes occur and the causes are poorly understood. During the past year, two studies were completed to (1) explore age changes in the relationship between speed of movement time and accuracy, and (2) the contribution of nerve function to age associated loss of strength.

A reciprocal tapping test was administered to participants in the Baltimore Longitudinal Study of Aging (BLSA). Movement time increased cross-sectionally and longitudinally with increasing age. After age 40, movement time increases with age was linked to decreased consistency of hitting the targets. Longitudinally, accuracy decreased with increasing age. Fitt's law accurately described the speed and accuracy of hand movements during adult aging, across cohorts and within participants.

Age associated muscle strength loss is attributed to decreasing muscle mass. Both strength and mass are dependent on muscle innervation. There is little direct human evidence demonstrating the association between age changes in nerve function and strength. Median and, to a lesser extent, ulnar nerves are the major nerves supplying grip strength. Grip strength and median and ulnar nerve conduction velocities were measured in BLSA participants. Median but not ulnar nerve significantly related to grip strength along with age and forearm circumference. Age and initial grip strength predicted rate of grip strength change with age. Motor nerves, muscle mass and age have significant, independent contributions to age associated levels of strength.

Z01 AG 00636-06 LSB

Study of Physical Activity in the BLSA

Fried, LP

No report for this project this fiscal year.

Z01 AG 00637-06 LSB

Gender Differences and Individual Variability in Human Aging

Brant, LJ; Pearson, JD; Morrell CH

Studies of gender differences and individual variability in age-related phenomena are being carried out to: 1) determine the "normal" range of variability in human aging, 2) identify potential sources of variability which may be responsive to intervention, and 3) determine if there are subgroups of individuals who are more susceptible or resistant to various aspects of aging. The research combines the use of sophisticated statistical methodologies and the unique time depth and multidisciplinary breadth of the existing BLSA data base to examine issues related to the concepts of "normal" and "successful" aging, as well as to increase the power of traditional research designs. The statistical methods used include longitudinal regression models, time dependent proportional hazards analysis, and finite mixture models. Major findings include: 1) the longitudinal rate of decline in pulmonary function is relatively constant with age in both men and women, percentile distribution curves have been calculated based on longitudinal methods that account for age-specific differences in variability (see Project Z01 AG 00634-06 LSB); 2) longitudinal patterns of change in blood pressure differ with age and gender, and individual patterns of change are highly variable; 3) hearing sensitivity declines more than twice as fast in men as in women at most ages and frequencies, individual hearing levels and longitudinal patterns are highly variable even though the BLSA study population is a highly selected group (see Project Z01 AG 00628-06 LSB); and 4) systolic blood pressure is an independent risk factor for hearing loss in the speech-range frequencies (see Project Z01 AG 00628-06 LSB). These findings represent significant contributions to the theoretical and methodological development of biomedical risk factor studies, as well as to an increased understanding of the dynamics of the aging process. Research is underway to develop more refined methods of studying variability in aging in order to develop theoretically and methodologically sound approaches to risk factor analysis which account for changes in an individual's covariates over time and the possibility that individuals differ in susceptibility or resistance to aging processes.

Z01 AG 00638-06 LSB

Health Promotion, Modifiable Risk Factors and Aging

Brand LJ

Unnecessary morbidity and mortality is an important problem which leads to increased health-care costs and can ultimately result in premature death. It has been estimated that approximately two thirds of mortality is due to potentially preventable causes - 1.2 million deaths (65%) and 8.4 million years of life lost before age 65 (63%). Principal factors associated with unnecessary morbidity and mortality include tobacco use, high blood pressure, improper nutrition, lack of screening and prevention services, alcohol abuse, and injury. This project uses longitudinal data from the Baltimore Longitudinal Study of Aging (BLSA) and other studies to examine the influence of modifiable risk factors on the occurrence of premature deaths and unnecessary morbidity and disability. Identification of risk factors can lead to primary prevention efforts. A major finding from the BLSA is that high systolic blood pressure is associated with the

occurrence of hearing loss in the speech-range frequencies (see Project Z01 AG 00628-06 LSB). This is the first documented modifiable risk factor other than noise exposure which has been identified for age-associated hearing loss. Information from these studies can have an impact on the development of primary and secondary prevention programs to improve longevity and quality of life for many Americans.

Z01 AG 00639-04

Individual Changes in Function with Age and Target Conditions

Verbrugge, LM; Gruber-Baldini, AL

The research analyzes longitudinal changes in functioning in 14 domains of activity and to study how these changes vary by sociodemographic and medical factors. The data for these analyses are derived from the "Activity Questionnaire II." It has been filled out by BLSA subjects at each visit since 1966. Subjects estimate the amount of time they spend on numerous specific activities, ranging from personal care to leisure. The data are unique for its time stretch (up to 25 years for some subjects) and its content (the comprehensive scope of activities). Analyses on this data set include examinations of the cross-sectional, longitudinal, and secular patterns by age and gender. Cross-sectional analyses reveal consistent age and gender differences for participation in and time spent doing various activities, especially work, housework, childcare, and various discretionary activities. Comparisons of longitudinal and cross-sectional results show evidence of secular changes in time spent doing work, housework, and childcare by women.

Examination of the effect of chronic conditions on time spent in activities revealed that the presence of a chronic condition (diabetes, hearing loss, hypertension, ischemic heart disease, musculoskeletal problems, pulmonary dysfunction, and visual acuity problems) increases the time spent in obligatory activities (personal care, sleep) and decreases time in discretionary activities (socializing, public service). The effect of a chronic condition on committed activities (housework, childcare) interacted with gender so that women increased time in these activities while men decreased their time; this may be a function of gender differences in perception of commitment of activities (e.g., women being more committed to housework).

Z01 AG 00640-03 LSB

Effects of Age on Muscle Strength, Body Composition and Health Status

Fozard JL; Hurley B

Age-related changes in muscle strength are an important contributor to frailty in the elderly. The time course and causes for these changes are poorly understood. This project examines the changes across the adult life span with an emphasis on what occurs early in middle age when the changes begin. The research consists of three parts.

First is an ongoing study in the BLSA comparing concentric (shortening) and eccentric (elongation) phases of movement assessed at slow, fast and zero (isometric) speeds. Almost 500 male and female subjects from the 20s through the 80s have been tested and longitudinal data collection is beginning. Age associated declines are found in cross-sectional analysis for both concentric and eccentric testing for all muscle groups and speeds. The cross-sectionally determined rate of decline is greater in the quadriceps femoris than for the biceps brachii. The rates of decline in concentric and eccentric strength tend to be parallel in men. Concentric

strength declined to a much greater extent than eccentric strength in women.

Second is an analysis of twenty-five years of muscle and power measurements in the BLSA. The initial analysis examines the issue of how qualitative changes in muscle mass are related to strength. Creatinine excretion declined in a manner similar to muscle strength, while body mass index, cross sectional area and lean body mass showed less change. The study suggests that measures that examine muscle contractile components may show a stronger association with strength than cross sectional muscle area.

Third are studies to examine the peripheral nerve contribution to strength loss. A protocol has been developed that explores motor unit function at different levels of muscle exertion in the quadriceps femoris. The technique shows reasonable test-retest reliability with a .1-.3 coefficient of variation. The size of recruited motor units increases at increasing percentage of maximal voluntary contraction. The study will examine the effects of the motor unit size and firing pattern.

Z01 AG 00641-03 LSB

Race & Gender Differences in Intracerebral & Carotid Arterial Velocity With Aging
Metter, EJ; Early C

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Z01 AG 00180-10 LPC

Stress, Coping and Personality in Aging Men and Women

McCrae, Robert R.

A longitudinal study of the continuity of personality and personality disorders was conducted in a sample of 1,917 men and women initially tested as college students. 24 years later, the individuals completed a measure of adult personality. Correlations showed that personality disorder symptoms in adolescence predict adult levels of personality traits, supporting the hypotheses that basic personality traits are related to personality disorders and show long term stability. Longitudinal research on personality, stress and coping will continue.

Z01 AG 00183-07 LPC

Basic Research in Personality

Costa, Paul T.

Personality can be defined in terms of enduring individual differences in emotional, interpersonal, experiential, and motivational styles. The five factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness provide a comprehensive taxonomy of personality traits for the description of personality in aging men and women. Two studies examined issues in personality structure. In the first, the five-factor structure of the NEO-PI-R was shown to be replicable in Chinese and Japanese data; in the second, positive and negative valence were located in the five-factor space.

Z01 AG 00184-07 LPC

Psychosocial Predictors of Mental and Physical Health

Costa, Paul T.

Normal personality dimensions can predispose individuals to certain forms of psychopathology, especially personality disorders. Studies in two samples examined relations between personality dimensions of the standard five-factor model and an alternative model designed specifically to capture dimensions of psychopathology. Correlations between the two models showed both overlapping and unique features; both sets of dimensions were useful in predicting personality disorder symptoms in a clinical sample. Efforts to relate basic research on personality to applications in clinical psychology and studies of the relation of personality to physical health will continue.

Z01 AG 00185-06 LPC

Early Markers of Alzheimer's Disease in Longitudinal Participants

Zonderman, Alan B.

Participants in the BLSA aged 60 and older were examined to detect changes in psychological, neurological, and neuropsychological tests related to early signs of Alzheimer's disease (AD). Annual neuroimaging replicated the relationship ($r = .54$) between cerebrospinal fluid (CSF) and age for both ventricular and sulcal CSF. Both white and gray matter volumes declined with age ($r = -.21$ and $-.11$), and ventricular CSF ratios were significantly higher for men than women.

Functional imaging data from PET Oxygen-15 water studies showed the expected pattern of brain activation during performance of verbal and spatial recognition memory tasks. Both tasks showed pronounced frontal and temporal brain activations, with greater right hemispheric activation for the spatial memory task and greater left hemispheric activation for the verbal task. Preliminary analyses by Statistical Parametric Mapping (SPM), a program widely used in the PET research community, suggest that the oldest group of men (70 and older) show less task-related activation than younger men and both older and younger women.

Z01 AG 00189-05 LPC

Giambra, Leonard M.

Attentional Processes in Normal and Impaired Elderly

We seek to understand the psychological and biopsychological aspects of normal and pathological aging in terms of attention and attentional processes. We are also concerned with applying that knowledge to develop strategies for improving attentional and cognitive functioning. We found that, when compared with controls, 14 men who developed dementia had significantly poorer sustained attention 4 to 12 years prior to the estimated date of onset of dementia. Furthermore, 13 of 14 demented but only 18 of 34 controls had sustained attention detection accuracy scores of less than 79%. We continue our 6 year longitudinal testing of sustained attention and 20 year longitudinal testing using our retrospective self-report questionnaire on spontaneous attention switching to the contents of consciousness.

Z01 AG 00724-03 LMG
Gene Specific DNA Repair
Vilhelm A. Bohr

We are studying the molecular biochemistry and fine structure of DNA repair. This includes work on the regulation and the mechanism of gene specific repair and transcription-repair coupling. We are studying DNA repair coupling with transcription with a view to clarify which gene products are involved and how these processes are regulated as compared to the DNA repair processes in the general, overall bulk of the genome. We use different approaches to the study of the regulation of gene specific DNA repair. They include the use of specific enzyme inhibitors, antisense systems, mutant cell lines, cell lines transfected with specific repair genes, fluorescence microscopy, and in vitro DNA repair assays. There are distinct differences in the efficiency of gene- and strand specific DNA repair dependent upon the type of DNA damage, and it is possible that the local degree of chromosomal distortion is the important element in determining the repair response chosen by the cell. We are suggesting that proficient DNA repair is necessary to secure genomic stability, and we find that certain regions of the genome that undergo translocation or rearrangements are poorly repaired.

Z01 AG 00725-03 LMG
Gene Specific DNA Repair Throughout the Cell Cycle
Vilhelm A. Bohr

Our studies are designed to understand the interrelationships between DNA metabolism, specifically DNA damage and its repair, and the cell cycle progression in mammalian cells. We are particularly interested in characterizing the gene- and strand-specific patterns of repair of various adducts in the different phases of the cell cycle; this will allow us to correlate DNA repair processes with transcription, replication, and mutagenesis. Determination of phase-specific repair processes will also shed light on the possible accumulation and distribution of DNA damage in non-cycling (differentiated and senescent) cells.

Z01 AG 00726-03 LMG
DNA Repair in Cancer and Senescence
David K. Webb

Our interest in understanding the complex interrelationships between DNA repair, cancer and senescence has led us to study the role of DNA repair in several human model systems which are pertinent to both cancer and aging. We have identified specific fine structure DNA repair phenotypes characteristic of a group of related heritable cancer prone and progeroid human syndromes. We have been able to determine that the tumor suppressor gene p53 most likely plays an active role in the DNA repair process via studies of repair in patients with LiFraumeni syndrome harboring a germ line mutation in p53. By investigating DNA damage induction and repair in progeroid syndromes such as Werner's syndrome, we can examine a human mutant which has several clinical manifestations concordant with normal human aging and also associated with an increased cancer incidence. Alzheimer's disease also provides a useful model system in which to study the role of DNA repair in a condition associated with senescence. We

have measured gene-specific repair in fibroblasts from patients with familial and sporadically occurring Alzheimer's disease.

Telomeric shortening is one of the age-associated genetic instabilities currently believed to be an important biomarker of aging and cancer. We have developed a novel method to measure DNA damage induction and repair in human telomeres and suspect that repair capacity in telomeres may be related to the genomic instability associated with normal human aging and perhaps with tumorigenesis. These studies are being extended with assays for telomerase activity.

Z01 AG 00727-03 LMG

Repair of Oxidative DNA Damage

Vilhelm A. Bohr

We have developed assays to detect oxidative lesions in specific genes and thus to quantitate their formation and repair. Oxidative DNA damage is generated by several different approaches including hydrogen peroxide, acridine orange, X-irradiation, irradiation with methylene blue, and treatment with 4NQO which forms at least one adduct with oxidative characteristics. The main lesion which we examine is 8-OH guanosine which can be detected by use of the FaPy glycosylase. This enzyme creates strand breaks in DNA at sites of the lesions, and the single stranded DNA can then be resolved on alkaline gels. We find that 8-OH guanosine is rapidly repaired in active genes in hamster and human cells.

While it has been a general notion that there is no DNA repair in mitochondria, we now find that these organelles do have repair capacity. They can not, however, repair all lesions. They are capable of repairing DNA lesions created by monofunctional alkylating agents, but not UV induced pyrimidine dimers. We find fast repair of oxidative damage in mitochondrial DNA, and the mechanism is under investigation. One question is whether the repair in mitochondrial DNA is transcription coupled. We are investigating whether the common deletions in mitochondrial DNA seen in senescence and other conditions could be due to a localized deficiency in DNA repair.

Z01 AG 00729-02 LMG

Genomic Instability

Vilhelm A. Bohr

Genomic instability is a general and characteristic feature of both cancer and aging. We are searching for the underlying mechanisms that are responsible for the development of the genomic instability. Replication errors in DNA, telomeric shortening, increased local DNA damage formation, and localized DNA repair defects, are all possible pathways that can lead to genomic instability. Recently, we have found situations where the genomic instability correlated with a deficiency in DNA repair. We are now examining these pathways in human cells from patients with aging or cancer associated genomic instabilities. a good example is hereditary, non-polyposis colorectal carcinoma (HNPCC), where a mismatch DNA repair defect has been found. We find a more generalized DNA repair defect. This will be further characterized and explored in other HNPCC cell lines. In other studies, we find a distinct shortening of the



telomeres in cell lines that have mutations or are deficient in the function of the tumoursuppressor gene, p53, which then leads to genomic instability.



Z01 AG 00055-06 LBC

Effect of Age on Osteogenic Activity

C. Tony Liang

The main goal of this study is to establish an animal model which can be used to define age-deficits in bone activity at the cellular and molecular levels and to design and test the effectiveness of novel treatment procedures for osteoporosis. A minor bone injury, initiated by aspiration of bone marrow, induces rapid bone formation in the marrow cavity followed by resorption of newly synthesized bone. Changes in the expression of osteoblast-related genes as well as growth factor genes that are associated with bone formation and resorption are also noted and correlate with changes in bone histology. Using this bone injury model, we have demonstrated multiple deficits in old bone. These include reduced bone formation, insufficient number of osteoprogenitor cells and osteoblasts, impaired expression of matrix proteins and altered expression pattern of growth factors and cytokines in old bone. Currently, we are using this model to test the effectiveness of various interventions including growth factors, stem cell replacement and minocycline, an antibiotic with anticollagenase activity, in restoring the ability of old bone to maintain bone mass.

