# Contents

# Foreword

The mission of the NIA is the *"conduct and support of biomedical, social and behavioral research, training, health information dissemination, and other programs with respect to the aging process and the diseases and other special problems and needs of the aged."*

Research on Aging Act of 1974, as amended in 1990 by P.L. 101-557.

The Intramural Research Program (IRP) of the National Institute on Aging (NIA) comprises nine scientific laboratories and a research program that include the scientific disciplines of biochemistry, cell and molecular biology, structural biology, genetics, behavioral sciences, epidemiology, statistics, and clinical research and the medical disciplines of neurobiology, immunology, endocrinology, cardiology, rheumatology, hematology, oncology, and gerontology. Medical problems associated with aging are pursued in-depth using the tools of modern laboratory and clinical research. The central focus of research is understanding age-related changes in physiology and the ability to adapt to environmental stress. This understanding is then applied to developing insight about the pathophysiology of age-related diseases. The program seeks to understand the changes associated with healthy aging and to define the criteria for evaluating when any change becomes pathologic. Thus, not only are the common age-related diseases under study (e.g., Alzheimer's disease, atherosclerosis, osteoarthritis, diabetes, cancer), but the determinants of healthy aging are also being defined.

The bulk of the NIA intramural research program is conducted at the Gerontology Research Center in Baltimore, Maryland. The Laboratory of Neurosciences operates basic and clinical research programs from the Clinical Center at the National Institutes of Health. The IRP provides a stimulating, academic setting for a comprehensive effort to understand aging through a multidisciplinary effort of investigator-initiated research. The program offers many excellent training opportunities in both laboratory and clinical medicine with a wealth of valuable resources. The NIA is committed to training researchers for lifetime careers in the biomedical and behavioral sciences.

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**Nikki J. Holbrook, Ph.D.**

# Laboratory of Biological Chemistry

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The interests of the Laboratory of Biological Chemistry (LBC) cover a wide range of topics devoted to understanding biochemical and molecular events contributing to basic mechanisms of aging, as well as the development of age-related disabilities and diseases. The LBC is currently comprised of three major research units/section, the Molecular Neurobiology Unit (MNU), the Cell Biology Unit (CBU) and the Gene Expression and Aging Section (GEAS). A common goal of these programs is the elucidation of critical events associated with various age related deficits that could serve as targets for therapeutic strategies aimed at preventing or delaying the onset of disabilities and disease processes.

The Molecular Neurobiology Unit studies the structure, metabolism, and expression of factors controlling neuronal functions and their involvement in aging and age-related diseases. These factors include the amyloid precursor protein (APP) and presenilins, both of which are important in the pathogenesis of Alzheimer's disease, as well as glutamate receptors, neurotrophic factors, and extracellular matrix proteins.

The Cell Biology Unit encompasses studies on cancer and aging, physiological and molecular aspects of cartilage and bone function, and the role of mitochondrial dysfunction in aging and cell death. The group studying cancer and aging is interested in the role of vascularization in controlling the rate of tumor growth in the elderly, and molecular events important in the initiation and progression of breast cancer. The group studying cartilage is interested in examining the role of chondrocyte apoptosis in the etiology of osteoarthritis and in engineering chondrocytes in order to establish the feasibility of a homologous cell-based cartilage repair therapy in the elderly. Studies on bone involve assessing the causes of age-related deficits in old bone, and the design of new therapies for the treatment of osteoporosis. The group on mitochondrial dysfunction and aging is studying the contribution of mitochondrial DNA deletions to the aging process, and the role of mitochondrial dysfunction in causing programmed cell death. Studies in the Gene Expression and Aging Section

are focused on signal transduction pathways involved in regulating cellular responses to stress, the influence of these responses on growth regulation and homeostasis, and their alterations with aging.

While the individual research programs within the LBC generally function as independent groups, they are highly interactive, conduct biweekly joint meetings, and engage in collaborative projects. The Laboratory is equipped with state-of-the-art instrumentation and an extensive computer network.

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**Keywords:**
cellular stress
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apoptosis
signal transduction

**Recent Publications:**

Gorospe M, et al. *Mol Cell Biol* 1996; 16: 6654-6660.

Guyton K, et al. *J Biol Chem* 1996; 271: 4138-4142.

Gorospe M, et al. *Oncogene* 1997; 14: 929-935.

Xu Q, et al. *J Clin Invest* 1996; 97: 508-514.

**Biography:** Dr. Holbrook received her Ph.D. from the University of South Florida, Tampa, Florida, in 1980. She completed postdoctoral training at Dartmouth Medical School and the National Cancer Institute. She moved to the NIA in 1986 to initiate a research program examining cellular responses to stress. She assumed the position of Chief of the Laboratory of Biological Chemistry in 1997.

**Cellular Response to Stress and Aging:** This research program focuses on cellular responses to stress and how they become altered with aging. The rationale for such studies is as follows: Aging is characterized by a general decline in most physiologic functions, and in particular, by a decreased capacity to maintain homeostasis during episodes of stress. These changes are believed to reflect the accumulation of damage to cells and tissues resulting from a variety of toxic factors, either produced endogenously during normal growth and metabolism, or derived from the environment. Normal function and survival are dependent on the cell's ability to resist or adapt to such stress and to repair or replace damaged molecules. Genetic systems have evolved to detect specific forms of damage and to activate the expression of genes whose products increase the resistance of the cell to damage or aid in its repair. The continued effectiveness of these genetic responses to environmental insults is likely to be a major factor in the resistance to disease and aging, and may be an important determinant of longevity.

**Signal Transduction Pathways Mediating the Response to Genotoxic/ Oxidative Stress and Consequences for Cell Survival:** A number of distinct pathways can be activated in response to stress, dependent on the nature of the insult. These include, but are not limited to, p53, the heat shock response, mitogen-activated protein kinase (MAPK) cascades, and NF*k*B. Although we have an interest in all of these pathways, much of our recent work has focused on the activation of the extracellular regulated kinase (ERK) and c-jun N-terminal kinase (JNK) MAPK cascades in response to oxidant injury. Efforts have concentrated on identifying the

initiating events and critical mediators involved in the response and determining the consequences of MAPK activation for cell survival. Through manipulation of the respective pathways we have shown that ERK activation is associated with enhanced survival, while JNK activation is associated with increased cell death following oxidant injury. Current studies are exploring downstream effectors of the ERK and JNK cascades, the interrelationships between MAPK cascades and other stress response pathways, and their role in *in vivo* models of stress.

**Roles of Specific Stress-Induced Gene Products:** More than 50 genotoxic stress-inducible genes have been identified in mammalian cells. Although the functions of many of these have not yet been identified, they are presumed to play an important role in determining cell fate. Depending on the particular stress or cell type examined, the response can range from proliferation and transformation, to transient or irreversible growth arrest, differentiation, or programmed cell death. Our research in this area examines the specific genes which are believed to mediate these differential effects. Our goal is to understand their regulation and determine their function during the stress response. Efforts over the past year have largely focused on the role of the cyclin-dependent kinase inhibitor p21/Waf1/Cip1 in mediating growth arrest and inhibiting apoptosis in various stress paradigms. Other gene products under study in this regard include the heat shock protein 70, p27/Kip1, and the growth arrest and DNA damage-inducible gene, GADD153.

**Age-Related Alterations in the Stress Response:** Aged cells and tissues exhibit a reduced ability to respond to environmental stresses. Studies in this project area are focused on identifying the causes for this altered responsiveness. We have recently demonstrated that aged hepatocytes show reduced activation of ERK in response to various stress stimuli including hydrogen peroxide, sodium arsenite, and heat shock. This results in reduced induction of ERK-regulated genes and is associated with reduced survival to arsenite treatment. Further studies will address which events lying upstream of ERK might be altered with aging, accounting for this change in responsiveness. Induction of heat shock proteins in response to heat is also reduced in aged cells. A long term goal is to devise strategies to up-regulate these stress responses in aged cells. Thus far, we have been successful in potentiating the heat shock response in aged hepatocytes using non-steroidal anti-inflammatory agents.

**Collaborators:** Robert Udelsman, M.D., Johns Hopkins University; John C. Lee, Smithkline Beecham Pharmaceuticals; Albert J. Fornace, Jr., NCI; George S. Roth, Laboratory of Cellular and Molecular Biology, NIA; Thomas W. Kensler, Johns Hopkins University; Prem Seth, NCI; Maurizio C. Capogrossi, M.D., Laboratory of Cardiovascular Science, NIA.

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**Keywords:**
mitochondria
cell death
apoptosis
Alzheimer's disease

**Recent Publications:**

Moyes C, et al. *Am J Physiol* 1997; 272: C1345-C1351.

Filburn CR, et al. *Mech Aging Dev* 1996; 87: 35-46.

**Biography:** Dr. Filburn received his Ph.D. at Purdue University and did postdoctoral training at Yale University studying regulation of cyclic nucleotide metabolism. He joined the NIA in 1973 as a Staff Fellow and continued studying hormonal regulation of cyclic nucleotide metabolism and action in the kidney and other systems. More recently he has focused his studies on the role of mitochondrial DNA damage and of mitochondrial dysfunction in cell death in aging and neurodegenerative disease.

**Mitochondrial Deletions in Aging:** A long-standing, still popular hypothesis about mechanisms of aging proposes that somatic mutations in mitochondrial DNA (mtDNA) increase with age and result in impaired energy metabolism in some, especially postmitotic, tissues. We have addressed this question in part by measuring deletions in animal models and in degenerating neuronal tissues of humans. Using PCR methodology we have shown that a 5.0 kb deletion in rat mtDNA increases markedly in liver and in specific regions of the brain over the 2 year lifespan of these animals. We found no association in the brain with loss of mitochondrial electron transport activity and increase in this deletion, possibly due to the fact that in no tissue do the levels of this or other mutations increase to the levels needed to impair energy metabolism. We have found a reduction in the buildup of this deletion in liver of rats placed on caloric restriction, a treatment that increases maximum lifespan and delays many of the effects of aging. Establishing the total mutational load due to deletions, duplications, and point mutations and determining whether this load actually causes impairment of mitochondria in old animals in a substantial way remains one of the major challenges in this field.

**Mitochondrial Dysfunction and Apoptosis in Neuronal Cells:** Impaired energy metabolism is known to sensitize neuronal cells to stresses, especially excitotoxins that can cause cell death. We are investigating the effects of reduced activity of components of the electron transport chain on cell survival using both rodent and human neuronal cell lines using specific inhibitors as well as cells harboring mitochondria with AD-associated mutations in cytochrome oxidase. In these studies we make extensive use of fluorescent techniques to assess *in situ* mitochondrial function, i.e., membrane potential and free radical production, as well as cell survival and death via apoptotic or necrotic processes. These studies also focus on the role of the mitochondrially-associated, antiapoptotic protein Bcl-$X_L$ in protection against various insults and its mechanism of action. For example, we have shown that Bcl-$X_L$ over expression in rat PC-12 cells protects cells from the death inducing effects of rotenone, a powerful mitochondrial inhibitor. More recently, we have begun studying the sensitivity to various stresses of human SH-SY5Y cells that have reduced cytochrome oxidase conferred to them by mitochondrial DNA from platelets of AD subjects. In addition, we are assessing the levels of AD-associated mutations in mtDNA-encoded cytochrome oxidase in white blood cells of subjects participating in the Baltimore Longitudinal Study of Aging. Lastly, we are overexpressing rat mitochondrial cyclophilin, a protein known to play a critical role in regulating mitochondrial membrane permeability in conditions of oxidative stress.

**Collaborators:** Richard Hansford, Ph.D., NIA; Wilhelm Bohr, Ph.D., NIA; John Kusiak, Ph.D., NIA; Bryan O'Connell, Ph.D., NIDR; Robert Davis, MitoKor, San Diego; Andrew Halestrp, University of Bristol, UK.

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**Recent Publications:**

Petersen E, et al. *Int J Imaging Syst Tech* 1997; 8: 285.

Zhao B, et al. *J of Neuroscience Research* 1997; 47: 253.

Horton WH Jr, et al. *Muscle & Nerve* 1998; 5: S79.

Horton WH Jr, et al. *In vitro Cellular & Developmental Biology* 1998; 43: 1.

**Biography:** Dr. Horton received his Ph.D. in Anatomy/Cell Biology from the University of Cincinnati. He carried out post-doctoral training in molecular biology at the University of North Carolina and the NIH. Dr. Horton worked at the Eli Lilly Company before moving to his present position in 1989.

**Cartilage Biology: Models & Mechanisms Related to Aging & Disease**: Cartilage undergoes degeneration with age resulting in osteoarthritis (OA). OA afflicts 60 million individuals in the U.S., most over the age of 60. The resident cells, chondrocytes, display genetic and biochemical alterations that likely contribute to disease progression. We are establishing model systems and studying mechanisms that contribute to these age-associated changes.

**Mechanisms Contributing to Age-associated Changes of Cartilage:** One dramatic change in cartilage with aging is a significant reduction in the number of chondrocytes. With time, this may result in too few cells to adequately replace the cartilage that is slowly degraded. We formulated a hypothesis that programmed cell death, or apoptosis might contribute to this loss of viable chondrocytes. We are the first group to provide direct evidence that articular chondroyctes die by apoptosis and that the incidence of apoptosis increases with age in animal models. We are now studying primary articular chondrocytes and various cell lines in culture to define the extracellular signals that initiate apoptosis and the intracellular molecules that mediate the signal to die. Our work on the role of apoptosis in the pathogenesis of degenerative diseases of aging such as OA may lead to a specific therapeutic target to prevent cell loss and maintain tissue function.

Evidence that age-associated OA has a genetic basis is limited. We have recently established that a polymorphic allele of the human aggrecan gene (which codes for the major proteoglycan of cartilage) is associated with individuals who have bilateral hand OA. This exciting finding supports the idea that a single gene defect may contribute to the pathogenesis of the most common form of OA.

Finally, the degeneration of cartilage is ultimately carried out by matrix metalloproteinases (MMPs). We have characterized a cell line (immortalized rat chondrocytes, IRC) that shows a pattern of MMP expression similar to what is observed in OA. Recently, we demonstrated that a particular porteinase (MMP-13) is up-regulated in IRC cells in response to cytokines. This cell line should be useful for studying the regulation of chondrocyte MMP expression and identifying inhibitors.

**Regulation of Collagen II Gene Expression:** Collagen II is the most abundant protein in cartilage and is expressd at high levels only by chondrocytes. We have identified sequences in the promoter and first intron of the collagen II gene that are important for its expression in chondrocytes and we are now characterizing the proteins that bind to these sequences.

**Tissue Engineering**: We are developing protocols and models to support tissue engineering therapies for cartilage disease. We are the first laboratory to demonstrate that chondrocytes isolated from the articular cartilage of mature animals and elderly OA patients can be induced to divide in culture. In addition, we have shown that these “secondary chondroprogenitor cells” will form hyaline cartilage *in vivo* and *in vitro*. The cells may be useful for the repair of degenerated cartilage in aged individuals.

Collaborative research has led to the development of a hollow fiber bioreactor (HFBR) that will support the 3-dimensional formation of cartilage from animal and human cells. The HFBR is compatible with analysis by nuclear magnetic resonance imaging and spectroscopy. This system will allow for the development of non-invasive methods to follow cartilage formation, maintenance, and response to growth factors and cytokines.

**Collaborators:** Dr. Karen Hasty, University of Tennessee; Dr. Kurt Doege, Shriner’s Hospital; Dr. Marc Hochberg, University of Maryland; Dr. Jordan Tobin, Dr. Richard Spencer, and Dr. Antonino Passaniti, NIA.

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**Keywords:**
neurodegeneration
Alzheimer's disease
amyloid
glutamate

**Recent Publications:**

Wolozin B, et al. *Science* 1996; 274: 1710-1713.

Bai G, et al. *J Biol Chem* 1997; 272: 5936-5942.

Zhao B, et al. *J Neurosci Res* 1997; 47: 253-263.

Iwasaki K, et al. *Molec Psychiatry* 1996; 1: 65-71.

**Biography:** Dr. Kusiak received his Ph.D. from the Biochemistry Department of the George Washington University School of Medicine and Health Sciences in Washington, D.C. He did postdoctoral work in the Developmental and Metabolic Neurology Branch of the National Institute of Neurological Diseases and Stroke (NINDS), NIH before joining the Macromoleclar Chemistry Section of the Laboratory of Cellular and Molecular Biology, NIA. He spent a sabbatical year in the Receptor Biochemistry and Molecular Biology Section, NINDS. In 1990, he joined the newly formed Molecular Neurobiology Unit, Laboratory of Biological Chemistry, NIA where he has continued to study neurodegeneration in aging and diseases of aging.

**Neurodegenerative Mechanisms in Aging and Alzheimer's Disease:** Neurodegenerative diseases of aging including Alzheimer's and Parkinson's Diseases have distinct pathologies but both exhibit severe neuronal cell loss. The etiology of these diseases is obscure although excessive oxidative stress, environmental factors, and genetic factors have been proposed as initiating elements. Recent clinical studies of Alzheimer's disease (AD) patients treated with anti-inflammatory and anti-oxidant drugs suggest a potential ability of these drugs to slow the progression of the disease. One of the hallmarks of the disease is the presence in brains of extracellular senile plaques. A major constituent of senile plaques is the Aß peptide derived from a larger precursor protein, the Amyloid Precursor Protein (APP). Clues to the disease process come from recent discoveries of mutations in the APP gene and in two genes, unrelated to APP, termed Presenilins 1 and 2 (PS-1, PS-2). Mutations in these genes are found in early-onset, familial forms of AD and in each case lead to an increase in the production of longer forms (1-42) of the Aß peptide which has a greater tendency to aggregate and form senile plaques. *In vitro* studies showed that the Aß peptide is toxic to neuronal cells and the cell death induced by Aß may be apoptotic in nature.

Glutamate receptors play a pivotal role in several brain functions. However, over-activity of these receptors can lead to excitotoxic neuronal cell death. The type of cell death may be either necrotic or apoptotic depending upon the receptor subtypes involved and the degree of receptor stimulation. Interestingly, the distribution of these receptors correlates with the areas of cell loss found in AD. The receptors are important in learning and memory, processes severely impacted in AD, and over-activation of these receptors is thought to initiate a common final pathway of neuronal cell death in both acute and chronic brain insults.

Work in this group has focused on two areas of research: (1) the role of APP and PS genes in the pathology of Alzheimer's disease and (2) the transcriptional regulation of expression of the NMDAR1 gene, a key subunit of all NMDA receptors.

**Amyloid Precursor Protein and Apoptosis in Alzheimer's Disease:** A major focus of this project is to discover the roles of APP and the PS in the etiology and pathology of AD and the mechanisms involved in the neuronal cell death induced by mutant forms of these proteins. One of the aims of our laboratory is to discover how APP or PS mutations lead to specific neuronal cell loss in AD. Previously we showed that over-expression of mutated forms of APP in stably transfected PC12 cells led to the increased production of intracellular, amyloidogenic C-terminal fragments of APP. This is accompanied by increased cell death over several days; this death appears to be apoptotic by several criteria. Recently, we showed that transient expression of mutated forms of PS-2 also increased the amount of apoptosis in growth factor-dependent PC12 cells. In this same model system, the over-expression of an antisense PS-2 construct reduced the amount of apoptosis induced by mutant APPs suggesting that the two proteins may share the same pathway of cell death.

Taken together, these results suggest that the selective neuronal cell loss in AD may be due, in part, to an apoptotic mechanism. This provides a rationale for targeting particular elements of an apoptotic pathway for therapeutic intervention in AD. We are generating adenoviral vectors for injection into rat brains in order to examine the *in vivo* effects of over-expression of APP mutations. We will examine the effects of over-expression in specific brain regions and the possible differential sensitivity of older animals to an increased A*ß* load. We also are generating transgenic mice conditional for expression of mutated APPs in order to examine questions about the dynamics and reversibility of A*ß* deposition.

**Transcriptional Regulation of NMDA Receptor Subunit Genes:** A major focus of this project is to discover the pathological roles that excitatory amino acid (glutamate) receptors play in neuronal cell loss in aging and AD and the mechanisms by which this cell loss occurs. One of our objectives is to determine how the NMDAR1 and other family member genes are regulated at the transcriptional level. Since neurons expressing NMDA receptors are lost in AD, it may be important to determine which factors are involved in regulating expression and consequent activities of NMDA receptors during development and in aging and disease. Another objective of this project is to determine the mechanism by which glutamate causes cell death and the role activation of glutamate receptors plays in initiating a genetic cascade of programmed cell death. We previously characterized the promoter region of the NMDAR1 gene and found that it contained several transcriptional elements in the proximal region responsible for both basal, inducible, and neuronal specific expression. In recent work, we showed that nerve growth factor and other neurotrophins were able to stimulate expression of NMDAR1 gene in PC12 cells. This stimulation was induced through activation of high affinity TrkA receptors and subsequent activation of a Ras/Raf MAP kinase pathway. In preliminary results, we showed that this activation may be mediated through both GSG and Sp1 transcription factor activation and that Sp1 may be a novel phosphorylation target of Erk. In other work, we characterized several promoters for the second family of NMDA receptor subunits and found that they share some similar characteristics with the NR1 promoter. Other elements in the NMDAR1 promoter are being studied including two CRE consensus sites in the proximal region which may have important roles in regulating expression.

We are examining several neuronal cell lines as potential models to study glutamate-induced excitatory neurotoxicity. We showed that glutamate treatment of a hippocampal cell line will cause cell death which can be prevented by the over-expression of Bcl-2 protein. The results suggest that this line may be a good model to study neuronal apoptosis. NT2-N cells express the NMDA subtype of receptor and may be a good model of glutamate-induced necrotic cell death.

**Collaborators:** Sangram Sisodia, Ph.D., Johns Hopkins University; Benjamin Wolozin, M.D., Loyola University; Andres Buonanno, Ph.D. and Mike Sasner, Ph.D., Laboratory of Developmental Neurobiology, NICHD; Lin Mei, M.D., Ph.D., University of Virginia; Stuart Lipton, M.D., Harvard University; Eva Eves, Ph.D., University of Chicago.


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**Keywords:**
osteoprogenitor cells
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bone injury repair
osteoporosis
intervention


**Recent Publications:**


Tanaka H, et al. *Bone* 1996; 18: 473-478.

Williams S, et al. *Bone* 1996; 19: 637-644.

Tanaka H, et al. *Mech Ageing Develop* 1996; 92: 1-10.


**Biography:** Dr. Liang received his Ph.D. from Kansas State University, Manhattan, Kansas, in 1972. After two years postdoctoral training at Duke University, he joined the NIA in 1974. He is a member of the American Physiological Society and the American Society of Bone and Mineral Research.

**Bone Biology and Aging:** Our main interests are to define the causes for age-associated deficits in bone remodeling activity and to develop novel treatment approaches for osteoporosis. In the past year, we have continued to focus on three areas.

**Effect of Matrix Proteins on Characteristics of Osteoprogenitor Cells:** The expression of extracellular matrix proteins declines in old bones and consequently, may lead to impaired development and function of old bone cells. To test this hypothesis, we examined the growth of osteoprogenitor cells in dishes coated with matrix proteins. We have shown that the number of osteoprogenitor cells is stimulated 50%, 30% and 15%, respectively, in culture dishes coated with laminin, type I collagen or fibronectin. Type IV collagen has no effect on osteoprogenitor cells. Increase in cell number can be attributed to the increase in colony number not the size of colonies. Since matrix proteins may also affect the osteogenic lineage of progenitor cells, we examined the number of colonies that express alkaline phosphatase, an indicator of osteogenic linage commitment. We found that the alkaline phosphatase positive colonies were reduced 35% and 25%, respectively, by laminin and type I collagen. However, these effects were the same whether cells are derived from adult or old bones. To assess the possibility that different matrix proteins may also affect lineage development of osteoprogenitor cells in culture, we determined the bone induction potential of cells grown in dishes coated with matrix protein. Bone forming activity of old osteoprogenitor cells in subcutaneous implants is approximately 1/6 that of adult progenitor cells. However, culturing of osteoprogenitor cells on matrix proteins has no apparent effect on bone induction potential regardless of the age of animals from which the cells are derived. We have

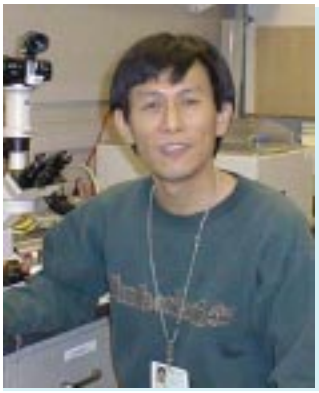
concluded that the decline in the expression of matrix proteins in senescence may impair the growth of osteoprogenitor cells but not the bone induction potential of old progenitor cells.

**Normalizing Bone Turnover Activity with Growth Factors:** Previously, we have shown that locally infused IGF-I can stimulate the expression of matrix proteins and increase trabecular bone volume in old femurs. In the past year, we examined the bone remodeling activity in old femurs after IGF-I treatment (50 ng/day, 14 days). We have observed that IGF-I treatment increases trabecular volume, trabecular number, and trabecular thickness, and decreases trabecular separation. IGF-I infusion increases the number of osteoblasts and osteoblast surface without significantly affecting the number of osteoclasts or osteoclast surface. Other parameters that assess the organic matrix (osteoid surface, osteoid volume) are also increased by IGF-I. Kinetic indices associated with bone formation, mineralizing surface, mineral apposition rate and bone formation rate, are elevated in IGF-I-treated femurs. Eroded surface, a resorption index, is not affected. We conclude that IGF-I treatment can improve trabecular bone status in old rats and that this effect is solely the result in changes in bone formation. Interestingly, it has been shown in clinical studies that subcutaneous injection of IGF-I at a low dose in elderly women can elevate serum markers of bone formation. At higher doses, IGF-I increases markers for both bone formation and resorption. The serum levels of IGF-I in research subjects treated with a low dose of IGF-I are elevated 100% which is similar to the estimated increase of IGF-I content in the marrow cavity in animals infused with 50 ng/ml of IGF-I.

**Phase II Clinical Trial with Minocycline in Treatment of Postmenopausal Osteoporosis:** We have shown that minocycline can prevent the loss of bone mass and trabecular bone in estrogen-deficient old rats by stimulating bone formation and, concurrently, inhibiting bone resorption. We have initiated a phase II clinical trial using minocycline to treat postmenopausal osteoporosis. Currently, human subjects with bone mineral density (either in the spine or hip) at 2.5-3.5 SD below the peak levels of young adults are being recruited for the study. Of fourteen subjects who passed the preliminary evaluation, four fit our criteria in further testing and were admitted to our clinical trial program. One subject developed an unrelated complication and was removed from the program. The treatment period will be for one year with 200 mg of minocycline by mouth given daily. Bone mineral density will be determined before the treatment, at 6-months, 12-months during the treatment and 4-months after the treatment. Serum and urine will be collected at 4-month intervals and markers of bone formation and resorption will be assayed during the treatment.

In the coming year, we are planning to expand our effort to recruit patients to the clinical trial with minocycline. In a parallel study, we will also examine the effect of minocycline on restoring the low bone mass in chronically estrogen-deficient old rats. Changes in femoral gene expression and alterations in bone turnover activity will be assessed to elucidate the mechanism of minocycline action.

**Collaborator:** Jay Shapiro, M.D., Johns Hopkins Bayview Medical Center.

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**Keywords:**
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**Recent Publications:**


Liu Y, et al. *Cancer Res* 1996; 56: 31-35.

Liu Y, et al. *J Biol Chem* 1996; 271: 3604-3607.

Liu Y, et al. *Free Radic Biol Med* 1996; 21: 771-781.


**Biography:** Dr. Yusen Liu received his Ph.D. in molecular biology and yeast genetics in 1991 from the Department of Fermentation Technology, Hiroshima University in Japan. He served briefly as an assistant professor in the same department, before joining NIA's Gene Expression and Aging Section in 1992, as a Visiting Fellow. In 1995 he was promoted to the position of Visiting Associate, and in 1996 to the position of Investigator. Dr. Liu's research is on signal transduction pathways involved in the stress response and their implications to the aging process.

**Signal Transduction Pathways Involved in the Stress Response and Aging**: Exposure of eukaryotic cells to harmful environmental conditions evokes alterations in gene expression. In mammalian cells, the stressful signals are mainly sensed by the cell membrane and transduced through posttranslational modifications of key regulatory molecules. Almost immediately after exposing cells to genotoxic agents, increases in the activities of a number of protein kinases can be detected. Activation of these primary protein kinases initiates the protein phosphorylation cascades, leading to the activation of a group of mitogen-activated protein (MAP) kinases including extracellular signal-regulated kinase (ERKs), c-Jun N-terminal kinase/Stress-activated protein kinase (JNK/SAPK) and the p38 MAP kinase. These MAP kinases can phosphorylate and activate

downstream protein kinases and a variety of transcription factors including Elk-1/TCF, c-Jun, ATF-2, and c-Myc, resulting in changes in gene expression. Altered gene expression can, at least in part, account for the variable phenotypical changes cells undergo after stress. Thus, in order to understand the molecular basis for the diversity in gene expression as well as phenotypical cellular outcomes, it is critical to understand the signal transduction pathways involved in the stress response and the transcription factors activated by these pathways.

Work in this group is aimed at understanding the molecular basis for the activation of MAP kinases in response to stress, and identifying the downstream targets regulated by them.

We have previously demonstrated that stressful stimuli can differentially activate ERK, JNK and p38 MAP kinases. Using sodium arsenite as a model for chemical stress, we have shown that in PC12 cells the activation of ERK is dependent on the activity of the proto-oncoprotein Ras, while activation of JNK and p38 does not require Ras activity. Similarly, activation of ERK is sensitive to pretreatment with the growth factor receptor blocker suramin, consistent with the model that ERK activation by arsenite involves activation of growth factor receptor tyrosine kinases. The fact that suramin has little effect on the activation of JNK and p38 suggests that growth factor receptor tyrosine kinases are not involved in their activation by arsenite. Recently, we extended our studies to signals downstream of ERK, and demonstrated that arsenite also activates the ERK-regulated protein kinase $p90^{RSK}$, a downstream serine/threonine kinase also known to be involved in transcriptional control. The $p90^{RSK}$ activation follows activation of ERK, and is correlated with the phosphorylation and degradation of the NF-κß inhibitor, Iκß , suggesting that it may play a role in regulating the activity of the transcription factor NF-κß . Supporting our hypothesis, very recently $p90^{RSK}$ has been demonstrated by others to be a physiological regulator of Iκß . We are currently testing the role of $p90^{RSK}$ in the activation of NF-κß in response to arsenite.

Since Ras plays a crucial role in activating ERK in response to arsenite and a growth factor receptor may be involved, it is likely that the adaptor protein Grb2 is involved in the activation pathway. Consistent with this, we found that arsenite strongly stimulates tyrosine phosphorylation of Shc and its interaction with Grb2. Furthermore, we have recently been able to co-immunoprecipitate a high molecular weight protein that is also tyrosine phosphorylated with kinetics similar to those of Shc. The identity of the high-molecular weight protein is currently unclear, but it may belong to the growth factor receptor tyrosine kinase family. We hypothesize that

arsenite activates a receptor tyrosine kinase through a mechanism independent of growth factors, resulting in its autophosphorylation on tyrosine residues. Shc binds to the phosphotyrosine residue through its SH2 domain, and itself becomes tyrosine phosphorylated. The phosphotyrosine residues then recruiting provide docking sites for Grb2 to the membrane, locating the Ras activator mSos to its target-membrane-associated Ras, leading to the activation of the ERK MAP kinase cascade. Currently, we are testing our hypothesis.

In future studies we plan to further establish the role of Shc and Grb2 in stress-induced ERK activation. We would also like to understand the identity of the tyrosine-phosphorylated high-molecular weight protein interacting with Shc. As a long-term goal of this program, we would like to establish a screening system to identify genes encoding substrates of JNK and p38. These should provide further insight into the functions of these pathways.

**Collaborators:** John C. Lee, Smith Klein Beecham Pharmaceuticals; Marvin O. Boluyt, University of Michigan School of Medicine; George S. Roth, Laboratory of Cellular and Molecular Biology, NIA; Nikki Holbrook, Laboratory of Biological Chemistry, NIA.


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**Keywords:**
angiogenesis
gene therapy
breast cancer
estrogen receptors


**Recent Publications:**


Cirielli C, et al. *J Neuro-Oncology* 1997; 31: 217-223.

Yang C, et al. *Cell Growth & Differentiation* 1996; 7: 161-171.

Jiang B-H, et al. *Cancer Res* 1997; 57: 5328-5335.

Pili R, et al. *Int J Cancer* 1997; 73: 258-263.


**Biography:** Dr. Passaniti received his Ph.D. at the University of Virginia and completed his post-doctoral training in cell biology at the University of Maryland and in tumor metastasis at the Johns Hopkins University. He joined NIA's Laboratory of Biological Chemistry in 1989 as a Staff Fellow and expanded on his work in tumor biology which now includes the investigation of anti-angiogenic therapeutics and mechanisms of breast cancer progression.

**The Biology of Cancer and Aging:** The goal of our studies is to understand the events and to elucidate the mechanisms contributing to age-related changes in tumor growth, progression, and angiogenesis. Using transplantable murine models to study tumor growth and angiogenesis, we have found significant age-related deficits in vascularization and are using an unique *in vivo* angiogenesis assay to evaluate angiogenic and anti-angiogenic factors. Our approach to study tumor progression is to determine the genetic and epigenetic events that control the loss of hormone responsiveness in a rat model of spontaneous breast cancer.

**Tumor Growth and Vascularization:** We are investigating several aspects of tumor vascularization: (1) inhibitors of angiogenesis that are isolated from tumor matrix, (2) tumor stromal cells and their role in tumor growth, (3) mechanisms of endothelial cell death, and (4) testing of angiogenic and anti-angiogenic agents using an *in vivo* assay.

Inhibitors of endothelial cell proliferation were found in extracellular matrix isolated from tumors grown in aged mice. These studies led us to design cell differentiation and apoptosis assays to isolate these factors. In collaboration with colleagues at our institute, we are also using chondrocyte differentiation assays to identify these factors. These factors may be important determinants of cellular differentiation and in the observed slower growth of tumors in aged mice. It is commonly believed that tumor-infiltrating cells contribute to the vascular response and to

tumor cell growth. We have been isolating stromal cells from tumors grown in animals of different ages and are now testing these cells for their biological activity using vascular and tumor cells as targets.

We have found that endothelial cell apoptosis is mediated by protein tyrosine phosphatases. Using fluorescent Tunel labeling to detect DNA fragmentation *in situ*, DNA laddering, and expression of the apoptosis-specific gene, TRPM-2, morphological changes diagnostic for apoptosis were correlated with the loss of tyrosine phosphorylation. Focal adhesion kinase, MAP kinase and cdc2 expression and phosphorylation were also examined. Actin filaments were found to depolymerize at the onset of apoptosis, an effect that could be prevented by inhibition of protein tyrosine phosphatases. We found that expression of the cyclin-dependent kinase inhibitor p21 was associated with survival of endothelial cells. Current efforts are examining the role of the bcl-2 family genes in endothelial cell apoptosis and how the expression of NFkB is related to phosphatase activation.

Using an *in vivo* angiogenesis assay, which we developed to investigate the response of aged mice to different angiogenic factors, we are testing novel chemotherapeutic drugs for anti-angiogenic (and therefore anti-tumor) activity. The use of angiogenic and anti-angiogenic adenoviral vectors in this assay has allowed us to study the role of VEGF, FGF, and the tumor suppressor gene p53 in tumor growth, vessel restenosis, and vascularization. This assay has also been useful for screening compounds that enhance angiogenesis, an application of particular interest in the aged because of the increased incidence of chronic wounds and diabetes.

**Breast Tumor Progression**: We have established a tumor progression model in aged rats in which estrogen receptor positive cells (ER+) become ER negative (ER-). We have observed this progression *in vitro* and *in vivo*. Using somatic cell fusion, we obtained hybrid clones that were predominantly ER+. The dominance of the ER+ phenotype implies that the ER+ fusion partner is contributing something missing from the ER- partner. These data suggest that a possible loss of tumor suppressor genes may regulate the progression. In these studies we have found that, unlike ER- cells, ER+ cells express more keratin and less vimentin, express desmosomal (junction) proteins, and respond to hormones and growth factors, including estradiol, dexamethasone, insulin, progesterone, and EGF. We are continuing studies to clone possible tumor suppressor genes and have completed our immediate goals by making a cDNA library from ER+ cells and investigating further phenotypic differences in these cells to allow the screening of suppressor genes. We have found that ER- cells are more invasive than ER+ cells and activate metalloproteinases. The ER+ cells are more sensitive to apoptosis than are ER- cells and we are looking

at the mechanisms of this change. In addition, we are using transfection of ER- cells with ER+ cell cDNA vectors and screening for the acquisition of specific proteolytic expression to identify putative tumor suppressor genes.

We have also analyzed the methylation status of the ER region encompassing exon 1 of the ER gene in primary rat mammary tumors to see if this is involved in tumor promotion in the aged rat. Our findings indicate that lower methylation is associated with increased tumor incidence. Hypomethylation occurs even in tumor-free mammary glands in aged rats, but not in middle-aged or young rats. Further studies in young rats induced with the mutagen DMBA have also shown lower methylation at this locus, even in normal glands, before tumor onset. Hypomethylation of the ER gene may, therefore, be a common event in mammary tumorigenesis in the rat and may be of predictive value as a marker of increased breast cancer risk. Continuing studies are aimed at investigating the role of dietary fat and calories in the regulation of ER methylation.

**Collaborators:** Maurizio Capogrossi, M.D., NIA; Walter Horton, Ph.D., NIA; Gregg Semenza, M.D., Johns Hopkins University; Leena Hilakivi-Clarke, M.D., Georgetown University; Josephine Egan, M.D., NIA.

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**Keywords:**
T lymphocyte activation
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immunology
oxidative stress

**Recent Publications:**

Isakov N, et al. *J Biol Chem* 1996; 270: 15753-15761.

Wange RL, et al. *Immunity* 1996; 5: 197-205.

**Biography:** Dr. Wange received his Ph.D. from the Department of Pharmacology at Vanderbilt University in 1991. He received his postdoctoral training at the Cell Biology and Metabolism Branch of the National Institute of Child Health and Human Development (NICHD) before becoming an Investigator in the Gene Expression and Aging Section of the Laboratory of Biological Chemistry in 1997. His research focuses on the signaling pathways involved in T lymphocyte activation.

**Aging and T Lymphocyte Activation**: A hallmark of aging in higher animals is a general decline in immune function. This is seen both as a decrease in the robustness of certain protective immune responses, and an increase in the occurrence of auto-immune responses. It has even been proposed that increased immune self-reactivity may play a causative role in aging, rather than being just another symptom of aging. It is our contention that a better understanding of the mechanisms by which the immune system becomes dysfunctional with age will enable the development of pharmacological interventions that will restore immune function, and perhaps alleviate some of the morbidity associated with aging.

T lymphocytes are vitally important in mounting an effective immune response. They not only orchestrate the immune response by regulating the activity of other components of the immune system, but also are directly involved in the surveillance and killing of infected or cancerous cells. Given the importance of T cells in initiating and maintaining the adaptive immune response, many of the studies aimed at understanding the defect underlying the decline in immune function that accompanies aging have focused on T cells. The fraction of T cells that are responsive to mitogenic stimuli declines with age. When compared to T cells from young animals, these unresponsive T cells exhibit aberrant biochemical responses to mitogens, including weak mobilization of intracellular calcium and aberrant phosphorylation of intracellular signaling proteins. This suggests that there are significant changes in the signal transduction pathways that

occur in T cells with aging. The goal of this research program is to characterize the signaling pathways that transduce proliferative signals received at the plasma membrane of T cells into the coordinated responses of proliferation and acquisition of effector function. We also want to identify which components of this pathway become altered with aging, and to understand the mechanisms that underlie these changes.

**T Cell Receptor Signaling**: In order to understand the nature of the signaling defects in T lymphocytes from aged animals, one must first understand the signal transduction pathways used by normal T cells. With this in mind, the laboratory is studying the molecules involved in normal TCR signaling. A key TCR-associated signaling molecule is the protein tyrosine kinase ZAP-70, which is required in order to signal through the TCR. Understanding the mechanism of action of ZAP-70 and how its activity is regulated is one of the priorities of this program. Precisely how ZAP-70 regulates T-cell proliferation is incompletely understood. We know that upon stimulation of the TCR that ZAP-70 is recruited to tyrosine-phosphorylated motifs within the TCR, whereupon ZAP-70 is tyrosine phosphorylated and activated by a heterologous kinase. But we know little else, except that there is no signaling through the TCR in the absence of ZAP-70. Even the substrates of ZAP-70 largely remain to be identified. One interesting question concerns the location of ZAP-70 before its recruitment to the TCR and whether or not release from this site is a regulated event. Although current models contend that ZAP-70 is freely cytosolic, observations in our laboratory and in others suggest that ZAP-70 may actually be associated with the cortical cytoskeleton in unstimulated T cells. Efforts are underway in the lab to assess this possibility. In addition we are using 2-hybrid and tribrid genetic screens to identify additional ZAP-70-associated proteins, which may play a further role in regulating or mediating ZAP-70 activity.

With advancing age there is also a general decline in the ability of cells to cope with oxidative stress. T cells are particularly susceptible to changes in oxidative tone, and there is some evidence of a redox sensitive signaling component to the TCR signaling pathway. In fact, oxidative stressors such as H2O2 or UV have been used to mimic the effect of TCR engagement, since many of the biochemical events initiated by TCR engagement can also be initiated by oxidative stress. The effects of these agents have been shown to be dependent on the presence of an intact TCR. Given the importance of ZAP-70 in mediating TCR signals, the role of ZAP-70 in propagating oxidative signals in T cells also needs to be assessed. We are currently using a ZAP-70 negative Jurkat T cell line to access the role of ZAP-70 in oxidative signaling. Preliminary results support a role for ZAP-70 in mediating these oxidative signals. Whether ZAP-70 localization or

activity is altered with age remains to be established, as does the physiological significance of the sensitivity of ZAP-70 activity to oxidative stimuli. These questions will be the subject of future investigations.

**CD28 Signaling**: T cell proliferative responses require engagement of a co-stimulatory receptor, such as CD28, in addition to TCR engagement. Stimulation of CD28 results in increased tyrosine phosphorylation of several substrates, as well as activation of phosphoinositide 3-kinase, sphingomyelinase and perhaps other enzymatic activities, ultimately causing the activation of transcription factors of the rel family. The precise signaling pathways that link CD28 engagement with transcriptional activation remain unclear, as does any role for CD28 in mediating the effect of aging on T-cell function. The lab is initiating a program that will induce somatic mutations within the pathways linking CD28 engagement and transcriptional activation. This project has the potential to identify signaling molecules previously unrecognized as playing a role in CD28 signaling, and will also provide null cells in which the functional elements of these unknown and known signaling molecules can be studied.

**Collaborators:** Joaquin Madrenas, M.D., University of Western Ontario; Lawrence Samelson, M.D., National Institute of Child Health and Human Development; John O'Shea, M.D., National Institute on Arthritis and Musculoskeletal and Skin Diseases; Robert Abraham, Ph.D., Mayo Clinic; Harvey Knull, Ph.D., University of North Dakota.

**Edward G. Lakatta, M.D., Chief**

# Laboratory of Cardiovascular Sciences

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The Laboratory of Cardiovascular Sciences (LCS) was established in 1985 as an outgrowth of the Cardiovascular Section of the Clinical Physiology Branch. LCS is presently organized into three sections: Cardiac Function, Membrane Biology, and Behavioral Hypertension. The Cardiac Function Section, which comprised the entire LCS at its incipience, is organized into six functional units, each headed by a tenured or senior scientist. The Membrane Biology Section was formerly in the Laboratory of Biological Chemistry and was assimilated into the LCS at the request of their scientists in 1991. The Behavioral Hypertension Section was formerly part of the Laboratory of Behavioral Science and joined LCS in 1997.

The overall goals of the Laboratory of Cardiovascular Sciences are (1) to identify age-associated changes that occur within the cardiovascular system and to determine the mechanisms for these changes; (2) to study myocardial structure and function and to determine how age interacts with chronic disease states to alter function; (3) to study basic mechanisms in excitation-contraction coupling and how these are modulated by surface receptor signaling pathways in cardiac muscle; (4) to determine the chemical nature and sequence of intermediate reactions controlling the movement of ions through ionic channels and pumps present in myocardium, and how these are affected by aging and disease; (5) to determine mechanisms that govern behavioral aspects of hypertension; (6) to determine mechanisms of normal and abnormal function of vascular smooth muscle and endothelial cells; and (7) to establish the potentials and limitations of new therapeutic approaches such as gene transfer techniques. In meeting these objectives, studies are performed in human volunteers, intact animals, isolated heart and vascular tissues, isolated cardiac and vascular cells, and subcellular organelles.

Each section/unit independently conceptualizes and implements its research portfolio. Opportunities for collaboration among units/sections, however, are fostered and encouraged. In addition to independent work, substantial interaction occurs among scientists both within and between

the sections/units. The stimuli for such interactions originate from individual scientists and from the Lab Chief, who commits substantial energy to encourage (but not to demand) these research collaborations. Consequently, many of the LCS projects become multi-faceted, spanning a range from humans to molecules. Using this approach, scientists recognize that future research advances require the integration of discoveries within and among individual research areas. The networking among individuals within LCS also extends to individuals in other institutes within the NIH, academic institutions, and industry. We believe that such networking among individual facets of the biomedical research community is required for integration of discoveries that is tantamount to practical application of these research discoveries. The broad overall LCS mission permits tenured scientists, senior fellows, and new fellows appointed to the Lab to chose their specific research projects. In other words, individuals are most productive when working on projects on which they develop their own "passion." The resultant LCS environment has become somewhat unique: it is not strictly akin to a university department in which each individual dictates his/her mission and applies for individual funding in order to implement the proposed program; however, neither is the LCS environment strictly "mission oriented" in the sense that each individual is mandated to work on a given project in a "top down" design. The LCS environment is best described as a balance between the above approaches; and in the broad sense, the collective research output of the Lab can be considered to be a "bottom up" approach. Specifically, most projects originate at the investigator level but are coordinated by the Lab/Section/Unit Chiefs to achieve a meaningful mosaic within the broad framework of the Lab mission.

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cardiovascular aging
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cardiac receptors
cardiac apoptosis
vascular cell
chemotaxis

**Recent Publications:**

Sollott SJ, et al. *Am J Physiol 271* 1996; 40: H896-H905.

Schulman SP, et al. *Circ* 1996; 94: 359-367.

Shah AM, et al. *Circ Res* 1997; 80: 688-698.

Pepe S, et al. *Circ* 1997; 5: 2122-2129.

**Biography:** Dr. Lakatta received his M.D. from Georgetown University School of Medicine, Washington, D.C. in 1970. His postdoctoral training included an internship and residency in medicine at Strong Memorial Hospital, University of Rochester School of Medicine; cardiology fellowships at Georgetown and Johns Hopkins University Hospitals; and basic research training at NIH and at the Department of Physiology, University College, London, England. He was section chief of the Cardiovascular Laboratory in the Clinical Physiology Branch from 1976 until 1985, at which time he founded the Laboratory of Cardiovascular Sciences.

**Cardiac Function Section Program**

Dr. Lakatta directs the Cardiac Function Section (CFS) which has a broad based research program ranging from studies in humans to molecules. The program is comprised of the following units:

**Human Cardiovascular Studies Unit:** This unit's studies deal with the interactions of age, lifestyle, and disease on cardiovascular structure/ function in humans. The study panel for the bulk of the studies is the Baltimore Longitudinal Study of Aging (BLSA). Initially, age-associated changes in cardiovascular structure and function are defined in healthy individuals and subsequent studies define mechanisms for these changes. Additional populations that provide a diversity of lifestyle and disease have been added to the study panel for specific projects. Acute or chronic interventions in these individuals or in the BLSA are utilized to determine the responsiveness of age-associated changes to pharmacological therapies or lifestyle changes, for example, exercise habits. Several areas of related research in animal tissue and cells implemented in other units of the Section complement these studies in humans.

**Molecular Cardiology Unit:** The main focus of this unit is to define the molecular bases of structure-function changes in the heart with aging. Regulation of cardiac cell growth and size is a major area of study within

this Unit, as one hallmark of advancing age is an increase in cardiac cell size. Many features of the *pattern* of age-associated changes in heart cells resemble the hypothyroid state. Thus, regulation of intracellular thyroid and retinoid (RxR) receptors has become a recent focus of this unit. As heart failure increases exponentially with age, studies of the transition from compensated cardiac hypertrophy to heart failure in animals of old age with hypertensive heart disease have been initiated. The focus of additional studies is early cardiac gene expression using an embryonic stem (ES) cell differentiation model system. In these studies, potential early cardiac gene transcription factors are identified and the proteins responsible for activating expression are sought using standard molecular biological techniques. These factors are then cloned and their role in regulating cardiac gene expression examined with respect to their potential contribution to the aging process.

**Excitation-Contraction Coupling Unit:** This unit's main research focus is on the control of cardiac cell regulation. Substantial evidence indicates that the triggering of sarcoplasmic reticulum calcium release in cardiac muscle depends upon the interaction of the L-type sarcoplasmic calcium channel (dihydropyridine receptor) and the sarcoplasmic reticulum (SR) calcium release (ryanodine receptor) via local calcium gradients. This unit has developed quantitative mathematical models that embody this "local control" hypothesis. To test the predictions of these models, we require the ability to alter the behavior of these channels, while preserving their natural geometrical relationship in the cardiac myocyte. To achieve this, models are developed in which the relevant proteins (DHPR, RyR, FKBP-12.6) are mutated by homologous recombination in mouse embryonic stem cells. Genetically engineered myocytes produced are studied by biophysical techniques (patch-clamp and confocal microscopy). Additional projects deal with identifying how cardiac cell regulatory mechanisms become altered with aging and disease (anoxia, ischemia, hypertension, heart failure). The initial mechanisms focus of this unit has broadened from the study of biophysical mechanisms in cardiac cells to endothelial and vascular smooth muscle cells (VSMC) as well. These studies, which combine fluorescence and confocal imaging, link strongly to projects within the Vascular Studies Unit.

**Receptor Signalling Unit:** The unit's focus is on elucidating distinct signal pathways for α and ß receptor subtypes and of opioid signal transduction pathways in the heart. The interaction of signals emanating from stimulation of these with other receptor-mediated signaling pathways

is also studied. Studies are designed to integrate information gleaned from electrophysiology, UV fluorescence, and confocal imaging, and probe novel intracellular regulatory mechanisms.

**Gene Therapy Unit:** Investigators in the unit engineer expression cassettes for insertion into non-replicative adenoviruses, which are then utilized in experiments to deliver genes to promote angiogenesis or to reduce restenosis following angioplasty. Growth factors, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF), and the metalloproteinase inhibitor, tissue inhibitor of metalloprotinease (TIMP), have been the initial thrust of this effort. The Gene Therapy Unit interacts with other LCS units/sections, serves as a resource for other GRC labs, and collaborates with industry and academic institutions in animal trials that employ gene targeted therapy.

**Vascular Studies Unit:** Research areas of this unit include matrix regulation of the differentiation status of vascular smooth muscle cells (VSMC), characterization of VSMC properties (migration, secretion, invasion) of dedifferentiated (modulated) *in vivo*, i.e., from neointimal lesions in restenosis injury, or from atherosclerotic plaque, and *in vitro*, i.e., in VSMC cells in tissue culture and various aspects of extracellular matrix remodelling. A major focus is directed at discovering novel aspects of growth factor receptor-coupled signaling pathways that regulate cell migration, and how these pathways change with age. Similar studies on signaling mechanisms of advanced glycation end-products (AGE) via their receptors (RAGE) on VSMC form an additional facet of the Unit's work. This Unit is highly interactive with other parts of a LCS-wide "vascular initiative" composed of Gene Therapy and Excitation-Contraction Coupling and Human Studies Units within the Cardiac Function Section of the Membrane Biology Section. The Vascular Unit also networks widely with academic institutions and industry.

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**Keywords:**
p53
apoptosis
gene therapy
angiogenesis

**Publications:**

Gorospe MC, et al. *Oncogene* 1997; 14: 929-935.

Pierzchalski P, et al. *Exp Cell Res* 1997; 234: 57-65.

Pili R, et al. *Cardiovasc Res* , In press.

Safi J, Jr. et al. *J Mol Cell Cardiol* 1997; 29: 2311-2325.

**Biography:** Dr. Maurizio C. Capogrossi received his M.D. from the University aLa Sapienza in Rome in 1975. One year later he moved to the United States to obtain further training. He was an Intern and a Resident in the Internal Medicine program at Emory University in Atlanta, Georgia. In 1982 he became a Staff Fellow in the Clinical Physiology Branch at the Gerontology Research Center and subsequently he was a Cardiology Fellow in the Johns Hopkins University program. In 1987 he became a tenured NIA Investigator and a faculty member in the department of Internal Medicine (Cardiology Division) at Johns Hopkins University. Dr. Capogrossi's research work has focused on the physiology of myocardial and endothelial cells. Since 1993 his interest has shifted to gene therapy to induce therapeutic angiogenesis and inhibit neointima development after vascular injury.

**Gene Therapy To Induce Therapeutic Angiogenesis:** The broad objective of this program is to perform tissue culture and preclinical experiments in animal models of myocardial and hindlimb ischemia to evaluate the therapeutic potential of gene therapy with angiogenic growth factors. Vascular cell's response to angiogenic growth factors, acidosis and hypoxia may help identify which strategy is more likely to induce therapeutic angiogenesis. *In vivo* experiments are aimed at characterizing clinically relevant animal models and at evaluating whether, and eventually under which conditions, adenovirus-mediated gene transfer of angiogenic growth factors induces therapeutic angiogenesis.

**Synergistic Effect of IGF-1 and bFGF on Microvascular Endothelial Cell Proliferation:** The effect of IGF-1 on endothelial cell function is poorly characterized and the objective of this study was to determine the effect of IGF-1 alone and in conjunction with bFGF and VEGF on the proliferation of human umbilical vein endothelial cells (HUVEC) and human microvascular endothelial cells (HMVEC). IGF-1 alone did not induce proliferation of either HUVEC or HMVEC and there was no

synergy between IGF-1 and VEGF on either HUVEC or HMVEC proliferation. In contrast IGF-1 had a marked synergistic effect with bFGF on HMVEC.

**Effect of Acidosis on Bovine Aortic Endothelial Cells Proliferation and Growth Factors Release:** Tissue ischemia stimulates angiogenesis and is associated with intracellular and extracellular acidification. However, the effect of acidosis on endothelial cell function is still unclear. We evaluated whether hypercarbic acidosis modulates bovine aortic endothelial cell (BAEC) proliferation and growth factor release into the conditioned medium. We found that acidosis inhibits BAEC proliferation in 10% FCS. Further, acidosis enhances growth factor release from BAEC and this is associated with increased cell proliferation when BAEC are cultured under starving conditions.

**Hypoxia Modulates Integrin Expression in Endothelial Cells:** Ischemia modulates endothelial function and stimulates new blood vessel growth. Since $\alpha_v ß_3$ and $\alpha_v ß_5$ integrins are involved in the angiogenic process we evaluated the effect of hypoxia on endothelial cell expression of these integrins. In addition, $ß_1$ subunit expression was assessed. HUVEC in complete medium were cultured in hypoxic conditions ($pO_2$<15 torr) for 24, 48, or 72 hours. Hypoxia had a significant effect to decrease $\alpha_v ß_5$ expression at all time points; $\alpha_v ß_3$ was not significantly different from normoxic control at 24 and 48 hours while it exhibited a 20% decrease which did not achieve statistical significance at 72 hours. $ß_1$ integrin expression remained unchanged under hypoxic conditions. Thus, hypoxia selectively decreases $\alpha_v ß_5$ expression and this may play a role in the angiogenic response to ischemia.

**Age-Dependence of Muscle Bioenergetic Recovery After Acute Ischemia:** We hypothesized that recovery of skeletal muscle metabolism after acute ischemia may be age-dependent. Male rats 2 and 20 months old underwent right femoral artery removal. Gastrocnemius muscle bioenergetics was evaluated by $^{31}P$ NMR on the operated and unoperated hindlimb 1 and 7 days after surgery. Peak heights of the phosphocreatinine (Pcr) and inorganic phosphate (Pi) resonances were obtained at 2 min intervals, at rest, during a 6 min period of electrical stimulation of the hindlimb and during at 12 min period of rest. PCr/(PCr+Pi) peak height ratios were evaluated prior to stimulation ($R_0$), when the ratio achieved a minimal value ($R_{in}$), and at the end of the recovery period ($R_{end}$). PCr/(PCr+Pi) ratios of the unoperated limbs were indistinguishable between groups and exhibited less decline with stimulation and faster recovery after stimulation than the operated limbs. In contrast, results for the operated limb show that 1 day after surgery both $R_0$-$R_{min}$ and $R_{end}$-$R_{min}$ are

lower in old than in young rats ($p<0.005$). Thus, following acute ischemia, muscle bioenergetic recovery is impaired in old vs. young rats.

**Adenovirus-Mediated Gene Transfer and Biosafety - Lack of Tumorigenicity Upon Transient versus Permanent Expression of Secreted and Non-Secreted Forms of Acidic Fibroblast Growth Factor:** Gene transfer of endothelial growth factors is being implemented as a possible therapeutic approach for the treatment of ischemic disorders. However, the role of growth factors on tumor growth has raised a biosafety issue. We tested the replication-deficient recombinant adenovirus (Ad) vectors coding for the signal sequence (Ad.CMV.sp+aFGF) and non-signal sequence (Ad.CMV.aFGF) forms of human acidic fibroblast growth factors both *in vitro* and *in vivo*. The results showed that phenotypic changes induced by adenovirus-mediated gene transfer of aFGF are transient, suggesting that transient expression of growth factors might induce only a transitory growth advantage, but not a stable transformation of normal cells.

**Adenovirus-Mediated Acidic Fibroblast Growth Factor Gene Transfer Increases Arteriole Density and Reduces the Risk Region for Myocardial Infarction in Rabbits - Evidence of Induction of Angiogenesis in the Non-Ischemic Heart:** The majority of patients with severe coronary artery disease have normal baseline myocardial blood flow. Therefore, interventions aimed at inducing therapeutic angiogenesis in these patients should cause new blood vessel growth in the heart in the absence of chronic ischemia. It was examined whether adenovirus-mediated gene transfer of acidic fibroblast growth factor ($aFGF_{1-154}$) in a discrete area of non-ischemic myocardium, next to a major epicardial artery, may induce neovascularization and whether by this approach it is possible to reduce the risk region for myocardial infarction upon coronary ligation near the injection site. The results showed that gene therapy with AdCMV.sp+$aFGF_{1-154}$ can induce angiogenesis in a discrete but critical area of myocardium in the absence of chronic ischemia. The newly formed collateral blood vessels provide anatomical basis for the reduction in the risk region for myocardial infarction upon subsequent coronary artery occlusion.