Z01 AG 00057-06 LBC

Cartilage Biology: Models and Mechanisms Related to Aging and Disease

Walter E. Horton Jr., PhD

Cartilage is a unique tissue that functions to cushion the impact between bones and is a target for degenerative changes that result in osteoarthritis (OA), an incurable disease that afflicts millions of elderly individuals. OA involves changes in expression of matrix proteins such as collagen II, the death of the only cell type in cartilage—the chondrocyte, and overexpression of specific matrix metalloproteases (MMPs) that contribute to the breakdown of the cartilage matrix. We are continuing to characterize transcriptional regulatory sequences in the collagen II gene. In addition to the intron enhancer, we have identified a region of the promoter that appears to be required for activation of transcription. This region contains putative binding sites for both SP-1 and bHLH transcription factors. We have made significant progress in studying the potential for cell-based cartilage repair. To date we have shown conclusively that adult rabbit articular chondrocytes can be phenotypically modulated and expanded *in vitro* with growth factors. Importantly, these cells retain the capacity to form hyaline cartilage when reintroduced into animals. Preliminary work suggests that a similar approach may prove feasible with human chondrocytes. Finally, we have shown that an immortalized chondrocyte line developed in our laboratory displays a similar pattern of MMP expression upon cytokine stimulation as has been described in human OA. These studies may lead to novel therapeutic approaches for OA.

Z01 AG 00058-06 LBC

Aging, Angiogenesis, and the Growth and Spread of Tumors

Antonino Passaniti

Age is the single greatest risk factor for the development of cancer. A reduction in host surveillance, longer exposure to carcinogens, and accumulation of mutations may all contribute



to age-associated cancers. However, adequate animal models to study these processes have not been available. We have developed improved methods to grow lines of human prostate cells as tumors. Lines of breast tumor cells arising spontaneously in aged female rats also have been developed. We also have developed a simple, quantitative assay to measure vascularization and assess angiogenic and potential anti-angiogenic factors. These systems provide the opportunity to investigate the genetic events underlying the development of these cancers and explore various methods of cancer prevention. Although the incidence of cancer increases with age, tumors of the prostate, breast, and ovary grow more slowly and progression is less aggressive in the elderly. Several mouse tumor models are being used to identify factors which may contribute to inhibition of tumor growth in aged animals. These factors are being tested in human prostate and rat breast tumor models in an effort to identify the source(s) of tumor growth inhibition with age. Programmed cell death (apoptosis) may be involved in age-dependent degeneration. Endothelial cell apoptosis occurs when cells are undergoing differentiation on Matrigel (an extract of basement membrane proteins) and when trophic factors are withdrawn. Matrigel prepared from old animals is especially potent at inducing apoptosis of endothelial cells. These protein preparations may be a useful source of negative regulators (from old hosts) or trophic factors (from young hosts) which may influence not only vascularization, but also tissue regeneration and repair. Some agents which we are testing, including a phosphatase inhibitor, orthovanadate, inhibit apoptosis of endothelial cells, promote vascularization in vivo, and may be useful agents to prevent age-associated tissue damage or degeneration.

Z01 AG 00059-05 LBC

Mitochondrial DNA Deletions: Role in aging and disease

Charles R. Filburn

Damage to mitochondrial DNA (mtDNA) may play an important role in the aging process or in age-associated neurodegenerative diseases. Recent studies indicate that mtDNA mutations cause myopathies, are involved in an increasing number of other diseases, including diabetes, and show marked increases in some human tissues with increasing age. We have identified and quantitated a 4.8 kb and a 3.7 kb deletion in rat and mouse, respectively and shown increases with age. Liver mtDNA from 24 mo diet-restricted rats were found to have an average of 1/10 deletion-containing genomes as ad libitum fed rats. Analogous studies are underway in mice and monkeys. These data support the suggestion that oxidative damage, which is reduced in diet-restricted, longer living animals, plays a role in generation of these mutations. MtDNA damage causing these mutations may be important in muscle weakness, neurodegeneration and other age-associated changes.

Z01 AG 00500-05 LBC

Regulation and Processing of Amyloid Precursor Protein Genes and Gene Products

John W. Kusiak

Alzheimer's Disease (AD) is a major neurological disorder of the elderly affecting as much as 50% of the population over 80. No cure for this disease is known and its etiology is obscure. However, several genetic loci have been identified in subsets of AD patients causing early onset forms of the disorder or increasing the risk of a late onset form. One of these mutations is in the gene coding for the amyloid precursor protein (APP). Normal processing of this protein generates a peptide fragment,



termed Ab, which is a major component of the senile plaques in brains of AD patients. These observations suggest an important role of APP in the causation of this disorder. Our work has focused on establishing a model system in which mutant APP genes are over-expressed in several types of cultured cells thought to be important in AD. We previously showed that neuronal-like PC-12 cells and endothelial cells over-expressing mutant APPs undergo morphological changes and shift the processing of APP towards production of larger carboxyl terminal amyloidogenic fragments within the cells. We have now determined by a combination of TUNEL staining and FACS analysis that as many as 20% of PC-12 cells expressing these mutant APPs undergo an apoptotic cell death. Conditioned media from these cells, containing slightly increased amounts of Ab peptide did not induce apoptosis in untransfected cells, suggesting an intracellular site for this effect. Scanning electron microscopic analysis of these cells revealed cell body compaction and membrane blebbing while transmission EM showed nuclear chromatin condensation at the periphery of the nucleus, both phenomenon characteristic of apoptosis. Over-expression of mutant APPs may cause apoptosis by inducing oxidative damage to cells since an anti-oxidant compound, L-cysteine prevents DNA laddering and cell death. We are currently examining other possible mechanisms of apoptosis in these cells due to mutant APP over-expression. Our emphasis is on examining intracellular calcium levels, pH changes, cell adhesion properties, and mitochondrial oxidative damage.

Z01 AG 00505-05 LBC

The Role of Excitatory Amino Acid Receptors in Alzheimer's Disease & Neurodegenerative Disorders

John W. Kusiak

Excitatory amino acids (EAAs) and their receptors play many important physiological roles in the central nervous system. They are involved in brain development, neural plasticity, and memory acquisition. In addition there is much circumstantial evidence suggesting a role for EAAs and their receptors in neurodegenerative disorders of aging, including Alzheimer's Disease. The receptors for EAAs exist in different functional forms due to the presence of multiple subunits and isoforms and their differential distribution in the brain. We propose the hypothesis that abnormal regulation of expression of EAA receptors or their persistent overactivity may be responsible for the neuropathology seen in these disorders. We previously cloned the promoter of the NMDAR1 gene and observed that a proximal region is sufficient to confer neuronal specific expression. We now show that both Sp1 and GSG motifs are required for basal activity suggesting control over NMDA receptor expression by immediate early genes. An Sp1-like and several other transcription factors cooperatively interact to control this activity. We show that CREB protein induces reporter gene activity. Various trophic factors also induce expression of a reporter gene including NGF and FGF. Interestingly, TPA stimulates expression greater than 5-fold suggesting a role for PKC in regulating NMDAR1 expression. A combination of cAMP and NGF treatment increases expression 7-fold suggesting phosphorylation events are also critical in regulating gene activity. The regulation of NMDAR1 gene expression appears to be complex and tightly controlled. We also cloned promoter regions for NMDAR2-A,B, and C genes and are currently characterizing them. Transgenic technology, utilizing *lacZ* promoter constructs will be used to confirm the neuronal specificity and regulation of this promoter. A neuronal cell culture model is being developed to examine receptor gene expression and signal transduction cascades involved in glutamate mediated cell death.



Z01 AG 00506-02 LBC

Apoptosis in Degenerative Diseases of Aging

Walter E. Horton Jr., PhD

The pathogenesis of Degenerative Diseases of aging such as Alzheimer's Disease (AD) and osteoarthritis (OA) involve the loss of important functional cell types. Apoptosis is a form of programmed cell death that is responsible for the elimination of many cell types during development. We have initiated studies into the role of apoptosis in age-associated processes. Previously we utilized an *in situ* method to demonstrate that samples from the hippocampus of AD patients show an increased incidence of apoptosis compared to age-matched controls. We have now carried out immunostaining of the sections to show that although neurons are involved, the majority of the dying cells are glial. Studies with chondrocytes have shown that several relevant signals can induce apoptosis in this cell type, including trophic factor withdrawal.



Z01 AG 00120-18 LN

Drug Development and Delivery to the Central Nervous System.

N. H. Greig

Strategies were developed to design novel, potent, selective, long-duration and centrally active therapeutics for the treatment of (i) Alzheimer's disease and age associated memory deficit, (ii) cancers of the brain, lymphatics, breast and ovaries, (iii) and (iii) drug abuse. Centrally active acetylcholinesterase inhibitors were developed as cognitive enhancers and modulators of beta-amyloid production and are being developed towards the clinical arena for Alzheimer disease. Lipophilic anticancer alkylating agents were developed for cancer treatment, inhibitors of dopamine reuptake and of butyrylcholinesterase are being developed for drug abuse.

Z01 AG 00126-14 LN

Cognition and Brain Function in Aging and Dementia as Assessed by Neuroimaging Techniques

C. Grady

Increasing perceptual difficulty is associated with increased activation of frontal cortex. Activation of rCBF during stimulus encoding was seen in young subjects in left frontal cortex and right hippocampus but not in old subjects, suggesting age-related impairment of learning processes. A study of visual selective attention showed that attention within a sensory modality is associated with increased activity in those brain areas that process the relevant stimulus features and decreased activity in brain areas that process input from other sensory modalities. A review of the literature revealed that some sparing of cognitive function early in Alzheimer disease is probably due to the efforts of the brain to compensate for loss of circuits, either through synaptic plasticity or reorganization of functional networks. White matter hyperintensities (on the MRI) are predictive of metabolic brain alterations and reductions in cognitive function, even in very healthy individuals. Patients with dementia of the Alzheimer type (DAT) who also have white matter changes on MRI, showed relatively lower metabolism in subcortical gray matter and calcarine cortex and smaller reductions in neocortical association regions, compared to DAT patients without white matter changes, suggesting that leukoencephalopathy alters the pattern of metabolic abnormality associated with DAT.

Z01 AG 00129-15 LN

Blood-Brain Barrier in Relation to Brain Function, Metabolite and Drug Delivery

Q.R. Smith

The identity and function of regulated blood-brain barrier transporters for essential nutrients and drugs were examined in relation to aging and Alzheimer's disease. The basic amino acid transporter of the blood-brain barrier was cloned and brain choline transport was shown to be maintained with age. The substrate selectivity of the choline and neutral amino acid transporters were examined and high affinity ligands for each transporter were identified. On the basis of structure/activity studies, new drugs were developed that showed enhanced uptake into brain via carrier-mediated transport. One such compound, D,L-NAM, exhibited 20-40 fold greater brain uptake than its clinical analog, L-melphalan, and was further developed for brain antitumor testing. A similar compound, 4-chloro-kynurenine, was tested as a neuroprotective agent against



excitotoxic brain damage. A sensitive secondary ion mass spectrometry method was developed to image metal distributions in human brain. Studies demonstrated no evidence for selective accumulation of aluminum in neurofibrillary tangle-bearing neurons in Alzheimer's disease.

ZO1 AG 00130-12 LN

Cognitive and Neurophysiological Function in Healthy Aging and Dementia

G. Alexander

C.L. Grady

M. Furey

The cognitive and neurophysiological effects of healthy aging and age-related dementias were investigated using standard neuropsychological tests, measures of component cognitive processing, and positron emission tomography with 18-F Fluoro-2-D-deoxyglucose. Healthy aging was associated with a specific pattern of cerebral metabolism (rCMRglc) showing frontal hypometabolism with relative increased activity in parieto-occipital association areas, basal ganglia, midbrain, and cerebellum. Two cerebral metabolic patterns characteristic of (dementia of the Alzheimer-type (DAT) were identified using regional covariance analysis of resting state rCMRglc. Expression of these patterns distinguished DAT patients from patients with frontotemporal dementia and were correlated with specific cognitive deficits in the DAT group. Premorbid intellectual ability was inversely correlated with rCMRglc in several regions of association cortex in DAT patients. Among DAT patients, early age at onset of dementia was related to greater impairment on measures of visuospatial function. DAT patients with a family history of dementia showed greater rCMRglc deficits in the frontal association regions. Further, a unique neuropsychological profile was observed for a subgroup of DAT patients with early prominent visual disturbances characterized by severe visuospatial difficulties but better memory performance than typical DAT patients. Studies of complex attention in DAT identified three similar impairments of shifting or divided attention. A measure of visuospatial attention during visual search showed incremental slowing of reaction time with parametric increases in attentional distracters. DAT patients showed less benefit than controls with decreasing cue size in a visual search task. Long-term memory and orientation decline with age among non-demented Down syndrome adults.

ZO1 AG 00132-11 LN

Cell Biology of Models for Human Brain Disorders

Z. Galdzicki

R. Pearce

S. Peng

R. Siarey

J. Stoll

Compared with cultured diploid hippocampal neurons, neurons from fetal trisomy 16 mouse (Ts16), a model for Down syndrome (DS), showed a decreased the voltage-dependent sodium current during activation, as well as fewer sodium channels assessed by binding of radiolabeled saxitoxin. mRNAs encoding the alpha and beta1 subunits of the sodium channels were not altered, indicating post-transcriptional dysregulation in channel formation.



High-voltage-activated calcium currents were larger in Ts16 neurons compared to controls. Ts16 neurons, correspondingly, bound more L-type calcium ligand, although mRNAs for channel subunits were normal. Thus, mental retardation in Down syndrome may be an ion channel dysfunction. The ultrastructural morphology of fetal Ts16 and diploid hippocampus and dorsal root ganglion did not differ, consistent with this interpretation.

Cultured Ts16 dorsal root ganglion (DRG) neurons were less able to adhere to laminin-coated dishes than diploid neurons. Differences depended on nerve growth factor (NGF). Septal neurons from the brain are NGF-dependent. In primary culture, a subpopulation of highly excitable Ts16 septal neurons had bigger inward currents during the action potential than highly excitable diploid septal neurons.

Z01 AG 00133-12 LN

Therapeutic Interventions in Patients with Alzheimer's Disease

H.C. Lee

U. Freo

M. Furey

Pharmacokinetic studies determined a plasma concentration of arecoline, a cholinergic agonist that improved memory in Alzheimer's disease (AD) patients and predicted an optimal dose in all subjects. Arecoline administration improved different cognitive functions at different doses. Verbal ability improved at low doses, whereas attention and visuospatial ability improved at higher doses. Improvement in cognition was not due to activation of the hypothalamic-pituitary-adrenal axis. Administration of physostigmine, an inhibitor of brain acetylcholinesterase, also showed a modest improvement in verbal memory in most AD patients. Memory enhancement was correlated with plasma butyrylcholinesterase inhibition but not with plasma physostigmine concentration. Based on reported reductions of in the concentrations of the cofactor tetrahydrobiopterin and catecholaminergic neurotransmitters in cerebrospinal fluid of AD patients, a therapeutic trial using tetrahydrobiopterin was initiated in AD patients.

Z01 AG 00134-12 LN

Brain Phospholipid Metabolism: Relation to Function, Aging and Disease

D. Purdon

M. Chang

C. Jones

E. Grange

O. Rabin

A mathematical model was used to derive equations to examine incorporation into and half-lives of long chain fatty acids within brain phospholipids under in vivo conditions. In rats, the model was combined with quantitative autoradiography and biochemical analysis to examine these parameters with saturated [9,10-³H]palmitic acid (³H]PAM) and unsaturated [1-¹⁴C]arachidonic acid (¹⁴C]AA) and [1-¹⁴C]docosahexaenoate acid (¹⁴C]DHA). Kinetic parameters were determined after we developed a method to measure specific activity relative to plasma fatty acid specific activity of brain acyl-CoA, the precursor for incorporation of fatty acids into brain



phospholipids. Low values for this dilution factor indicated marked recycling of fatty acids within phospholipids (e.g. half-life of 40 min for arachidonate in phosphatidylinositol). This recycling reflects activity of phospholipase A2 in signal transduction, and could be inhibited by manoalide, a specific inhibitor of this enzyme. Fatty acid incorporation into brain occurs at the level of brain synapses, as shown following fractionation of brain. Ischemia contributes to release of fatty acids and an increased level of arachidonyl-CoA via activation of phospholipases, products which contribute to neuronal death. Chronic sensory deprivation is associated with reduced membrane remodeling and signal transduction, as measured with the fatty acid method. The method was extended to monkeys following the synthesis of fatty acids labeled with carbon-11, and using positron emission tomography (PET).