**Gene Therapy to Inhibit Intimal Hyperplasia After Endovascular Injury:** Vascular smooth muscle cells (VSMCs) play a major role in the arterial wall response to injury. VSMCs are normally present in the arterial tunica media where they regulate vascular tone and blood flow. In the vessel wall, VSMCs are surrounded and separated from other cells by extracellular matrix (ECM). Arterial injury leads to proliferation of medial VSMCs and migration of these cells from the media to the intima. These

steps are dependent on the local degradation and remodeling of the ECM. Our studies examined the role of adenovirus (Ad)-mediated wild-type p53 (AdCMV.p53) and tissue inhibitor of metalloproteinase 2 (AdCMV.hTIMP-2) overexpression in vascular smooth cells (VSMCs). In preliminary experiments AdCMV.p53 failed to induce VSMC apoptosis *in vitro* and inhibition of intimal hyperplasia in the rat model of neointima development after carotid injury. In contrast, AdCMV.p53 induced melanoma cell apoptosis. Therefore, the role of p21, a p53-effector gene was examined in the different responses of VSMC and melanoma cells to AdCMV.p53. The results show that the failure of AdCMV.p53 to induce VSMC apoptosis is associated with enhanced p21 expression in these cells. In contrast, in melanoma cells AdCMV.p53 results in apoptosis and does not increase p21. In additional experiments it was shown that Ad-mediated p21 overexpression before exposure to AdCMV.p53 protects melanoma cells from the apoptotic effect of this viral vector. The results support the view that p21 plays a fundamental role in the decision fork between programmed cell death and survival and account for the failure of AdCMV.p53 to inhibit neointima development. In a different study, the effect of AdCMV.hTIMP-2 on VSMC function *in vitro* and on neointimal development *in vivo* was assessed. Previous studies in the rat model of carotid injury indicated that vascular injury increases activation of matrix metallo-proteinase 2 (MMP2) during the time VSMCs migrate to the intima. TIMP-2 is a physiologic MMP2 inhibitor and AdCMV.hTIMP-2 inhibits MMP2 activity and SMC invasion in cultured VSMC. In this study, 6 month old rats underwent balloon injury of the common carotid artery. The vessel wall was infected either with AdCMV.hTIMP-2 or with the control vector AdCMV.null at the time of balloon injury. AdCMV.hTIMP-2 transgene expression in VSMCs *in vivo*, was shown by immunohistochemistry 5 days after injury and infection. At 4 days post-injury and infection, intimal cell number was decreased by 36% in AdCMV.hTIMP-2 *vs* AdCMV.null infected carotid arteries. At 8 days after injury and infection, AdCMV.hTIMP-2 induced a 50% reduction of neointimal area *vs* AdCMV.null. In contrast to the changes seen in the neointima, no difference was seen in medial wall area. Thus, Ad-mediated TIMP-2 overexpression in the rat model of carotid artery injury inhibits VSMC migration and decreases the severity of neointimal formation.

**Collaborators:** I. Kovesdi, GenVec, Inc., Rockville, Maryland; P. Anversa, New York Medical College, New York; L. Becker, Johns Hopkins University, Baltimore, Maryland; W. Stetler-Stevenson, NIH, Bethesda, Maryland; A. Felici, Instituto Dermopatico dell' Immacolata, Rome, Italy; Richard Spencer, Kenneth Fishbein, Kimberlee Potter, and Pavel Shkarin, Laboratory of Cellular and Molecular Biology, NIA.

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**Recent Publications:**

Byrne E, et al. *J Appl Physiol* 1996; 81: 743-750.

Schulman S, et al. *Circulation* 1996; 94: 359-367.

Swinne CJ, et al. *Am J Cardiol* 1996; 78(9): 1070-1073.

Pearson JD, et al. *J Gerontol: Med Sci* 1997; 52A: M177-M183.

**Biography:** Dr. Jerome Fleg received his M.D. from the University of Cincinnati in 1970. After completing training in Internal Medicine and Cardiovascular Disease at Washington University in 1977, he assumed his current position in NIA's Laboratory of Cardiovascular Science. His research interests include normative aging changes in cardiovascular structure and function, silent myocardial ischemia, and congestive heart failure.

**Effects of Age, Gender, Lifestyle and Disease on Cardiovascular Structure and Function**: Advancing age in humans is accompanied by significant changes in the cardiovascular system and, all too often, by the development of cardiovascular disease. A major challenge undertaken by our laboratory is to define normative aging changes in cardiac and vascular structure and function and their modulation by lifestyle variables and disease. To accomplish this ambitious task, we utilize a wide variety of noninvasive testing methodologies at rest and during exercise.

Early M-mode echocardiographic studies in our laboratory, pioneered by Drs. Gary Gerstenblith and Edward Lakatta, demonstrated that normative aging was accompanied by a thickening of the left ventricular (LV) muscular wall and a reduction of early mitral value closure slope analogous to the findings in mild hypertension. These findings have led us to conceptualize that aging is a muted form of hypertension. In industrialized societies, a 20-30 mm Hg rise in systolic blood pressure (SBP) typically occurs across the adult lifespan in subjects who remain normotensive by clinical criteria. The etiology of this SBP rise involves a gradual replacement of elastic fibers in the vascular media by less distensible collagen and calcium. Recent studies in our laboratory are quantifying these age-associated changes in arterial stiffness using pulse wave velocity and applanation tonometry of the large arteries. These studies have demonstrated a 200-500% increase in stiffness across the adult life span. Two-dimensional echocardiographic determination of LV mass in these same subjects has

revealed that arterial stiffness, especially the late systolic augmentation of arterial pressure quantified by applanation tonometry, is an independent determinant of LV mass, beyond the effect of SBP. These studies, therefore, support the hypothesis that age-associated increases in arterial stiffness are responsible in part for the mild LV hypertrophy and substantial reduction in early diastolic LV filling rate seen with aging. To test this hypothesis, we have designed short-term drug interventions and longer-term exercise training interventions to determine whether arterial stiffness can be reduced, both in normal older subjects and individuals with congestive heart failure. Although the exercise training studies are still in progress, a recently completed study has shown that acute infusion of the vasodilator sodium nitroprusside to normal older subjects dramatically reduced their resting arterial stiffness and improved their LV performance during exhaustive cycle exercise to levels typical of unmedicated young individuals.

Another major goal of our laboratory is to determine the mechanisms for the well known decline in maximal aerobic capacity ($VO_{2max}$) seen with aging. In an early study, we found that normalization of treadmill $VO_{2max}$ for total body muscle mass nearly eliminated the age-associated reduction in $VO_{2max}$, inferring that the loss of muscle tissue with age contributes importantly to the decline in $VO_{2max}$. We have employed gated cardiac blood pool scans with the isotope technetium-99m to quantify LV performance at rest and during maximal upright cycle exercise and its modulation by age, gender, lifestyle variables and cardiovascular disease. Our initial investigation using this techniques demonstrated that stroke volume at peak exercise was preserved across age by a greater reliance on LV dilatation to compensate for reduced systolic emptying. More recently we have found that this age-associated LV dilatation during exercise is more prominent in men than women despite similar impairment in emptying. Endurance trained older subjects utilize both larger end-diastolic LV volumes and enhanced LV emptying to augment stroke volume during exercise to a greater degree than untrained individuals.
Simultaneous monitoring of oxygen consumption throughout these exercise cardiac blood pool scans has allowed us to examine the relative importance of cardiac versus peripheral factors in the age-associated decline in aerobic capacity and its modulation by endurance training. A recent investigation using this methodology suggests that declines in cardiac output and arteriovenous oxygen difference contribute nearly equally to this decline in aerobic capacity with aging. Similarly, the marked augmentation of peak $VO_2$ in endurance trained older subjects relative to their sedentary peers is accomplished to a similar extent by enhanced cardiac output and peripheral oxygen extraction.

We have also utilized pharmacological probes to further define mechanisms for the decline in maximal exercise cardiac performance with age and their potential for modulation. For example, beta adrenergic blockade during exhaustive cycle ergometry in younger subjects markedly reduced their maximal heart rates and systolic emptying and augmented their exercise-induced LV dilatation, producing a profile similar to that of older unmedicated subjects. These data support our hypothesis that an important mechanism for the age-associated reduction in maximal cardiac performance is reduced beta adrenergic responsiveness.

**Collaborators:** Edward Shapiro, M.D., Gary Gerstenblith, M.D., Lewis Becker, M.D., Steven Schulman, M.D., Johns Hopkins University; Leslie Katzel, M.D., Andrew Goldberg, M.D., University of Maryland at Baltimore; James Hagberg, Ph.D., Stephen Porges, Ph.D., University of Maryland, College Park; Yoji Nagai, M.D., E. Jeffrey Metter, M.D., NIA.


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**Biography:** Dr. Michael Crow received his Ph.D. in Physiology and Biophysics from Harvard University in 1981 and did postdoctoral studies in cellular and molecular biology of skeletal muscle development at Stanford University. In 1984, he joined the Faculty of the Department of Pharmacology at the University of Texas, Houston and moved to his current position in the NIA in 1991, shifting research interests from skeletal muscle to smooth muscle and cardiomyocyte cellular and molecular biology.

**Vascular Smooth Muscle Cell Biology**: We study the behavior of isolated vascular smooth muscle cells (VSMCs) and cardiomyocytes. In the past year, our work has been concentrated on three distinct areas.

**Intracellular Signaling Pathways Regulating VSMC Migration**: The migration of vascular smooth muscle cells (VSMCs) is a key event in the pathogenesis of many vascular diseases. Migration of resident VSMCs

**Recent Publications:**

Bilato CB, et al. *J Clin Invest* 1997; 96: 1905-1915.

Bilato CB, et al. *J Clin Invest* 1997; 100: 1-12.

Long X, et al. *J Clin Invest* 1997; 99: 2635-2643.

Pauly RR, et al. Methods in Cell Biology (Eds.) HL Sweeney and C Emerson, *Academic Press,* San Diego, CA 1998; 52: 133-154.

requires that the cells undergo a phenotypic switch from a contractile to synthetic/proliferative state. We previously showed that a key factor in this switch was the ability of VSMCs to activate the multifunctional protein kinase, calcium/calmodulin-dependent protein kinase II (CamKII). Our current work is focused on identifying the intracellular targets for CamKII, its upstream regulation, and its unique role in ß3 integrin-mediated signaling of ß1 integrin function. We have shown that nonmuscle myosin light chain kinase is inhibited by CamKII and that this inhibition is important to the mechanism by which CamKII regulates PDGF-directed migration. In addition, we have shown that platelet derived growth factor (PDGF)-stimulated CamKII occurs through a signaling pathway different than that employed by other receptor agonists and requires the small GTPase protein, $p21^{rac}$, and the generation of reactive oxygen species. This unique pathway for activating CamKII provides additional nodes at which migration can be regulated by the availability of co-stimulatory growth factors, such as basic fibroblast growth factor (bFGF). Recently, we demonstrated that occupancy of ß3 integrin complexes is also required for CamKII activation and VSMC migration and that signaling from ß3 integrins to CamKII occurs through a bFGF-dependent signaling pathway. Occupancy of ß3-containing integrins in VSMCs not only regulates migration by facilitating CamKII activation but also by suppressing non-integrin signaling pathways for migration. In fact, the migration of VSMCs that lack functional ß3 integrins, such as those from aged animals, is not regulated by CamKII and may possibly underlie the exaggerated response of aged vessels to endothelial denudation. Our results represent the first demonstration of how outside-in signaling by ß3-containing integrins modulates a specific growth-factor stimulated signaling pathway. They identify a unique intracellular signalling network in VSMCs that is triggered by chemoattractant recognition and modulated by growth status, secretion of growth factors and ECM components, and ECM-VSMC interactions.

**Advanced Glycation Endproducts, Their Receptors, and Vascular Disease**: Advanced glycation endproducts of proteins (AGE) accumulate in the plasma and in tissues with age and at an accelerated rate in diabetes. In isolated vascular cells, AGEs induce a proxidant stress, leading to activation of pro-inflammatory events such as increased activity of MAPK and NF-kB, increased monocyte chemoattractant protein-1 (MCP-1) production, and increased PDGF B chain activity, all of which have been implicated in vascular lesion development. We have demonstrated that many of the effects of AGEs on gene expression are mediated through a unique immunoglobulin-type receptor called RAGE. We have constructed epitope-tagged wild type and mutant RAGE molecules and have shown that transfection of wild type receptor leads to increased MAPK activity

and MCP-1 RNA and protein levels in response to AGEs. Mutant receptors in which the cytosolic tail has been removed, however, do not result in increased MCP-1 production, but in fact block the ability of co-transfected wild type receptors to signal. These observations demonstrate that RAGE acts not merely as an AGE-binding protein but a bona fide transmembrane receptor, engaging intracellular signaling molecules to affect changes in gene expression and protein production and secretion. Current studies are concentrated on exploiting the truncated receptor as a dominant negative to block the effects of RAGE-mediated signaling during vascular lesion development in transgenic mice. In addition, interaction cloning techniques are being used to identify intracellular proteins associated with the receptor.

**Cardiomyocyte Apoptosis**: Cardiac cell loss marks the transition from hypertrophy to heart failure and is the likely result of chronic myocardial ischemia and cell hypoxia. Cell loss is due predominantly to the death of cardiac myocytes and is mediated in part by apoptosis. Because adult cardiac myocytes are terminally differentiated cells, the effects of such loss can never be fully compensated. The identification of the intracellular signaling events and extracellular factors that regulate this process and the development of strategies to prevent such loss is, therefore, likely to have important beneficial consequences. We have adopted an experimental system to induce cardiomyocyte cell death by apoptosis that involves exposing neonatal cardiomyocytes to prolonged hypoxia. We have shown that there is increased expression and transactivating ability by the tumor suppressor gene, p53, that accompanies the onset of apoptotic cell death in these cells. Forced expression of p53 with a recombinant adenoviral vector was sufficient to induce cardiomyocyte, but not cardiac fibroblast, apoptosis. Forced expression of p21/WAF1, a downstream target for p53 transactivation, also resulted in apoptosis, as did the incubation of normoxic myocytes with bafilomycin, an inhibitor of membrane-associated proton pumps which, in other cell types, leads to intracellular acidification. Apoptosis induced by p53, p21, and bafilomycin was effectively prevented or delayed by exposure to the hypertrophy-inducing factor, phenylephrine (PE), occurring through a PI3-K-dependent pathway. Our current studies are directed at understanding how p53/p21 engage the “death machinery” in cardiomyocytes and in developing viral vectors to counteract the effects of p53.

**Collaborators:** Maurizio Capogrossi, NIA; Piero Anversa, New York Medical College, Valhalla, NY; David Stern, Anne-Marie Schmidt, Columbia University, NY; Scott Blystone, Fred Lindberg, Washington Univ., St. Louis, MO; Jonathan Fox, Univ. of Pennsylvania, Philadelphia, PA.


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**Recent Publications:**


Kaddoura S, et al. *Circ* 1996; 93: 2068-2079.

Wankerl M, et al. *J Mol Cell Cardiol* 1996; 28: 2139-2150.

Wong K, et al. *Circ* 1997; 96: 2239-2246.

Martin XJ, et al. *Mol Cell Biochem* 1996; 157: 181-189.


**Biography:** Dr. Boheler received his B.Sc. from Duke University and his Ph.D. from the University of California, San Diego. After completing a post-doctoral fellowship at Unit 127 of the National Institutes of Health and Medical Reseach (INSERM) in Paris, France, he was appointed Assistant Professor (Lecturer) at Imperial College School of Medicine in the Department of Cardiothoracic Surgery, London, United Kingdom. In October of 1996, he joined the NIH to head the Molecular Cardiology Unit of the Cardiac Function Section of the Laboratory of Cardiovascular Science.

**Research:** The focus of our research over the past several years has involved examination of the expression and regulation of a number of proteins involved in regulating calcium movements in cardiac myocytes, including the sarcoplasmic reticulum calcium ATPase (SERCA), phospholamban (PLB), the Na/Ca exchanger (NCX) and the sarcoplasmic reticulum calcium release channel (Ryanodine Receptor). The work has involved examination of the spatial and temperol expression of these mRNAs and proteins in the developing myocardium. Using simpler *in vitro* models, the regulation of presence of the mRNAs encoding some of these gene products has been studied through examination of signal transduction pathways. Isolation and characterization of the promoter regions for human SERCA2 gene and rat NCX are underway. And, in the case of cardiac hypertrophy, we have examined the effects of angiotensin converting enzyme inhibitors on their ability to improve diastolic dysfunction in the rat myocardium. Our recent work is focused on use of an *in vitro* differentiation model of embryonic stem cells and embryonic carcinoma cells in an attempt to further understand the consequences of development and of altered gene expression on function of these proteins.

**Spatial and Temporal Analyses:** These studies have been performed in close collaboration with the laboratory of Dr. Antoon Moorman, Amsterdam. With the development of molecular cell markers specific for contraction and relaxation, functional aspects of myocardial differen-

tiation have been addressed through the use of *in situ* hybridization. We have reported how expression of SERCA2 and PLB in the rat may partly explain why the embyronic atrium and ventricle function essentially as they do in the adult. SERCA2 is expressed in a craniocaudal gradient; whereas that of PLB is expressed in a gradient essentially opposite to that of SERCA2. Accumulation of the NCX and RyR transcripts also occurs very early, similar to that for SERCA2, but do not show gradients of expression. With development SERCA2 and PLB expression increase during late fetal and perinatal development; whereas that for NCX decreases at or around birth in a compartment dependent manner. Its expression is however increased with aging.

**Signal Transduction Pathways Mediating SERCA2 and PLB Expression:** Using a model of neonatal rat cardiomyocytes, we have been able to determine that adrenergic agonists can play a critical role in regulation SERCA2 and PLB mRNA accumulations. The pathways have some overlap, in that activation by $\alpha$ adrenergic agonists and protein kinase C isoforms reduces both their expressions in a time and dose dependent mechanism probably through activation of the MAP kinase system. Beta adrenergic activation only results in decreased SERCA2 mRNA expression through a pathway that requires extracellular calcium and entry via the voltage dependent sarcolemmal calcium channel. The regulation also appears to be primarily transcriptional based on transfection data of the human SERCA2 genomic constructs linked to reporter sequences.

**Expressional Analysis of Cardiac NCX in Development and Senescence:** We have examined the mRNA expression of the Na/Ca exchanger (NCX) in rat heart during perinatal development and with aging. NCX is highly expressed in late fetal and neonatal rat hearts, decreasing to adult levels by 20 days after birth. The lowest level of accumulation is seen in 6 and 18 month old animals. In the 24 month old senescent rat, NCX expression is increased by almost 50% above that seen at 6 and 18 months ($p<0.05$) but is not different from that at 15 neonatal days. Results from nuclear run-on assays indicate that NCX expression during the perinatal period is regulated at least partially through transcriptional mechanisms. Relatively high transcriptional activity is seen at birth but by 20 post-natal days, no transcriptional activity from NCX can be detected. During development, there are no major changes seen in the use of the five identified transcription start sites, nor is there any major difference in the splicing patterns seen in the 5' untranslated regions. We have identified the presence of 5 different splicing variants in the cytosolic loop of the coding region, three of which have not been previously described in heart. We have also recently cloned a 2.8 kb fragment containing the putative cardiac NCX1 promoter and a consensus thyroid hormone responsive element which we are now examining.

**Embryonic Stem Cells and Myocardial Development:** This new research area involves a model of *in vitro* differentiation of cardiomyocytes originating from embryonic stem cells (R1) and embryonic carcinoma cells (P19). The research is aimed at understanding the developmental processes involved in cardiac myocyte differentiation and development. We are differentiating pluriopotent ES and EC cells in the presence of various growth factors to monitor the development of atrial versus ventricular like cells. To identify atrial versus ventricular like cells, expression vector constructs are being made that link atrial and ventricular markers to the green florescence protein (GFP). These constructs will be introduced into the cells and positive transformants identified through neomycin resistance selection. In concert with this project is the analysis of various promoter regions of α and beta myosin heavy chain, human SERCA2 and rat NCX as we attempt to determine which transcription factors are responsible for their induction at specific times of development. From this work, we hope to use various molecular techniques to identify and analyze various transcription factors and growth factors that promote cardiac cell division and differentiation and importantly, the sequence of their activation and inhibition.

**Collaborators:** Professor Magdi H. Yacoub, Imperial College School of Medicine, United Kingdom; Professor Antoon F.M. Moorman, University of Amsterdam, The Netherlands; Professor Alan Williams, Imperial College School of Medicine, United Kingdom; Dr. Kenneth MacLeod, Imperial College School of Medicine, United Kingdom; Dr. Ketty Schwartz, INSERM 153, France; Dr. Anne-Marie Seymour, University of Hull, United Kingdom; Professor Antonio Zorzano, University of Barcelona, Spain; Dr. Thomas Eschenhagen, University of Hamburg, Germany; Dr. Anna Wobus, Institut fur Pflanzengenetik und Kulturpflanzenforschung, Germany.

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**Recent Publications:**

Stern, MD et al. *Biophys J* 1996; 70: 2100-2109.

Rios, E et al. *Annu Rev Biophys Biomol Struct* 1997; 26: 47-82.

Stern, MD et al. *J General Physiology* 1997; 110: 415-440.

**Biography:** Dr. Stern studied theoretical physics at Princeton and received an M.D degree from University of Pennsylvania. Following internship, he was a Staff Fellow in the Laboratory of Technical Development of the National Heart Lung and Blood Institute (NHLBI), where he invented a method to measure tissue microvascular blood flow using laser light scattering. Following an Internal Medicine residency at University of Michigan and Cardiology fellowship at Johns Hopkins, Dr. Stern joined the faculty in Cardiology at Johns Hopkins in 1981. His research on laser light scattering fluctuations in cardiac muscle, in collaboration with the Laboratory of Cardiovascular Science at GRC, led to the discovery that apparently resting heart muscle produces continuous, random asynchronous subcellular waves of contraction, which proved to be due to propagated calcium release from the sarcoplasmic reticulum. This led directly to his present interest in the basic mechanism of cardiac excitation-contraction coupling. His studies on the physiology of excitation-contraction coupling in single cardiac myocytes during extreme hypoxia led to the finding that reoxygenation injury is due to calcium shifts brought about by ionic conditions created during a vulnerable period of complete energy depletion. In parallel with this work, Dr. Stern carried out mathematic modeling of the basic mechanisms of sarcoplasmic reticulum calcium release. Based on this work he proposed, in 1992, the *local control* hypothesis of excitation contraction coupling, which has become the leading theory of this process. In 1996, Dr. Stern joined LCS full time as a member of the Senior Biomedical Research Service.

**Calcium Microdomain Signaling in Intracellular Communication**: The heartbeat is initiated by the release of calcium from stores in the sarcoplasmic reticulum (SR). It is now well established that the trigger for this release is the entry of a much smaller amount of calcium through voltage-controlled L-type calcium channels in the cell membrane. This is the mechanism of *calcium-induced calcium release* (CICR), which is known to be mediated by ryanodine receptors, which

are calcium sensitive calcium channels located in the membrane of the SR. Similar ryanodine receptors are located on intracellular calcium stores in a wide variety of cell types, where their function is not yet understood.

The release of SR calcium is a tightly controlled and smoothly graded function of the trigger calcium; this is paradoxical since CICR is an intrinsically self-reinforcing process which might be expected to lead to an all-or-none response. A possible resolution of the paradox is based on the fact that the L-type trigger channels and the SR release channels are known to be localized to opposite sides of the 15 nm dyad junctions between the cell membrane and the SR membrane. This means that the trigger for CICR is not whole cell calcium, but rather the local calcium microdomain generated in the neighborhood of the triggering channel. We have shown mathematically that the interaction between the stochastic gating of individual channels and the fluctuating calcium microdomains which they generate can give rise to smoothly graded and controlled calcium release in the whole cell aggregate, even though individual release events may be nearly all-or-none. This is the *local control* hypothesis, which implies that whole cell calcium release depends critically on the details of the gating and ion permeation of the trigger and release channels, and on the local geometrical relationship between them. Over the past several years, considerable evidence has accumulated showing that this is the case. We have recently constructed a similar local-control model of the role of CICR in skeletal muscle excitation-contraction coupling. This model successfully explains many paradoxical observations, and leads to the insight that collective behavior of mesoscopic arrays of calcium-coupled release channels, which we term *couplons*, may be the basic functional unit of EC coupling.

In order to test the local control hypothesis more definitively, we hope to develop a model in which the full machinery of excitation-contraction coupling (junctions, ryanodine receptors, L-type calcium channel, auxiliary junctional proteins) is expressed and in which the components and the signals that control their localization can be manipulated genetically. This is the major project of our laboratory at the present time  Since cardiac myocytes are terminally differentiated and non-dividing, they cannot be used directly. In general, cultured cell lines do not form SL/SR junctions even when expressing the channel proteins. Our present approach to the problem is to make use of the well developed technique of gene targeting in mouse embryonic stem (ES) cells, together with *in vitro*  differentiation of ES cells into embryoid bodies which contain beating cardiac myocytes. We have successfully established culture techniques which promote cardiac differentiation in a high percentage of embryoid bodies, and have demonstrated calcium sparks and waves, which are produced by RyR-mediated intracellular calcium release, in cells as early as 7 days of differ-

entiation. These studies will be continued to characterize the biochemistry, ultrastructure and EC coupling physiology of these cells. These baseline studies will define that model and lead to increased understanding of the development of cardiac-type EC coupling. We will then use homologous recombination methods to obtain cardiac myocytes in which the key protein domains responsible for calcium sensing and release, and others (such as the enormous 2 megadalton “foot process” of the ryanodine receptor) whose function is unknown, have been altered. More importantly, we hope to discover the signals which give rise to the organized geometrical structure of the dyad junction, and to alter it in order to test the sensitivity of coupling to geometry which is predicted by the local control theory. A combined approach utilizing ES cell techniques, confocal calcium measurement, patch clamp and mathematical modeling will be used.

Since ryanodine receptors are ubiquitous, it is likely that the insights gained from this program will be important for understanding the way in which spatial and temporal localization of intracellular calcium signals leads to their diversity of function in many cell types.

**Collaborators:** Heping Cheng, Kenneth Boheler, LCS; Eduardo Rios, Department of Physiology and Molecular Biophysics, Rush University; Phillip Palade, University of Texas, Galveston; Michal Horowitz, Hebrew University, Jerusalem.


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**Keywords:**
2-adrenergic receptor
cAMP dependent protein kinase
pertussis toxin-sensitive G proteins
cardiac contractility


**Biography:** Dr. Rui-Ping Xiao has been working in the Laboratory of Cardiovascular Sciences since February, 1990. She was trained as a physiologist and pharmacologist at Tong-Ji Medical University, China, and at the University of Maryland, where she received her M.D. and Ph.D., respectively. Her scientific focus has been related to receptor-mediated transmembrane signal transduction in the cardiovascular system. The mechanistic and multidisciplinary nature of her research has made the past few years particularly fruitful. The breadth of Dr. Xiao’s work covers four different areas:

**Recent Publications:**

Xiao R-P, *Am J Physiol* 1997; 272: H797-H805.

Pepe S, *Circulation* 1997; 95: 2122-2129.

Xiao R-P, *J Physiol* 1997; 500: 331-342.

Zhou Y-Y, *Am J Physiol* 1997; 273: H1611-H1618.

Korzick DH, *Am J Physiol* 1997; 272: H590-H596.

1) Signal transduction mechanisms which underlie the distinct actions of ß-adrenergic receptor (ßAR) subtype stimulation in cardiac myocytes; 2) age- and heart failure-related alterations in cardiac responses to ßAR subtype stimulation; 3) interaction of the ß-adrenergic signalling pathway with other cardiac sarcolemmal receptor mediated signaling pathways (e.g., opioid, adenosine, and acetylcholine receptors); and 4) the physiological role of protein kinase-phosphatase in cardiac functional regulation (e.g., regulation of cardiac calcium influx via L-type calcium channels by Ca/calmodulin-dependent kinase or cAMP-dependent kinase).

Our recent studies have systematically documented the distinctly different cardiac response to $ß_2$- versus to $ß_1$-adrenergic stimulation. By taking advantage of genetic manipulations, including transgenic mice overexpressing cardiac $ß_2$ARs and $ß_1$AR or $ß_2$AR knockout models, we have revealed that cardiac $ß_2$AR couples to two functionally opposing G protein families, i.e., a stimulatory G protein and inhibitory G proteins, $G_{i2}$ and $G_{i3}$. The dual coupling of $ß_2$AR to $G_s$ and $G_i$ not only reveals a new level of complexity of cardiac ßAR signal transduction, but also provides new insights for understanding the physiological and pathophysiological significance of the differential regulation of ßAR subtypes. Because the diminished ßAR contractile response in failing or aging hearts is accompanied by a selective down-regulation of $ß_1$AR, without loss of $ß_2$AR, considerable effort has also been put on the potential role of $ß_2$AR activation for improving cardiac performance under these conditions. In canine and human failing cardiomyocytes, the efficacy of $ß_2$AR stimulation is markedly increased as compared to that of normal cells. The up-regulation of $ß_2$AR-directed signaling relative to $ß_1$AR may be beneficial, because it provides inotropic support without promoting sarcoplasmic reticulum (SR) $Ca^{2+}$ overload and spontaneous SR $Ca^{2+}$ release. The cellular logic for the multiplicity of ßAR may partly reside in the different arrhythmogenic properties of ßAR subtypes. In light of these recent findings, an increase in the ratio of $ß_2$AR/ $ß_1$AR and $G_i$ amount might reflect important adaptive changes associated with heart failure and cardiac aging. In addition, the novel signaling mechanism of $ß_2$AR stimulation, i.e. coupling of $ß_2$AR to $G_i$ proteins, represents a potential target for therapeutic interventions to attenuate the inhibitory pathway thereby extending and augmenting the action of various therapeutic agents.