ZO1 AG 00135-12 LN

Molecular Biology of Brain Aging and Disease

K. Chandrasekaran

M.C. Bennett

R. Fukuyama

Studies on patients with Alzheimer's disease (AD) using positron emission tomography show impaired brain glucose utilization as an early manifestation. We have identified a molecular mechanism involving mitochondrial and nuclear genetic systems of oxidative phosphorylation (OXPHOS) that may relate to this impairment. Post-mortem brains from AD patients showed 40% - 60% decreases in levels of mRNA for mitochondrial DNA (mtDNA) encoded - cytochrome oxidase (COX) subunits I, III and NADH-dehydrogenase subunit 1 as well as for nuclear DNA (nDNA) encoded F_1F_0 -ATPase subunit in midtemporal cortex (association cortex showing reduced metabolism in life) not in the unaffected primary motor cortex (primary cortex comparatively spared), compared with control tissue. There was no difference in expression of the mitochondrial 12S rRNA (mitochondrial transcript) gene, nuclear lactate dehydrogenase subunit B (a marker of glycolytic metabolism) gene, nor of nuclear beta actin gene. COX enzyme activity was decreased by 34% in temporal association neocortex of AD brains as compared with control cortex. The question of whether the impairments in mitochondrial oxidative metabolism are a primary or secondary to other pathologic changes in AD remains to be answered. Direct cytochrome oxidase inhibition in the rat, induced by continuous infusion of sodium azide, resulted in neuronal death when combined with administration of a low dose of corticosterone.

ZO1 AG 00401-12 LN

Cerebral Chemistry in Dementia, Aging and their Treatments

H.U. Shetty

The project entails identification of metabolic defects associated with the pathophysiology of Down syndrome (DS). Subsequently, the implicated metabolic processes will be probed in Alzheimer's disease (AD) and in healthy aging. Development of bioanalytical techniques to study brain chemistry is an integral part of this study. Profiles of polyols in DS were examined in view of the functional abnormalities that constitute its phenotype. Abnormal levels of polyols lead to neurological disorders and cataract formation in humans (diploids). Several polyol species in cerebrospinal fluid (CSF) and plasma from DS and age-matched control subjects were



quantitated by a mass spectrometric technique based on generation of a unique fragment ion. The CSF concentration and the CSF to plasma concentration ratio of myo-inositol were significantly elevated in DS. Other polyols in CSF and plasma myo-inositol were unaltered. A positive correlation between the level of CSF myo-inositol and age was observed in controls and not in DS. myo-Inositol plays a central role in signal transduction and osmoregulatory processes in the brain. Analysis of polyols in AD showed no significant increase in CSF myo-inositol. However, a correlation between the levels of myo-inositol in CSF and plasma was lost in AD as in DS. Physostigmine is a candidate cholinergic drug for treating AD subjects. The plasma concentration profile of this drug is routinely being obtained by a HPLC technique developed in this laboratory. Additionally, structure analysis of rat brain phosphatidylcholine (PC) and phosphatidylinositol was carried out. Two polyunsaturated molecular species of PC into which radiolabeled arachidonate incorporated with high specific activity were identified. These molecular species may play an important role in signal transduction in the brain.

ZO1 AG 00403-10 LN

Genetic and Nongenetic Factors in Alzheimer's Disease

M. Schapiro

Family history studies showed a familial aggregation of Alzheimer disease among first-degree relatives of Alzheimer's disease probands compared with controls. A case report shows that dementia in Down syndrome may occur without mental retardation. DNA repair studies show a G2 excision-repair deficiency in Down syndrome and sporadic and familial Alzheimer disease. APOE4 allele in Down syndrome increases the age-related incidence of dementia and shortens life expectancy.

ZO1 AG 00404-09 LN

Functional Interactions among Brain Regions in Aging and Dementia

B. Horwitz

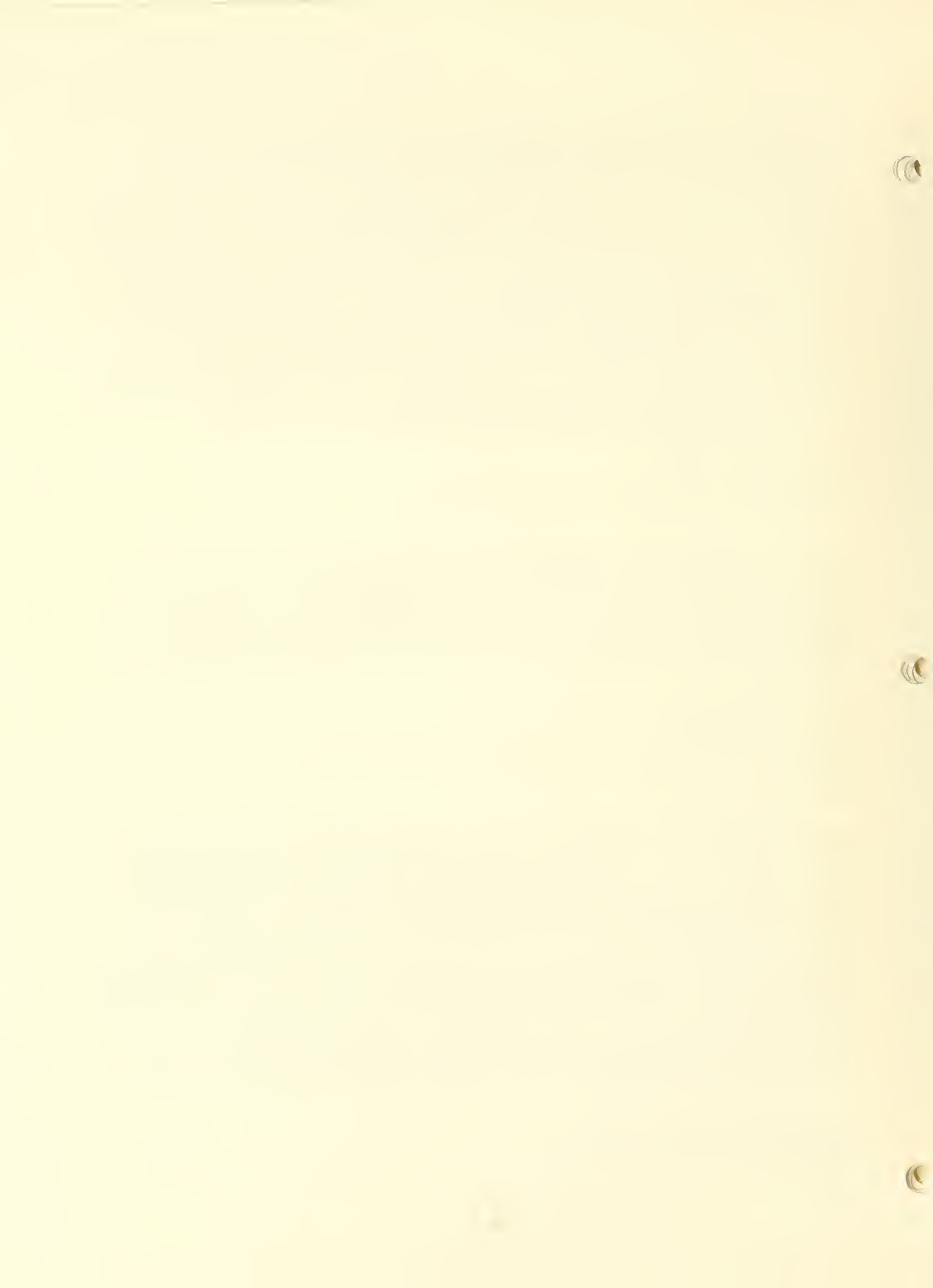
A.R. McIntosh

A correlation method was developed to examine functional interactions between brain regions, by correlating either regional cerebral metabolic rates for glucose or regional cerebral blood flows, as determined by positron emission tomography (PET) in humans. In humans in whom regional cerebral blood flow (rCBF) was measured with PET during a face matching task, correlations between visual brain areas and frontal regions were reduced in patients with mild dementia of the Alzheimer type (DAT) compared to controls. A rationale for use of neural modeling techniques with functional neuroimaging data was developed. A systems-level neural network model, fitted to rCBF PET data, permitted determination of the brain regions and their interactions that were involved in a working memory for faces task. A multiple regression/discriminant analysis involving PET regional interdependencies distinguished men and women on the basis of their inter- and intrahemispheric functional relations.

ZO1 AG 00406-05 LN

Mechanisms for Alzheimer's Disease

S. Rapoport



J. VanMeter

Comparative anatomic data suggest that a telencephalic system of brain regions underwent selective expansion during primate evolution, including the neocortex and connected non-neocortical regions such as posterior hippocampus and amygdala. Molecular changes during evolution likely made this system vulnerable to the neurodegeneration of Alzheimer's disease, as neuropathological and functional imaging studies using positron emission tomography (PET) show that the affected regions become abnormal earlier and more extensively in disease than do non-system regions. Functional changes occur at the level of the synapse in these regions. Accordingly, a model and method using activation brain blood flow studies with PET in individual subjects was designed to examine synaptic integrity in vivo, and to see how synaptic failure can be reversed with modulatory drugs during cognitive or psychophysical activation of the affected regions. The affected regions also demonstrate a reduced critical temperature of membrane lipids, hypothesized to cause membrane instability and ascribed to reduced concentrations of phosphatidylethanolamine.

Z01 AG 00407-04 LN

Neuroanatomy and Neuropathology of Aging Primate Brain

D. Brady

K. Hatanpaa

R. Fukuyama

There was a correlation between neuropathology and apoptosis (programmed cell death) in the hippocampus of Alzheimer's disease (AD) brains, but no evidence of apoptosis in cells with neurofibrillary tangles. The perirhinal, middle and inferior temporal cortices exhibited more dystrophic neurites than the superior temporal cortex, with laminae 2-3 most severely affected. We developed several approaches to elucidating the molecular basis of selective regional vulnerability in AD. Monoclonal antibodies were generated against the entorhinal cortex or basolateral amygdala using the SOFISTIC technique, which labeled subcellular compartments of neurons as well as neurofibrillary tangles. Brain specific expression of human microtubule-associated protein 1A (MAP1A) gene was demonstrated and assigned to human chromosome 15. In situ hybridization revealed a deficit in cytochrome oxidase (COX) activity and of messenger RNA (mRNA) expression in association brain regions in AD, in neurons which were stained by an antibody for paired helical filaments (PHF), a constituent of NFTs. Thus, neurons expressing PHFs retain some capacity for oxidative phosphorylation. A brain bank was established to provide appropriate tissue for neuropathological studies in AD. AD patients with clinically leukoencephalopathy revealed on postmortem severe cortical amyloid angiopathy without involvement of white matter vessels or atherosclerosis.

Z01 AG 00409-02 LN

Genetic Determinants of Neurodevelopmental Disorders in Humans

M. Schapiro

Human models exist in which genetic or hormonal dysfunction results in specific brain changes leading to specific mental retardation syndromes. Correlational analysis and multiple regression



and discriminant function analyses of brain glucose utilization data as measured with positron emission tomography (PET) show that brain language areas are disrupted in young adults with Down syndrome. Down syndrome subjects use similar regions to process language as controls, but to a lesser extent. Subjects with Turner syndrome (45,X), including mosaics, had reduced volume of the hippocampus as measured with magnetic resonance imaging, and impairment of memory and visuospatial abilities. Mosaic Turner syndrome subjects had volumes of left cerebral hemisphere and subcortical nuclei, and metabolism of left middle temporal region and right/left metabolic asymmetry ratios in parietal region intermediate between full Turner syndrome subjects and controls. The cognitive profile in fragile X syndrome is distinct from that in other forms of mental retardation. In this syndrome there is an enlarged brain on MRI and altered functional metabolic interactions among frontal and subcortical brain regions; focal changes in brain structure and metabolism may be related to cognitive changes.

ZO1 AG 00410-02 LN

Risk Factors for Vascular Dementia

H.C. Lee

A. Dani

T. Strassburger

Well-treated hypertensive but otherwise healthy people demonstrated cerebral atrophy on magnetic resonance imaging (MRI), manifested by lateral ventricle enlargement and left hemisphere atrophy. Brain glucose utilization as measured with positron emission tomography was reduced in territories of perforating arteries (thalamus and lenticular nucleus bilaterally). Well-treated hypertensives also had an abnormal circadian blood pressure variation, although their average 24 hour ambulatory blood pressure was well controlled compared with healthy controls.

We compared the clinical course, cerebral morphometabolic data, and postmortem examination in dementia of the Alzheimer type (DAT) patients with and without white matter change (leukoencephalopathy) on MRI. Extensive white matter changes were found in patients with slowly progressive dementia clinically indistinguishable from DAT. These patients differed from DAT patients without white matter change in their pattern of cerebral glucose consumption, suggesting that the white matter change is a process different from Alzheimer's disease. Postmortem studies of brains of three such patients showed Alzheimer's disease and severe amyloid angiopathy. In healthy subjects with white matter changes on MRI, the volume of the changes correlated with increased ventricular volume, reduced brain volume, and reduced cognitive scores. Subjects with greater than 0.5 % white matter hyperintensity volume also had lower whole brain and frontal lobe glucose metabolism, higher systolic blood pressure, and larger ventricular volume than age-matched controls.

ZO1 AG 00411-06 LN

Magnetic Resonance Imaging of Brain Anatomy in Aging and Dementia

J. Krasuski

J. VanMeter

B. Horwitz



As measured by volumetric magnetic resonance imaging (MRI), there was significant sexual dimorphism in the age-related decrease in brain volumes in healthy human subjects. The aging effect brain atrophy was significantly greater in males than females in whole brain, frontal and temporal lobes, but greater in females than males in hippocampus and parietal lobes. Discriminant analysis of MRI volumes completely separated Dementia of the Alzheimer type [DAT] subjects from healthy age- and sex-matched controls.

ZO1 AG 00413-01 LN

Molecular Biology of Cell Death and Neurodegeneration

H. Harris

Programmed cell death (apoptosis) is of central importance in many age-related degenerative processes. Interactions between cells and their extracellular matrix are critical to many cellular processes including differentiation, and survival. Loss of contact with extracellular matrix can induce apoptosis. We demonstrate apoptosis in PC12 cells deprived of extracellular matrix adhesion and we report that nerve growth factor (NGF) treatment greatly accelerates this process. Plating PC12 cells on agarose-coated dishes effectively blocks cell adherence. During the initial 24 hours of culture, 30% of the cells grown on agarose undergo apoptosis while cells plated on collagen-coated surfaces maintain nearly 100% viability. Apoptosis of adhesion-blocked cells continued over the next 72 hours. Addition of NGF paradoxically accelerates apoptosis in nonadherent cells while inducing proliferation and differentiation of adherent cells. Both NGF and fibroblast growth factor induced apoptosis while epidermal growth factor (which induces proliferation but not neuronal differentiation of PC12 cells) was far less potent. We investigated the nature of the signaling associated with NGF in nonadherent PC12 cells. Immunoblotting with anti-phosphotyrosine antibodies revealed striking differences in the pattern of tyrosine phosphorylation induced by NGF in adherent as compared to nonadherent cells, suggesting that NGF signaling is altered in nonadherent PC12 cells.

ZO1 AG 00414-01 LN

In Vivo Brain 1H and 31P Magnetic Resonance Spectroscopy in AD and Down Syndrome

W. Huang

Clinical protocols have been written to use magnetic resonance spectroscopy (MRS) to examine brain myoinositol levels in healthy human subjects, and in patients with Down syndrome and Alzheimer's disease. The method employed will be ¹H MRS. Whereas global brain concentrations of phosphorus metabolites have been reported by us to be unchanged in Alzheimer disease patients, the possibility that local changes occur in brain regions which demonstrate reduced glucose metabolism is being evaluated with ³¹P combined spectroscopic imaging.

ZO1 AG 00415-01 LN

Brain Activation during Passive Stimulation with Pharmacological Modulation, in Relation to Age and Disease

M.J. Mentis

A. Polles



L. Beason-Held

Single-subject activation studies involving passive stimuli that were varied in measurable ways (parametrically) were designed to examine brain networks and synaptic integrity in healthy subjects and subjects with Alzheimer's disease (AD) and Down syndrome (DS), and to see how pharmacological agents can modify these parameters. Regional cerebral blood flow (rCBF) was measured during stimulation, using positron emission tomography (PET) with 15O-water or functional magnetic resonance imaging (fMRI). Stimulation involving alternating flashes to both eyes, at different frequencies, demonstrated increasing rCBF responses with frequencies to 7 Hz in the primary visual cortex of normal subjects, but only to 4 Hz in AD patients; and responsiveness at 1 Hz in the middle temporal cortex of normal but not of AD subjects, indicating selective pathology in the magnocellular compared with the parvocellular visual pathway in AD. Data were analyzed by a new regions of interest method involving coregistration of PET and MR, taking into account atrophy. In another study, textured visual patterns were presented passively to young healthy subjects in relation to pattern organization, while using 15O-water PET. Two visual textures with the same black-white load produced different rCBF response patterns in the visual cortex. The observations were confirmed by fMRI. Thus, there are distinct form networks involved in texture perception, which can now be examined in noncompliant subjects.

ZO1 AG 00416-01 LN

Dementia in Down Syndrome

M. Schapiro

Longitudinal measurements of cognition, brain glucose metabolism as determined with positron emission tomography (PET), and brain anatomy using magnetic resonance imaging, were conducted in five Down syndrome subjects initially older than 40 years of age. Changes in memory test scores over 5 years preceded the appearance of overt dementia in two subjects, indicating that a pre-dementia prodrome can be identified in subjects certain to develop Alzheimer type dementia. Predementia changes also could be identified by submitting older, non-demented subjects to audiovisual stimulation while measuring regional glucose metabolism with PET.

ZO1 AG 00417-01 LN

Pharmacological Modulation and Cognitive Function in Alzheimer's Disease

M. Furey-Kurkjian

U. Freo

J. Van Meter

Measurements of regional cerebral blood flow (rCBF) during cognitive activation, and the effect of drugs which modulate synaptic function on the rCBF responses, can be used to examine synaptic integrity in the brain in health and disease. In healthy subjects performing an active working memory for faces task, rCBF effects of physostigmine, an inhibitor of acetylcholinesterase, were measured by positron emission tomography with 15O-water.



Significant effects were demonstrated and were shown to become stable within 40 minutes after establishing a steady-state plasma concentration of physostigmine by controlled infusion, derived by experimentally determined pharmacokinetics (Ann Rep Z01 AG 00133-LN). No habituation with repeated testing was evident. Subjects performing the task had increased rCBF in occipitotemporal visual brain regions and in right prefrontal cortex. Physostigmine reduced rCBF increments in the right prefrontal cortex in relation to a decrease in reaction time during the working memory task, indicating that the right prefrontal cortex is involved in effort and subject to cholinergic modulation. Whereas young and old subjects showed differences in resting-state rCBF, consistent with an aging effect, while performing the working memory task during physostigmine infusion, both groups showed similar reductions in reaction time. Unlike the young subjects, the old subjects failed to activate frontal and posterior cingulate regions during the task.

Z01 AG 00418-01 LN

Late Onset Depression And Other Behavioral Changes in Old Age, With or Without Dementia

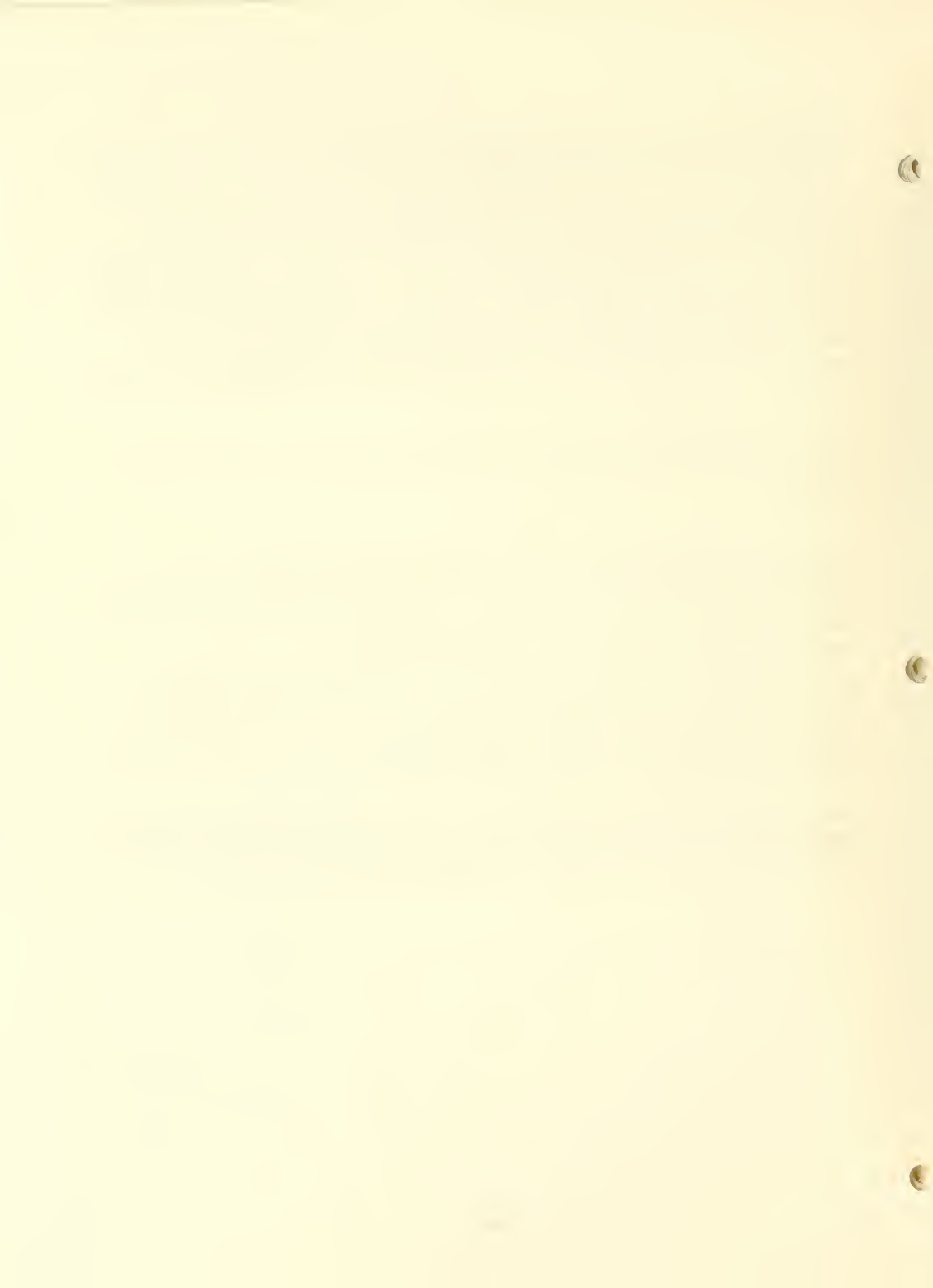
JS Krasuski

M Mentis

Positron emission tomography (PET) studies of Alzheimer's Disease (AD) patients with Delusional Misidentification Syndromes identified reduced glucose metabolism in orbitofrontal, anterior cingulate and temporal brain structures compared to matched AD patients and controls. Delusion formation was independent of cognitive abnormalities.

Compared with controls, elderly patients with late onset depression without cognitive impairment, and without "secondary depression", were found by PET to have increased brain glucose consumption in the medial and lateral temporal regions, orbitofrontal and lateral prefrontal regions of the right hemisphere, and decreased consumption in both thalamic nuclei, compared with matched healthy controls. This patient group may represent a distinct depressive subtype reflected in laterality of the brain metabolic abnormalities.

Neuropsychiatric rating scales were introduced to quantify behavioral abnormalities in aging and dementia, and to relate these to regional changes in brain metabolism as measured with PET. Behavioral changes likely are a major cause of institutionalization of AD patients.



Z01 AG 00226-12 LCS

Excitation-Contraction Mechanisms in Isolated Cardiac Myocytes

A. M. Janczewski

Contraction of the cardiac muscle is activated by a transient increase in intracellular $[Ca^{2+}]_i$, initiated by Ca^{2+} influx through voltage-gated Ca^{2+} channels of the surface membrane, which induces Ca^{2+} release from the sarcoplasmic reticulum (SR), apparently the major source of activator Ca^{2+} . An amplification of the transmembrane Ca^{2+} current (I_{Ca}) by the SR Ca^{2+} release, i.e. the gain of release, is thought to be determined both by characteristics of the I_{Ca} and by the SR Ca^{2+} load. However, the effects of changes in SR Ca^{2+} loading on the control of release by Ca^{2+} influx via I_{Ca} , and on an impact of released Ca^{2+} on SR Ca^{2+} release mechanism remain poorly understood. Last year, we reported a linear load/gain relationship under experimental conditions in which the SR Ca^{2+} load was varied (by up to 60%) while, using steady steps to a fixed potential, the voltage-gated activation of the Ca^{2+} channels was presumed constant. We focused this year on the gain function of SR Ca^{2+} release graded both by the SR Ca^{2+} loading and by the voltage-dependent activation of the Ca^{2+} channels, at potentials spanning the cardiac action potential. We also examine the age-related changes in the mechanisms that regulate the gain function of SR Ca^{2+} release. Isolated rat ventricular myocytes are voltage-clamped and dialyzed with Ca^{2+} indicator, indo-1. Ca^{2+} influx via the sarcolemmal Na^+-Ca^{2+} exchanger is inhibited by $0 Na^+$ in the patch pipette. Under these conditions, the I_{Ca} (Ca^{2+} -sensitive current) is graded by the voltage step potential, from $-30 mV$ to $+30 mV$, and provides an exclusive trigger for the SR Ca^{2+} release and a sole source of changes in the SR Ca^{2+} load. The SR Ca^{2+} load is graded by the duration of conditioning voltage steps and verified by changes in amplitudes of the Ca^{2+}_i transients elicited by SR Ca^{2+} depletions using caffeine. The SR Ca^{2+} release is assessed from amplitudes and rates of rise of the I_{Ca} -dependent Ca^{2+}_i transients. The gain of release is indexed by ratio of the amplitude of the Ca^{2+}_i transient and the corresponding influx via I_{Ca} , integrated during the time to peak Ca^{2+}_i . The results obtained so far in myocytes isolated from young rats (2-3 months) show that changes in the SR Ca^{2+} loading achieved under the present experimental conditions induce marked, quantitative changes in the gain index of SR Ca^{2+} release, but fail to alter its voltage-dependent changes. These results indicate that a tight control of SR Ca^{2+} release by voltage-gated Ca influx via I_{Ca} is maintained at various degrees of SR Ca^{2+} loading, and provide further evidence against strong positive feedback of released Ca^{2+} on the SR Ca^{2+} release mechanism.

Z01 AG 00231-11

Regulation of Energy Metabolism in Aging and Disease: Cardiovascular

D. Zorov

This project examines mitochondrial functioning in old age and in pathological states in which decreased energy transduction by mitochondria may compromise tissue survival. We have focused this year on mitochondrial functioning in the hearts of rats made diabetic by treatment with streptozotocin and studied in the chronic stage of the disease (6-8 weeks after treatment). We have extended last year's findings on the relative inability to elevate intramitochondrial



free Ca^{2+} ($[\text{Ca}^{2+}]_m$) of cardiac myocytes from diabetic rats, when subjected to electrical stimulation. This was studied using indo 1:AM-loaded and Mn^{2+} -quenched individual cardiac myocytes, using fluorescence microscopy. Stimulation (4 Hz) in the presence of elevated superfusate Ca^{2+} concentration (3.5mM) gave significantly smaller values of $[\text{Ca}^{2+}]_m$ in myocytes from diabetic animals, when compared with controls. Isolation of cardiac mitochondria from the two groups of animals revealed no differences in the kinetics of Ca^{2+} uptake or release, however, when studied using metallochromic indicators. It is concluded that the failure to elevate $[\text{Ca}^{2+}]_m$ in cells from the diabetic animals, which is of a magnitude such that it would be expected to limit the activation by Ca^{2+} of pyruvate dehydrogenase and the tricarboxylate cycle, derives from a failure of excitation-contraction coupling in this model.

Z01 AG 00261-07 LCS

Nitric Oxide (NO) Modulation of Cardiac Myocyte Signaling and Contraction

S. J. Sollott

Because cardiac myocytes *in vivo* are in such close proximity to endothelium, the effects of endothelial products on cardiac myocyte contractility may be important in myocardial function. Recent studies suggest that both endocardial endothelium and coronary vascular endothelium influence myocardial contraction, but the mediators responsible and their mechanisms of action are not well defined. These effects are apparently mediated by the release of at least three diffusible substances from endothelial cells: 1) endothelium-derived relaxing factor(s) (EDRF, including nitric oxide, NO), 2) endothelin-1, and 3) a novel stable factor, recently described by our laboratory, that acts predominantly by reducing the response of cardiac myofilaments to calcium. While much remains unknown about the mechanisms each of these factors, the most controversy probably surrounds contradictory reports regarding the effects of NO on myocardial contraction. We examined whether the apparent variability of myocardial responses to NO among recent studies could be due, in part, to a dependence on the (pre-existing) balance of cGMP- and cAMP-dependent-protein kinase (PKG, PKA) signaling pathways. In isolated rat cardiac myocytes we observed that the NO donor S-nitroso-N-acetyl-penicillamine (SNAP) significantly attenuated contraction and relaxation, without changing the Ca^{2+} transient, effects analogous to those observed previously with 8-Br-cGMP (*Circ. Res.* 1994;74:940). In cells where PKG has been pharmacologically inhibited, the negative effect of 8-Br-cGMP is completely eliminated. In contrast, in these PKG-inhibited cells, SNAP caused *increases* in twitch- and Ca^{2+} -transient amplitudes, accelerated relaxation, and Ca^{2+} loading; this positive inotropism was abolished in cells where both PKG and PKA have been pharmacologically inhibited. Thus, when PKG is blocked, an effect of NO to stimulate PKA is unmasked. Cells pretreated with a guanylate cyclase inhibitor to block NO-induced stimulation of cGMP production also demonstrated significant increases in twitch- and Ca^{2+} -transient amplitudes after SNAP. We interpret these results to indicate that NO stimulates both PKG and PKA pathways with opposing effects on contractile activation, with the negative contractile (*myofilament*) effects of PKG masking the positive effects of PKA in these experiments. Thus, changes in the balance of PKG/PKA activation, via NO exposure,



could serve a genuine physiological modulatory role in myocardial contraction and relaxation.

Z01 AG 00266-04 LCS
Ion Transport Mechanisms
J. P. Froehlich

A host of cellular activities depend on the ATP-dependent ion pumps for their maintenance or regulation. Our laboratory is engaged in transient state kinetic studies whose primary goal is the development of a transport model that explains how substrate and protein conformational energy is utilized to achieve unidirectional cation flux in primary active transport systems.

Titration of iodoacetamide spin-labeled Ca^{2+} -ATPase from skeletal muscle sarcoplasmic reticulum (SR) with AMPPCP revealed the presence of high (50 mM) and low (650 mM) affinity nucleotide binding sites in a ratio of 3:1. Phosphorylation of Ca^{2+} -ATPase with saturating ATP labeled only 50% of the total site population. Concurrent P_i production exhibited a burst phase equal to one-half of the total phosphoenzyme. Dephosphorylation by ADP revealed the presence of roughly equal amounts of E_1P and E_2P , despite the rapid accumulation of E_2P and its slow reversal to E_1P . Solubilization of Ca^{2+} -ATPase with C_{12}E_8 markedly reduced the level E_1P , indicating acceleration of its conversion to E_2P . This behavior is incompatible with a functional unit consisting of a simple Ca^{2+} -ATPase monomer.

Results obtained from parallel rapid mixing and electron paramagnetic resonance (spin label) experiments were used to construct a tetrameric model for the Ca^{2+} pump in skeletal muscle SR. In this scheme, staggered activation of the subunits by Ca^{2+} and ATP in the pre-steady state leads to the formation of a steady state complex in which each of the four principal intermediates of the transport cycle reside on a separate subunit. Unidirectional cation flux is enhanced by conformational coupling between the subunits permitting the exchange of energy from states of high energy potential (e.g., E_1P) to those of low energy potential (e.g., E_2P). The mechanical analog of this model is the "ratchet and pawl" in which the subunit-subunit interactions cooperate to prevent pump reversal.