**Collaborators:** Dr. Robert J. Lefkowitz, Howard Hughes Medical Institute; Dr. Walter J. Koch, Duke University Medical Center; Dr. Ruth Altschuld and Charlene Hohl, Department of Medical Biochemistry, Ohio State University; and Dr. E-G. Krause, Max Delbruck Center of Molecular Medicine, Department of Cardiology, Berlin, Germany; Dr. Brian Kobilka, Howard Hughes Medical Institutes, Stanford University Medical Center.

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**Keywords:**
excitation-contraction coupling
calcium
nitric oxide
chemotaxis

**Recent Publications:**

Sollott SJ, et al. *Am J Physiol* 1996; 271: H896-905.

Miyashita Y, et al. *Am J Physiol* 1996; 271: H244-H255.

Shah AM, et al. *Circ Res* 1997; 80(5): 688-698.

Irani K, et al. *Science* 1997; 275(5306): 1649-1652.

**Biography:** Dr. Sollott received his M.D. from the University of Rochester and completed his residency in internal medicine at a Cornell University program. He subsequently completed his cardiology fellowship at Johns Hopkins University and an NIH medical staff fellowship at LCS, GRC. His research attempts to bridge interests spanning basic and clinical science to therapeutics.

We are studying structure and function of cells from the cardiovascular system along two principal and distinct lines: 1) mechanisms of cardiac contractility, and 2) cellular changes after vascular injury. An underlying theme in both of these areas involves the pursuit and development of single cell biophysical methods to overcome certain limitations and complexities inherent in *in vivo* and in multicellular *in vitro* experimental systems, to gain an understanding of basic cell biological processes that may have implications for the pathophysiology and treatment of human disease.

**Mechanisms of Cardiac Contractility**: Principal research efforts, often employing these newly-developed biophysical methods, have focused on the regulation of contractility in intact cardiac myocytes, with particular emphasis on modulation of myofilament contractile activation by novel signaling pathways, for example, via alterations in the balance of specific kinase/phosphatase pathways, via cross-talk between the cGMP- and cAMP-dependent pathways, and via endogenous nitric oxide-dependent mechanisms. Recent work has focused on novel mechanisms of recruitment of contractile activation underlying the Frank-Starling response.

**Cellular Response to Vascular Injury**: The other major research direction involves the investigation of basic cellular responses of vascular smooth muscle cells during gradient-directed chemotaxis, in order to gain insight into fundamental events in the pathogenesis of vascular disease. These experiments with vascular smooth muscle cells have enabled an understanding of how focal receptor-tyrosine-kinase activation coordinates

the cascade of signaling traffic and the reorganization of the cytoskeleton, leading to directed migration. Migration of vascular smooth muscle cells from the arterial media to the intima is a key event in the pathogenesis of occlusive vascular disorders, including atherosclerosis and post-angioplasty restenosis. We found that a unique intracellular $Ca^{2+}$-signaling profile is initiated via extracellular cues provided specifically by gradient exposure to PDGF, achieving an apparent threshold for activation of CaM kinase II (requisite during VSMC chemotaxis), and this phenomenon mediates VSMC chemotaxis. Differences in this specific $Ca^{2+}$ signaling paradigm among individual cells underlies the asynchronous occurrence rate of chemotaxis seen in VSMC populations. Work is continuing to establish the mechanisms and coordination of subcellular $Ca^{2+}$-microdomains, compartmentalization of CaM kinase II activation and cytoskeletal rearrangements.

These ideas have been applied to the search for strategies to ameliorate the complications of vascular injury. We found that nanomolar levels of paclitaxel (taxol) blocked chemotaxis of VSMC in culture via specific interference with microtubule function, without killing cells. Subsequent *in vivo* experiments showed that paclitaxel, given systemically to rats at doses achieving blood levels some 2 orders of magnitude below that used in oncologic therapeutics (i.e., averaging well below peak levels of 50 nM), reduces the extent of neointimal proliferation following balloon injury by 70-80% without apparent toxicity. Currently, studies in larger mammals are under way to determine the feasibility of initiating a clinical trial in humans. Also, collaborative efforts are under way pursuing local paclitaxel delivery schemes, such as paclitaxel-coated stents, for this purpose. A microtubule-stabilizing-agent use-patent has been obtained for the applications of paclitaxel in treatment of atherosclerosis and restenosis, and a CRADA has been established with private industry partners.

**Collaborators:** Salvatore Pepe, Ph.D., Baker Medical Research Institute, Melbourne, Australia; Kaikobad Irani, M.D., Johns Hopkins University; Pascal Goldschmidt-Clermont, M.D., Ohio State University; Jay L. Zweier, M.D., Johns Hopkins University; Ajay M, Shah, M.D., University of Cardiff, Wales, United Kingdom; Dilip Kittur, M.D., Sc.D., Johns Hopkins University; Robert S. Danziger, M.D., Columbia University; David Wink, Ph.D., NHLBI; Antoine Younes, Ph.D., Universite d’Auvergne Clermont, Aubiere, France.

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**Keywords:**
cell calcium
cardiac electrophysiology
confocal microscopy
contraction mechanics

**Recent Publications:**

Cheng H, et al. *Cell Calcium* 1996; 20: 129-140.

Sollott SJ, et al. *Am J Physiol* 1996; 40: H896-H905.

Xiao R-P, et al. *Am J Physiol* 1997; 41: H797-H805.

Wheeler DM, et al. *Anesthesiology* 1997; 86(1): 137-146.

**Biography:** Dr. Harold A. Spurgeon received his Ph.D. in Medical Physiology from Loyola University Stritch School of Medicine, Chicago, in 1972, and completed postdoctoral training at the University of New Mexico, Albuquerque. He joined the Intramural Research Program in 1974 where he worked on problems related to cardiac muscle mechanics and innervation control.

**Recent Research:** Because the unique characteristics of our implementation of a system to measure calcium, cell shortening, and membrane current/voltage, we measure the true instantaneous values associated with each of these parameters, without "time smearing" during a given excitation/contraction cycle due to averaging effects. This approach has allowed us to investigate the codependency of cell length, free calcium, or potential, in a two dimensional model. Clearly there is a time-dependence as well, important in understanding the interaction between time-dependent changes in the extent of cell shortening and free intracellular calcium.

Confocal imaging opens up exciting windows into physiological questions. For example, calcium "sparks" recently described in *Science*, raise questions about the role of transmembrane currents accompanying microburst releases of calcium in highly discrete regions of the cell. We hope to determine conclusively whether the sparks result in an evoked localized calcium current triggered by the local release of calcium from (most likely) the sarcoplasmic reticulum, or whether the sparks are themselves triggered by localized transient increases in calcium conductance leading to localized SR release.

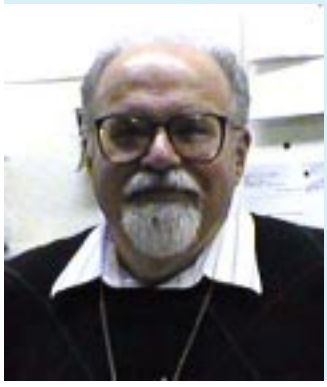
Image processing is in its infancy. Individual images contain large amounts of information which must basically be hand-tweaked to extract even a small fraction of the information contained. As imaging of a variety of types becomes even more prevalent, expert systems of software and hardware offer the only currently identifiable relief in processing this mountain of data. We will need to develop and integrate these approaches

to increase our data reduction efficiency. Quantification of image characteristics has been likewise largely confined to “looks like, bigger than,” although very recently more quantitative descriptors have begun to appear. To understand the physiology, functional descriptors need to be developed as well. Pattern recognition techniques and fractal reduction may prove useful here.

In addition to the core technical development tasks and assorted research projects, I have become involved in human studies. By adding pulsewave measurements to two existing multi-center studies being conducted by NHLBI and our Human Studies Unit, we gain a relatively low cost access to approximately 3800 subjects already screened as hypertensive. We will be able to follow these patients in a blind interventional study where the intervention planned is prescribed exercise. We hope to produce a more comprehensive picture of arterial stiffness, aging, and disease, particularly in the important area of structure/function relationships as they relate to disease outcomes. By building a unified database across several of these studies, the bias inherent in single population studies should be eliminated. In the BLSA population in particular, both prospective and retrospective data are available for disease outcomes, but the somewhat biased population demographics preclude more global interpretations. We hope as well to get better data relative to dietary sodium intake in at least a subset of these populations, for the role of increased sodium in modifying vascular stiffness is not clear.

Further investigations in the pharmacology of $ß_1$ and $ß_2$AR are being extended to address, in the dog model, the changes in phosphorylation of key cellular proteins by $ß_1$ and $ß_2$AR. Of particular importance is definition of the $ß_2$ inhibitory pathway in this model. The preponderance of evidence points to a non-cAMP mediated mechanism for $ß_2$ activation because there is no evidence to date showing increased cAMP production in the adult dog heart by I.C. zinterol, $ß_2$ agonist, and no phospholamban phosphorylation. In collaboration with Dr. George Krause, who has developed a monoclonal antibody for the ß-subunit of the L-type calcium channel we will further define ß-adrenergic subtype actions on the cardiac L-type calcium channel.

**Collaborators:** Gary Gerstenblith, M.D., Johns Hopkins School of Medicine; Peter Snell, Ph.D., University of Texas Southwestern; Kim Sutton-Tyrrell, Ph.D., University of Pittsburgh; Leslie Pruitt, Ph.D., Stanford University; Paul Ribisl, Ph.D., Wake Forest University; Mary O'Toole, Ph.D., University of Tennessee; Peter Vaitkevicius, M.D., Johns Hopkins School of Medicine; Richard Havlik, M.D., Epidemiology Demography and Biometry Program, IRP, NIA; Mark Lane, Ph.D., Laboratory of Cellular and Molecular Biology, IRP, NIA; Salvatore Pepe, Ph.D., Baker Research Institute, Australia; A.J. Shah, M.D., University of Wales; Constantine Bogdanov, Ph.D., Cardiology Research Center, Moscow; G. Krause, Ph.D., Max Delbruck Center for Molecular Cardiology, Berlin.

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**Keywords:**
thermoregulation
hemodynamics
microcirculation
angiogenesis


**Recent Publications:**


Talan MI, et al. *Physiol Behav* 1996; 60: 1285-1289.

Talan MI, et al. *Exp Gerontol* 1996; 31: 687-698.

Talan MI, *Ann NY Acad Sci* 1997; 813: 95-100.

Shefer VI, et al. *Exp Gerontol* 1997; 32: 325-332.


**Biography:** Dr. Talan was trained as a physician at the First Leningrad Medical School in Russia. He received his Ph.D. in Physiology at the Pavlov Institute of Physiology in Russia where he continued to work as a scientist before coming to the NIA in 1980. His studies at the NIA in the area of thermoregulation, regulation of hemodynamics, and operant conditioning of autonomic function evolved to his present interests concerning (1) the effects of thermoregulatory responses to cold on the development of hypertension and other cardiovascular risk factors; and (2) microcirculation and stimulation of angiogenesis.

**The Mechanisms of Cold-Induced Hypertension**: A number of epidemiological observations reported an increased incidence of adverse cardiovascular events and high prevalence of elevated arterial blood pressure during the winter. The entire population is affected by this annual rhythm but the elderly are the most vulnerable to the negative effects of seasonal changes. The colder ambient temperature was implicated as the single most important factor responsible for this effect, but the mechanisms of seasonal hypertension and elevation of other cardiovascular risk factors remain poorly understood. This program was set up to develop an experimental animal model of cold-induced hypertension and to investigate the mechanisms responsible for elevation of blood pressure and other risk factors during cold acclimation.

The immediate goal of this project is to define the time course and parameters of changes in arterial blood pressure associated with acclimation to different environmental temperatures and to measure accompanied changes in plasma volume and rheological characteristics of blood in adult and aged Wistar rats.

Adult and aged Wistar rats acclimated to thermoneutrality, i.e. temperature that does not requires any metabolic expenditure to maintain body temperature (26EC), were exposed to cold (6EC) for 9 weeks followed by 5 weeks of rewarming (26EC). In adult rats the elevation of systolic blood pressure started two weeks after beginning of cold exposure and reached 30 mmHg above the control level after six weeks of exposure. The elevation of blood pressure was preceded by a 50% plasma volume expansion and was accompanied by an increased water consumption and elevation of whole blood viscosity. During the five weeks of rewarming plasma volume and water consumption returned to normal but blood pressure and blood viscosity remained elevated. Cold exposure of aged rats did not result in elevation of the systolic blood pressure, blood viscosity or plasma volume expansion, however, before cold exposure these parameters were already higher in aged than in adult rats.

We believe that cold-induced elevation of blood pressure in rats represents the first naturalistic experimental model of volume-associated hypertension that will facilitate the development of treatment and prevention of this debilitating condition. We will be carrying out systematic studies to further understand the mechanisms by which naturally occurring physiological responses to cold might contribute to changes in circulating plasma volume, blood viscosity, and eventually lead to morphological vascular changes characteristic for hypertension.

**Collaborators:** Natalya Roukoyatkina, Ph.D., Behavioral Hypertension Section, LCS; Joseph Rifkind, Ph.D., and Ranjeet Ajmani, Ph.D., Molecular Dynamic Section, LCMB.

**Jeffrey Froehlich, M.D.**
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**Keywords:**
ion transport
relaxation
restenosis
local delivery

**Recent Publications:**

Miyashita Y, et al. *Am J Physiol* 1997; 272 : H244-H255.

Kane DJ, et al. *Biochemistry* 1997; 36(43): 13406-13420.

Hartung K, et al. *Biophys J* 1997; 72(6): 2503-2514.

**Biography:** Dr. Froehlich received his M.D. degree from the University of Chicago in 1969 and completed 3 years of postgraduate research in the Department of Biophysics before joining the NIH as a Commissioned Officer in the USPHS. In 1985 he was named chief of the Membrane Biology Section which became part of the Laboratory of Cardiovascular Science, NIA, in 1990. At the NIH, he collaborated with Robert Berger to develop a rapid mixing, chemical quenched-flow device that has been extensively used for kinetic characterization of the ion motive ATPases, an interest that evolved from his postgraduate work. Although internationally recognized for his contributions to the field of active transport in subcellular organelles, he has also studied $Ca^{2+}$ transport in vascular smooth muscle cells, focusing on $ß_2$-agonist-mediated relaxation and aging. Recently, he has begun to examine the vascular smooth muscle response to mechanical injury in a CRADA-supported program aimed at developing pharmacological approaches for the prevention of restenosis following angioplasty.

**Ion Motive ATPases**: The ion motive ATPases represent an important class of enzymes that couple ATP hydrolysis to unidirectional, uphill cation transport. A major theme in Dr. Froehlich's research on these enzymes in eukaryotic cells has been the role of quaternary (subunit-subunit) protein interactions in the mechanism of ion-dependent ATP hydrolysis. Using rapid mixing techniques (quenched-flow and stopped-flow mixing), he has identified specific features of the pre-steady state and steady state kinetic behavior of these enzymes that reflect the presence of subunit-subunit interactions in the catalytic mechanism. These interactions impose constraints on the timing of the reactions in the conformationally coupled subunits, forcing them to occur sequentially rather than simultaneously. In a dimeric enzyme, the leading protomer binds the transported ion and moves it across the membrane in advance of these events in the neighboring protomer. A central problem has been to understand why the system operates this way as opposed to rapidly transporting all of the

bound cations across the membrane, yielding the highest catalytic efficiency. An answer to this problem may be found in the conservation of free energy which can be transferred between adjacent subunits residing at different energy levels. Another explanation involves the minimization of repulsive forces between like charges which arise from complexation of the transported cation with the pump protein. These and other issues related to the oligomeric behavior of these enzymes are being explored by a variety of special techniques including rapid chemical quenching, the laser flash/lipid bilayer technique and time-resolved electron spin resonance. Future studies involving prokaryotic ion motive ATPases, which exhibit similar kinetic behavior, will allow testing of these hypotheses by site-directed mutagenesis.

**Vascular Smooth Muscle Relaxation Mechanisms:** Older individuals manifest an increase in systolic blood pressure together with increased arterial stiffening and reduced vasorelaxation in response to $ß_2$-adrenergic agonists. Dr. Froehlich has proposed a common etiology for these changes based on altered smooth muscle $Ca^{2+}$ metabolism and has tested this hypothesis using freshly-isolated rat arterial cells loaded with a cytoplasmic $Ca^{2+}$ indicator and ratiometric video fluorescence imaging. The $ß_2$-agonist, isoproterenol (ISO), was found to mediate smooth muscle relaxation by reducing $Ca^{2+}$ influx and by decreasing $Ca^{2+}$ stores in the sarcoplasmic reticulum (SR). These effects resulted from cyclic AMP-dependent stimulation of the $Na^+/K^+$ pump and secondary activation of the $Na^+/Ca^{2+}$ exchanger. Older cells exhibited larger SR $Ca^{2+}$ stores following ISO, which reduced the $Ca^{2+}$ buffering capacity of SR and increased the probability of enhanced vascular tone. Increased arterial tonus might explain the arterial stiffening and rise in systolic blood pressure associated with aging. The single cell model affords a unique opportunity to explore the relationship between intracellular $Ca^{2+}$ and contractility in smooth muscle pharmacomechanical coupling.

**Local Drug Delivery and Restenosis:** A complication facing 40-50% of the patients undergoing percutaneous transluminal coronary angioplasty (PTCA) for symptomatic coronary artery disease is restenosis, a condition associated with neointimal proliferation and late vascular remodeling. Efforts to develop a pharmacological approach for the prevention of restenosis have focused on paclitaxel, an anti-neoplastic drug with proven efficacy at preventing vascular smooth muscle cell migration and proliferation. Paclitaxel coated directly onto metallic intracoronary stents was shown to produce a significant reduction in neointimal hyperplasia and lumenal encroachment in minipigs without the thrombotic complications commonly associated with some polymeric local delivery systems. Future research will concentrate on defining a safe and effective therapeutic dosing range in preparation for clinical trials.

**Collaborators:** R. W. Albers, NINCDS, NIH; A. Heldman, J. Brinker, D. Kittur, R. Hruban, G. Jenkins, Johns Hopkins University; K. Fendler, K. Hartung, E. Bamberg, R. Clarke, Max-Planck-Institute for Biophysics; K. Altendorf, S. Droese, Osnabrueck University; W. Epstein, University of Chicago; K. Taniguchi, Hokkaido University.


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**Keywords:**
vascular injury
restenosis
drug delivery
gene therapy


**Recent Publications:**


Yamamoto H, et al. *Exp Cell Res* 1996; 222: 125-130.

Li Z, et al. *Am J Path* 1996; 148: 121-128.

Miyashita Y, et al. *Am J Physiol* 1997; 272: H244-H255.


**Biography:** Dr. Cheng received her Ph.D. in biochemistry from Wayne State University. After completing her postdoctoral fellowship at The Johns Hopkins University, she joined the NIA in 1977. Her present interests focus on using gene therapy and paclitaxel to reduce neointimal development after vascular injury in animal models of restenosis.

**Animal Model of Restenosis:** Mechanical injury of the blood vessel wall causes de-endotheliazation, mural thrombosis, platelet activation, thrombin generation, and the release of growth factors and cytokines. These events lead to vascular smooth muscle cell migration, proliferation and extracellular matrix deposition that are the hallmarks of restenosis. Our laboratory has been involved in developing the rat and pig models of restenosis and using these models to understand *in vivo* proteolytic cascades and intracellular signal transduction. In addition, we use these models to develop therapeutic strategies such as local drug delivery and gene therapy to treat restenosis.

**Local Drug Delivery:** Local delivery of therapeutic agents to the arterial wall represents a new strategy for the treatment of fibroproliferative vascular disease, including restenosis after percutaneous transluminal angioplasty and arterio-venous bypass grafting. A major advantage of local delivery is the ability to achieve effective tissue concentrations of the drug while avoiding the toxic effects associated with systemic dosing. We developed biodegradable microbeads as a mechanism of local delivery. Our microbeads are formed by the complex coacervation of collagen and chrondroitin sulfate. Microbeads formed by this process are particularly suitable for entrapping proteins and hydrophobic com-

pounds. We have shown that paclitaxel, an antineoplastic agent, inhibited neointimal hyperplasia resulting from balloon catheter injury when administered systemically to rats. The ability of locally-administered paclitaxel to prevent the neointimal hyperplastic response was tested by incorporating the drug into sustained-release biodegradable microbeads which were applied to the adventitial surface of the rat carotid artery immediately following balloon injury. Locally-delivered paclitaxel produced a dose-dependent and site-specific inhibition of neointimal growth. This inhibitory effect could be achieved without a detectable inflammatory response and without evidence of medial wall cell death at low paclitaxel concentrations. These results demonstrate complete inhibition of neointimal hyperplasia by extravascular paclitaxel applied directly over the site of injury. Local delivery of paclitaxel in biodegradable polymer matrices may have application in the creation of arterio-venous shunts and in other surgical grafting procedures where stenosis is a complication. Our current efforts concentrate on the effect of paclitaxel coated stents on pig coronary arteries.

**Gene Therapy:** The migration of vascular smooth muscle cells (VSMCs) is an important and essential step in restenosis after balloon angioplasty. This step is dependent on the local degradation and remodeling of the extracellular matrix. We have shown that vascular injury increased the expression and activation of matrix metalloproteinase 2 (MMP-2) during the migration of VSMC to the intima in the rat carotid artery injury. Adenoviral vectors are a very useful tool for the transfer of genetic material to the vessel wall. In order to inhibit VSMC migration from media to the intima, we constructed a replication-deficient adenovirus vector carrying the cDNA for human tissue inhibitor of metalloproteinase-2 (AdCMV.hTIMP-2). TIMP-2 is a physiologic MMP-2 inhibitor. AdCMV.hTIMP-2 was previously shown to inhibit MMP-2 activity and SMC invasion in cultured VSMC. Recently, we have shown that medial VSMCs exhibit positive staining for human TIMP-2 protein after vascular injury and infection with AdCMV.hTIMP-2. In addition, we demonstrated that localized arterial infection with AdCMV.hTIMP-2 at the time of vascular injury significantly reduced the number of cells in the intima and neointimal formation. These results demonstrate the important role of TIMP-2 in regulating migration of VSMC, and suggest either TIMP-2 alone or in combination may have therapeutic potential for treating restenosis after vascular injury.

**Collaborators:** Kam Leong, Ph.D., Johns Hopkins University.

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**Keywords:**
carbon dioxide
endogneous digitalis-like factors
hypertension
sodium

**Recent Publications:**

Anderson DE, et al. *Journal of Hypertension* 1996; 14: 1073-1079.

Fedorova OV, et al. *American Journal of Hypertension* 1996; 9: 1126-1131.

Dhokalia A, et al. *Psychosomatic Medicine* 1997, In Press.

Fedorova OV, et al. *American Journal of Hypertension* 1997; 10: 929-935.

**Biography:** Dr. David E. Anderson received his Ph.D. from the University of Oregon in 1966. He developed his career interest in the environmental and behavioral origins of hypertension and on the nature of the mediating physiological mechanisms at The Johns Hopkins University School of Medicine (1968-1981) and the University of South Florida (1981-1987). During that period, he was a recipient of an NIH Research Career Development Award (1983-1987) and the Pavlovian Award for Biological Science in 1985. He came to the National Institute on Aging in 1987, and was appointed Chief of the Behavioral Hypertension Section of the Laboratory of Cardiovascular Sciences in 1997.

**The Behavioral Origins of Hypertension:** The goal of the Behavioral Hypertension Section is to clarify how interactions of the individual with the external environment contribute to the development of chronic hypertension. It is known that intermittent behavioral stress can potentiate a sodium-sensitive experimental hypertension in large laboratory animals. This form of hypertension involves sustained renal sodium retention, but its development is not prevented by renal denervation. The finding that sustained suppression of breathing is characteristic of hypertensive animals led to a focus in this Section on behaviorally-induced increases in $pCO_2$ and its effects on renal regulation of sodium, sodium pump inhibitors and blood pressure. A fundamental hypothesis that directs experimental work is that behaviorally induced breathing suppression increases plasma volume via increases in $pCO_2$, carbonic acid formation, hydrogen ion concentrations, and increased renal sodium-hydrogen exchange, and potentiates the hypertensive effects of a high sodium diet.

**Behaviorally Induced Respiratory Suppression and Blood Pressure Regulation**: In support of this view, one series of studies with laboratory micropigs showed that anticipatory behavioral stress can increase $pCO_2$ acutely, decrease plasma pH, increase plasma bicarbonate, and decrease hematocrit, indicative of plasma volume expansion. Consistent with this finding is the associated observation that endogenous digitalis-like factors

(EDLF) which are sensitive to plasma volume changes are also increased under these conditions. A parallel series of studies with healthy humans showed that comparable effects could be produced by self-regulated inhibition by breathing maintained by a respiratory gas monitor and feedback system.

More recent studies have shown that high resting end tidal $CO_2$ is a risk factor for sodium sensitivity in older, and to a lesser extent, in younger humans. These studies also showed that urinary EDLF excretion was higher in subjects with high end tidal $CO_2$. Resting end tidal $CO_2$ is correlated with $pCO_2$ in humans with healthy lungs and can be measured nonintrusively and continously. Studies have shown that high resting $PetCO_2$ is stable over time, and highly positively correlated with the tendency to worry and experience negative affects. Thus, high resting $pCO_2$ may be an indicator of chronic stress in humans.

**Endogenous Digitalis-like Factors in Blood Pressure Regulation:** Studies in this Section also focus on the role of EDLF in blood pressure regulation. EDLF are of interest in this laboratory because they vary with plasma volume and can inhibit sodium/potassium pump activity in vascular smooth muscle. Previous work in this laboratory showed that an endogenous marinobufagenin-like bufodienolide is a more rapid and powerful vasoconstrictor than an endogenous ouabain-like cardenolide, and that plasma concentrations of each are stimulated by saline-induced expansion of plasma volume. Moreover, a high sodium diet can sustain increases in urinary marinobufagenin excretion for at least weeks. Studies have also shown that the bufodienolide has a greater affinity for the $\alpha$-1 isoform of Na,K-ATPase, which is concentrated in vascular smooth muscle membranes, and that the cardenolide has a greater affinity for the $\alpha$-3 isoform, which is concentrated in neural membranes. Thus, the two EDLF may have different primary sites of action and different roles in blood pressure regulation. Studies will be conducted to determine the effects of administration of EDLF antibodies and natural protective ligands of Na, K-ATPase in various models of hypertension.

**Ongoing Studies**: Current work focuses on the development of a model of behavioral hypertension in the rat which will test the hypothesis that sodium sensitivity is a function of habitual breathing pattern and chronic increases in $pCO_2$. Rats maintained in a metabolic chamber are trained to self regulate breathing rates for up to eight hours per day, to avoid onset of

aversive bright light. Blood pressure is monitored continuously 24 hr per day via telemetry. It is hypothesized that chronic breathing inhibition will potentiate the development of experimental hypertension in rats on a high sodium diet, but that this form of hypertension will not occur in sodium-fed rats who maintain high breathing rates or in breathing-suppressed rats on a low sodium diet. The role of EDLF, sodium pump activity and sodium-hydrogen exchange in the development of this form of hypertension will be analyzed experimentally.

Finally, studies are in progress with participants in the Baltimore Longitudinal Study on Aging to examine the relationships between breathing patterns, resting $pCO_2$, plasma volume, renal functions, and long-term regulation of blood pressure. Taken together, these studies may clarify how habitual patterns of behavioral interactions contribute to sodium sensitivity and the development of hypertension, and provide the basis for prevention or delay of this common cardiovascular disorder and its sequelae.

**Collaborators:** Alexei Y. Bagrov, M.D., Ph.D., Sechenov Institute of Evolutionary Physiology, Russian Academy of Sciences, St. Petersburg, Russia; A. Dhokalia, Ph.D., Laboratory of Cardiovascular Sciences, NIA; Olga V. Fedorova, Ph.D., Laboratory of Cardiovascular Sciences, NIA; Richard G. Weissman, Ph.D., Laboratory of Cardiovascular Sciences, NIA.

**George S. Roth, Ph.D., Acting Chief**

# Laboratory of Cellular and Molecular Biology

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The Laboratory of Cellular and Molecular Biology (LCMB) was established in the early 1980's by Dr. Gunther Eichhorn. Dr. George Roth was transferred from the Clinical Physiology Branch in 1984 to initiate and direct the Molecular Physiology and Genetics Section. In subsequent years, the Molecular Dynamics Section was established by Dr. Joseph Rifkind, the Nuclear Magnetic Resonance Unit by Dr. Richard Spencer, and the Drug Development and Design Unit by Dr. Nigel Greig. The Gene Expression and Aging Section, directed by Dr. Nikki Holbrook was also a component of LCMB from 1995 until 1997 when Dr. Holbrook became Chief of the Laboratory of Biological Chemistry. Dr. Eichhorn retired in 1994, phasing out many of his activities but remaining as a Scientist Emeritus. Since that time, Dr. Roth has served as Acting Chief, LCMB.