Z01 AG 00270-06 LCS
Age-Associated Changes in Vascular Stiffness Properties
E. Lakatta

Although normative aging is accompanied by increases in arterial stiffness, the relationship between stiffness and cardiac structure and function is not known. The ultimate goals of this project are to determine how arterial stiffness properties influence myocardial structure and function and predict cardiovascular morbidity and mortality. Several sub-projects are described below. **A.** To determine whether arterial stiffness per se exerts an independent influence on left ventricular (LV) mass, we derived LV mass index (LVMI) from echocardiograms and arterial stiffness from pulse wave velocity (PWV) and applanation tonometry-derived augmentation index (AGI) in 133 normotensive BLSA subjects age 23-87 yrs. Age-associated increases were seen in LVMI ($r=0.24$), systolic blood pressure (SBP, $r=0.31$), PWV ($r=0.66$) and AGI ($r=0.53$). By multiple regression analysis, AGI ($p=.004$), SBP ($p<.03$) and male sex ($p=.04$)

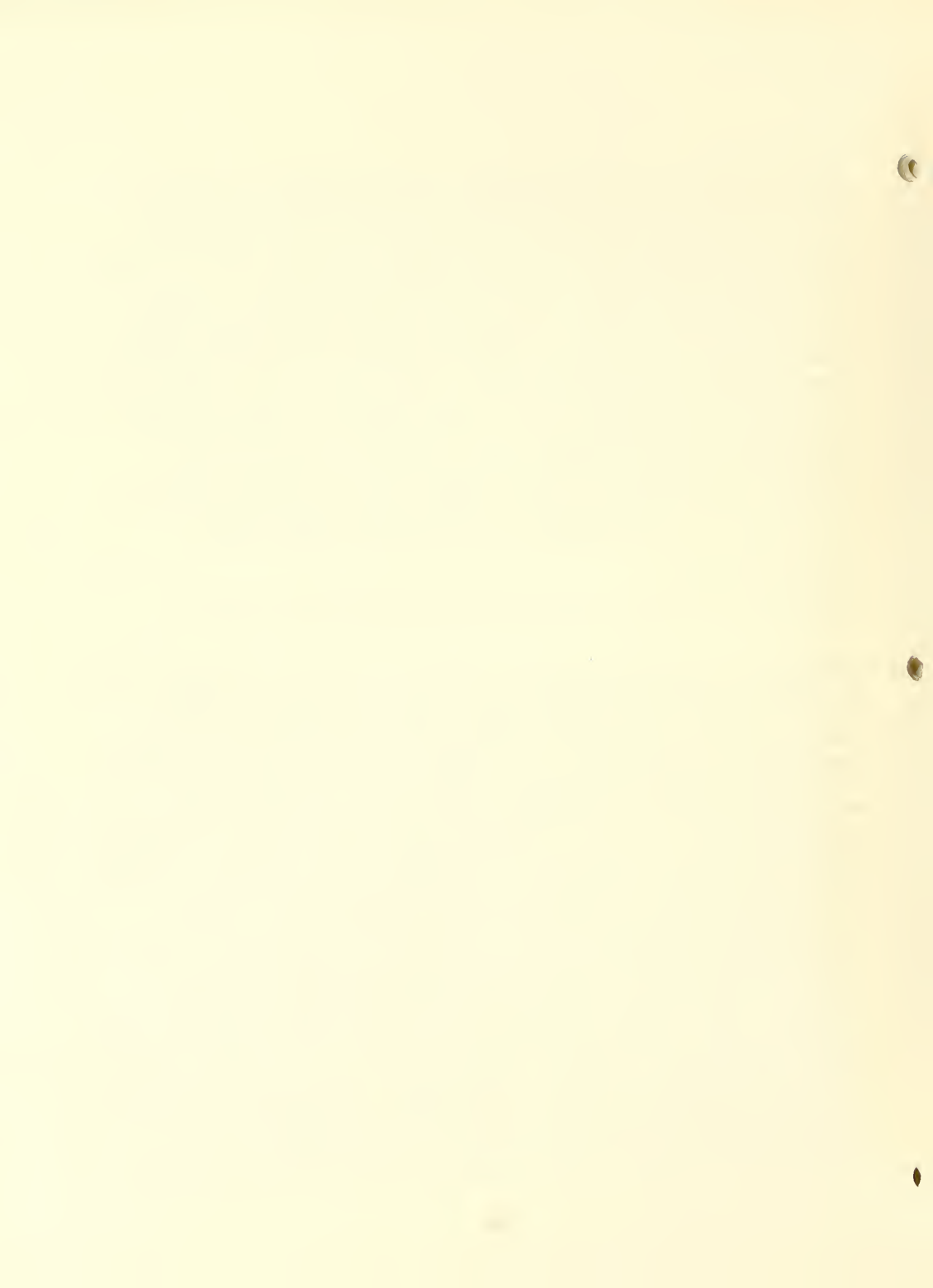


but not age or PWV were independent predictors of greater LVMI. **B.** To test whether the blunted LV emptying and LV ejection fraction (LVEF) response to exercise in older versus younger subjects can be ameliorated by reducing central arterial stiffness, we performed maximal upright cycle exercise in 9 healthy subjects 64-82 years old and 8 subjects <40 years old before and after administering the vasodilator sodium nitroprusside (N) intravenously. N reduced LV end systolic volume (ESV) and increased LVEF at peak effort in these older subjects, eliminating age differences in these variables noted prior to N. **C.** We will test the hypothesis that several months of home-based aerobic exercise training can reduce arterial stiffness in an NIH-sponsored multicenter trial of 810 individuals 35-75 years old. **D.** We have recently completed a study examining the effect of age, sodium intake, body composition and physical activity on arterial stiffness and LV mass in 2 populations in Taiwan. (Cf Contract #N01-AG-02-2118) **E.** A multicenter study to determine whether arterial stiffness is an independent risk factor for cardiovascular events in elderly free-living persons is being developed by the Epidemiology Demography Biometry Program of the intramural NIA. **F.** A method for calculation of characteristic impedance of the arterial tree from non-invasive measurements of central arterial pressure and flow has recently been developed.

Z01 AG 00274-05 LCS

Vascular Remodeling and Cell Differentiation During Injury, Disease, and Aging
M. Crow

The migration of vascular smooth muscle cells (VSMCs) from the media to the intima and the remodeling of the vascular extracellular matrix (ECM) are interrelated key pathogenic features of a number of life-threatening vascular conditions, such as atherosclerosis and restenosis following balloon angioplasty. Remodeling of the vascular ECM also occurs with aging and involves changes that are likely to contribute to the increased incidence and severity of vessel disease in aging animal populations. VSMCs in vivo are surrounded by and embedded in ECMs that must be traversed during migration, a process that involves regulated extracellular proteolysis. We have previously shown that activation of matrix metalloproteases (MMPs), particularly that of MMP-2 or type IV collagenase, occurs is required for the in vitro migration of VSMCs across an ECM barrier. We now demonstrate that activation of MMP2 occurs following balloon injury and that the activator of MMP2, known as membrane type-MMP (MT-MMP), is upregulated in vivo in response to vessel injury and in vitro in response to ligand engagement of $\alpha 2\beta 1$ integrin. Given that expression of $\alpha 2\beta 1$ is increased in VSMCs in vivo following vessel injury, signaling through this integrin may link injury to activation of the MMP cascade. In addition to acting as a barrier for cell movement, the ECM may also regulate VSMC differentiation. The molecular mechanisms controlling VSMC differentiation are unknown but may involve the family of ID proteins (for inhibitor of DNA binding or differentiation). ID proteins are helix-loop-helix proteins that act in many in other cell systems as transdominant suppressors of differentiation. We show that many ID isoforms, including a unique splice variant of ID3, are expressed in dedifferentiated VSMCs. Forced expression of, at least, one of these IDs, namely rat ID1, blocks VSMC differentiation. Current studies are examining the effects of the splice variant of ID3 which we have cloned



from proliferating VSMCs. The 2nd helix of the HLH domain, a region critically important for the function of ID, is disrupted in this variant. We have shown that this variant does not block the transactivating action of the basic-HLH protein, myoD, in 10T1/2 or the growth-suppressing effects of the retinoblastoma gene product in SAOS cells. In fact, the splice variant of ID3 acts as a dominant inhibitor of ID3 in SAOS proliferation suppression assay.

Z01 AG 00802-04 LCS

Effects of Age and Conditioning Status on Rest and Exercise Cardiac Performance

J.L. Fleg

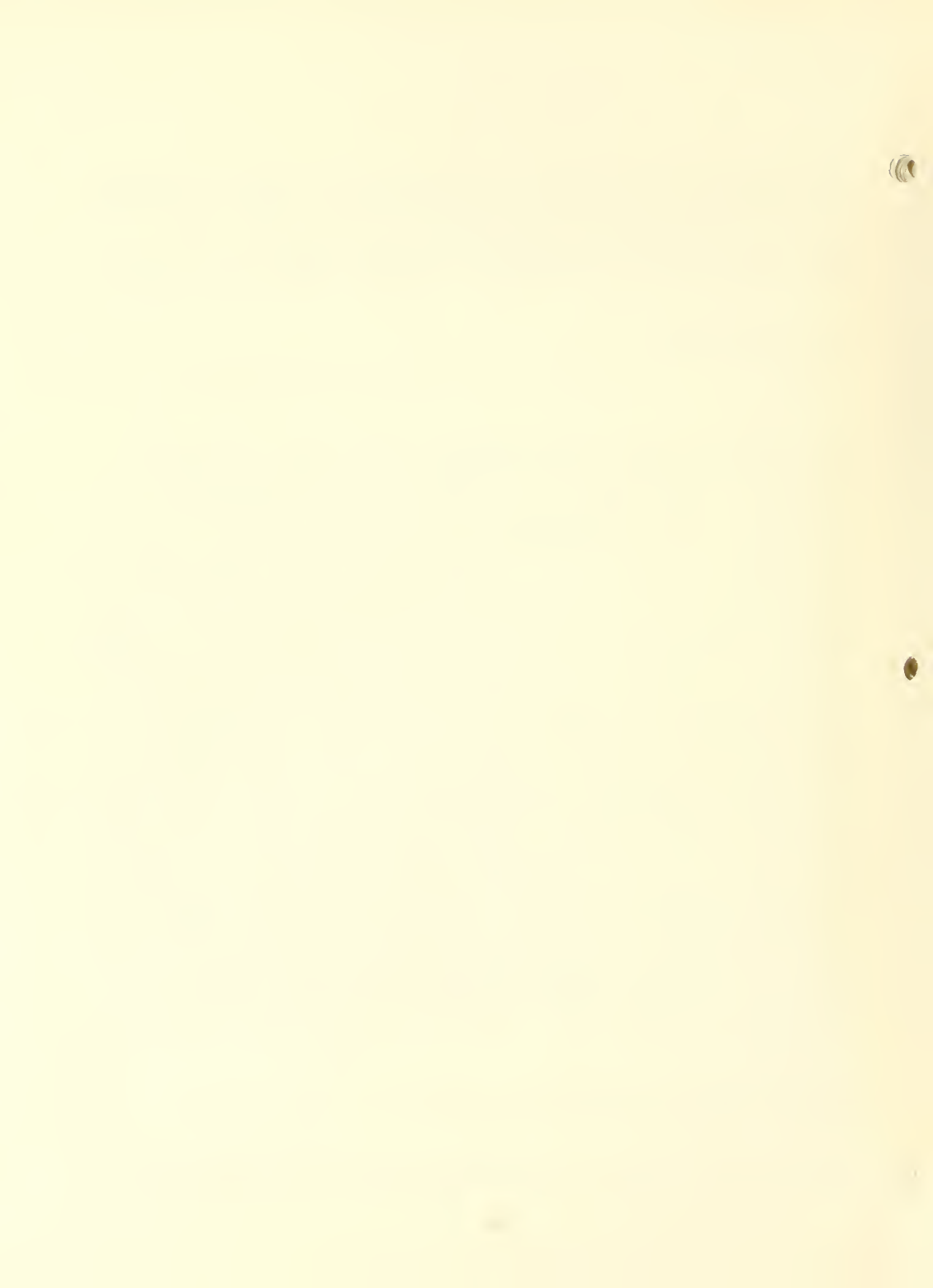
A longstanding goal of the Laboratory has been to determine the effect of age, gender, cardiovascular (CV) disease, and lifestyle variables on cardiac performance, both at rest and during exhaustive dynamic exercise. To accomplish this mission, we have utilized several techniques including gated cardiac blood pool scanning, Doppler echocardiography, and measurement of maximal aerobic capacity (VO_{2max}). These studies are predominantly focused on the Baltimore Longitudinal Study of Aging (BLSA), a panel of about 1000 community dwelling volunteers ages 20 to over 90 years. Recent examples of these studies are shown. **A.** To determine whether the beneficial effects of exercise training on CV performance in older individuals depend on the baseline fitness level, we performed rest and maximal exercise gated radionuclide ventriculography in 10 sedentary men aged 60 ± 2 years before and after 6 months of aerobic exercise training and in 8 endurance trained men of similar age before and after detraining for 12 weeks. At peak cycle exercise after the intervention, all of the initial intergroup differences in CV performance were abolished, indicating that CV performance in older adults is modulated in large part by physical conditioning status. **B.** To determine the relationship between left ventricular (LV) filling variables and LV structure, we performed by Doppler echocardiography, in 289 healthy, normotensive subjects age 20-89 yr (mean 52 ± 18) from the Baltimore Longitudinal Study of Aging. Age-associated decreases in peak E and E/A ratio and increases in LV mass index, relative wall thickness (RWT), the ratio of LV diastolic wall thickness to cavity size, peak A, atria filling fraction (AFF) and isovolumic relaxation time ($r=0.28$), each $p < .0001$, were observed. By multiple regression analysis, independent determinants of reduced E/A ratio were age ($p < .0001$), systolic blood pressure (SBP, $p < .01$) and heart rate ($p < .001$). AFF was directly related to age ($p < .0001$), SBP ($p < .02$) and RWT ($p = .05$). Neither LV mass index, cavity size or posterior or intraventricular septal wall thickness was an independent predictor of E/A ratio or AFF. Thus, age-associated changes in diastolic LV performance are not primarily determined by the modes LV Hypertrophy which accompanies normative aging.

Z01 AG 00803-04 LCS

Age-Associated Patterns of Gene Expression in the Heart

M. Boluyt

Cardiac hypertrophy is a hallmark of aging. Hypertrophied hearts of aged mammals resemble



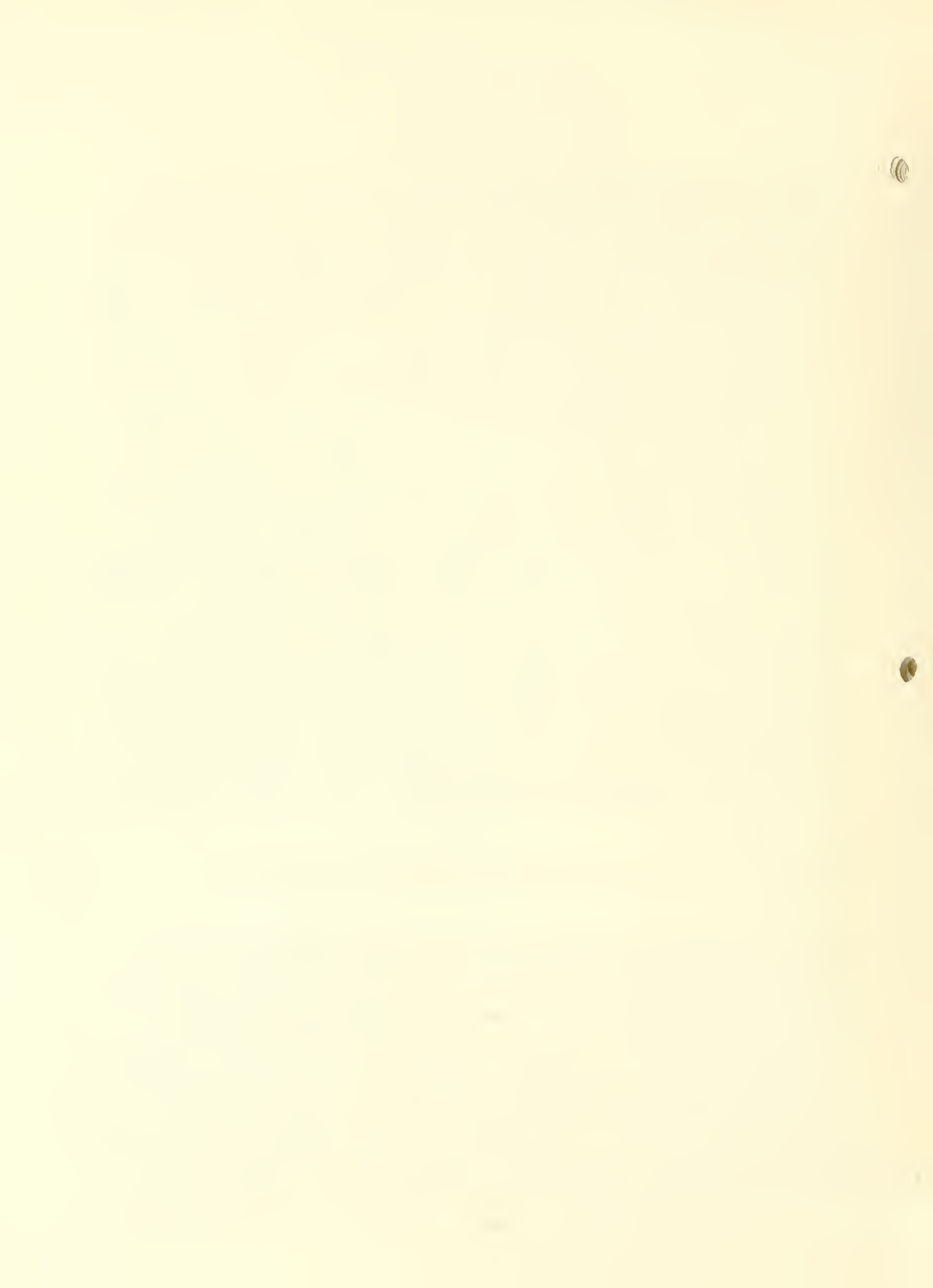
those of hypertensive younger rats in many respects, including changes in expression of a number of specific genes. It was not known, however, whether adrenergic receptor mediated hypertrophy shared common features of gene expression with aging and hypertension. We sought to examine the influence of adrenergic receptor stimulation on cardiac gene expression during hypertrophy using specific agonists and antagonists. Secondly, in an effort to approach mechanisms regulating expression of specific genes, we determined whether injection of DNA directly into the beating ventricle would be feasible in rats of advanced age. Male Wistar rats received either 2.4 mg/kg isoproterenol (ISO)/day, 9.9 mg/kg/day propranolol (PROP), both ISO and PROP, or vehicle (NaCl) via subcutaneously implanted osmotic pumps. In ISO rats, the ventricular weight/body weight ratio was increased by 27% after 1 day and peaked on day 3 (+40%). The levels of atrial natriuretic factor (ANF) and fibronectin (FN) mRNA in the left ventricles were elevated 20-fold and 13-fold in ISO rats, respectively, peaking at 3-days of infusion. Levels of transforming growth factor β 1 (TGF β ₁) mRNA were elevated 2-fold after 3 days of ISO infusion. The abundance of skeletal α -actin (SK) mRNA increased 4-fold after 1 day of ISO, and declined thereafter. ISO infusion decreased sarcoplasmic reticulum Ca²⁺ ATPase (SERCA) and preproenkephalin (PNK) gene expression by \approx 50% and induced a myosin heavy chain (MHC) isogene switch favoring β -MHC. PROP partially inhibited the ISO-evoked increases in ANF and FN mRNA, completely prevented the ISO-induced changes in TGF β ₁ and SERCA mRNA, but had no effect on the ISO-stimulated changes in SK and PNK gene expression. Results of experiments using irreversible inhibitors of α - and β -adrenergic receptors in conjunction with aortic constriction, indicated that these manipulations altered the levels but not the direction of gene expression and did not diminish the magnitude of hypertrophy. These results demonstrate that chronic ISO infusion elicits alterations in cardiac gene expression that are qualitatively identical to those observed in the hypertensive heart, but differ somewhat from those in senescent hearts. Luciferase activity and X-gal staining after injection of DNA into hearts of aging rodents demonstrated that this approach will be useful to determine mechanisms regulating transcription of genes in the heart during aging.