The interests of the Laboratory are relatively broad with a major focus on basic mechanisms of aging and age-related diseases. The Molecular Physiology and Genetics Section plays a central role in examining aging processes at levels ranging from the molecular to the behavioral, with coordination by Drs. Roth and Donald Ingram, respectively. Much of this research involves age changes in regulations of physiological and behavioral functions utilizing whole animal and cellular models of hormone and neurotransmitters signal transduction. Since 1987, however, their most visible project has been an examination of the effects of caloric restriction on the aging of primates. The Molecular Dynamics Section, under Dr. Rifkind, examines the role of oxygen and oxyradicals in biological systems and their involvement in the aging process. Collaboration among the LCMB sections and units on age-related projects also involve Dr. Spencer's Nuclear Magnetic Resonance Unit, while their major emphasis is on imaging, metabolic studies of chondrocytes and spectroscopic studies of muscle metabolism under various conditions. The Drug Design & Development Unit, headed by Dr. Greig, attempts to develop novel agents to combat diseases of the nervous system with particular emphasis on Alzheimer's disease.

In addition to these major independent projects, a number of collaborative studies are underway in the Laboratory of Cellular and Molecular Biology. Regular meetings of the various organizational units and special interest groups (such as the Basic Mechanisms of Aging and Imaging groups) are held. LCMB personnel are also actively involved in educational studies and lectures for fellows.

## Laboratory of Molecular and Cellular Biology Staff

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| Carol Lindsay | Secretary |

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signal transduction
dopamine receptors
G proteins

**Recent Publications:**

Kelly JF, et al. *Proc Nat Acad Sci USA* 1996; 93: 6753-6758.

Kitano S, et al. *Biochem Biophys Res Com* 1996; 225: 122-127.

Chernak JM, et al. *Mol Brain Res* 1997; 44: 113-124.

Shinkai T, et al. *J Neurosci Res* 1997; 47: 393-399.

**Biography**: Dr. George S. Roth received his Ph.D. from Temple University School of Medicine in 1971. After postdoctoral training at the Fels Research Institute, he became a Staff Fellow at the Gerontology Research Center (formerly National Institute of Child Health and Human Development), receiving tenure in 1976 and becoming Chief, Molecular Physiology and Genetics Section in 1984 and Acting Chief, Laboratory of Cellular and Molecular Biology in 1994. He is very active in the biogerontological community, serving as Chair of the Gordon Conference on the Biology of Aging in 1985, various offices in the Gerontological Society of America, and receiving the Research Award of the American Aging Association in 1981 and the Sandoz Prize for Gerontological Research in 1989.

**Basic Mechanisms of Aging; Signal Transduction Models and Interventions:** We are studying basic mechanisms of aging from the molecular to the behavioral levels, with particular emphasis on functional regulation by hormones and neurotransmitters. Our recent work is concentrated in four distinct areas.

**Loss of Dopaminergic Motor Control During Aging:** Loss of striatal $D_2$ dopamine receptors contributes substantially to reduced motor control in the elderly. Such receptor loss is due both to the death of some receptor-containing neurons and decreased expression of the receptor gene in the surviving neurons.

Loss of neurons has also been implicated in a number of neurodegenerative diseases including Alzheimer's, Huntington's and Parkinson's. We have recently quantitated the concentrations of actual $D_2$ receptor-mRNA containing neurons in animals of different ages. An approximately 25% loss of cells occurs, enough to account for roughly half of the receptor loss observed over the adult lifespan (which is 40-50%).

Interestingly, we have also observed apoptotic neurons in the adult rat striata but at an extremely low frequency; 2-4 per 100,000. Although this figure appears at first glance to be physiologically of little importance, if clearance times for dying neurons are on the order of hours as has been reported for some cell types, apoptosis could represent an important mechanism of neuronal loss during aging.

Current studies in our laboratory are attempting to elucidate possible age changes in transcriptional control mechanisms for the $D_2$ receptor gene, determine the relationship between decreased expression of the gene and neuron death, and ameliorate the age-related loss of motor control by transfection of living rodents with attenuated adenoviral vectors containing the gene. Although, we have not yet identified any transcription factors whose binding to the $D_2$ receptor gene promoter region changes with age, we have been successful in constructing viral vectors containing the gene. When injected into striata of living rats and mice, mRNA is transcribed and translated into receptors capable of binding $D_2$ receptor ligands. The next step will be the determination of whether increasing receptor levels by this method can restore the impaired motor function of aged rats.

**Age- and Alzheimer's Disease-Related Changes in Striatal Muscarinic Receptor-G Protein Coupling**: We have been investigating mechanisms underlying defects in muscarinic cholinergic receptor-G protein coupling found in aging and Alzheimer's disease. Muscarinic cholinergic pathways play a key role in learning and memory processes. Using the rat striatum model, we have shown that aging is associated with reduced muscarinic receptor-augmented stimulation of low Km GTPase activity and that this change is correlated with an increase in membrane cholesterol/ phospholipid molar ratio and a reduction in membrane bilayer width measured by small angle X-ray diffraction. We have also shown that there is an age-related decrease in basal and muscarinic agonist-induced GTP binding to the G protein subunit Gaq/11 which mediates signaling via the second messengers IP3 and DAG. In a series of studies utilizing sucrose density gradient centrifugation of detergent solubilized receptor-G protein complexes we have demonstrated a mean age-related decrease in the molecular mass of complexes, a finding which may be explained by a higher proportion of receptors and G proteins in the uncoupled state. In Alzheimer's disease, we have shown that there is a similar but more profound reduction in agonist-stimulated low Km GTPase activity.

Using a rodent fetal cortical cell model system in collaboration with Dr. Mark Mattson of the University of Kentucky, we have shown that exposure to amyloid ß peptide produces a reduction in GTPase activity which can be attenuated by preincubation with antioxidants. In our most recent studies, we have shown that 4-hydroxynonenal (HNE), a highly reactive aldehyde by-product of oxyradical-induced membrane lipid peroxidation, may mediate this effect, since there is an HNE-related decrease in muscarinic as well as metabotropic glutamate receptor-stimulated GTPase activity associated with the formation of Gaq/11-HNE adducts, and which can be prevented by preincubation with glutathione.

**Impaired Stimulation of DNA Synthesis in Hepatocytes of Aged Rats**: Altered control of DNA synthesis and cell division results in a number of age-associated disorders including impaired wound healing, tissue regeneration immune response, and cancer.

Stimulation of DNA synthesis by various agents including catecholamines and growth factors is markedly reduced in primary cultures of hepatocytes obtained from aged rats when compared to younger counterparts. Such impairment is not the consequence of receptor loss. Moreover, since very different signal transduction pathways are employed by G protein linked receptors and those mediated by tyrosine kinases, the defect would appear to be at a very fundamental level. Results to date indicate that increased expression of sdi-l/p21, an inhibitor of cyclin-dependent kinases, is not responsible. However, decreased stimulation of the MAP kinase pathway (including ERK2), possibly due to elevated levels of MAP kinase phosphatase, may also play a role. In addition, cells of aged rats appear to shift to other growth factor responsive pathways. Thus, examination of signal transduction components in various pathways which mediate DNA synthesis will continue in an effort to comprehensively define the pattern of age change.

**Collaborators**: Donald Ingram, Ph.D., Mark Lane, Ph.D., Jeremiah Kelly, M.D., Yongquan Luo, Ph.D., Yolanda Mock, Ph.D., Regis Perichon, Ph.D., Sugata Ray, Ph.D., Hiroyuki Umegaki, M.D., Ph.D., Yoshikage Yo, Ph.D., Nikki Holbrook, Ph.D., Yusen Liu, Ph.D., John Kusiak, Ph.D., NIA; B. Wolozin, Loyola University, Chicago; Mark Mattson, University of Kentucky.


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brain aging
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**Recent Publications:**


Ingram DK, et al. *Ann NY Acad Sci* 1996; 786: 348-361.

Jucker M, et al. *Behav Brain Res* 1997; 85: 1-25.

Kuo H, et al. *J Gerontol: Biol Sci* 1997; 52: B146-B151.

Shimada A, et al. *Neurobiol Aging* 1997; 18: 329-333.


**Biography:** Dr. Ingram was trained in psychology and gerontology at the University of Georgia where he received his Ph.D. in 1978. From 1978-79 he served as a National Institute of Mental Health-supported postdoctoral fellow in behavior genetics at the Jackson Laboratory. He came to NIA in 1980 as a Staff Fellow in the Laboratory of Behavioral Sciences and then moved to the MPGS in his current tenured position in 1985. His work has concerned development of behavioral assays of aging in rodents and recently in primates with focus on motor and memory performance as well as assessment of various pharmacologic, genetic, and nutritional interventions that impact beneficially on brain aging.

**Behavioral Neuroscience of Aging:** Aging occurs at multiple levels of biological organization. Behavior represents the integration of multiple aging processes that reflect the functional capacity of the organism. We have developed a battery of cognitive and motor tests to assess neurobiological mechanisms of age-related behavioral impairments in rodents and to evaluate interventions that purport to alter these impairments.

Regarding age-related decline in memory performance, we have focused on the cholinergic and glutamatergic systems and their interaction. For cholinergic interventions, we have collaborated with Dr. Nigel Greig to develop a novel class of cholinesterase inhibitors, that are long-acting, highly specific for acetylcholinesterase, with a wide range of therapeutic efficacy and low toxicity within this range. For glutamatergic interventions, we are examining manipulations of the glycine and polyamine sites on the N-methyl-D-aspartate (NMDA) glutamate receptor as well as generators of nitric oxide (NO) that is activated through the NMDA receptor. We have found that combinations of the glycine agonist, D-cycloserine, and the polyamine agonist, spermine, can act synergistically to improve learning performance. NO donors are also being assessed to overcome age-related learning impairments. In collaboration with Dr. Hideki Kametani, age-related changes in NMDA-stimulated NO release is being assessed using *in vivo* microdialysis.

Collaborating with Dr. Peter Mouton, we have also begun to examine the role of estrogen in preserving memory in a mouse model. In addition to the behavioral analysis, the latter project is part of a larger collaboration with Drs. Mouton and Mathias Jucker that involves quantitative morphometrics using unbiased stereology. Specifically, we are assessing age-related changes in the numbers of neurons, synapses, and glia, in the hippocampus of mice from different genders and strains including transgenics and knock-outs. The objective is to relate specific neuromorphometric parameters to age or treatment-induced changes in cognitive performance.

Regarding age-related motor impairment, we have focused on the loss of striatal dopamine $D_2$ receptors. Collaborating with Drs. George Roth, Hiroyuki Ikari, and Hiroyuki Umegaki, we have developed an adenoviral vector that can induce genetic transfer of the $D_2$ receptor into rat brain and produce functional changes mediated through this receptor. We are currently assessing the use of positron emission tomography (PET) to image vector-mediated production and decline of $D_2$ receptors in rat brain.

In collaboration with Drs. M.G. DiSimoni, D. Taub, P. Baskar and D. Longo, we are investigating the age-related increase in brain inflammatory response to elucidate its role in neurodegeneration. Inflammation has been strongly implicated in the pathophysiology of Alzheimer's disease, and the use of nonsteroidal anti-inflammatory drugs to treat this disease appears to have a strong potential. For our project, we are exploring age-related changes in the number of microglia and astroglia as well as glia-mediated alterations in cytokine production in response to injury. Although hippocampal microglia do not appear to increase with age, endotoxin induced release of microglia-produced cytokine, such as IL6 and TNFa do appear to increase with age. This project involves several techniques, including immunocytochemistry, polymerase chain reaction (PCR), glia culture, and quantitative morphometrics requiring unbiased stereology.

Thus, our research program applies a range of approaches from molecular biological techniques to behavioral analysis for examining possible mechanisms of age-related neurobiological changes that reduce functional capacity at advanced ages and for identifying possible treatments.

**Collaborators**: M.G. DiSimone, Ph.D., Mario Negri Insti. of Pharmacol., Italy; Hiroyuki Ikari, M.D., Ph.D., Hiroyuki Umegaki, M.D., Nagoya U. School of Medicine, Japan; Mathias Jucker, Ph.D., University of Basel, Switzerland; Hideki Kametani, Ph.D., Fukuoka Prefectural University, Japan; Edythe London, Ph.D., NIDA; Peter Mouton, Ph.D., Johns Hopkins U. School of Medicine; Dan Longo, M.D., Padmavathi Baskar, Ph.D., Joseph Rifkind, Ph.D., George Roth, Ph.D., Dennis Taub, Ph.D., William Wallace, Ph.D., NIA.


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calorie restriction
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**Recent Publications:**


Lane MA, et al. *Proc Natl Acad Sci* 1996; 93: 4159-4164.

Sell DR, et al. *Proc Natl Acad Sci* 1996; 93: 485-490.

Lane MA, et al. *J Clin Endocrinol Metab* 1997; 82(7): 2093-2096.

Verdery RB, et al. *Am J Physiol* 1997; 273: E714-E719.


**Biography:** Dr. Lane received his Ph.D. from the Pennsylvania State University in 1991 as a pre-doctoral NIA Training Fellow at the Penn State Gerontology Center. Dr. Lane came to the Gerontology Research Center, NIA as an IRTA postdoctoral fellow to pursue his interests in interventions targeting physiological aging. Following his postdoctoral training, Dr. Lane remained at NIA where he is currently a Senior Staff Fellow in the Laboratory of Cellular and Molecular Biology at the GRC. His work at the GRC has focused on aging and caloric restriction in nonhuman primates. Particular emphasis is placed on the effects of nutritional intervention on aging and age-related disease and development of primate models of human aging process, as well as studying the effects of this intervention on physiological aging and age-related diseases. An additional aspect of this research focuses on developing noninvasive biomarkers of aging that can be used to evaluate the effects of anti-aging therapies, including calorie restriction.

**Calorie Restriction in Primates:** Among gerontologists calorie restriction (CR) is widely recognized as the only intervention proven to consistently extend lifespan and maintain physiological function in many systems at more youthful levels. CR also delays the onset and slows the progression of many age-related diseases, including cancer. This nutritional intervention is among the most powerful and versatile experimental tools for the study of aging processes and age-related

diseases in experimental animal models, and possibly humans. The diverse beneficial effects of CR have been extensively documented in short-lived species including rats, mice, hamsters, spiders, flies, and fish. However, the effects of CR on longer-lived species more closely related to humans are not known. If it is shown the CR has beneficial effects in longer-lived species similar to those reported in rodents, the implications for human aging are significant.

With colleagues George Roth, Chief, and Donald Ingram, Research Psychologist, of the Molecular Physiology and Genetics Section, the main project of the laboratory involves studies of CR in long-lived nonhuman primates with an aging colony of nearly 200 rhesus and squirrel monkeys. Monkeys in several age groups representative of the species life span are being studied. Experimental groups are approximately equally divided between freely eating controls and monkeys receiving 30% less calories per day. The main hypothesis being tested is whether, as extensively reported in rodents and other short-lived species, CR will extend lifespan and slow aging in longer-lived species more closely related to humans. Another major focus of the laboratory is investigation of the biological mechanisms which underlies the anti-aging and anti-disease effects of CR.

Work in the laboratory initially focused on establishing a nonhuman primate model of CR. Since previous studies were limited to rodents and other short-lived species the safety and efficacy of this paradigm in long-lived mammals was not known. We have shown that caloric intake can be reduced by about 30% with no apparent adverse effects in monkeys. For example, CR monkeys do not exhibit any signs of increased stress or prolonged distress such as elevated blood pressure, lethargy, as loss of appetite. Further, we have not observed increased behavioral abnormalities in these monkeys, compared to controls. In establishing a CR model in monkeys we have shown that most primate physiological responses to this nutritional paradigm are in agreement with the extensive findings reported in rodents. Current research in the laboratory is focused in three main areas; elucidation of possible metabolic mechanisms of CR, amelioration of age-related diseases by CR, and development of primate biomarkers of aging.

**Metabolic Mechanisms of CR:** Even if CR is proven to extend lifespan in primates, it is unlikely that 30% reduction in caloric intake will become a widespread practice in humans. However, elucidation of underlying biological mechanisms of CR could make possible novel interventions with beneficial effects on aging and age-related diseases that are not dependent on reduced food intake. Studies in the laboratory related to possible mechanisms of CR utilize both monkey and rodent model

systems. We have demonstrated that reductions in metabolic rate, body temperature and glucoregulation are among the earliest changes to occur during CR. Ongoing studies of these metabolic adaptations involve several cohorts of young and old monkeys and focus on assessment of metabolic rate, body temperature, glucoregulation, and endocrine regulation of metabolism. Also, in conjunction with several collaborators, we are actively investigating oxidative stress, mitochondrial metabolism and damage, fat metabolism and genetic factors related to metabolism and physiological stress during short-term CR. Studies in rodent models are focused on the possible relationship of glucose and insulin metabolism to the underlying mechanism of CR. Pilot studies utilizing glucose analogues, which are incompletely metabolized by the body, suggest that it may be possible to "mimic" certain physiological responses to CR such as reduced body weight, body temperature, and fasting insulin levels without significantly reducing food intake. Future studies will involve assessment of the effect of glucose analogues on aging processes and lifespan and on the development of additional "CR mimetic" agents. One final line of investigation focuses on insulin signaling during CR. Recent studies in nematodes have suggested the possible relationship between regulation of lifespan in this species and genes homologous to components of the insulin-signaling pathway in mammals. Preliminary findings suggest that CR alters at least one of these mammalian genes in this pathway. Future work will focus on further investigation of this pathway during aging and CR.

**Amelioration of Age-Related Disease:** Recent work has focused on nutritional modulation of risk factors associated with several age-related diseases including diabetes, cardiovascular disease, menopause and osteoporosis. Our group and others have reported that CR lowers fasting glucose and insulin levels and increases insulin sensitivity, suggesting that this intervention may have beneficial effects in preventing diabetes. We recently reported that CR lowered serum triglycerides and increased the levels of a high density lipoprotein subfraction ($HDL2_b$) that is protective against cardiovascular disease in humans. More in-depth studies of both diabetes and cardiovascular disease are underway including investigation of the effect of CR on Syndrome X. This syndrome, a clustering of metabolic abnormalities such as hypertension, hypertriglyceridemia, and insulin resistance, is known to be associated with increased cardiovascular disease risk in humans.

Little is known regarding the effects of this nutritional intervention on osteoporosis or menopause. However, rodents on CR have lower bone density, but remain reproductively capable longer and do not exhibit significant bone loss in old age. In humans, reduced body weight and intake may be related to lower bone mass and altered reproductive cycling.

Current findings show that CR  does not lower peak bone mass and that bone density is slightly lowered at only one of several skeletal locations examined. Our findings also show that CR does not alter menstrual cycling or reproductive hormones and that markers of calcium metabolism and bone turnover are not disturbed  Ongoing studies will determine if CR alters the timing of menopause or the rapid acceleration of bone loss that occurs after menopause in humans. Future studies are also planned to focus on the relationship of body weight to bone density in this model by simulating increased biomechanical stress to compensate for the reduction in body weight seen in CR monkeys.

**Biomarkers of Aging**: Noninvasive biomarkers of aging are being developed to test whether or not the rate of aging has been altered in monkeys on CR. In addition to their utility in our CR studies, noninvasive markers of primate aging could be employed to evaluate a broad spectrum of anti-aging strategies in humans and other species. The recent popularity of anti-aging therapies, such as DHEAS and melatonin, underscores the need for objective criteria by which to evaluate the efficacy of proposed treatments related to aging processes. We have established a strategy for evaluating candidate markers and have identified several that may prove useful in a variety of species. These include serum markers such as dehydroepiandrosterone-sulfate (DHEAS) and pentosidine a collagen cross-link product measured in skin samples. Several other markers are currently under study. Recently, we have shown that CR slows the age-related decline in serum DHEAS levels and studies of pentosidine accumulation in rhesus monkeys on CR are underway.

**Collaborators:** Roy Verdery, M.D., Ph.D., University of Arizona; Joseph Kemnitz, Ph.D., University of Wisconsin Regional Primate Center; Richard Weindruch, Ph.D., University of Wisconsin; William Rumpler, Ph.D., and David Baer, Ph.D., USDA Human Nutrition Research Ctr. Beltsville; Byung P. Yu, University of Texas Health Science Ctr., San Antonio; Richard Feures, National Center for Toxicological Research; Eric Poehlman, University of Vermont.


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**Keywords**:
drug design
acetylcholinesterase
butyrylcholinesterase
Alzheimer's disease


**Recent publications:**


Yu QS, et al. *Med Chem Res* 1997; 7: 116-122.

Haroutunian V, et al. *Mol Brain Res* 1997; 46: 161-168.

Badio B, et al. *Biochem Pharmacol* 1997; 53: 671-676.

Wang Y, et al. *J Clin Invest* 1997; 99: 2883-2889.


**Biography:** Nigel Greig was trained as a pharmacologist with a background in chemistry and physiology and gained his Ph.D. from the University of London, England. Leaving the Cancer Chemotherapy Department of the Imperial Cancer Research Fund, London, he joined NIA in 1982. His initial studies focused on optimizing the delivery to and action of drugs within the brain. This resulted in the development of drug candidates for the treatment of brain tumors, and cancers of the breast, lymphatics and ovaries, as well as agents for the treatment of drug abuse and AIDS dementia complex, and technology for the delivery of neuropeptides, antisense and proteins to the brain. This work has evolved into his present interest, the design and development of drugs and diagnostics for the treatment of Alzheimer's disease.

**Design of Drugs and Diagnostics**: The goal of the Drug Design & Development program is to develop novel agents against rate-limiting steps involved in the pathophysiology of nervous system diseases, with particular interest in Alzheimer's disease (AD). Although the neuropathological quantification of beta-amyloid plaques and neurofibrillary tangles in the AD brain is the basis for confirming disease diagnosis after death, it is the neocortical synapses rather than the plaques and tangles that correlates best to psychometric indices of cognitive performance in AD. The loss of cholinergic synaptic markers in selected brain regions remains one of the earliest events leading to AD, with the cholinergic system being the most affected of the neurotransmitters and intimately involved in memory processing.

Our efforts have focused on augmenting the cholinergic system, but maintaining the normal temporal pattern of neurotransmitter release by selectively inhibiting the enzyme acetylcholinesterase (AChE), acetylcholine's degrading enzyme, in brain. Extensive studies involving chemistry, X-ray crystalography, biochemistry and pharmacology resulted in the development of "selective cholinesterase inhibition technology" (SCIT). This has provided us the basis for the development of novel drugs

to selectively and reversibly inhibit either AChE or butyrylcholinesterase (BChE), in either the brain or periphery for an optimal duration for the potential treatment of a variety of diseases, including AD and other dementias as well as myasthenia gravis and glaucoma.

The targeting of selective and site-directed drugs to specific enzymes rather than to receptors is a conceptually attractive method to optimize drug action. The formation of reversible drug-enzyme complexes allows selective enzyme inhibition over a long duration that is independent of the pharmacokinetic half-life of the drug. Once the drug has formed a slowly reversible drug/enzyme complex to inhibit its function, the presence of free drug is no longer required for continued action. In contrast, receptor stimulation requires the continued presence of drug, and its time-dependent maintenance at the target. The former method, targeted enzyme inhibition, enhances specificity and reduces toxicity, and has resulted in several novel compounds with dramatic sustained cognitive action from once daily dosing with wide therapeutic windows and minimal toxicity. For example, the drug, phenserine, a long-acting and brain-directed, selective AChE inhibitor has completed FDA required preclinical toxicological assessment and an IND is being prepared to support assessment of its clinical utility in patients with AD. Other novel agents from SCIT have demonstrated considerable activity in reducing beta-amyloid precursor protein levels, the source of the AD neurotoxin *ß* -amyloid, in both *in vitro* and *in vivo* studies, and likewise are being developed as potential therapeutics. Whereas yet other agents are being developed as potential imaging probes, to quantitate lowered AChE and elevated BChE levels associated with the AD brain, as early diagnostic tools.

Further studies are elucidating the mechanism by which nicotine protects neuronal cells from the toxicities associated with insults, such as from beta-amyloid and gp120. In this regard, novel subtype-selective nicotinic receptor channel modulators are being developed in collaborative studies with John Daly, Ph.D., NIDDK. Studies also are elucidating the mechanism by which HIV-infected immune cells cross the blood-brain barrier to gain access to and infect the brain, to characterize potential target for interventive therapy and treatment of AIDS dementia complex.

Among its many roles, BChE is a critical and rate-limiting enzyme in the metabolism of a number of drugs, including cocaine. In collaborative studies with Charles Schindler, Ph.D., and colleagues, at the National Institute on Drug Abuse (NIDA), we have demonstrated that we can increase the metabolism of cocaine, both *in vitro* and *in vivo*, by manipulating plasma BChE levels to increase its clearance and alter its

metabolic profile to favor less toxic metabolites. Furthermore, we can substantially reduce cocaine's psychomotor stimulatory action by exogenous BchE administration. Collaborative studies with Amy Newman, Ph.D., and colleagues, NIDA, are additionally elucidating mechanisms to reduce cocaine's euphoric actions by inhibiting its binding to the dopamine re-uptake transporter with novel tropane analogues, which, likewise, are being developed as potential therapeutics for the treatment of cocaine abuse.

Finally, collaborative studies with Josephine Egan, M.D., NIA, are being undertaken on type II diabetes, a disease that appears to be caused by a relative refractoriness of the insulin receptor to its ligand and a deficiency in its normal release. We seek to optimize the performance of pancreatic islet cells in elderly rodents with peptides that stimulate insulin release in the development of novel therapeutics for the treatment of type II diabetes.

**Collaborators:** Arnold Brossi, Ph.D., University of North Carolina, Chapel Hill, NC; Debomoy Lahiri, Ph.D., University of Indiana, IN; Vahram Haroutunian, Mount Sinai School of Medicine, NY; Marvin Hausman, M.D., Axonyx Inc., NY; John Daly, Ph.D., NIDDK, NIH; Amy Newman, Ph.D., and Charles Schindler, Ph.D., NIDA, NIH; Donald Ingram, Ph.D., and William Wallace, Ph.D., Molecular Physiology and Genetics Section, and Josephine Egan, M.D., Diabetes Unit, NIA, NIH.

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**Keywords:**
magnetic resonance imaging and spectroscopy
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cartilage
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**Publications:**

Horska A, et al. *Magnetic Resonance in Medicine* 1996; 36: 232-240.

Vittone J, et al. *Metabolism* 1997; 46: 89-96.

Spencer RGS, et al. *Am J Physiol* 1997, 272: H409-417.

Peterson E, et al. *Intl J Imag Sys Tech* 1997, Vol 8(3): 285-292.

**Biography:** Dr. Spencer obtained his Ph.D. in Medical Physics from the Massachusetts Institute of Technology (MIT) in 1987, and his M.D. from Harvard Medical School in 1988. He was a postdoctoral fellow at the Francis Bitter National Magnet Laboratory at MIT, and is a Diplomate of the American Board of Internal Medicine. Dr. Spencer joined the NIA in 1991, as Chief of the Nuclear Magnetic Resonance Unit.

**Nuclear Magnetic Resonance Unit**: The interests of the Nuclear Magnetic Resonance (NMR) Unit are primarily in imaging (NMRI) and metabolic studies of chondrocytes in culture and in cartilage, and spectroscopic studies of muscle metabolism under a variety of pharmacologic and physiologic conditions. Particular emphasis is placed on biological response modifiers and gene therapy interventions. Methodology development in magnetic resonance imaging and spectroscopy is also ongoing.

**A Bioreactor System for Magnetic Resonance Microimaging and Spectroscopy of Chondrocytes and Neocartilage (with Walter Horton, LBC):** Osteoarthritis is the leading cause of joint pathology in the older population. One possible approach to control this disease is the use of chondrocyte transplantation. Accordingly, we have begun a detailed exploration of cartilage growth and development in a hollow-fiber bioreactor specially designed for NMR studies. This system permits cells and the three-dimensional matrix that they elaborate to be studied longitudinally for several weeks in a non-invasive manner. Ultimately, we hope to define appropriate conditions for neocartilage development in osteoarthritic joints *in vivo*.

In cartilage developing from whole chick sterna, we have carried out detailed correlations between histology and NMR microimages. NMRI revealed the development of stromal layers between growth units of neocartilage centered about each hollow fiber. Density images show decreased mobile water content in these layers. Just outside the fiber

walls, we find high proton density with relatively low mobility. Mobility increases with distance from the hollow fibers within the growth units, corresponding to differences in cell size and density. In magnetization transfer contrast images, we find that the lowest $k_m$ values correspond to areas of high proteoglycan concentrations. These are prevalent in the mid-regions of the growth units. In contrast, the stromal layers and the regions around the fibers which are relatively proteoglycan-poor show the highest $k_m$ values, potentially indicating greater collagen-water interactions.

We are also using $^{31}P$ NMR to gain insight into metabolic adaptations as chondrocytes mature. We have been able to establish the presence of phosphocreatine in this system, and have demonstrated a decrease in intracellular pH during early development of the tissue. This is consistent with the known tendency for developing chondrocyte-cartilage systems to become increasingly dependent on anaerobic metabolism.

In addition, we are investigating the effects of biologic response modifiers on neocartilage development. Using NMRI, we have found that matrix proliferation from human articular chondrocytes is accelerated by addition of the combination of insulin-like growth factor-1 (IGF-1) and transforming growth factor-ß (TGF-ß), or the addition of the combination of IGF-1 and connective tissue growth factor, to the growth medium. Studies of the interactions of these growth factors and cytokines are ongoing.

**Angiogenesis in Rats as a Function of Age, and in Response to Gene Therapy (with Maurizio Capogrossi, LCS):** Atherosclerosis is a critical factor in the development of both peripheral vascular disease and cardiac ischemia. One approach to control the ischemic vascular disease is application of angiogenic factors delivered through genetically altered viral vectors. Therefore, we have utilized NMR spectroscopy (NMRS) methods to measure high-energy phosphate metabolites in muscle distal to experimental femoral artery resection in rats.