Z01 AG 00804-04 LCS

Cardiac Gene Expression During the Transition to Heart Failure in Advanced Age

M. Boluyt

Heart failure is characterized by impaired cardiac function and increased fibrosis and muscle stiffness. We showed previously that expression of transforming growth factor β 1 (TGF β ₁) and genes encoding extracellular matrix (ECM) components is markedly upregulated during the transition from stable hypertrophy to heart failure in aged spontaneously hypertensive rats (SHR). Since matrix accumulation is influenced by angiotensin II and membrane type matrix metalloproteinases (MT-MMP), our objectives were 1) to determine whether chronic treatment with an angiotensin converting enzyme inhibitor (captopril) would prevent the increases in expression of ECM genes associated with failure, 2) to determine whether MT-MMP is expressed in rat heart, and if so, 3) whether its expression is modulated during pressure overload hypertrophy, and 4) to investigate and characterize a non-genetic model of heart failure. We studied hearts from 18-24 mo SHR with signs of failure (SHR-F), without failure (SHR-NF), SHRs treated with captopril (2g/l in drinking water) beginning at 12 mo of age (SHR-Rx) and



age-matched normotensive (WKY) rats. In SHR captopril completely prevented the respective 4-fold and 1.4-fold increases in levels of α_1 (III) collagen and TGF β_1 mRNA that were exhibited by untreated SHR of advanced age. MT-MMP expression was studied in hearts of control and aortic constricted (AC) rats without failure. In hearts of young AC rats the expression of MT-MMP mRNA was elevated 1.7-fold at 1 hour post-AC and this elevation was sustained through 3 days of AC. Chronic AC of young rats was used as a non-genetic model of failure, with heart failure observed in 50% of rats by 11 mo of age. In AC rats with heart failure, TGF β_1 mRNA levels were elevated 1.25-fold ($p < 0.05$) compared to AC rats without failure. In contrast to the SHR model, no increase in $\alpha 1$ (I) collagen or fibronectin mRNA, only a small (1.5-fold) increase in $\alpha 1$ (III) collagen mRNA and a significant decrease in the level of the sarcoplasmic reticulum calcium ATPase (SERCA) mRNA were observed in failing compared to non-failing AC hearts. **CONCLUSIONS:** 1) Chronic captopril treatment prevented the increases in ECM gene expression that occur in untreated SHR of advanced age, suggesting a causative role for the renin-angiotensin system in fibrotic lesioning of the failing heart. 2) The rapid and sustained increase in expression of MT-MMP mRNA in hearts of AC rats implicates it in remodeling of heart. 3) Despite extensive fibrosis in AC rats with failure, the mRNA levels of ECM genes were not increased as in the SHR model, suggesting that the mechanisms underlying fibrosis differ in the two models.

Z01 AG 00806-03 LCS

Role of Extracellular ATP in Growth of Cardiac Myocytes and Fibroblasts

J-S. Zheng

ATP is now widely recognized as an extracellular ligand for membrane bound P₂-purinergic receptors. Since ATP is coreleased with norepinephrine (NE) from sympathetic nerve terminals in the heart, it may modulate the effects of NE on cardiac cells. We have previously shown that extracellular ATP induces expression of immediate-early genes (IEGs) and inhibits hypertrophic growth of neonatal rat cardiac myocytes. Since cardiac fibroblasts (CAFB) constitute > 70% of the cell population in the mammalian heart, and since Northern blot analysis showed the expression of P_{2Y} purinergic receptor mRNA in CAFB, it was hypothesized that ATP might also exert effects on CAFB via P₂-purinergic receptors. To determine whether CAFB respond to P₂-purinergic agonists, *c-fos* expression was studied in cultured neonatal rat CAFB (passage 3). In response to micromolar quantities of ATP, levels of *c-fos* mRNA were elevated 8-10 fold at 30 min. Relative potencies for *c-fos* induction in CAFB were UTP = ATP > ATPrS > ADP >> adenosine, consistent with P₂-purinergic receptor involvement. ATP increased Ca_i to peak levels in 10-30 sec and maintained an elevated Ca_i of lesser magnitude for up to 10 min. BAPTA-AM completely inhibited the ATP-stimulated induction of *c-fos*. Downregulation or inhibition of protein kinase C with phorbol ester and staurosporine, respectively, partially inhibited the ATP-mediated increase in *c-fos* mRNA. Western blot analysis demonstrated tyrosine phosphorylation of mitogen-activated protein kinase by 10 min treatment of either NE or ATP. These data show that ATP stimulates *c-fos* expression via P₂-purinergic receptors, and that P₂-purinergic receptor stimulation activates multiple second messenger pathways in CAFB. To assess the impact of ATP on CAFB growth, synthesis of DNA and protein was estimated. While NE increased incorporation of labelled precursors into cellular DNA and protein 2-3 fold, ATP inhibited both



basal and NE-stimulated incorporation of both precursors. Although UTP was equipotent for induction of *c-fos*, it did not inhibit incorporation of label into either DNA or protein. These data demonstrate that CAFB respond to P₂-purinergic agonists and suggest that distinct P₂-purinergic receptors subtypes mediate induction of *c-fos* and inhibition of DNA and protein accumulation.

Z01 AG 00807-03 LCS

Treatment of Restenosis After Angioplasty With Microtubule Stabilizing Agents

J. Kinsella, Research Physiologist, LCS

Significant improvements in the primary success rate of various medical and surgical treatments of atherosclerotic disease have been made in the last few years. Yet recurring failures continue in 30 to 50% of the patients after balloon angioplasty, bypass surgery, and endarterectomy because of late restenosis of the treated vessel. The restenosis is a result of a complex series of fibroproliferative responses to the vascular injury that results in vascular smooth muscle cell (VSMC) proliferation, migration, neointimal accumulation, and secretion of extracellular proteins. Microtubules are likely involved in controlling or moderating critical intracellular mechanisms necessary for the VSMC fibroproliferative response. We hypothesize that stabilizing microtubules with taxol or other agents may disrupt the mechanisms involved in the fibroproliferative response of the smooth muscle cells and therefore limit the cellular response to injury. We found that taxol, a microtubule stabilizing agent, inhibited VSMC proliferation, migration, and invasion in vitro. In vivo, taxol prevented neointimal VSMC accumulation in the rat carotid artery after balloon dilation and endothelial denudation injury. The peak blood levels measured in the experimental animals that was effective in inhibiting neointimal formation were 100 to 1000 times lower than the levels measured for the treatment of tumors in comparable assays. We subsequently have found that another microtubule stabilizing agent, D₂O (heavy water) also inhibited VSMC proliferation, migration, and invasion in vitro and neointimal formation in vivo. These experiments suggest that taxol, D₂O, or other pharmacologic agents that stabilize microtubules may have therapeutic value in preventing human restenosis after balloon angioplasty, bypass surgery, and endarterectomy.

Z01 AG 00809-03

Dietary Fatty Acid Regulation of Myocardial Function and Influences on Aging

S. Pepe

This research program was commenced in 1993 to identify: whether the composition of myocardial membrane phospholipid fatty acids can be altered by modification of the type of dietary fat intake; to ascertain if this alters cardiac function in aging and discern the nature of the underlying molecular mechanisms. It has been shown previously that in studies with rats that the vulnerability to arrhythmic stimuli increased with age and fish oil diet rich in omega-3 PUFAs (FO) abolished this effect whereas a diet rich in saturated fat (SAT) exacerbated arrhythmogenesis. In isolated working rat hearts, myocardial O₂ consumption, especially after ischemia, was distinctly high in SAT hearts but markedly reduced in FO hearts that had high O₂-energy utilization efficiency. This was not due to any change in basal O₂ consumption but rather was indirectly found to be related to altered intracellular Ca⁺⁺ homeostasis as when hearts were



perfused with ruthenium red, to block mitochondrial (MITO) Ca^{++} entry, the thermodynamic efficiency increased in SAT hearts. We observed that myocardial membranes in this dietary model, with increased age (6 vs 24mo), had increased omega-6 PUFA content but markedly reduced omega-3 PUFA content. SAT diet augmented this effect whereas no major change in these fatty acids with increased age occurred with FO. We also observed that in isolated cardiac myocytes, FO confers resistance against an age-linked increase in Ca^{++} -intolerance and arrhythmogenesis, whilst SAT exacerbates these age-linked effects. In isolated smooth muscle cells, SAT diet augments and FO attenuates age-related increases in cytosolic Ca^{++} transient. Recently, studies were conducted to define the less efficient use of O_2 at the MITO level and test whether this was related to increased Ca^{++} cycling by MITO in SAT hearts compared to FO. The respiratory control ratio, an index of the degree of coupling and thermodynamic efficiency, was raised in MITO from FO hearts. Ca^{++} -dependent activation of MITO pyruvate dehydrogenase and $[\text{Ca}^{++}]_{\text{MITO}}$ was significantly greater in preps from 24mo rats vs 6mo and this effect was augmented in SAT groups vs FO. It is concluded that decreased membrane fluidity with aging or SAT diet contributes to increased MITO H^+ and Ca^{++} cycling with decreased thermodynamic efficiency. The physicochemical state of cell and intracellular membranes, modified by aging and dietary lipids, regulates a range of intracellular effectors which alter inter-organelle communication and subsequently their response in the etiology of cardiovascular pathology. The effect of increased omega-3 PUFA content of phospholipids may thus have important beneficial consequences on cardiac mechanical & metabolic function with aging.

Z01 AG 00811-03 LCS

Gene Therapy of Coronary Artery Disease

M.C. Capogrossi

Our studies are aimed at evaluating whether gene therapy with replication-deficient, recombinant adenovirus vectors can be used to induce angiogenesis and restore blood supply to ischemic tissues and to prevent and treat restenosis after angioplasty. For the studies on angiogenesis, we have constructed adenoviral vectors which carry the cDNA for different angiogenic growth factors: VEGF₁₆₅, aFGF₁₋₁₅₄, recombinant secreted aFGF₁₋₁₅₄ modified by the addition of the signal peptide for secretion from FGF-4, bFGF, PD-ECGF. The vectors which carry the cDNAs for VEGF and for aFGF induce endothelial cell proliferation and differentiation in vitro as well as angiogenesis in vivo when coinjected subcutaneously with reconstituted basement membrane proteins (Matrigel) in mice. Further, preliminary results show that autologous endothelial cells infected ex vivo with AdCMV.VEGF₁₆₅ and subsequently injected into the iliac artery supplying the ischemic limb in a rabbit model of hindlimb ischemia induce angiogenesis in vivo. The construction of the remaining vectors has been completed only recently and the vectors are being evaluated for their biologic effects in vitro. For the studies on restenosis after angioplasty we have constructed adenoviral vectors which may act through one of the following steps: (1) selectively enhance endothelial cell regrowth at the site of vascular injury. (AdCMV.VEGF₁₆₅ and AdCMV.PDECGF). (2) induce vascular smooth muscle cell death or growth arrest. (AdCMV.WAF-1, AdCMV.IGF1R-AS and AdCMV.MKP-1). In addition an adenovirus vector which carries the cDNA for human wild type p53 has been obtained from Dr. Vogelstein (Johns Hopkins University). (3) inhibit vascular smooth muscle cell migration from the media to



the intima (AdCMV.TIMP-2). The above vectors are presently being evaluated to determine their biologic effects in vitro. Preliminary results show that AdCMV.p53 inhibits vascular smooth muscle cells (VSMC) proliferation while AdCMV.TIMP-2 inhibits VSMC invasion in the Boyden chamber.

Z01 AG 00815-03 LCS

Signal Transduction Pathways Involved in Vascular Smooth Muscle Cell Migration

M. Crow

The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular disorders. We have previously shown that VSMC migration is suppressed in growth-arrested VSMCs due the failure of these cells to activate calcium/calmodulin-dependent protein kinase (CamKinase) II in response to the chemoattractant, platelet-derived growth factor (PDGF). This was convincingly demonstrated by overexpressing constitutively activated Cam Kinase II in VSMCs and showing these VSMCs were capable of migrating toward PDGF even when the cells had been growth-arrested. We have recently demonstrated that autocrine stimulation of VSMCs by basic fibroblast growth factor (bFGF) is required for VSMCs migration and Cam Kinase II activation. bFGF's effect on migration is likely due its role in enabling PDGF to activate CamKII since 1) bFGF antibodies block activation of CamKII in response to PDGF, while the migration of VSMCs expressing constitutively active CamKII is not affected by these antibodies; and 2) the ability of exogenous bFGF to stimulate migration in growth-arrested cells is blocked by CamKII inhibition. In vivo, the ability of VSMCs to respond to PDGF and migrate is also controlled by interactions between VSMCs and the extracellular matrix (ECM). Our recent studies have shown that occupancy of avb3 integrin, which has been shown by other members in the laboratory to upregulated in vivo in response to vessel injury, is required for VSMC migration in vitro and for the activation of Cam Kinase II in response to PDGF. The migration of stably transfected VSMCs or VSMCs infected with a recombinant adenovirus expressing constitutively activated Cam Kinase II is unaffected by reagents that block avb3-ECM interactions. These results demonstrate that multiple intracellular signaling pathways triggered by chemoattractant recognition and integrin-ECM interactions are essential for migration and define one possible mechanistic link between integrin-mediated events and chemoattractant/growth factor signaling.

Z01 AG 00816-03 LCS

Nuclear Transcription Factors and Skeletal and Cardiac Muscle Aging

M. Crow

We have identified a number of genes whose expression in the heart change with aging. The age-associated shift of cardiac myosin heavy chain isoform expression (aMHC to bMHC) is similar to that observed in the hypothyroid heart and indicative of a constellation of changes in the aging heart in which thyroid-dependent gene expression appears to be depressed. Numerous studies, however, have failed to conclusively demonstrate an age-associated decrease in plasma levels of thyroid hormones and infusion of thyroid hormones to levels many times that in younger animals fails to fully restore thyroid-dependent gene expression



and contractile function. Because the effects of thyroid hormones are mediated by a heterodimeric transcription complex of thyroid hormone receptors (TRs) and retinoid X receptors (RXRs), we measured the levels of TRs and RXRs in the young adult (2 and 6 mo) and aging (24 mo) rat heart. The TRs are encoded by the erbA gene: erbAa1 and erbAb1 are splicing variants that encode functional TRs, while erbAa2 is a splicing variant that encodes a TR variant that does not bind triiodothyronine. The RXRs, on the other hand, are encoded by three separate genes, RXRa, RXRb, and RXRg. No significant changes were observed in the mRNAs for the two functional TRs, erbAa1 and erbAb1. On the other hand, the mRNA levels for both RXRb and RXRg decreased significantly between 6 mo and 24 mo. age, while that of RXRa remained relatively constant. In addition, using a monospecific antibody, we have also observed a significant decrease in RXRg protein levels with age. These results suggest that some of the age-related decline in cardiac gene expression may be due to decreased thyroid hormone-dependent intracellular signalling, due to decreased formation of the receptor-transcription complex necessary for thyroid dependent gene transcription.