In our first series of experiments, we investigated angiogenesis as a function of animal age and days after femoral artery resection without addition of growth factor. NMR spectra of the gastrocnemius muscle of the anesthetized rat were collected at rest, during a period of intense muscle stimulation, and during recovery from stimulation. We have found that over a period of weeks following femoral artery resection, 2 month old rats recover muscle metabolic reserve significantly more rapidly than 20 month old rats. This likely reflects loss of angiogenic potential with age.

Modulators of angiogenesis have vast potential for treatment of arterial vascular disease. Accordingly, we have performed a set of experiments involving application of vascular endothelial growth factor (VEGF) just prior to femoral artery resection. Distal muscle bioenergetics was then assessed over a period of weeks. All NMRS measurements incorporated physiologic stress in order to probe vascular reserve. We found that VEGF acted to help normalize the pattern of high energy phosphate response to muscle stimulation and recovery, indicating an increase in the rate of development of perfusing vessels.

Extensions of this work which are underway include variations in the timing and other important elements of VEGF therapy delivery. We also plan to implement NMR imaging methods to more directly look at increased blood flow to the ischemic limb.

**Collaborators:** Eric McFarland, M.D., Ph.D., University of California at Santa Barbara; Walter Horton, Ph.D., Laboratory of Biological Chemistry, NIA, NIH; Maurizio Capogrossi, M.D., and Jerome Fleg, M.D., Laboratory of Cardiovascular Sciences, NIA, NIH; George Weiss, Ph.D., Division of Computer Resources and Technology, NIH; George Roth, Ph.D. and Donald Ingram, Ph.D., Laboratory of Cellular and Molecular Biology, NIA, NIH.

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**Keywords:**
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**Publications**:

Balagopalakrishna C, et al. *Biochemistry* 1996; 35(20): 6393-6398.

Hengemihle JM, et al. *Phys & Behavior* 1996; 59: 153-156.

Balogopalakrishna C, et al. *Oxygen Transport to Tissue* 1997; 118: 337-345.

Balogopalakrishna C, et al. *Mol Cell Biochem* 1997; 170: 85-89.

**Biography**: Dr. Joseph M. Rifkind received his Ph.D. in Physical Chemistry from Columbia University in 1966. He obtained postdoctoral training in protein chemistry at the University of Minnesota and joined the Gerontology Research Center of what was then part of National Institute of Child Health and Human Development in1968. He is a member of the American Chemical Society, the Biophysical Society, the American Association for the Advancement of Science, the Gerontological Society of America, the International EPR (ESR) Society, and the International Society on Oxygen Transport to Tissue.

**Molecular Dynamics Section**: The Molecular Dynamics Section under the direction of Joseph Rifkind is studying the role of oxygen in biological systems and how it influences the aging process. Our current focus is on the detrimental effects of oxyradicals produced in erythrocytes under hypoxic conditions. This program is being pursued simultaneously on three different levels.

1. We are studying the mechanism whereby oxyradicals are produced under hypoxic conditions. Using electron paramagnetic resonance combined with visible spectroscopy fluorescence spectroscopy and molecular dynamics simulations, we are studying the hemoglobin autoxidation process that produces oxyradicals. Enhanced protein fluctuations for partially oxygenated hemoglobin results in the nucleophilic displacement of oxygen as a superoxide. This superoxide formed in the heme pocket can (i) pick up an additional electron from nearby amino-acids producing protein radicals, (ii) react with the heme resulting in the formation of heme degradation products, or (iii) leak out of the globin.

2. We are studying how these processes produce cellular damage despite the presence of antioxidants and the enzyme systems designed to protect from oxidative stress. Under hypoxic conditions, there is an enhanced affinity of hemoglobin for the erythrocyte membrane. The superoxide that

is liberated from hemoglobin bound to the membrane is relatively inaccessible to cytoplasmic superoxide dismutase and ideally located to damage the red cell membrane. This hypothesis is supported by the formation of protein crosslinks and a decrease in red cell deformability when red cells are incubated under hypoxic conditions. An additional source for membrane damage is the accumulation of hydrophobic heme degradation products in the membrane. The hemoglobin membrane binding site is on the membrane band 3, which is also the anion channel, capable of transporting superoxide out of the red cell where it can damage lipoproteins and endothelial cells. We are studying these reactions and have found that red cells do induce oxidation of low density lipoproteins. These modified lipoproteins were shown to induce aortic smooth muscle cell proliferation, suggesting a possible relationship to the pathophysiology of the atherosclerotic process.

3. Impaired red cell deformability found to be induced under hypoxia is also associated with subject aging. We are very interested in understanding altered deformability in the aged erythocyte as well as other decrements in blood rheology. Our studies suggest a link with oxidative stress, which could originate in hypoxic induced oxyradical production. These changes can influence the ability of the organism to maintain an adequate supply of oxygen resulting in possible functional decrements. We are investigating the relationship between decrements in blood rheology and function using subjects from the Baltimore Longitudinal Study of Aging. We have, in collaboration with LPC, found some relationships between cognitive function and increases in mean corpuscular volume and other alterations in red cell properties, which should influence flow through the microcirculation. We are also, with the LSB, investigating the relationship between blood flow in the brain and our hemorheological measurements. Studies are also being initiated with LCS to determine the effect of exercise on changes in blood rheology.

**Collaborators:** P.T. Manoharan, Indian Institute of Technology, Madras, India; Avraham Mayevsky, Bar Ilan University, Israel; Victor McDonald, Walter Reed Army Institute of Research; David Danon, Weissman Institute, Rechovot, Israel; Paul Costa, Laboratory of Personality and Cognition, NIA; Jerome Fleg, Laboratory of Cardiovascular Science, NIA; Jeffrey Metter, Longitudinal Studies Section, LCI, NIA.

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# Laboratory of Clinical Investigation

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The Laboratory of Clinical Investigation (LCI) chiefly focuses on clinical research issues of importance in gerontology. Much of the clinical work deals with volunteers on the Baltimore Longitudinal Study of Aging (BLSA), but additional clinical research protocols are also ongoing.

The **Applied Physiology Section** (APS) is concerned primarily with the physiology and pathophysiology that links normal changes in aging with two of the most common diseases of the elderly, osteoporosis and osteoarthritis. These diseases are among the most common causes of morbidity in the elderly population and their costs, both economic and personal, have been well documented. The effort in osteoporosis research on the BLSA volunteers pointed out the need for studying women who will be going through the menopause, and following the changes in bone mineral, hormones, lipids, and body composition that they will undergo as they traverse the menopause. This led to the third major effort in the section with the collaboration of our colleagues, especially in the Endocrinology Section and the Longitudinal Studies Section, the Perimenopausal Initiative. An additional effort that involves both osteoporosis and osteoarthritis is the cross-cultural investigation of these diseases in genetically, geographically, and culturally diverse populations, and comparing results around the world with those of the BLSA volunteers. In all these efforts we have used an epidemiological approach to identify risk factors associated with the diseases, biological measurements to ascertain the importance of hormones and biomarkers in the pathophysiology of these processes, physiological measurements to determine correlates with these processes, and genetic epidemiology to measure the familial components. Through the better understanding of the normal and pathological processes involved, we would hope to be able to develop a strategy for disease prevention and optimization of function in the elderly.

The **Diabetes Section** (DS) focuses on improving present methods for treating type 2 diabetic patients. Diabetes mellitus is one of the most prevalent diseases among the elderly. Approximately 40% of all adults

over the age of 65 have diabetes or elevated fasting glucose. Diabetes is also a comorbid condition in other conditions of the elderly, especially cardiovascular disease. By definition, diabetes mellitus is a group of metabolic diseases characterized by high blood sugar resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes is characterized by both defects. It is generally accepted that it is the elevated sugar which leads to the complications of diabetes. Therefore, we in the Diabetes Section feel that our endeavors should be directed towards improving insulin secretion or restoring insulin action. Despite the fact that 3 new agents have become available in the past eighteen months to treat type 2 diabetes, they have proven less than adequate at normalizing blood sugars.

The **Endocrinology Section** (ES) conducts and facilitates (by collaboration with other intramural and extramural entities) research aimed at understanding the particulars of changes in regulation of hormones during the normal aging process. It explores the relationships of hormone secretion to states of nutrition and health and the interrelationships among various hormone axes during aging. ES elucidates the influence of alterations of endogenous hormone activity on risk factors for susceptibility to chronic diseases associated with aging. Current efforts focus on changes in the growth hormone and reproductive hormone (sex steroids) axes. Finally, the ES conducts research investigating the clinical utility and risk/benefit ratios of rationally selected hormone replacement interventions, designed to reverse documented age-related alterations of hormone balance.

The **Longitudinal Studies Section** (LSS) has a twofold mission. The first is to manage the operations of the Baltimore Longitudinal Study of Aging (BLSA), a multidisciplinary longitudinal study of human aging. Research on aging using this open panel of research volunteers is performed by scientists based in several NIA intramural research laboratories and numerous outside collaborators. The second is to perform research with the BLSA using both existing data and data from newly initiated projects.

**BLSA Operations:** LSS staff schedules and manages the activities of the men and women research volunteers during their biannual two and half-day visits during which time the volunteers participate in numerous research studies. LSS staff conducts the clinical evaluations that establish health status of all active participants on every visit. The results are used in many investigations and also are used to determine the safety of research procedures for various participants. The results of the clinical evaluations are given to participants and to their physicians if requested by the participant.

Between visits, LSS staff maintain communication with participants, provide information about the findings of the study to participants both individually and by means of a periodic participant newsletter. They also maintain periodic contact with those who either are unable or unwilling to come in for regularly scheduled visits. LSS staff manages the recruitment of new research volunteers from a large group of applicants on a waiting list. LSS staff employs numerous mechanisms to learn about deaths in the study sample, obtain information about deceased BLSA participants and manage the autopsy program.

**BLSA Research:** LSS was given the responsibility to analyze, report and recommend continuing, changing or stopping a number of existing research projects without active investigators. Most had been started in the 1960s or 1970s and had either been recently discontinued or were ongoing. Project areas for which longitudinal analyses and reports were completed included: pulmonary function; hearing and vision, reaction time, reciprocal movement speed, nerve conduction velocity, power and strength measurements, self-reported participation in physical activities, blood pressure, and a variety of studies using clinical data.

New studies were initiated in the areas of prostate aging and disease, neuromuscular changes with age, hearing, physical functioning and disability and age differences in the dynamics of cerebral blood flow. All were designed to take advantage of the unique BLSA longitudinal database and all required the development of research teams from other laboratories and outside collaborators.

LSS staff developed a number of statistical approaches that facilitated the analysis of longitudinal data and have applied these approaches to a number of historical data sets in the BLSA.

The **Metabolism Section** (MS) has played a critical role in evaluating diagnostic standards and in determining whether an adjustment for age is appropriate. In two areas, diabetes and obesity, the standards in general use to define these diseases have not been age-adjusted during the adult years of life. The primary technique used to establish standards has been the relationship between levels (fasting glucose and glucose tolerance for diabetes and the Body Mass Index for obesity) and the subsequent development of complications that are strongly related to the diseases. The BLSA and the Follow-up Study of the National Health and Nutrition Examination Survey-I have provided unparalleled data sources for this effort. In both areas, the analyses suggest that adjustment of standards for age is required. In further studies in collaboration with other intramural and extramural scientists, factors influencing glucose/insulin homoeostatic mechanisms and quantification of the obese state are under study.

## Laboratory of Clinical Investigation Staff

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**Keywords:**
osteoporosis
osteoarthritis
menopause
body composition

**Recent Publications:**

Hirsch R, et al. *Ann Rheum Dis* 1996; 55: 25-29.

Muller DC, et al. *Aging Clin Exp Res* 1996; 8: 13-21.

Martin K, et al. *J Rheumatol* 1997; 24: 702-707.

Lethbridge-Cejku M, et al. *J Rheumatol* 1996; 23: 1943-1947.

**Biography:** Dr. Tobin received an A.B. from Columbia College in 1959 and an M.D. from New York University School of Medicine in 1963. He trained in Internal Medicine at Bellevue Hospital and in Diabetes at NIH with Dr. Andres from 1965-1967, and as an NIH Special Fellow at the EP Joslin Research Labs from 1967-1969. In 1969, he returned to the NIH to the Metabolism Section of the Laboratory of Clinical Physiology of National Institute of Child Health and Human Development (NICHD), later NIA. In 1981, he became Chief of the Human Performance Section and in 1986, Chief of the Applied Physiology Section of the Laboratory of Clinical Physiology. He is a past Chair of the Clinical Medicine Section and past President of the Gerontological Society of America.

The Applied Physiology Section (APS) is primarily concerned with the physiology and pathophysiology that links normal changes seen with aging with two of the most common diseases of the elderly, osteoporosis and osteoarthritis. These diseases are among the top causes of morbidity in the elderly population and their costs, both economic and personal, have been well documented. The effort in osteoporosis research on the BLSA volunteers pointed out the need for studying those women who will be going through the menopause, and following the changes in bone mineral, hormones, lipids, and body composition that they will undergo as they traverse the menopause. This led to the third major effort in the section with the collaboration of our colleagues, the Perimenopausal Initiative. In all these efforts, we have used an epidemiological approach to identify risk factors associated with the diseases, biological measurements to ascertain the importance of hormones and biomarkers in the pathophysiology of these processes, physiological measurements to determine correlates with these processes, and genetic epidemiology to measure the familial components. Through the better understanding of the normal and pathological processes involved we would hope to be able to develop a strategy for disease prevention and optimization of function in the elderly.

**Osteoporosis:** The APS effort in osteoporosis research includes studies on the BLSA volunteers, as well as specific populations. These include sunlight deficient elderly, and in collaboration with the Endocrine section, studies on elderly volunteers with low sex hormones and IGF-I who are part of a double-blind study of sex and growth hormone treatment. Our approach is multifactorial, with the assessment of bone mineral density at multiple sites being the central parameter, and physiological (i.e., strength, obesity, metabolism), biochemical (ionized calcium and markers of bone turnover), hormonal (PTH), and vitamin (Vitamin D and D3) parameters measured to relate to bone status and rates of bone change. The recognition that bone loss occurs in males as well as in females is an important aspect of this work, and the potential for increased morbidity from hip fractures in males becoming more important as more men live to an age at which hip fracture (the incidence rate is approximately half that in women) is more common. The understanding of the processes responsible for the age-associated bone loss is important for differentiating this loss from the disease osteoporosis if one is to be able to suggest preventive and treatment strategies. Future work includes the longitudinal assessment of biochemical and hormonal changes now that a sufficient number of volunteers have had repeat visits, as well as the longitudinal assessment of rates of change in bone density. These are both extensions of the initial cross-sectional work.

**Osteoarthritis:** The research on osteoarthritis (OA) in the BLSA started with an epidemiological description of the prevalence of hand OA in the original male cohort, and was expanded to include a longitudinal study of the development of hand OA in males and females, as well as the development of an axis of radiographic changes of individual features of the disease. Recent work has focused on the epidemiology of knee OA, as well as the development of an atlas of radiographic knee changes, the familial association of OA, the association with hand OA (polyao), its relationship to symptoms of pain, and biochemical and hormonal, physiological, and life style risk factors associated with abnormal xrays. Genetic studies of OA have included both epidemiological and molecular approaches. The familial association of OA of the hand and polyarticular OA was demonstrated in the family members of the BLSA with clinically relevant correlations between brothers, sisters, and sister-brother pairs for OA in each of the hand joints, in multiple joint site OA in the hands, and in polyarticular OA (including the knee joint). On a molecular level, we examined the association between alleles of the polymorphic gene controlling aggrecan protein, one of the most common proteins associated with cartilage, and bilateral hand OA. Men who have one of the most common alleles of this aggrecan gene have a five-fold greater likelihood of having moderate to severe hand osteoarthritis in multiple joints on both hands then men who do not.

**Perimenopause:** On the average, a woman in this country experiencing the menopause still has more than one-third of her life ahead of her. The development of cardiovascular disease, the leading cause of death in U.S. women, and osteoporosis and musculoskeletal impairment, common conditions that predispose to frailty in older women, is accelerated in the menopausal state. Cross-sectional studies suggest that the menopausal transition is associated with changes in the endocrine-metabolic milieu, body composition, uterine function, cardiovascular risk profile, and psychosocial well being. In 1993, the BLSA initiated a study of the perimenopause by starting to recruit a cohort of 100 White and 100 African-American women 45-55 years old. All recruits are healthy, nonsmoking women who are experiencing monthly menses at enrollment and who do not receive hormonal therapies. In addition to the bi-annual inpatient BLSA visits, these women receive quarterly outpatient visits until menses have ceased for 2 years, or hormone replacement is begun. These visits include a menopausal symptom questionnaire, endocrine profiles, anthropometry, dual energy x-ray absorptiometry, bone biochemistries, and psychosocial assessments. To date, 91 White women and 10 African-American women have been enrolled in the BLSA and have been followed from 3 to 45 months. Results on 21 women seen at least 3 times who were clearly premenopausal, showed no significant changes in the percent of their total body that was fat, their waist circumference or their waist-hip ratio. In contrast, the 24 women who could be characterized as perimenopausal, just starting the transition to menopause, demonstrated significant increases in the percent of their weight that was fat, increases in their waist circumference, and increases in their waist-hip ratio. The premenopausal women also had significant increases in the bone mineral density of the lumber spine, while those who were perimenopausal had significant bone loss, with a significant difference between the two groups. These differences indicate that changes in body composition and bone mass start before the cessation of menses, and that the early identification of women who will undergo these changes is possible.

**Collaborators:** M.C. Hochberg, M.D., M. Lethbridge-Cejku, Ph.D, and K. Martin, M.D., University of Maryland; M. Bellantoni, M.D., M. Blackman, M.D., and W.W. Scott, Jr., M.D., Johns Hopkins University; W. Horton, Ph.D, S.M. Harman M.D., R. Andres, M.D., D. Elahi, Ph.D., P. Costa, Ph.D, R. Hirsch M.D., NIH.

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**Keywords:**
GLP-1
Exendin-4
insulin
islets of Langerhans

**Recent Publications:**

Wang Y, et al. *J Clin Invest* 1997; 99: 2883-2889.

Wang Y, et al. *Mol Cell Endocrinol* 1996; 116: 81-87.

Perfetti R, et al. *J Gerontol: Biological Sciences* 1996; 51: B331-B336.

Perfetti R, et al. *Am J Physiol* 1995; 269: B983-B990.

**Biography:** Dr. Josephine Egan received her M.D. from University College in Galway, Ireland in 1978. She is a board certified endocrinologist who received her endocrine training at the University of Virginia, Charlottesville. She has been at the GRC since July, 1990 and on the tenure track since July, 1994. Her early work from her fellowship related to investigating and quantitating insulin release from individual beta cells in the islets of Langerhans. Using this methodology, she outlined the abnormalities that occur in the aging beta cells of rats. More recently she has been working on ways to reverse these abnormalities and on ways to increase insulin secretion in Type II diabetes mellitus.

**Aging and Type II Diabetes**: The goal is to design new drugs to restore glucose sensitivity to the beta cells in Type II diabetes and to prevent deterioration of the beta cells which seems an inevitable occurrence in aging. The general strategy is to outline the abnormalities that occur in aging and Type II diabetes in beta cells and the search for agents that can alter these processes. The approach is to take the agents that have been first tested in beta cell lines into animal models of aging and diabetes, and with the information gained from the animal models, go as quickly as possible directly into the human situation.

Type II diabetes develops, for the most part, because insulin becomes less effective at its target tissues with increasing age, adiposity and changing lifestyle. This puts increased demand on the beta cells of the pancreas which then must supply more insulin. When supply cannot keep up with demand, blood sugars rise which then lead to complications such as blindness, nephropathy and neuropathy as a direct result of the elevated blood sugars. With increasing age, beta cells respond less well to glucose stimulus. They also do not replicate at the same rate as beta cells in younger animals. Thus, in principle, we need to find agents that would restore glucose responsivity to the beta cells and that would prevent the decrease in replication that occurs in aging mammals.

**Design of Drugs of Potential Use in Type II Diabetes**: We have been concentrating on a group of peptides known as incretins. They are released from the gut in response to food and they augment the insulin response to glucose. One of these peptides, GLP-1, is effective at increasing insulin release when given systemically even in long-standing Type II diabetes. It also appears to be a trophic agent to the pancreas in pharmacological doses. This is a major difference from other agents that are presently used to treat diabetes as studies show that even with good control of blood sugars, there is an inexorable decline in beta cell function. GLP-1 has a short half-life and consequently has to be given at least three times a day subcutaneously to maintain high insulin levels in the blood. We are presently working with a peptide called Exendin-4 which is secreted in the saliva of the Gila monster (a lizard) and which is 53% homologous to human GLP-1. It also is very effective at inducing insulin release and, of great significance, when given subcutaneously or intraperitoneally, it has a much longer biological action than GLP-1. We are presently involved in animal testing of this compound and hope to be involved in the human testing. We are also testing Exendin-4 that has been "humanized" i.e. we are replacing the amino acids of Exendin-4 with those of GLP-1 and hope to find out where the crucial amino acids that are responsible for the prolonged biological activity of Exendin-4 lie. Current efforts show that GLP-1 is a true growth factor for beta cells in the pancreas and perhaps is involved in cell differentiation in other organs besides pancreas.

**Collaborators:** Drs. Joel Habener, Doris Stoffers and Dariush Elahi, Massachusetts General Hospital; Drs. Seamus Shreenan and Anthony Pick, University of Chicago Medical School; Drs. Marie Byrne and Burkgard Goke, Marlburg, Germany; Dr. Nigel Greig, Laboratory of Cellular and Molecular Biology, NIA; Dr. Andrej Janczewski, Laboratory of Cardiovascular Sciences, NIA; Dr. Andrew Young, Amylin Pharmaceuticals, San Diego.


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**Keywords:**
insulin
receptors
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insulin resistance


**Recent Publications:**


Kole HK, et al. *J Biol Chem* 1996; 271: 14302-14307.

Kole HK, et al. *J Biol Chem* 1996; 271: 31619-31626.

Perfetti R, et al. *Endocrinology* 1997; 138: 1829-1835.


**Biography:** Dr. Bernier received his Ph.D. from the University of Montreal, Canada, in 1983, and completed two postdoctoral fellowships. The first one was held in Unit 162 of the National Institutes of Health and Medical Research (INSERM) in Lyon, France, and the second one at the Johns Hopkins University School of Medicine in Baltimore. He was an assistant professor of Biochemistry at McGill University in Canada before coming to the NIA in 1990. His research interest concerns molecular aspects of insulin receptor signal transduction. He is a member of the American Diabetes Association.

**Molecular Aspects of Insulin Receptor Signaling**: Diabetes mellitus is one of the most prevalent illnesses among the elderly and is a comorbid condition in other diseases of the elderly. It is the hyperglycemia per se that leads to most of the complications of diabetes. The cause of diabetes mellitus is a diminished ability of the beta cells of the pancreas to release insulin in response to blood glucose, and a decreased insulin response at the target tissues. Treatments presently available to lower blood glucose are less than adequate. One goal of the Diabetes Section is to improve our understanding of factors regulating insulin action at the target cells in order to provide new insight into effective treatments for this and other metabolic-related diseases, including obesity.

There is a large body of evidence supporting the concept of glucose toxicity, in which hyperglycemia causes the deterioration of insulin secretion from the pancreas and insulin action in peripheral tissues (e.g., adipose, heart and skeletal muscle). This resistance to insulin action has been linked to an increase in glucose metabolism through the hexosamine biosynthetic pathway. Because the effect of high concentrations of glucose and glucose metabolites on the expression of genes important for regulation of adipose cell functions remains largely unknown, we have undertaken a study to look at the regulation of the transcription factor CCAAT/enhancer-binding protein (C/EBP) alpha by glucose and glucosamine, a product of the hexosamine pathway, in a cultured adipose

cell line that is known for its high responsiveness to insulin. This transcription factor has been implicated in the establishment and maintenance of energy homeostasis in adipocytes by controlling the expression of several gene products includeing the insulin-responsive glucose transporter GLUT4, lipid-synthesizing enzymes, and leptin. Our initial results indicate that the decrease in transcription and mRNA accumulation of the C/EBP-alpha gene caused by high concentrations of glucose or glucosamine is accompanied by a proportional reduction in the expression of the GLUT4 gene. We have also used a cell line that stably expresses a portion of the promoter for C/EBP-alpha gene fused to a reporter gene and demonstrated that promoter activity was decreased by these treatments. It is now recognized that the adipose tissue plays major endocrine roles in addition to its function as an energy storage depot. We are carrying out a study aimed at evaluating the effect of hyperglycemia or glucosamine on known molecular regulators of adipocyte metabolic and endocrine functions in rats. Progress in our understanding of factors regulating obesity-related insulin adipose cell physiology will allow the development of effective interventions for resistance, a major contributor to morbidity and mortality in the U.S. and other Western societies.

Despite close similarities in the structure of their receptors, insulin and insulin-like growth factor 1 (IGF-1) have different physiological functions. Differential interaction of the receptor intracellular domains with effector proteins may provide one mechanism by which insulin and IGF-1 signaling diverges. We have found that a discrete non-catalytic region of the carboxyl-terminal domain of the insulin receptor appears to contribute to the specific enhancement of insulin receptor activity, while having no effect on IGF-1 receptor function. Current efforts are concentrated on the characterization of the functional importance of this region of the insulin receptor in receptor signaling by using a minigene approach. The ability of this stably expressed receptor domain to enhance selectively insulin responsiveness with respect to receptor activation and function toward signaling intermediates and DNA synthesis will be evaluated. Another goal of this project is to further define the specific nature of insulin signaling regarding programmed cell death (apoptosis). Apoptosis is a natural phenomenon that plays a major role in normal turnover of cells. Insulin has been shown previously to rescue cells from apoptotic death. However, the concentrations of insulin used in these studies were supraphysiologic and could have exerted protection by activating the IGF-1 receptor, whose role as a survival factor has been well documented. Our preliminary experiments indicate that insulin possesses antiapoptotic properties but utilizes a signaling pathway that differs from that of IGF-1. This finding strengthens the notion that divergence in the signal cascade between these two hormones could originate from distinct

intrinsic properties of each receptor. A better understanding of the nature of these properties may represent a target for the development of selective receptor activator drugs. Protein tyrosine phosphatases (PTPases) have been found to dephosphorylate key tyrosyl residues from the insulin receptor kinase domain, thereby causing an inactivation of the receptor kinase activity and insulin action. Thus, PTPases may oppose tyrosine kinase-mediated insulin signaling and contribute to insulin resistance. Indeed, altered PTPase activity has been noted in different tissues from diabetic rats and in humans. Therefore, the development of PTPase inhibitors that can selectively block insulin receptor dephosphorylation might have therapeutic value. Our previous work has shown that a tris-sulfotyrosyl-containing peptide, 3S-peptide-I, whose primary sequence is identical to that of the kinase domain of the insulin receptor, exerts a potent inhibition against members of the PTPase family. In cultured cells that were semi-permeabilized, the addition of 3S-peptide-I was found to greatly reduce tyrosine dephosphorylation of preactivated insulin receptors, but not that of EGF receptors. We have also modified 3S-peptide-I by incorporating a stearyl moiety at the N-terminus of the peptide. This derivative has been shown to enhance insulin-stimulated receptor activation and signal transduction in intact cells. In contrast, ligand-stimulated EGF receptor functions were not affected by stearyl-3S-peptide-I. Thus, it appears that this peptide selectively enhances insulin signal transduction by specifically inhibiting dephosphorylation of the insulin receptor in intact cells. This result indicates that 3S-peptide-I or a derivative thereof may be a valuable tool in the identification, purification and characterization of PTPase(s) acting on the insulin receptor. Moreover, the administration of this or other related compounds in animal models of obesity and Type II diabetes would provide some insight into whether improvement of glucose homeostasis can be obtained. This research effort may help develop new agents that have potential therapeutic value for the treatment of diabetes.

**Collaborators:** Jeremy Tavare, Ph.D., University of Bristol, UK; Lance Macaulay, Ph.D., CSIRO Division of Biomolecular Engineering, Australia; M. Daniel Lane, Ph.D., Johns Hopkins University, Baltimore.

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**Recent Publications:**

Bellantoni MF, et al. *J Clin Endocrinol Metab* 1996; 81: 2848-2853.

Dobs AS, et al. *J Clin Endocrinol Metab* 1996; 81: 4108-4112.

Vittone J, et al. *Metabolism* 1997; 46: 89-96.

**Biography:** Dr. Harman is a 1970 graduate of the M.D., Ph.D. program at the State University of New York Health Sciences Center at Brooklyn. He trained in Internal Medicine at the Yale-New Haven Hospital, and in Endocrinology at National Institute of Child Health and Human Development (NICHD) as a Clinical Associate in the laboratory of Dr. Griff T. Ross. Dr. Harman is board certified in Internal Medicine and Endocrinology. He joined the Endocrinology Section, Laboratory of Clinical Physiology in 1974 (now Laboratory of Clinical Investigation), where he and his colleagues have helped elucidate the normal changes occurring with age in reproductive, growth, thyroid, and adrenal hormones and conducted investigations of hormone replacement in the elderly.

**Changes in Hormone Regulation with Aging and Utility of Hormone Replacement Interventions:** Research in the Endocrinology Section (ES) has documented alterations in hormone balance during the normal aging process by measuring changes in dynamic hormone secretory patterns in women and men using sensitive, state of the art methods. This work also explores the relationships of hormone secretion to states of nutrition and health and interrelationships among various hormones. Studies also elucidate the influence of alterations of endogenous hormone activity on risk factors for age-related chronic diseases. Finally, the ES conducts research on the clinical utility and risk/benefit ratios of hormone replacement interventions designed to reverse age-related alterations of hormone balance. The ES has maintained a close and consistent collaborative interaction with senior investigators at the Johns Hopkins University School of Medicine (Dr. Marc R. Blackman and Dr. Michele F. Bellantoni).

With aging, there are alterations in hormone secretion and in body composition. Loss of muscle mass may lead to reduced strength and functional capacity. Increased central fat may be associated with deterioration in lipid profiles and glucose tolerance (risk factors for heart disease). Aging is also associated with reductions in cardiac function and

fitness, immune function, and thinning of skin, all of which may have hormonal components. Decreases with age in the sex steroid hormones, testosterone (T) in men and estradiol ($E_2$) in women appear to play a role in the changes in body composition that occur with aging. Pituitary growth hormone (GH) decreases percent body fat and works via the intermediate hormone, insulin-like growth factor-I (IGF-I), to maintain calcium and nitrogen balance, and increase bone and muscle mass. Cortisol, the major steroid secreted by the adrenal cortex generally opposes the actions of GH and may increase slightly with age. Our work has demonstrated retention of a GH response to GH releasing hormone (GHRH), the brain peptide that stimulates GH secretion, an intact response of IGF-I to GH, and, more recently, improved muscle strength and lipoprotein patterns with no apparent changes in body composition, blood pressure, or glucose tolerance in men over 65 years of age treated with GHRH. Currently work examines the effects of hormone replacement for 26 weeks in healthy women and men >65 years old. Volunteers are randomized to treatment with GH, sex-appropriate steroid hormones, both GH and sex steroid, or placebos only and studied intensively at baseline and at 26 weeks. Studies include overnight blood sampling for GH, cortisol, and LH secretory profiles assessment of thyroid and reproductive hormones and leptin. Every 4 weeks, we measure glucose, CBC, and IGF-I, and serum T (men) or serum $E_2$ (women). Muscle-related endpoints include strength, muscle mass by magnetic resonance imaging (MRI), and muscle biopsies (for histology, histochemistry, and molecular responses). Additional endpoints include body composition defined by multiple procedures, whole body protein synthesis by $^{13}C$-leucine uptakes, cardiovascular function and anatomy, and vascular reactivity. Bone metabolism is assessed from biochemical measures. Metabolic measurements include lipid profiles and glucose and insulin during an oral glucose tolerance test. Subsidiary studies examine immune function at baseline and in response to immunization and psychological function and quality of life. Because this large study remains in progress (100 subjects enrolled to date), treatment groups are still masked. Thus, analyses have been restricted to exploring relationships among variables at baseline. Mathematical analysis (deconvolution) of overnight secretory profiles reveals that secretion of cortisol appears to be directly proportional to secretion of GH. Because cortisol acts to break down lean tissue and bone and GH to build them up, the observation that their secretory rates are linked suggests the presence of a protective compensatory mechanism in the elderly to keep these opposing hormone influences in balance. We also find that plasma levels of leptin, the fat cell hormone that inhibits appetite, is directly and independently related to adiposity (% body fat) rather than to age, sex or levels of other hormones. Thus, leptin may serve as a biomarker of total adiposity in elderly, as well as young women and men.

The ES also participates as an active contributor to ongoing studies of estrogen replacement therapy in women, longitudinal assessments of the physiology of the perimenopause, and longitudinal studies of testosterone and other steroid hormones in aging men and their relationship to prostate disease. These investigations are carried out in collaboration with other intramural investigators (Dr. Metter, and Dr. Tobin, LCI) and with extramural investigators (Dr. Blackman, Medicine, JHU and Dr. Bellantoni, Geriatric Medicine, JHU).

Future research will examine the effects of augmenting GH secretion with GH releasing peptide (GHRP), a secretagogue which produces a more physiologic pattern of GH secretion than does GH treatment. Studies will examine the interaction of GHRP and sex steroids on bone in women and men with osteoporosis and the responses of normal and failing hearts in older patients to GHRP intervention. A collaborative study with the Laboratory of Clinical Immunology examining effects of DHEA (dehydroepiandrosterone) on T- and B-cell responses to immunization in the elderly is also planned.

**Collaborators:** Marc R. Blackman, M.D.; Michele F. Bellantoni, M.D., Jocelyn Jayme, M.D., Johns Hopkins University; Kieran O'Connor, M.D., Endocrinology Section, LCI, NIA; Jordan Tobin, M.D., Applied Physiology Section, LCI, NIA; Jeffrey Metter, M.D., Longitudinal Studies Section, LCI, NIA; Lawrence Jacobs, M.D., University of Rochester School of Medicine.

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**Keywords:**
aging
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**Recent Publications:**

Deeg DDH, et al. *Handbook of the Psychology of Aging* 1996; 129-149.

Metter EJ, et al. *J Gerontol: Biol. Sci* 1997; 52: B267-B276.

Carter HB, et al. *JAMA* 1997; 277: 1456-1460.

Morrell C, et al. *J Acoust Soc Am* 1996; 100: 1949-1967.

**Biography:** James L. Fozard received his Ph.D. in Experimental Psychology from Lehigh University in 1961 and completed a two year postdoctoral fellowship at the Massachusetts Institute of Technology in 1966. From 1967 to 1978 he was a research psychologist with the Department of Veterans Affairs Normative Aging Study, Co-director of the Boston Division of the Boston/Bedford Geriatric Research, Educational and Clinical Center (1976-1978) and a member of the Psychiatry Department Faculty at Harvard Medical School (1968-1979). From 1978 to 1985, he was the Director of the Patient Treatment Service in the Office of Geriatrics and Extended Care, Department of Veterans Affairs. He joined the NIA in 1985 as Head of the Baltimore Longitudinal Study of Aging.

**Prostate Aging and Disease:** This BLSA project has a retrospective arm and a prospective arm that is scheduled to run through 2003.The goal of the project is to characterize normal aging in the prostate and to identify transitions to prostate disease, particularly benign prostatic hyperplasia (BPH) and prostate cancer. In addition to evaluating hypotheses about the natural history of prostate aging and disease, the goal of the research is to use information about structure and function of the prostate for early detection of prostate disease. Clinical evaluations of prostate growth and function have been made in over 800 men with and without prostate disease and the availability of stored sera and genetic material permits analysis of known and newly developing risk factors. The prospective arm of the study consists of the men currently in the BLSA plus those ranging in age from 30 to 79 who enter the BLSA through 2003.

Baseline demographic information includes age, race, and information on variations in male hormone levels. Longitudinal data on male hormone levels is obtained from stored sera. In FY1997, all usable data on several male hormones has been placed into a computer-based file and initial analyses have begun. Sera from the prospective part of the study are frozen and stored and will be assayed as funds allow.

Prostate growth information in the retrospective part of the study comes from serial analyses of changes in serum levels of prostate specific antigen (PSA) and clinical evaluations including digital rectal examinations (DREs). In the prospective part of the study growth is also being assessed with magnetic resonance imaging (MRIs). To date, the major accomplishments have come from analyses of PSA levels which show that PSA increases linearly a bit faster over a period of years in men who develop BPH than in those who do not. The rate of change is still greater in men who develop prostate cancer, and the increases go up exponentially 5-7 years before diagnosis. Papers published in FY 1997 further show that when the ratio of free to total PSA is computed, the ability to distinguish changes in PSA levels between men who develop prostate cancer and those who do not increases from 4-5 years to about 10 years prior to diagnosis. Analyses of a subset of the men who developed prostate cancer show that the ratio is lower in men who have clinically defined aggressive tumors. Thus, rate of PSA increase (>0.75 units/year) identifies those likely to have prostate cancer and a low percentage of free PSA identifies those with aggressive disease whose cancer needs aggressive treatment.

Alterations in prostate structure or function are studied in relation to the possible development of prostate disease, particularly BPH. Currently, the MRI data are being analyzed to estimate prostate volume as well as the percentage of epithelial and stromal tissue. Symptoms associated with BPH are assessed with the standard AUA symptom questionnaire and with measures of urine flow and post-void residuals. Current analyses of cross sectional data indicate that, as expected, flow rate decreases with older age and that the distribution of positive responses to questionnaire queries about urinary symptoms increases. Except for limited symptom questionnaire data from the retrospective arm of the study, all of these measurements were initiated in the prospective arm of the study.

Diagnosis and tracking of prostate disease is based on current clinical practice. In the prospective arm of the study, participants who require further clinical evaluation and prostate biopsy are offered that evaluation free of charge at the Johns Hopkins Department of Urology.
We attempt to obtain all pertinent medical records about diagnoses and treatment of prostate disease. In the prospective part of the study we attempt to obtain and preserve the prostate of deceased participants on whom an autopsy was performed.

Genetic factors contributing to prostate disease are being studied. Starting in FY1997 a case-control study of four genes that may contribute to prostate cancer began. BLSA men who have prostate cancer are compared to age matched controls that, on the basis of longitudinal clinical observation, were judged to have a low probability of having prostate

cancer. The four genes are: the mu-class glutathione S-transferase (GST) gene, GSTM1; the pi-class GST gene GSTP1; the human androgen receptor gene hAR; and the inherited prostate cancer susceptibility gene PRCA.

A study of familial genetics in BPH is under review. The experimental subjects, BLSA participants with early onset BPH, will be identified on the basis of MRI and/or PSA data; first degree relatives will be studied to identify genes linked to prostate growth.

**Hearing and Aging:** Assessments of hearing thresholds for pure tones in consenting BLSA participants using a Bekesy audiometer began in 1965. An expanded hearing protocol was introduced in 1991 that included pure tone audiometry using more contemporary psychophysical procedures, acoustic reflex measures, speech perception in noise and a self-report of hearing difficulties. It continued until 1995 when it was necessary to discontinue the procedure after loss of the technician. A proposal to resume the protocol with an additional assessment of vestibular function is under review at present. The proposed protocol would be used with BLSA participants who have consented to be part of the autopsy study. There is considerable interest in studying the pathology of the auditory system in relation to hearing performance. Current activity is directed at analyzing and reporting the data from the expanded hearing protocol.

The published results of the BLSA longitudinal data on pure tone audiometry have become standard reference data. Data published in FY1997 provide longitudinal and cross-sectional percentiles of age-related changes and differences for men and women ranging in age from the 20s to the 80s. These data are valuable to persons concerned with assessing the effects of long term noise exposure on hearing of persons of different ages. Noise-induced hearing loss produces hearing loss greater than one would expect on the basis of aging alone and the projections allow one to estimate the excess hearing loss associated with noise exposure or other exogenous factors that would contribute to hearing loss.

The analyses of the data on self-reported hearing complaints (Hearing Handicap Inventory) indicate that the number of difficulties in hearing increase with aging as would be expected. However, when individual differences in pure tone hearing thresholds are taken into account, the importance of age as such is considerably diminished. The same is true when considering the relationship between self-reported hearing loss and speech perception and noise. Both are imperfectly correlated with age, and when all are considered together, the major factors determining self-reported hearing difficulties is hearing loss, not aging.

The analyses of the acoustic reflex data show that middle ear disorders are a minor contributor to the age associated changes in hearing that have been found. This result is consistent with earlier reports on small samples.

**Collaborators—Prostate Aging and Disease:** E.J. Metter, L.J. Brant, R. Andres, S.M. Harman, NIA; H. B. Carter, A.W. Partin, J.I. Epstein, D.W. Chan, P.C. Walsh, W. Isaacs, M. Blackman, Johns Hopkins Univ.; Joan Bailey-Wilson, NCGR; J.D. Pearson, H. Guess, Merck Laboratories.

**Collaborators—Hearing and Aging:** E.J. Metter, L.J. Brant, Kaori Nagai, NIA; Sandra Gordon-Salant, University of Maryland; C.H. Morrell, Loyola College, Baltimore, MD; J. D. Pearson, Merck Laboratories.


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**Keywords:**
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**Recent Publications:**


Kawas C, et al. *Neurology* 1997; 48: 1517-1521.

Stewart K, et al. *Neurology* 1997; 48: 626-632.

Carter HB, et al. *Urology* 1997; 49: 379-384.

Pearson JD, et al. *Urology* 1996; 48(6A): 4-9.


**Biography:** Dr. E. Jeffrey Metter received his M.D. from the University of California, Los Angeles in 1971. He completed a medical internship and neurology residency at the Mayo Graduate School of Medicine, Rochester, Minnesota in 1976. He returned to Los Angeles, where he became a staff neurologist and chief of the stroke rehabilitation ward at the Veterans Administration Medical Center, Sepulveda, California. He was also on the full time faculty in the Department of Neurology, UCLA School of Medicine. In 1987, he joined the National Institute on Aging as the physician for the Baltimore Longitudinal Study of Aging.

**Health Evaluation in the Baltimore Longitudinal Study of Aging (BLSA):** A clinical evaluation unit, under Dr. Metter's supervision, is responsible for the health evaluations in the BLSA. The characterization of the health status of all subjects is important to many of the researchers and projects within the study. Starting in 1985, the BLSA health evaluation has undergone major changes to improve medical information collection. The most substantial change occurred between 1988 and 1990, when the BLSA

initiated extensive use of nurse practitioners and physician assistants (NP/PA) to perform the history and physical examinations, rather than medical staff fellows. Subsequently, revisions have occurred in health questionnaires, medication and diagnosis listing.

We continually try to improve the quality of the clinical evaluation. We continue to assess quality assurance across the questionnaires, maintain staff training, and monitor and improve staff cooperation so that reliability and consistency of the clinical evaluation remains at a high level over time. This effort seems successful as over the past several years, staff has turned over, and new staff have easily adjusted and adapted to the unique needs of the BLSA. As new research questions are developed by scientific staff, we add new dimensions to the evaluation. We try to do this so those existing questionnaires are not changed, to maximize the longitudinal capabilities of the health data.

The unit is also responsible for the day to day health requirements of the participants during their visit. The unit tries to maintain and improve as necessary the high level of nursing and technical support, and to maximize the good will between the staff and the BLSA participants. The technical support includes health screening for a number of research protocols and assisting researchers in project development as it applies to unit interaction with the research. To meet these ends, the NP/PA and nursing staff have established quality assurance in the evaluation program. They have regularly scheduled meetings to discuss evaluation problems and related issues. A protocol manual was prepared describing most of the procedures and questionnaires. Ongoing efforts are designed to maximize the participant well-being, with continued monitoring of forms, records, protocols and comments of research participants.

**Neuromuscular Changes with Age:** The purpose is to characterize and explain age-associated losses of muscle strength. We seek to understand the time course of strength loss, factors that contribute to the loss, and to what degree the exercise response differs between old and young individuals. Our research has three main components.

1. Characterization of longitudinal strength changes in the BLSA. This consists of two parts. From 1960 to 1985, strength and power were measured in BLSA participants using in-house constructed equipment that measured isometric strength and power in the upper extremities. The purpose was to determine long term longitudinal changes (up to 25 years) in strength and power, and to relate these changes to changes in muscle mass, peripheral nerve function, daily and physical activity, and aerobic fitness. Starting in 1992, strength has been measured using a state of the art isokinetic dynamometer (Kin-Com). This equipment allows for the

measurement of both concentric and eccentric strength at multiple velocities in both the upper and lower extremities. The specific purposes are to determine age-associated maximal force production of the upper and lower body musculature during the concentric and eccentric phases of exertion, at fast, slow and zero speed, and determine the angle of greatest force; determine relationships between changes in strength with age and changes in lean body mass, fat mass, bone mineral density, glucose homeostasis, functional abilities, physical activity and nutritional state.

2. Comparison of exercise response to resistive strength training in young and old subjects. This project is being completed under contract with the University of Maryland, College Park, Dr. Ben Hurley, principal investigator. The specific purposes are: (1) determine the relationship between changes in lean body mass or muscle mass and changes in glucose regulation with age and strength training. (2) To determine if changes in strength or muscle mass can predict changes in total or regional bone mineral density. (3) To determine what factors best explain strength losses associated with aging and detraining and strength gains associated with strength training.

3. Examination of the motor unit and its relationship to muscle strength and exercise response. A protocol has been developed that explores motor unit function at different levels of muscle exertion in the quadriceps. The goal of this project is to understand the changes that occur in motor units with aging, and the effects of these changes on muscle strength and how these changes affect the exercise response. Over the past 20 years, *in vivo* techniques have allowed for the direct examination of the motor units in humans. Most studies that have examined age-related changes in motor units have focused on old versus young rather than examining the entire adult life span. They do not allow for an assessment of where during the life span these changes begin, or the association between the motor units and strength. The ongoing study addresses these issues.

**Race and Gender Differences in Intracerebral and Carotid Arterial Velocity with Aging**: This project is studying intracerebral blood flow velocity and resistance, and carotid blood flow velocity using doppler ultrasonographic techniques in BLSA participants. The goal is to determine whether differences in these parameters may explain racial and gender differences in stroke and coronary heart disease, and whether changes in arterial characteristics are associated with fitness and frailty.

We have found that intimal-media thickness of the common carotid artery increases with age concomitant with dilatation. Changes in wall thickness are associated with increasing risk for the development of coronary heart disease after adjusting for age. In addition, age changes in flow velocities in the carotid artery are poorly correlated with the flow velocities in the

middle cerebral arteries. We have also compared different measures of arterial stiffness across age and explored which measures are most related to the development of coronary heart disease. The development of a noninvasive technique to assess vascular stiffness, a major determinant of cardiovascular disease risk, may be valuable for identifying the effects of interventions aimed at modifying cardiovascular risk.

**Collaborators - Neuromuscular Changes with Age:** Robin Conwit, M.D., Johns Hopkins Bayview Medical Center; William Brown, M.D., Tufts University; Daniel Stashuk, Ph.D., University of Waterloo, Ontario, Canada; Benjamin Hurley, Ph.D., University of Maryland, College Park.

**Collaborators - Race and Gender Differences in Intracerebral and Carotid Arterial Velocity with Aging:** Christopher Earley, M.D., Ph.D., Johns Hopkins Bayview Medical Center.


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**Keywords:**
diabetes
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insulin
nutrition


**Biography:** Dr. Andres received his medical degree and residency training at Southwestern Medical College in Dallas. His postdoctoral fellowship began at Johns Hopkins in 1950 and he has maintained his academic appointment there as Professor of Medicine. He came to the NIH in 1962 to be the Clinical Director and Assistant Chief of the Gerontology Unit in Baltimore, initially when it was in the National Heart Institute, then in the National Institute of Child Health and Human Development, and now in NIA. Dr. Andres is past president of the Gerontological Society, a member of the American Society of Clinical Investigators and the Association of American Physicians, and the recipient of the Kleemeier Award, the Allied-Signal Achievement Award in Aging, the Enrico Greppi Gerontology Prize (Italy), and the Rank Prize in Nutrition.

**Recent Publications:**

Muller DC, et al. Muscle Mass: Its Measurement and Influence on Aging. The BLSA. *Nutrition Assessment of Elderly Populations* 1995; pp. 50-62.

Andres R, et al. Body Weight and Age. *Comprehensive Textbook of Eating Disorders and Obesity* 1995; pp. 65-70.

Muller DC, et al. *Aging Clin Exp Res* 1996; 8: 13-21.

Edelstein S, et al. *Diabetes* 1997; 46: 701-710.

**Glucose/Insulin Homeostasis and Aging:** Several diverse research approaches are in progress in order to understand the role of aging in the progressive changes occurring in this complex metabolic axis. (1) Factors influencing the age changes in fasting glucose and in glucose tolerance have been shown to be obesity and a central pattern of fat deposition, physical inactivity, dietary variables, physical inactivity, and a number of distinct diseases and medications associated with aging. (2) The glucose clamp technique (hyperglycemia and hyperinsulinemic/euglycemic) was devised in order to quantify, in intact humans, (a) beta cell responsiveness to glucose and to incretins (GIP and GLP) and (b) sensitivity of body tissues to insulin. (3) The implications of elevated fasting glucose and glucose tolerance values for the development of the characteristic complications of diabetes are being quantified in participants in the Baltimore Longitudinal Study of Aging. The development of coronary artery disease, the overt diabetic state, and all-cause mortality are under study. (4) The diagnostic cutpoints for the "impaired" state and for diabetes, recently recommended by the American Diabetes Association, are being carefully examined with reference to the possibility that an adjustment might be required for older men and women. Data from the BLSA, the Rancho Bernardo Study, and the National Health and Nutrition Examination Survey III are being collated.

**Interactions of Aging, Obesity, and Mortality**: There is continuing controversy over recommended weight-for-height in men and women and whether or not these standards need to be age-specific. The NHANES I Follow-up Study provides an unparalleled data set to examine the association between Body Mass Index at age 55-74 years at entry being and subsequent mortality over the next 20 years in white and black men and women. In addition, collaboration with the Applied Physiology Section, some 40 years of anthropometric measurements have been used to generate equations for the computation of percent body fat using DEXA scanning as the gold standards.

**Collaborators:** Dr. Dariush Elahi, Massachusetts General Hospital; Dr. Elizabeth Barrett-Connor, University of California, San Diego; Dr. Katherine Flegal, National Center for Health Statistics; Drs. John Sorkin and Andrew Goldberg, University of Maryland; Dr. Jordan Tobin, Applied Physiology Section, LCI, NIA; Dr. Josephine Egan, Diabetes Section, LCI, NIA; Dr. Ballentine Carter, Johns Hopkins; Dr. Judith Hallfrisch, Beltsville Human Nutrition Research Center, USDA; Dr. Katherine Tucker, Human Nutrition Research Center, Tufts University.

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# Laboratory of Genetics

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The Laboratory of Genetics was established in Autumn, 1997 by David Schlessinger, with a Human Genetics Unit, a Transcription Remodeling and Regulation Unit initiated by Weidong Wang, and a Gene Recovery and Analysis Unit headed by Ramaiah Nagaraja. A fourth unit, the Developmental Genomics Section, is programmed to start in mid-1998.

The interests of the Laboratory are based on the view that aging has genetic determinants as an integrated part of human development, with a profound dependence on the interplay of synthetic and degradative processes that are initiated in utero. Three major types of study are projected:

1. Transitions between immortal and mortal cells, particularly at the level of large-scale regulatory phenomena at the level of chromatin. For example, the transition of immortal embryonic stem cells to mortal differentiating cells is a fundamental feature of the initiation of aging in metazoans. The genes specifically activated and repressed during such transitions are being studied in mice, both by differential assays of gene expression in 3.5 days post coitum (dpc) mouse embryos and by the analysis of differential function of mutant and unmutated helicases that are affected in premature aging syndromes (the latter in the Unit on Transcription Remodeling and Regulation).

2. Cohorts of genes involved in the development of selected “nonrenewable” systems. To understand and ultimately try to compensate for loss of cells and tissues during aging, the examples of skin appendage and pronephros-kidney development are being studied. Studies start from human or mouse hereditary defects that have been attributed to single genes, such as the ectodysplasin-A involved in X-linked ectodermal dysplasia or the emx2 gene required for kidney formation.

3. Genes involved in embryonic events that prefigure aging-related phenomena. For example, the Human Genetics Unit is involved in studies of overgrowth syndromes, in which the set point of size of tissues and organs is determined in fetal life; and in studies of premature ovarian failure, in which the aging phenomenon of early menopause is determined by an increased rate of follicular atresia during fetal life.

The laboratory is equipped with state-of-the-art resources for genomic approaches in the Gene Recovery and Analysis Unit, including large-insert clones and recovery methods, high throughput sequencing, nuclear fractionation and chromatin analysis techniques, and the potential to make and analyze high-quality cDNA libraries from very few cells from subregions of embryos. It also benefits from collaborative efforts with other groups and resource providers both within NIA and at a number of extramural sites in the United States and abroad.

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### Human Genetics Unit

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**Keywords:**
X chromosome
gigantism/overgrowth syndromes
ectodermal dysplasia
premature ovarian failure

**Recent Publications:**

Pilia G, et al. *Nature Genetics* 1996; 12: 241-247.

Kere J, et al. *Nature Genetics* 1996; 13: 379-380.

Nagaraja R, et al. *Genome Research* 1997; 7: 210-222.

Srivastava AK, et al. *PNAS* 1997; 94: 13069-13074.

**Biography:** Dr. Schlessinger received his Ph.D. from Harvard University in 1960. Following postdoctoral training at the Pasteur Institute in Paris, he joined Washington University in St. Louis, where he served as Professor of Molecular Microbiology, Genetics, and Microbiology in Medicine until his move to NIA in September, 1997. He has contributed both to microbial and human genome studies. He has served as President of the American Society for Microbiology in 1995, and as the Director of the Human Genome Center at Washington University from 1987-97. During his tenure as Center director, he oversaw the development of the X chromosome map and of much related technology, with the concomitant finding of a number of disease genes. He is currently a councillor of the Human Genome Organization.

**Human Genetics Unit:** The program is designed to complement studies by many groups in lower animal models and in fibroblast senescence with corresponding studies of embryonic events critical for the aging of specialized mammalian cells and concomitant aging-related phenomena.

1. Studies at the level of gene regulation in chromatin. Projects are designed to understand tissue- and developmentally-restricted expression of the genes in which mutation causes the inherited conditions SGBS or EDA (see below). Promoter and enhancer element function will be analyzed in those instances and in another in which a gene (SYBL1) is expressed on X but not on the Y homologue; it may be repressed by nearby Y heterochromatin. The regulatory processes in all these cases involve features of chromatin; analyses of open and closed chromatin are projected for the genes recovered in chromatin form in artificial chromosomes.

2. Cohorts of genes involved in selected processes, using a “genome approach” to developmental phenomena. The approach starts from human inherited conditions and relevant embryological studies in mouse models (where sets of genes from embryonic stages can be easily mapped in the

genome and localized in sections, and knockout technologies are available). Examples include:

Premature ovarian failure. A set of translocation breakpoints in a “critical region of the X chromosome” are associated with POF. We are analyzing the breakpoints to look for genes or structural features in the chromosomal DNA that can limit ovarian function. In correlated developmental work, systematic studies are beginning of gene cohorts specifically expressed during the development of the kidney and urogenital tract, including ovary and testis.

Hypophosphatemic rickets, X-linked. The responsible gene has been sequenced. It encodes a putative endopeptidase (with an as-yet unknown substrate), and is expressed along the kidney-urogenital developmental axis and in bone precursors. The gene and its protein would be investigated developmentally and biochemically; the HYP mouse has been shown to be an expermental model for the human disease.

Simpson-Golabi-Behmel syndrome (SGBS). Gigantism and overgrowth, particularly of mesoderm-derived tissues and organs, results from mutational lesions in a matrix glycoprotein, glypican 3. The speculative model for the etiology of the disease sees the determination of the set point for organ size as based on IGF2 and related features of growth hormone action. Tests and extensions of this hypothesis are based on developmental studies, including the generation of a mouse model.

X-linked anhidrotic ectodermal dysplasia (EDA). The gene provides an entree to an embyonic branch point that leads to teeth, hair follicles, and sweat glands. The Tabby mouse has been shown to be an experimental model for the human condition, and interacting genes can be found both by genomic approaches and by genetic studies of some of the other 150 inherited ectodermal dysplasias.

The projected work will depend on the Gene Recovery and Analysis Unit and collaborating groups, both for the developmental analysis of gene cohorts and for studies of physiology in aging populations with the aim of facilitating long-term patient benefit.

**Collaborators:** Professor J.M. Cantu, University of Guadalajara Medical School; Dr. Michele D’Urso, International Institute of Genetics and Biophysics, Naples; Professor Raj Thakker, M.D., Royal Postgraduate Medical School, London; Professor Antonino Forabosco, University of Modena; Dr. Giuseppe Pilia, Italian Research Council, Cagliari; Dr. Juha Kere, University of Helsinki; Dr. Anand Srivastava, Greenwood Genetics Center.

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**Keywords:**
chromatin-remodeling
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SWI/SNF
helicase

**Recent Publications:**

Wang W, et al, *Genes & Dev* 1996; 10: 2117-30.

Wang W, et al, *Embo J* 1996; 15: 5370-82.

Wang W, et al. *Proc Nat Acad Sci* 1998; 95: 492-498.

**Biography:** Dr. Wang was trained as a biochemist and a molecular biologist at both UCLA, where he obtained his Ph.D., and Stanford University, where he worked as a postdoctoral fellow. His research has focused on the regulation of mammalian gene expression at the chromatin level. He has purified to homogeneity one of the first ATP-dependent chromatin-remodeling complexes in mammals, and has subsequently cloned all the subunits within one complex. His current projects include purification of novel tissue-specific chromatin-remodeling complexes, and investigation of how these complexes are involved in development and differentiation.

**Structural and Functional Studies of Mammalian SWI/SNF-Related Chromatin-Remodeling Complexes:** The establishment and maintenance of transcriptionally active and inactive chromatin structure in higher eucaryotes play a key role in gene regulation during development, differentiation and adaptation to environmental stimuli. Evidence accumulated during the last two decades indicates that chromatin structures are remodeled when multipotent precursor cells develop into terminally-differentiated cells. However, the underlying mechanism of chromatin remodeling is poorly understood, primarily because molecules that remodel chromatin structures have been discovered only recently. Several molecules identified so far are all multisubunit complexes, but little is known about their structures, functions and mechanism of action. It is not even clear how many chromatin remodeling complexes exist in any given species. The investigation of the chromatin remodeling process will be an exciting field for the next decade.