Z01 AG 00817-03 LCS

Vascular Smooth Muscle Cell Function and Age-associated Hypertension

J. P. Froehlich

Systolic hypertension, arterial stiffening and diminished β_2 -adrenergic function are common in older individuals. The etiology of these conditions may involve similar elements affecting intracellular ion metabolism and contractility. To investigate changes in smooth muscle function with age, we have chosen a single cell model from the rat tail artery which retains many of the functional characteristics of smooth muscle in intact tissue.

Previous work from this laboratory has shown that the β_2 -agonist, isoproterenol (ISO), activates the redistribution of intracellular Ca^{2+} from the sarcoplasmic reticulum (SR) to the extracellular space via the sarcolemmal (SL) $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Maximum stimulation by ISO led to a 69% decrease in the amplitude of the cytoplasmic $[\text{Ca}^{2+}]$ spike elicited by the agonist, phenylephrine (PE), and abolished smooth muscle contraction. An analysis of the PE-induced $[\text{Ca}^{2+}]_i$ signal completed this year revealed that, in addition to lowering peak $[\text{Ca}^{2+}]_i$, ISO also produced a 39% decline in the amplitude of the tonic phase. Since the latter is dependent upon extracellular Ca^{2+} , these results suggest that ISO reduces Ca^{2+} inside the cell by decreasing Ca^{2+} influx through SL Ca^{2+} channels in addition to triggering active Ca^{2+} efflux.

Maximum stimulation by ISO (1 mM) depleted SR Ca^{2+} stores in SMC from 30 month old rats to 37.6% of pre-ISO levels compared to 30.7% in 6 month old rats ($p < .05$). Investigations this year showed that the larger SR Ca^{2+} stores in old SMC following exposure to ISO generate larger PE-induced contractures compared to young SMC. These results suggest that the ISO-dependent difference between the Ca^{2+} stores in young and old SMC is physiologically significant. We propose that normal α -agonist activity in the presence of reduced β_2 -adrenergic function may increase vascular tonus in old animals, contributing to the rise in systolic blood pressure and arterial stiffness.

Z01 AG 00818-02 LCS

Mechanisms of Signal Transduction of Cardiac Opioid Receptor Stimulation

S. Pepe

Opioid peptides (OP) are coreleased with catecholamines (CA) from nerve terminals in the heart cardiac myocytes also produce and secrete opioids and have opioid peptide receptors (OPR). Thus we proposed that OP also may have a postsynaptic cardiac intracellular signalling role which involves a potent "cross-talk" with the β -adrenergic receptor (β -AR) stimulation pathway. The focus of this work was to identify whether OPR stimulation influences the effects of β -AR stimulation and the nature of the mechanism(s) of interaction. In an intact isolated heart preparation (pretreated with 6-hydroxy-dopamine to deplete CA from peripheral nerve endings) peak systolic pressure was increased to 217% of control by the CA norepinephrine (NE; 10^{-7} M), addition of the δ -OPR agonist leucine enkephalin (LE; 10^{-8} M) resulted in a marked reduction in developed pressure to 66% of control within 15-25min. The OPR antagonist naloxone (10^{-8} M) added to the LE+NE buffer rapidly reversed the LE effect (< 1 -2min) to 188% of control systolic pressure. Although 10^{-8} M LE potently inhibited the positive inotropic effect of NE including the stimulated increase in cAMP, alone, at this concentration it had no effect on systolic pressure nor tissue content of cAMP. A non-hydrolyzable analog of cAMP, CPTcAMP, at 3×10^{-5} M increased systolic pressure to 176% of control but LE (10^{-8} M)+CPTcAMP could not counter act the positive inotropic effect. Similarly during perfusion with forskolin, which raised systolic pressure to 200%, 10^{-8} M LE also had no effect. Following pretreatment with pertussis toxin, (which catalyzes the adenine nucleotide ribosylation of G_i/G_o -protein α subunits and inhibits the response to agonists), LE could no longer inhibit the effects of β AR stimulation. Similar results were observed in single cardiac myocytes in which cytosolic Ca^{2+} and I_{Ca} could be measured. We conclude that the potent effects of LE are due to a specific "cross-talk" of the LE signalling cascade on the β AR stimulation pathway and mediated by a pertussis toxin-sensitive G protein involved in the inhibition of adenylyl cyclase. This interaction may thus regulate the magnitude of β -adrenergic effects on cardiac work and provide protection by preventing metabolic substrate supply/demand imbalance during intense cardiac stress such as exercise or ischemia.

ZO1 AG 00821-02 LCS

Chronic Regulation of Mitochondrial Content in Muscle

C. Moyes

We are studying the chronic regulation of mitochondrial enzyme activity in muscle, with a view to understanding the decreased capacity of skeletal muscle to respond to endurance training in old age. We aim to identify signals which allow the cell to sense increased energy demand and respond with increased expression of mitochondrial proteins encoded by the nuclear and mitochondrial genomes. This year we have studied C2C12 cells in culture (a mouse muscle-derived cell line) and have shown that differentiation from a tumor-like to a myocyte-like morphology, in response to serum-starvation, precedes the induction of mitochondrial enzyme activity. Similarly, in NIH 3T3 cells, which we have investigated because they show a very large (up to 40-fold) induction of mitochondrial enzymes upon differentiation into an adipocyte-like phenotype, the induction of lipogenic capacity precedes the mitochondrial changes. Thus, it is likely that the energy-demand of differentiation induces the mitochondrial enzyme synthesis. We

established that inner mitochondrial membrane enzymes with subunits coded for on mt-DNA (Complexes I and IV) show a lag in synthesis during differentiation in both cell types, whereas nuclear encoded matrix enzymes (citrate synthase, NAD-isocitrate dehydrogenase, pyruvate dehydrogenase, 3-hydroxyacyl CoA dehydrogenase) increase in a linear way. Succinate dehydrogenase (Complex II), which is a membrane enzyme with no mt-DNA-encoded subunit, behaves like the matrix enzymes, suggesting that it is the distinction between mt-DNA and nuclear DNA which is important, rather than the localization of the protein. Mitochondrial membrane enzyme activities are still much lower in differentiated C2C12 cells than in mouse soleus muscle, raising the question of an additional role of membrane lipid in organizing functional enzyme complexes - which we are investigating. Northern blots for COX II - a subunit encoded on mt-DNA - shows that this message tracks the copy number of mt-DNA in differentiating C2C12 cells, suggesting a rather straight-forward mode of control, at least in this instance. Northern blots for other representative enzymes are in progress.

Z01 AG 00822-02 LCS

Advanced Glycation Endproducts, Their Receptors, and Vascular Disease

M. Crow

The blood vessels of aging animals are characterized by a number of histological changes, including an increase in the number of vascular smooth muscle cells (VSMCs) and monocytes in the intima. These changes are likely to contribute to the increased occurrence and severity of vascular disease that is associated with aging. Advanced glycation endproducts of proteins (AGE) accumulate in the plasma and in tissues with age and at an accelerated rate in diabetes and in advanced atherosclerotic lesions. A receptor for AGE (RAGE) has been identified and cloned and we have previously shown that RAGE is expressed in the vessel wall by the endothelium and by intimal vascular smooth muscle cells, indicating that they are likely targets of AGEs. RAGE expression is also dramatically upregulated following vessel injury with the increase in expression confined to the developing neointima. Incubation of quiescent intimal VSMCs and endothelial cells with AGE-albumin leads to a receptor-dependent intracellular oxidant stress and induces NF- κ B activity. In VSMCs, AGEs also induce expression of monocyte chemoattractant protein-1 (MCP-1) and PDGF B chain, which are leukocyte and VSMC chemoattractants, respectively.

These changes in transcription factor and gene expression are inhibited by soluble RAGE, which prevents AGE binding to RAGE, and by the antioxidants, probucol and N-acetylcysteine. In intimal VSMCs but not in endothelial cells, crosslinking of RAGE with affinity purified antibodies to the receptor also results in activation of NF- κ B and increased MCP-1 and PDGF expression. Transfection of medial VSMCs which express low levels of the endogenous receptor with wild type and in vitro mutated RAGEs is currently being conducted to identify signal transduction pathways for AGE-mediated changes in gene expression in the cell. Overall, these studies demonstrate that VSMCs are targets for AGE and that the consequences of this interaction could result in increased VSMC migration and monocyte infiltration, a likely prelesional event in atherogenesis.

Z01 AG 00823-02 LCS

Coordination of Vascular Smooth Muscle Cell Signaling During Chemotaxis

S. J. Sollott

We have recently demonstrated in the vascular smooth muscle cell (VSMC) that Ca^{2+} -signaling regulates both Boyden assay chemotaxis, and the prerequisite calcium/calmodulin-dependent protein kinase II (CaMK II) activation, in response to PDGF. Since significant heterogeneity in the rate of chemotaxis is evident among proliferating VSMCs (i.e., only 5-10% chemotaxis at 4 hrs), we sought to understand how intracellular calcium (Ca_i) signaling is regulated among these cells and coordinated with other intracellular signaling pathways including kinase trafficking, and the resulting morphological polarization and reorganization of the cytoskeleton during chemotaxis. We have developed a novel microscope-adapted Boyden chamber which allows simultaneous measurement of locomotion and Ca_i in individual cells during chemotaxis (through filters with 8 μm pores). In addition, the ability to perfuse this chamber enables periodic intervention with experimental agents throughout the assay. Experiments with this chamber have demonstrated that significant differences in the spatio-temporal patterns of Ca_i exist between migrating and non-migrating cells. There are two distinct, sequential cellular Ca_i signaling phases required for VSMC chemotaxis: (1) an initial phase (time frame of minutes), with a relatively synchronous, transient increase in Ca_i in most cells, associated with CaMK II activation, and (2) a delayed phase (time frame of hours), with asynchronous and heterogeneous increases in Ca_i among cells which precede (and whose timing apparently determines) VSMC chemotaxis. During the initial phase, focal adhesion kinase (pp125^{FAK}), and phosphatidylinositol 3-kinase (PI3K)(which we found requisite for chemotaxis), translocate to the activated cell membrane "patch" over filter pores, and this polarization may be important in coordinating cytoskeletal remodeling during chemotaxis. The actin-cytoskeleton normally undergoes dynamic reorganization during chemotaxis. This occurs as a series of transitional phases from organized actin stress fibers in the stationary cell to various forms of membrane ruffling in the migrating cell, accompanied by the formation of a cortical actin shell during actual PDGF-gradient chemotaxis, with the eventual reformation of stress fibers upon completion of migration, beyond the PDGF-*gradient*. The delayed-phase Ca_i -increase in individual cells coincides with abrupt pore transmigration (a process prevented in cells with Ca_i buffered by an intracellular Ca^{2+} -chelator) and could provide the signal necessary for the generation of sufficient force by contraction of the cortical actin shell to overcome the cellular resistance to passage through the pore. Preliminary evidence, consistent with CaMK II co-association with actin stress fibers, suggests a plausible mechanism whereby local or global Ca_i activation, via CaMK II, could signal and coordinate dynamic actin reorganization. Thus, the close coordination of Ca_i and specific kinase activation and perhaps trafficking appears critical in the orchestration of cytoskeletal dynamics responsible for PDGF-directed VSMC chemotaxis.

Z01 AG 00826-01 LCS

Gene Therapy of Cancer

M. C. Capogrossi

Gene transfer techniques may provide efficient treatment for a variety of malignant neoplasms. A replication-deficient adenovirus (Ad) vector which carries the cDNA for wild-type p53

(AdCMV.p53) was tested for its *in vitro* and *in vivo* effects on the growth of murine melanoma cell and prostatic cancer cells. Cells infected with AdCMV.p53 were growth inhibited. DNA laddering using agarose gel electrophoresis and *in situ* labeling of DNA fragmentation (TUNEL) showed that AdCMV.p53-infected cancer cells underwent apoptosis. Nude mice injected subcutaneously with melanoma cells developed localized tumors. These tumors were subsequently infiltrated either with AdCMV.p53, with AdCMV.NLSβgal (2x10⁹ pfu), or with saline alone. One week after infection melanomas exposed to AdCMV.p53 were significantly smaller than control tumors. A similar result was observed with prostatic cancer cells infected *ex vivo* and subsequently injected subcutaneously in mice. Therefore, Ad-mediated wild-type p53 overexpression resulted in melanoma and prostatic cancer cell apoptosis *in vitro* and inhibition of tumor growth *in vivo*. These gene therapy approaches may be useful in targeting melanoma and prostatic tumors in a clinical setting. A preliminary study has been performed to determine whether intravenous basic fibroblast growth factor (bFGF) protects, against bone marrow hypoplasia following radiation therapy. The results show that in C3H mice bFGF enhanced LD₅₀ at 30 days in a dose-dependent fashion. Additional experiments will examine in a similar animal model the therapeutic potential of an Ad vector which carries the cDNA for bFGF. In summary, gene therapy with Ad vectors may provide a novel approach to induce programmed cell death of cancer cells. In addition, gene transfer of bFGF may enhance the resistance of bone marrow cells to radiation therapy. The biosafety and clinical applicability of the above approaches will be examined in future studies.

Z01 AG 00827-01 LCS

Epidemiology of Silent Myocardial Ischemia in Apparently Healthy Subjects

J. Fleg

Despite the decline in the age-specific death rates from coronary artery disease (CAD) since the late 1960's, CAD continues to kill more Americans than any other disorder, particularly among the elderly. A multifaceted program to define the prevalence, risk factors for, and prognostic significance of silent myocardial ischemia (SI), a marker for significant CAD in apparently healthy individuals has been developed. **A.** To determine the long term prognostic significance of SI in apparently healthy subjects, we examined the incidence and predictors of future coronary events (CE) over a mean follow-up of 10.9 years, in 407 asymptomatic volunteers aged 40-96 years from the Baltimore Longitudinal Study of Aging (BLSA). By proportional hazards analysis, a concordant positive ECG and thallium response, indicative of SI, was a significant predictor of CE, independent of conventional risk factors. Thus, exercise-induced SI is a potent risk factor for future CE among apparently healthy older adults. **B.** Although the risk factors contributing to overt coronary artery disease (CAD) have been extensively investigated, very little is known regarding the risk factors for SI in apparently healthy subjects. To address this question we performed tomographic thallium myocardial perfusion scans and assessment of standard CAD risk factors in 281 asymptomatic subjects. By multivariate analysis, older age, male gender, low HDL-cholesterol and elevated waist-hip ratio were associated with exercise-induced SI, as defined in A above. These data may be used to identify an asymptomatic population at high risk for SI, who may benefit from aggressive risk factor modification. **C.** To examine the importance of Apo E as

a genetic marker for the development of early CAD, manifest only by exercise-induced SI, we performed Apo E phenotyping on 172 asymptomatic volunteers ages 46 to 79 who also underwent maximal treadmill exercise testing. The Apo E4 allele was present in 44% of men with ischemic ST segment depression on exercise ECG compared with 17% of men with normal exercise tests, $p < .001$ despite similar lipid profiles in the 2 groups. Thus, the Apo E allele appears to be a genetic marker for exercise-induced SI and may allow identification of asymptomatic individuals at high risk for developing overt CAD.

Z01 AG 00828-01 LCS

Local Delivery of Therapeutic Agents for the Prevention of Restenosis

M. Jenkins

Restenosis following angioplasty remains a major factor limiting the long-term success of this form of therapy. Many of the failures of pharmacological therapy for restenosis in animal models are related to systemic intolerance or difficulty in providing controlled administration of drugs for adequate periods of time. Therefore, our studies have been directed towards the development of local, sustained-release delivery vehicles and the use of adenoviral-mediated gene transfer. We have previously reported that taxol, significantly inhibited intimal thickening following balloon injury to the rat carotid artery when given systemically intraperitoneally. In order to locally deliver taxol and other drugs at high concentrations locally and limit potential systemic side effects, we have developed sustained-release biodegradable microcapsules to encapsulate bioactive compounds. Applying these microcapsules loaded with taxol to the adventitial side of the rat carotid artery following balloon injury resulted in a dose-dependent inhibition of neointimal regrowth (restenosis) without a host inflammatory response. In addition to taxol, peptides that inhibit matrix metalloproteinase (MMP) activation are also being tested. We have also begun to develop a technique for intravascular drug delivery using polymeric films coated onto intraluminal stents. Preliminary results reveal that tantalum and stainless steel stents provide a satisfactory adhesive surface for drug-containing polymeric films. Using the pig coronary artery model of restenosis, future studies will determine whether polymeric coated stents impregnated with taxol or the MMPI provide a useful mechanism for the prevention of restenosis. Intraluminal adenoviral-mediated gene transfer has become an attractive alternative to achieve localized and controlled modulation of the vessel wall following vascular injury. In order to achieve targeted expression of genetic material in the pig coronary artery, we conducted experiments using a new flow-through catheter, the Dispatch catheter (Boston Scientific, Inc.). Using the reporter gene LacZ and this delivery system, we were unable to achieve efficient localized or reproducible transfection. Additional studies are planned to evaluate other catheter systems and techniques for intra-coronary gene delivery.

Z01 AG 00829-01 LCS

Mechanism of Age-Associated Decrease in Cardiac β -Adrenergic

R-P. Xiao

While a large body of evidence has demonstrated that cardiac responses to β_1 - or mixed β -adrenergic receptor (β AR) stimulation decrease with aging, the biophysical cellular mechanisms underlying the

age-associated changes are not well understood. In addition, possible age effects on β_2 RA stimulation is unclear. Our recent studies have shown that the distinct effects of β AR subtype stimulation in cardiac myocytes are mediated by different signalling pathways. Specifically, β_2 AR stimulated positive inotropic effect is dissociated from β_2 AR-induced increase in cAMP. In addition, β_2 AR is simultaneously coupled to Gs as well as a pertussis toxin (PTX) sensitive G protein. Therefore, β_1 AR and β_2 AR stimulation in heart cells may be differentially affected by aging. In the present studies, the ability of β AR subtype stimulation to modulate cardiac Ca^{2+} metabolism and contractility was examined in single rat cardiac cells from hearts of a broad age range (2-4, 6-8, and 24 mo). Under control conditions, there was no systematic age difference in L-type Ca^{2+} current (I_{Ca}), cytoplasmic Ca^{2+} (Ca_i) transient or contraction. However, a generalized diminution in the response of all these parameters to a β_1 AR agonist norepinephrine (NE) occurred with aging. In additional studies we found that the contractile response to β_2 AR stimulation by zinterol (ZINT) is also markedly decreased with aging. Furthermore, PTX specifically potentiates the positive inotropic effect of β_2 AR, but not β_1 AR stimulation in both young and old groups (2 and 24 mo). However, the potentiating effect of PTX is not altered by aging, suggesting that the age-associated diminishment in β AR subtype stimulated positive inotropic effect is not caused by a up-regulation of PTX-sensitive G protein(s) with aging. Taken together, the results of our studies show that the positive inotropic effects of both β AR subtypes stimulation are decreased with aging. The age effects on β AR subtype stimulation may be mediated by multiple mechanisms, including changes in receptor, Gs or β AR-Gs-adenylyl cyclase coupling. For example, β AR subtypes could be differentially desensitized in aged hearts by β -adrenergic receptor kinase (β ARK) induced phosphorylation. Further studies are required to test these hypothesis.

Z01 AG 00830-01 LCS

Distinct Mechanisms underlie β_1 - and β_2 -adrenergic Actions in Canine Heart Cells

R-P. Xiao

The β -adrenergic receptor (β AR) mediated modulation of myocardial performance is a major component of cardiovascular reserve function. While there are several different types of β AR, those in the myocardium are primarily β_1 AR. However, now strong evidence suggests that both β_1 AR and β_2 AR subtypes coexist in the hearts of various mammalian species, and that stimulation of both β AR subtypes play a significant role in the regulation of cardiac performance. Because the reduced contractile response to β AR stimulation in both aged and failing hearts is accompanied by a substantial loss of β_1 AR, with no loss of β_2 AR, the potential role of β_2 AR activation for improving cardiac performance has received considerable attention. Recently, we demonstrated that both β_1 AR and β_2 AR functionally coexist in cardiac myocytes and that β_2 AR stimulation augments L-type Ca^{2+} current (I_{Ca}), cytosolic Ca^{2+} (Ca_i) transient, and contraction. However, the actions of β AR stimulation on cardiac Ca^{2+} metabolism and contractility are largely dissociated from cAMP production and phospholamban phosphorylation in rat heart myocytes. Pertussis toxin (PTX) pretreatment specifically potentiates the β_2 AR stimulated increases in Ca_i transient, contraction and I_{Ca} . In the present study, we found that while β_2 AR stimulation by zinterol does induce an positive inotropic effect and increases in I_{Ca} and Ca_i transient, it has no effect on cellular cAMP production or on phospholamban phosphorylation in canine myocytes. These results strongly suggest that cAMP signalling pathway may not be involved in canine cardiac β_2 AR stimulation. Furthermore,



while the augmentation of I_{Ca} induced by β_1 AR agonist, NE, is completely blocked by a specific peptide inhibitor of cAMP-dependent protein kinase (PKI, 50 μ M in pipette filling solution), β_2 AR stimulated increase in I_{Ca} by zinterol persists in the presence of PKI. In addition, a G protein inhibitor, GDP β S (5 mM), included in pipette filling solution completely abolished the actions of β_2 AR as well as β_1 AR stimulation on I_{Ca} . Taken together, we conclude that while the effect of β_1 AR stimulation on I_{Ca} is due exclusively to cAMP-dependent protein phosphorylation, the effect of β_2 AR stimulation on I_{Ca} may be mediated by non-cAMP-dependent G protein(s)-coupled signalling pathway(s) in canine ventricular myocytes.

Z01 AG 00831-01 LCS

Remodeling of the rat arterial wall during aging

Z. Li

Peripheral vascular resistance increases with age resulting in the development of systolic hypertension. This phenomenon is associated with arterial stiffening which is modulated by vascular tonus as well as by structural changes in the arterial wall. The goal of these studies is to characterize the structural changes within the rat aortic wall during aging and to elucidate the mechanism(s) responsible for age-associated remodeling of the vasculature.

Tail cuff systolic blood pressure in Fisher 344XNB rats increased by 33.8% ($p < .001$) between 6 and 30 months of age without a change in diastolic pressure. Morphometric analysis of the aortic wall revealed a significant age-associated increase in vessel diameter (25.6%) and wall thickness (38.4%) and a decrease in medial wall cellularity (18.4%). In old animals, frequent breaks in the elastic laminae were observed together with a 5-fold increase the subendothelial intimal space and the occasional appearance of smooth muscle cells. Immunohistochemical studies revealed increased expression of ICAM-1 and TGF- β in the thickened intima of old rat aortae. Consistent with this, the levels of fibronectin and TGF- β in aortic extracts from old rats were significantly elevated. Compared to young rats, zymograms of tissue extracts from old rats showed increased activity of both the activated and inactivated forms of matrix metalloprotease-2 (MMP-2).

The increased expression of adhesive molecules in old animals may attract monocytes to the vessel wall, providing a source of mitogenic factors and cytokines for extracellular matrix (ECM) remodeling. Accompanying the increase in TGF- β and activated MMP-2 in senescent aortae, smooth muscle cell migratory activity and ECM protein secretion are enhanced. These structural modifications, leading to an increased wall thickness and decreased cellularity, are likely to be important factors contributing to the increase in arterial stiffness and peripheral resistance associated with vascular aging.

Z01 AG 00832-01 LCS

Role of STAT Proteins in Vascular Disease

J. Kinsella Research Physiologist

The vascular smooth muscle cells (VSMC) are the predominant cell in the medial layer of the normal vascular wall. With injury to the vessel wall, such as during atherosclerosis and after balloon angioplasty, the local release of growth factors (e.g. PDGF) and cytokines (e.g. g-IFN) acts as a stimulus for smooth muscle cells to produce protein products, to migrate and to proliferate. A

transcriptional pathway shared by both g-IFN and PDGF, as well as other cytokines and factors, is p91 (STAT1a: signal transducer and activator of transcription). p91 becomes tyrosine phosphorylated directly or through JAK family kinases by plasma membrane receptors, translocates to the nucleus, and binds specific DNA sequences in gene promoter regions. We propose that g-IFN released from T-cells within atherosclerotic lesions increases the expression of p91 in smooth muscle cells and that other locally released factors such as PDGF may activate p91. The combination of increased p91 expression and activation leads to a different program of gene activation of the smooth muscle cells within the atherosclerotic vessel compared to normal tissue. PDGF-BB, a mitogen and chemoattractant for smooth muscle cells thought to play a critical role in the pathology of vascular disease, activated the p91 or STATa (signal transducing and activator of transcription) family of proteins by tyrosine phosphorylation. We found that unstimulated VSMCs, isolated from rat aortae, express p91 (STAT1a) in the cytoplasm. Within 10 min after VSMCs stimulation by PDGF-BB (40 ng/ml) but not PDGF-AA or bFGF, p91 becomes tyrosine phosphorylated and translocates to the nucleus of the cell. In electrophoretic mobility shift experiments, we found that protein extracts from PDGF-BB treated cells contained GAS (g-IFN-activated-site) and SIE (sis-inducible-element) oligomer binding protein(s). When we added anti-p91 antibody to the cell protein extract, the DNA oligomer-protein-antibody complex was further retarded compared to the GAS and SIE oligomer-protein binding complex alone. We concluded that a DNA binding hetero- or homodimer of p91 was produced by PDGF-BB activation of VSMC. Increasing the VSMC expression of p91 by treating the cell for 18 hr with g-IFN, increased the both the intensity of cell staining and the intensity of the GAS and SIE binding complexes. This study demonstrates that VSMCs express p91, that g-IFN increases smooth muscle cell p91 expression, and that PDGF receptor activation elicited a p91 tyrosine phosphorylation concurrent with its nuclear translocation. Activated p91 may then bind to specific DNA sequences (GAS and SIE) in the promoters regions of targeted genes, such as c-fos or ICAM-1 or potentially JE, scavenger receptor, and VCAM-1, involved in both normal or pathological control of smooth muscle cells.

Z01 AG 00833-01 LCS

Sodium and Potassium Ion Transport ATPase

J.P. Froehlich

The Na^+/K^+ pump is involved in the regulation of cell osmolarity, electrical activity, and the energized transport of ions and solutes across the plasma membrane. Our laboratory utilizes high-resolution, time-resolved methods to characterize the dynamic behavior of the enzymatic, electrical and conformational reactions catalyzed by the Na^+/K^+ pump. Our objective is to understand how these events are coordinated in order to accomplish active Na^+ and K^+ transport by this pump.

Detergent-solubilized membrane fragments containing eel electric organ Na^+/K^+ -ATPase have been used to identify the conformational interactions between catalytic (a) subunits during ATP-dependent cycling. Native membranes exhibit similar steady state levels of the principal Na^+/K^+ -ATPase phosphoenzyme intermediates, E_1P and E_2P , despite the rapid conversion of E_1P to E_2P and slow hydrolysis of E_2P (which favor E_1P accumulation). Exposure to detergent, which produces a monomers, depletes E_1P by increasing its turnover rate. This implies that the steady state formation of E_1P is stabilized by intersubunit conformational interactions which are relieved by detergents. These quaternary interactions may have a critical role in energy conservation during the transport



cycle.

Computer analysis of the enzymatic reactions and transient state pump currents produced by Na⁺/K⁺-ATPase-containing membrane fragments from electric organ and pig kidney have demonstrated that electrogenic Na⁺ transport is delayed with respect to K⁺-activated E₂P hydrolysis. This implies that K⁺ is bound to the pump and is translocated before Na⁺ is released. This behavior conflicts with the traditional pump model (Albers-Post scheme) in which K⁺ binding takes place after Na⁺ is released (ping-pong mechanism).

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Effects of NOS inhibitor DPI and of Hypoxia on Vascular

R. Ziegelstein

The endothelium plays a central role in the control of vascular tone by producing vasoactive mediators like nitric oxide (NO). NO is generated from the amino acid L-arginine by the enzyme NO synthase (NOS), which requires NADPH and O₂ and the cofactors FMN, FAD, heme, tetrahydrobiopterin and thiol. Among the five classes of NOS inhibitors which have been described are the flavoprotein binders like diphenyleneiodonium (DPI). DPI and its analogues are also inhibitors of the enzyme NADPH oxidase, which has recently been suggested as a possible oxygen sensor in several cell types. Inhibition of this enzyme by DPI irreversibly inhibits the hypoxic chemoreceptor response in the rat carotid body and blocks the effect of hypoxia on an outward K⁺ current in cultured pulmonary neuroepithelial bodies. We recently showed that DPI, like the arginine analogue and NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME), inhibits endothelium-dependent relaxation to acetylcholine (ACh) in isolated rat aortic rings (n = 10), which is mediated by NO (*Circulation* 1994;90:1-459). In addition, we have shown that both DPI and L-NAME produce an additional contraction of aortic rings partially precontracted with prostaglandin (PG)F_{2α}. While the onset and magnitude of this contraction are similar for both NOS inhibitors (+145 ± 38% for L-NAME vs. +92 ± 24% for DPI at 1 minute, P = NS), the L-NAME contraction is sustained throughout the period of exposure (+234 ± 39% at 15 minutes) while that due to DPI is transient and followed by a more prolonged relaxation to a minimum tension of -27 ± 19% at 15 minutes of continued exposure (n = 11, P < 0.001 vs. L-NAME). This relaxation is endothelium-independent and may be mediated by a fall in vascular smooth muscle pH_i (see below) which may alter myofilament sensitivity to Ca²⁺. Thus, while these two NOS inhibitors have similar effects on both endothelium-dependent vasodilation to ACh, the addition of DPI to PGF_{2α}-precontracted rings reveals a *vasodilation* not found with L-NAME that may account for the different duration of the constriction each inhibitor initiates.

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Intracellular signalling pathways regulating cardiomyocyte hypertrophy

M. Boluyt

The intracellular signaling mechanisms involved in regulating cardiac myocyte hypertrophy have remained elusive. Studies of proliferating cell types have identified a family of 70 and 85 kDa protein kinases (p70^{S6K}) that phosphorylate ribosomal S6 protein and regulate translation of mRNA, thereby allowing cellular growth and cell cycle progression. Our purpose was to determine whether



p70^{S6K} plays a role in α_1 -adrenergic receptor (α -AR) mediated cardiomyocyte hypertrophy. Hypertrophy of neonatal rat cardiac myocytes was induced in culture with 20 μ M phenylephrine (PE) and was assessed by ¹⁴C-phenylalanine (¹⁴C-Phe) incorporation into TCA-precipitable protein and planimetric evaluation of two-dimensional cell area. Immune-complexed p70^{S6K} phosphotransferase activity was measured using synthetic RRRLSSLRA as a substrate. Treatment of myocyte cultures for either 20 min or 24 hours with PE increased p70^{S6K} activity >3-fold. Exposure of cells to PE for 72 hours resulted in a \approx 2-fold increase in ¹⁴C-Phe incorporation and a \approx 50% increase in myocyte cell area. Rapamycin, a specific inhibitor of p70^{S6K} activation, completely inhibited the PE-stimulated increases in p70^{S6K} activity, incorporation of ¹⁴C-Phe into cardiomyocyte protein, and myocyte cell area. Inhibition by rapamycin was dose dependent, and was reversed by FK506, a related immunosuppressant that competes with rapamycin for an FKBP binding site. Wortmannin (1 μ M) inhibited the PE-induced increase in p70^{S6K} activity, suggesting involvement of a phosphoinositide 3-kinase. It is concluded that α_1 -AR stimulation increases p70^{S6K} activity and that activation of p70^{S6K} is required for α_1 -AR-mediated hypertrophy of cultured cardiac myocytes.

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The Effect Of Age On Restenosis After Vascular Injury

M. Jenkins

Percutaneous transluminal coronary angioplasty (PTCA) has become an attractive alternative for treating coronary artery disease (CAD) in older individuals because it is associated with less morbidity and mortality than bypass surgery. However, restenosis following PTCA, occurring in 30-50% of all patients, is the major factor limiting long term success of this procedure. Although advanced age is a major risk factor for atherosclerosis, it is not known if the elderly are at more risk for restenosis than younger populations. Therefore, elucidating the effect of advanced age on restenosis will provide important information in advising older individuals on the long term sequelae of PTCA. Aging is known to result in changes in the vessel wall which might alternatively effect the response to vascular injury. Such changes include an increase in vessel wall diameter, thickness and stiffness. In addition to these changes, aging also increases the expression of growth factors such as TGF- β_1 , the PDGF receptor BB. Therefore, we hypothesized that these changes would alter the response to vascular remodelling following injury and possibly impart an additional risk to restenosis. In order to investigate the effect of age on restenosis, we chose the rat carotid artery model of restenosis. In contrast to previous studies using injury to the rat aorta as a model of restenosis, our results revealed that at early time points following injury, vascular smooth muscle cell (VSMC) migration and intimal thickening was greater in young animals without significant differences in medial VSMC proliferation. However at later time points, no age-associated differences were observed. Mechanisms underlying the initial delay in VSMC migration in old animals are not completely understood, but may be partly due to differences in extracellular matrix (ECM) composition, interactions of the ECM with VSMCs in the vessel wall, and the elaboration of proteases required for VSMC migration. These studies highlight important age-associated differences in the response to vascular injury which will be exploited to further characterize the cellular and molecular mechanisms that underlie restenosis.



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