My research project has focused on one type of chromatin remodeling complex. The mammalian version of SWI/SNF complex. The SWI/SNF complex, originally identified in yeast, functions as a chromatin remodeling machine in signaling pathways that lead to activation of gene expression. The complex was also reported as a part of yeast RNA polymerase II holoenzyme. In Drosophila the complex is required for

control of important developmental regulators, such as homeotic genes and segmentation genes. In mammals, the SWI/SNF-related complexes appear to be involved not only in gene regulation, but also in targeting of HIV integration, in tumor suppression by interacting with RB protein, and in T cell leukemia. It was shown recently that one complex is essential for viability of a mouse embryonal carcinoma cell line. I have completely purified not one, but several distinct mammalian SWI/SNF-related complexes. By microsequencing, my colleagues and I have cloned all 10 subunits from the major complex of human KB cells. Six of them belong to five different multigene families. In one case, three members of the same gene family have different tissue expression patterns, suggesting the existence of tissue-specific chromatin remodeling complexes. Cloning of these new subunits and their family members has paved the way for future investigation of the roles of chromatin-remodeling complexes in development, differentiation and other biological processes in mammals.

My research project is directly related to several human diseases, including aging-related diseases such as Werner's syndrome. At least one subunit of the human SWI/SNF complex belongs to a huge family of ATP-dependent helicases. About half of the human helicases discovered to date are related to human diseases, which include the Werner's Syndrome gene (WRN), Cockayne's Syndrome (ERCC6), Xermaderma pigmentosum, Bloom's Syndrome and ATR-X (X-linked mental retardation with L-thalassemia) Syndrome. Many of the gene products have only been identified recently and their mechanism of action are not known. Studies of SWI/SNF-related complexes provide a way to approach the analysis of function of these helicases, and can facilitate clinical research on pathological aspects of these diseases.

**Collaborators:** Dr. Gerald Crabtree, Stanford University; Dr. Robert Tjian, University of California, Berkeley; Dr. Matthew Scott, Stanford University; Dr. Jerry Workman, Penn State University; Dr. John Tamkun, University of California, Santa Cruz; Dr. Jacques Cote, Laval University Cancer Research Center; Dr. Bradley Cairns, Harvard Medical School; Dr. Kristine Swiderek, City of Hope Hospital, Los Angeles; Dr. Terry Magnuson, Case Western Reserve University; Dr. Michael Carey, University of California, Los Angeles.

**Dennis D. Taub, Ph.D., Acting Chief**

# Laboratory of Immunology

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The interests of the newly-formed Laboratory of Immunology (LI) cover a wide range of topics devoted to a greater understanding of the biological, biochemical, and molecular alterations in immune functions that occur within individuals during both normal and pathology-associated aging processes. The Laboratory comprises four major research units, the Clinical Immunology Section (CIS), the B-Cell Development Section (BCDS), and the laboratories of Drs. Nan-Ping Weng and Albert Nordin. A common goal of these research programs is the elucidation of the age-related deficits in immune function that could be potentially targeted by various therapeutic strategies.

The Clinical Immunology Section is currently examining (1) a role for various cytokines, hormones, opioids, and chemokines in leukocyte trafficking, cellular activation, and apoptosis; (2) the biological and molecular mechanisms of HIV-1 entry and propagation in Th1/Th2 subsets and mononuclear cells obtained from young and elderly individuals; and (3) the preclinical and clinical development of immunologically-based protocols focusing on the promoting cellular responses in elderly populations with the ultimate goal of improving the innate immune function of aged individuals.

The B-Cell Development Section is currently examining (1) a number of protein-conjugate vaccines for *Streptococcus pneumoniae* for use in various immunoglobulin transgenic and knockout animal models as well as in the highly susceptible elderly population and (2) the escape of autoreactive B cells in normal mice through the rearrangement and overexpression of various immunoglobulin H and L chains. Within the laboratory of Dr. Albert Nordin, studies are underway examining the role of $p27^{Kip1}$ and MARCKS in cyclin-dependent kinase regulation of the cell cycle and their possible association with the decline in T-cell proliferation observed with advanced aging. Within the laboratory of Dr. Nan-Ping Weng, studies are focused on the molecular examination of telomere length, telomerase activity, and the various factors and genes that appear to be differentially regulated during human lymphocyte development, differentiation, and activation.

## Laboratory of Immunology Staff

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**Keywords:**
chemokines
T cells
expression
HIV
costimulation
G protein


**Recent Publications:**


Taub DD, et al. *J Immunol* 1996; 156: 2095-2103.

Murphy WJ, et al. *J Immunol* 1996; 156: 2104-2111.

Taub DD, et al. *J Leuk Biol* 1996; 59: 81-89.

Hesselgesser J, et al. *Curr Biol* 1997; 7: 112-121.


**Biography:** Dr. Dennis D. Taub received his Ph.D. from the Department of Microbiology and Immunology at Temple University School of Medicine in Philadelphia in 1991. He subsequently entered the laboratory of Dr. Joost J. Oppenheim as a staff fellow at the National Cancer Institute in Frederick, Maryland. From 1994-1997, Dr. Taub headed the tumor vaccine monitoring laboratory within the Clinical Services Program at the National Cancer Institute. In early 1997, he moved to the newly-formed Laboratory of Immunology at the National Institute on Aging as the Acting Chief of the Clinical Immunology Section.

**Chemokines and Lymphocyte Function:** The recruitment of lymphocytes into inflammatory sites requires several activation events including endothelial cell activation by inflammatory cytokines, the expression of adhesion molecules, cellular adhesion, diapedesis, and migration via established chemotactic gradients. Over the past 10 years, members of the *chemokine* super family have been shown to induce adhesion, chemotaxis, activation, and degranulation of human and rodent leukocytes and lymphocytes both *in vitro* and *in vivo*. The Clinical Immunology Section is currently examining a role for chemokines in lymphocyte costimulation/activation and as immunodjuvants in vaccine-based studies with hapten-carrier protein complexes. In addition, the laboratory is also examining the ability of various chemokines and other G-protein receptor ligands to modulate other T, B, and NK cell effector functions as well as antigen-presenting cell activities. We believe that a better understanding of the complexities of leukocyte extravasation and the mediators that induce cell trafficking and activation will greatly assist our ability to orchestrate, regulate, and control various pathological disease states associated with aging as well as our understanding of normal leukocyte trafficking.

**Chemokine Receptors, Th1/Th2 Clones, and HIV Pathogenesis**: Recent studies have shown that HIV-1 utilizes cell surface-bound CD4 molecules as well as chemokine receptors to enter and subsequently infect

human T lymphocytes. Our laboratory has demonstrated that human T cells and antigen-specific T-cell clones express significant levels of several of the high affinity G protein-linked chemokine receptors on their surface which mediate T-cell migration, adhesion, degranulation, and intracellular calcium mobilization. Examination of a panel of human Th1 and Th2 clones has also revealed the differential expression of several distinct chemokine receptors on the surface of these T cell subsets suggesting that the differential expression of these receptors on T-cell subsets may not only facilitate selective T-cell trafficking but may also mediate the selective entry of HIV-1 into T-cells. However, despite these differences in chemokine receptor expression, our studies have demonstrated that human Th0, Th1, and Th2 clones are all capable of being infected with the various T-cell tropic strains of HIV-1. HIV-1-infected Th1 clones are rapidly infected with HIV-1; however, they also exhibit a rapid (1-5 day) Fas-mediated apoptosis *in vitro* compared to infected Th2 clones (4-21 days). The increased expression of Fas ligand on the surface of Th1 but not Th2 clones post HIV-1 infection may possibly explain the more rapid turnover of this $CD4^+$ T cell-subset in HIV-infected people. Further examination of the various apoptotic signaling differences between human Th1 and Th2 clones revealed that all human Th1 but not Th2 cells are susceptible to activation-induced cell death (AICD). In addition, the majority of human Th1 clones expressed low levels of the anti-apoptotic protein, bcl-2, making them more susceptible to various apoptotic stimuli as well as HIV-1-induced T-cell death. In contrast, human Th2 clones, the majority of which express high levels of endogenous bcl-2, were less susceptible to apoptotic stimuli and HIV-1-mediated cytopathic effects. Thus, the protection of bcl-2-expressing T cells from HIV-induced cell death suggests that apoptosis not only contributes to cell killing by HIV infection but may also permit the selective destruction of Th1 cells in the periphery leading to a systemic Th2 response. As AIDS in the elderly population continues to increase in number and as a percentage of all new AIDS cases, it has been hypothesized that T-cells obtained from elderly patients have an increased susceptibility to HIV-1. Over the next year, the Clinical Immunology Section will directly examine this question. We believe that understanding the immunophysiology of HIV-1 infectivity of young and old T cells may provide new insight into HIV-1 pathology.

**Clinical Monitoring and Preclinical Protocol Development:** The Clinical Immunology Section will also continue its involvement in the preclinical and clinical development of immunologically-based protocols focusing on promoting T-cell responses in elderly patients. Peripheral blood leukocytes obtained from normal healthy volunteers or elderly patients treated with various human hormones such as growth hormone (GH), prolactin (PRL) and DHEA will be examined for alterations in

innate immune function and leukocyte trafficking. In addition, a clinical trial examining the *in vivo* immunoadjuvant effects of PRL in elderly patients and a joint NIA-NIDA trial examining the *in vitro* and *in vivo* immunological status of opiate addicts pre- and post-methodone intervention are currently being planned. Preclinical studies from this laboratory have already revealed that GH, PRL, and DHEA provide costimulatory signals during T cell activation both *in vitro* and *in vivo*. We believe that additional immunological research on hormone-immune cell interactions may provide insight into the various homeostatic mechanisms that control immunocompetence during aging.

**Collaborators:** Nicholas Lukacs, Ph.D., Robert Strieter, M.D., University of Michigan; Larry Kwak, M.D., Ph.D., Robert Fenton, M.D., Ph.D., NCI; Richard Horuk, Ph.D., Berlex Pharmaceuticals; Milan Fialo, M.D., UCLA; Stefan Brocke, Ph.D., NINDS, NIH; Francis Ruscetti, PhD., NCI, NIH.


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**Keywords:**
streptococcus
vaccine
phosphocholine
transgene


**Biography:** Dr. James Kenny received his Ph.D. from the University of California, Los Angeles, in 1977. Having completed his postdoctoral training at the University of Michigan, he joined the Department of Microbiology at the Uniformed Services University for the Health Sciences in 1979 and moved to the National Cancer Institute-FCRDC in 1986. In 1996 he moved to the NIA's Laboratory of Immunology as Chief of the B-Cell Development Section. He is a member of the American Association of Immunologists.

**B-Cell Development:** We are studying B cell development in transgenic, knock-in, and knockout mice expressing rearranged genes that encode for the heavy (H) and light (L) chains of antibodies having specificity for phosphocholine. In the past several years, our work has been concentrated in three distinct areas.

**Recent Publications:**

Kenny JJ, et al. *J Immunol* 1996; 157: 1054-1061.

Young HA, et al. *Blood* 1997; 89: 583-595.

Guo W-X, et al. *Int Immunol* 1997; 9: 665-667.

**Vaccine Development**: Infection caused by *Streptococcus pneumoniae* is responsible for 40,000 deaths each year in the U.S.A. Most of these deaths are among the aged, but a significant number of deaths also occur in children less than two years of age and in immune deficient patients. These highly susceptible human populations are also the least responsive following immunization with the current group-specific carbohydrate based vaccine. The B Cell Development Section is working on the development of a broad spectrum protein-conjugate vaccine for *S. pneumoniae*. Success in immunodeficient animal models has been obtained using phosphocholine (PC) conjugated to immunogenic protein carriers. PC is found on the cell wall of all strains of *S. pneumoniae* although, it may not be exposed on many highly encapsulated serotypes. We are developing a vaccine for a phase I human trial that will use PC conjugated to tetanus toxoid. This is currently being tested in animal models for potential as a broad-spectrum vaccine against infection with *S. pneumoniae*.

**Immunoglobulin Transgenic Mice as Models for Vaccine Development**: We have developed a large number of transgenic mice expressing rearranged H and L chain genes that encode for anti-PC antibodies. Transgenic mice expressing one of the four variant forms of the same H chain gene ($V_H1$) as well as mice expressing both H and L chain anti-PC Ig-transgenes have been tested for protection against challenge with *S. pneumoniae*. None of the mice exhibit much innate protection before immunization but all strains show some protection following immunization. The protection seen in transgenic mice was less than that seen in immunized transgene negative controls. This is not surprising since the transgene positive mice cannot undergo class switching following immunization; thus, they produce only IgM anti-PC antibodies while the control mice make high levels of IgG anti-PC antibodies, which are known to be much more protective than IgM anti-PC antibodies.

**The Autoreactive Nature of PC-Specific B Cells**: Several years ago, we demonstrated that PC-specific B cells would not develop in the peripheral lymphoid organs of x-linked immune deficient mice (xid) mice that expressed transgenes encoding anti-PC antibodies. However, these B cells developed normally in the bone marrow of xid mice. We hypothesized that this might be due to clonal deletion of these PC-specific B cells following stimulation by an autologous PC-antigen. In the normal mouse, this same stimulation appeared to result in positive selection of PC-specific B cells. We demonstrated that the PC-specific B cells in H + L transgenic xid mice could be rescued by over expressing the bcl-2 proto-oncogene. This suggested that the xid PC-specific B cells are induced to die by apoptosis,

whereas, B cells expressing a functional form of Bruton's tyrosine kinase (btk) could somehow escape this tolerance induction. Subsequent studies using anti-PC transgenic mice crossed onto a Rag-2 knockout background demonstrated that PC-specific B cells expressing wild type btk cannot develop in the absence of a functional recombinase unless they coexpress bcl-2. Even in the presence of bcl-2, these B cells appear to arrest at an immature stage of development, but they can up regulate their sIgM receptors and migrate from the bone marrow to the periphery. These studies have further demonstrated that most combinations of the $V_H1$ H chain with a variety of L-chains leads to early arrest of B-cell development in the Rag-2 knockout mouse. These data suggest that PC-specific B cells may escape tolerance in normal mice by rearranging and expressing more than one H or L chain gene.

Future studies will be aimed at analysis of the anti-PC response in humans and in transgenic mice expressing human immunoglobulin H-chain genes. We hope to demonstrate that human anti-PC antibodies can provide protection against a broad spectrum of *S. pneumoniae* serotypes. Additional studies will involve analysis of the competition between B cells expressing variant forms of the $V_H1$ H chain for long-term survival in peripheral lymphoid tissues.

**Collaborators:** Johanas Reim, M.D., Johns Hopkins University; Howard Dintzis, Ph.D., Johns Hopkins University; Howard Young, Ph.D., NCI-FCRDC; James Mond, M.D., Ph.D., Uniformed Services University for the Health Sciences; Andy Lees, Ph.D., Virion Inc; Brian Rogerson, Ph.D., Trudeau Institute; Dan Schulze, Ph.D., University of Maryland at Baltimore; Lenni Shultz, Ph.D., Jackson Laboratories; J. Latham Claflin, Ph.D., University of Michigan; Phil Tucker, Ph.D., University of Texas.

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**Keywords:**
cell cycle regulation
cyclin-dependent kinases
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senescent T-cells

**Recent Publications:**

Kwon TK, et al. *Cell Growth Differ* 1996; 7: 1305-1133.

Kwon TK, et al. *Gene* 1996; 180: 113-120.

Kwon TK, et.al. *J Immunol* 1997; 158: 5642-5648.

**Biography:** Dr. Albert A. Nordin received his Ph.D. from the University of Pittsburgh School of Medicine in 1962. After completing postdoctoral training at Pittsburgh, he was appointed to the Faculty of the School of Medicine. In 1966 he joined the faculty of the University of Notre Dame, South Bend and moved to his present position at the National Institute of Child Health and Human Development (NICHD) in 1972, now NIA. He was a Visiting Scientist of the Swiss Institute for Experimental Cancer Research, Lausanne in 1969-1970 and Guest Professor of the Basel Institute of Immunology, Basel in 1975 and 1980-1981.

**Cell Cycle Regulation**: **T-Cells**: We are investigating the intracellular molecular events associated with the cyclin-dependent kinase (cdk) system that induces resting T cells to enter and progress through the cell cycle. A unique advantage of the T-cell system for analysis of cell cycle regulation is that T cells spontaneously arrest in a resting stage ($G_0$)*in vivo*. Following activation, $G_0$ T cells enter the $G_1$ phase of the cell cycle and progress through the other phases of the cell cycle (S, $G_2$ and M) in an orderly fashion regulated in large part by a family of phosphorylating enzymes known as the cdks. We have shown that resting T cells express low or no detectable levels of cyclins or cdks and little if any related kinase activity. On the other hand, resting T cells contain high levels of a protein that inhibits the enzymatic activity of a variety of cdks including those essential for the cells to progress through the $G_1$ phase of the cell cycle. Upon activation, the expression of this inhibitor, $p27^{Kip1}$, ceases and the existing protein is degraded allowing the activated cells to enter and progress through $G_1$. We have cloned, sequenced and characterized the promoter region of the $p27^{Kip1}$ gene. The activity of this promoter region, which is essential for the expression of the gene, is down regulated to a large extent by a T-cell growth factor (IL-2) that is itself induced soon after the activation of resting T cells. Withdrawal of growth factor results in a rapid increase of $p27^{Kip1}$ and the cells cease proliferation and enter the resting state. We have shown that the decline in T-cell proliferation associated with advancing age is in part associated with a persistent expression of $p27^{Kip1}$ which inhibited a significant portion of the activated

T cells from progressing through the $G_1$ phase of the cell cycle. The promotor region of $p27^{Kip1}$ in T cells from older mice is less responsive to inhibition induced by IL2. We are attempting to elucidate the mechanism of this effect.

**Non-Lymphoid Cells**: The role of the cyclin-dependent kinase inhibitors (cki) in regulating the progression of various other cell types through the $G_1$ phase of the cell cycle has been investigated. Staurosporin treatment of breast carcinoma cell lines has been shown to arrest cells at several points within the cell cycle. We have shown that treatment with low doses of staurosporin induced a complete $G_1$ block of a representative breast carcinoma cell line. The arrest correlated with a significant reduction in the kinase activity of the $G_1$ associated cdks (cdk2, 4 and 6) which correlated with an increase in the transcription of two cki genes, p18 and $p27^{Kip1}$, and accumulation of high levels of these proteins in the $G_1$ arrested cells. Inhibition of the cdks prevented the phosphorylation of retinoblastoma protein, which in the underphosphorylated state binds several factors essential for cells to complete $G_1$ and enter the S phase of the cell cycle. In addition to the under phosphorylation of retinoblastoma protein, we discovered a novel substrate for the $G_1$ associated cdks in both breast and lung epithelial derived carcinoma cells. This 88kDa protein was recently identified as myristoylated alanine rich protein C kinase substrate (MARCKS). The protein is heavily phosphorylated in breast and lung tumors as compared to that in normal lung or mammary ductal epitheleal cells. Since protein kinase C is classically associated with signal transduction and the cdks with cell cycle regulation, it will be of interest to determine if MARCKS may represent a substrate at the confluence of these major cellular mechanisms. Studies are in progress to determine the significance of this substrate in human tumor development in regards to the cyclin-dependent kinase system of cell cycle regulation.

**Characterization of $p27^{Kip1}$ cdk2 Binding Domain**: The cdk2 binding domain of $p27^{Kip1}$ was determined by *in vitro* techniques to be located within the amino acid sequence 53-85. A series of point mutations were generated within this region and further localized the domain to amino acid residues 62-75. Mutations within two motifs, EDE and GXY, completely inhibited binding to cdk2. A mutation at residue 64 phenylalanine to alanine (F64A) also showed a significant reduction in the capacity of this mutant $p27^{Kip1}$ protein to inhibit the kinase activity of cdk2. The cellular response to the introduction of the F64A mutant form of $p27^{Kip1}$ was compared to that of $p27^{Kip1}$ wild type by transfecting HeLa cells with constructs of full-length sense and antisense coding sequences. Over expression of the F64A mutant form of $p27^{Kip1}$ bound significantly

lower levels of cdk2 as compared to wild type and did not effect the cdk2 related kinase activity of the transfected HeLa cells. Over expression of wild type $p27^{Kip1}$ resulted in a reduction of the level of cdk2 kinase activity and effectively suppressed the growth of the transfected HeLa cells.

**Collaborators:** Edward W. Gabrielson, M.D., Joel Shaper, Ph.D., and Nancy Shaper, Ph.D., Johns Hopkins University.


## Nan-Ping Weng, M.D., Ph.D.
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**Keywords:**
lymphocyte lifespan
telomere
telomerase
immune senescence


**Recent Publications:**


Weng N, et al. *J Exp Med* 1996; 183: 2471-2479.

Palmer LD, et al. *J Exp Med* 1997; 185: 1381-1386.

Weng N, et al. *J Immunol* 1997; 158: 3215-3220.


**Biography:** Dr. Weng received his M.D. from Shanghai First Medical College, China, in 1984 and Ph.D. in Immunology from Baylor College of Medicine in 1993. He obtained postdoctoral training at Baylor College of Medicine and at the National Cancer Institute, and joined the Laboratory of Immunology at the Gerontology Research Center in 1997.

**Research:** The research focus of this laboratory is on the molecular and cellular mechanisms of lymphocyte lifespan and replicative senescence. The function of the immune system is dependent on a high degree of lymphocyte division during development, differentiation and activation. In particular, substantial cell division that occurs in the course of somatic mutation and subsequent clonal expansion of antigen-specific lymphocytes is essential for an effective immune response to pathogens. Molecular analysis of factors or genes that are differentially regulated in young and senescent lymphocytes will enhance our understanding of immune senescence, and provide a rational basis for developing strategies of experimental and clinical intervention.

**Regulation of Telomere Length and Telomerase Activity During Human Lymphocyte Development, Differentiation, Activation and Aging:** Telomeres are specialized terminal chromosomal structures that have been implicated in the integrity of the chromosome and in cellular senescence. The inability of DNA polymerase to completely replicate the ends of chromosomes results in a loss of 50-200 bp telomere repeats with each cell division in normal human somatic cells. It has thus been

proposed that a minimal length of telomeres is essential for cellular replication and telomere reduction is a mechanism for limiting replicative lifespan. In contrast, germline and malignant cells have infinite lifespan and maintain telomere length due partly to a ribonucleoprotein enzyme, telomerase, which is capable of synthesizing telomeric repeats. The selective presence of telomerase activity in the germline and malignant cells but not in most normal human somatic cells has been hypothesized as a basis for the immortality of the germline and of malignant cells. Our study demonstrated that the telomeric length is longer in naive than in memory T cells, reflecting their replicative history *in vivo* and paralleling replicative capacity *in vitro*. Furthermore, we proved that telomerase expression is not restricted to germline and malignant cells, but is also expressed in normal human somatic cells, T and B lymphocytes, in which telomerase activity is strictly regulated in development, differentiation, activation, and aging. Finally, we demonstrated that the expression of telomerase RNA template (hTR) is also regulated in parallel to telomerase activity in lymphocyte development, differentiation, and activation. Currently, we are analyzing the role of telomerase in lymphocyte function.

**Molecular Analysis of Differential Gene Expression in Young and Senescent Human $CD4^+$ T Lymphocytes:** The long term goal of this study is to elucidate the molecular mechanisms of replicative lifespan and senescence in human lymphocytes. The possible control mechanisms may include cytokine production and utilization, cell cycle regulation, and functions of telomere and telomerase. Using an *in vitro* model, we analyzed gene expression in young and senescent $CD4^+$ T cells by differential display technique. Three cell populations were used for such analysis: freshly isolated naive and memory $CD4^+$ T cells and $CD4^+$ cells that have been cultured to the point of senescence or loss of replicative response to stimulation by either anti-CD3/CD28 or by PMA/ionomycin. We have identified dozens of transcripts that are present in one population but not in others, and found that many of them are new genes by sequencing analysis. Currently, we are characterizing the expression, regulation and eventually elucidating the functions of these differentially expressed genes.

**Collaborators:** Richard J. Hodes, M.D., National Cancer Institute; Carl H. June, M.D., Naval Medical Research Institute.

**Vilhelm A. Bohr, M.D., Ph.D., Chief**

# Laboratory of Molecular Genetics

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The Laboratory of Molecular Genetics (LMG) investigates the molecular basis for aging and age-dependent diseases, notably cancer. Studies focus on DNA related mechanisms such as genomic instability, DNA repair, DNA replication, and transcription. We consider the increased DNA damage accumulation in senescence as the major molecular change with aging, and this DNA damage may eventually inactivate individual genes and lead to a deterioration of the organism which is characteristic of the senescent phenotype. The goal of LMG is thus to understand the underlying mechanisms involved in DNA damage formation and its processing as well as the changes that take place with aging and that make aging cells susceptible to cancer. DNA repair is likely to play a critical role, and we have a special interest in the fine structure of DNA repair which includes the study of the DNA repair processes in individual genes. We are investigating the molecular mechanisms involved in DNA repair and in genomic instability in normal, senescent and cancer cells. We are studying the molecular biochemistry of the DNA repair processes nucleotide excision repair and base excision repair in *in vitro* systems, in fractionated cell extracts, and in intact cells. We are also interested in the molecular processes that interact with the DNA repair processes. They include transcription, replication, somatic mutation and mitochondrial alterations.

The accumulation of DNA damage with age could be a result of a gradual decline in DNA repair capacity. Work from this and other laboratories suggests that this decline is not readily detectable in the overall genome, but may rather be a decline in the fine structure or transcription coupled component of the DNA repair process.

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Balajee AS, et al. *Proc Nat Acad Sci* 1997; 94: 4306-4311.

Gilchrest B, et al. *FASEB J* 1997; 11: 322-330.

Orren D, et al. *Mol Biol Cell* 1997; 8: 1129-1142.

Croteau DL et al. *J Biol Chem* 1997; 272: 25409-25412.

**Biography:** Dr. Bohr received his M.D. in 1978, Ph.D. in 1987, and D.Sc. in 1987 from the University of Copenhagen, Denmark. After residencies in neurology and infectious diseases at the University Hospital in Copenhagen, Dr. Bohr did a postdoctoral fellowship with Dr. Hans Klenow at the University of Copenhagen. He then worked with Dr. Philip Hanawalt at Stanford University as a research scholar during 1982-1986. In 1986 he was appointed to the National Cancer Institute (NCI) as an investigator, becoming a tenured Senior Investigator in 1988. Dr. Bohr developed a research section in DNA repair at the NCI. In 1992 he moved to the NIA to become Chief of the Laboratory of Molecular Genetics.

Dr. Bohr has conducted clinical studies (infectious diseases and oncology), but worked most extensively in basic research. His main contributions have been in the area of DNA repair. He has worked on many aspects of DNA damage and its processing in mammalian cells. He developed a widely used method for the analysis of DNA repair in individual genes and found that active genes are preferentially repaired. This observation was a major advance in the clarification of the tight interaction between DNA repair and transcription, a process termed transcription-coupled repair. In recent years numerous papers from his laboratory have focused on mechanisms of DNA damage processing, particularly on nucleotide excision repair and transcription coupling. A main interest now is to elucidate those processes in relation to aging.

**DNA Repair Processes**: Several types of DNA lesions have been observed in mammalian DNA and they are removed by a number of different DNA repair pathways. One important pathway is nucleotide excision repair (NER), which removes and replaces bulky lesions, such as UV-light induced pyrimidine dimers. Damaged bases are removed as nucleotides, typically as oligonucleotide fragments. This pathway involves several of the xeroderma pigmentosum DNA repair proteins. Another important DNA repair pathway is base excision repair (BER), which removes single damaged bases as free bases, and replaces them. Base

excision repair removes a large number of minor lesions from DNA, many that had been caused by oxidative modification. A third important pathway of DNA repair is mismatch repair, which occurs during DNA replication. Finally, a fourth pathway is recombination repair.

In the Laboratory of Molecular Genetics we mainly focus on NER and BER. We are interested in some of the subcomponent DNA pathways: gene-specific DNA repair and transcription-coupled repair (TCR). TCR reflects the tight interaction between DNA repair and transcription that leads to the highly efficient removal of lesions from the transcribed strand of active genes. Gene-specific DNA repair occurs at the nuclear matrix, where a number of repair proteins are recruited early in the repair process.

**Transcription-Coupled Repair:** Research in TCR has recently become a very fast moving area. There has been excitement about the discovery of a close molecular linkage between the processes of DNA transcription and repair. The most dramatic proof of this linkage was the finding a few years ago that the basic transcription factor, TFIIH, which is involved in transcription of DNA into mRNA, contains at least two DNA repair proteins among its seven subunits. The linkage between transcription and repair is now understood to be so close that they overlap extensively. Much evidence supports the idea that the TCR pathway and the gene-specific DNA repair pathway are different from the DNA repair pathways that operate in the rest of the genome, which consists of transcriptionally inactive bulk DNA.

Despite the interest in TCR, questions about the exact mechanism remain unanswered: What is the signal for TCR? Is the signal a RNA polymerase that is stalled at a lesion? What is the signal from the transcription complex to the repair proteins? Which lesions are repaired by TCR? Can oxidative DNA damage, thought to accumulate with aging, be repaired by TCR? To answer these questions about the mechanism of TCR, we are taking a number of approaches. DNA repair is studied in intact cells, in situ, in tissue culture, in cell extracts, or using purified components. DNA damage is induced by exposure of cells or purified DNA to various types of DNA damage or cellular stress.

**DNA Repair and Aging**: DNA damage is observed to accumulate with senescence. Subtle defects in DNA repair that arise during aging could explain this observation. The question of whether or not DNA repair declines with aging has been of great interest, and is a central concern of our research. We have many projects that address this issue. Whereas there is no hard evidence for a decline in the efficiency of the general genomic, DNA repair during aging, there is some evidence for a decline in the DNA

repair fine structure: gene specific repair, or TCR. This is currently being investigated.

**Oxidative DNA Damage and Mitochondrial Functions:** Reactive oxygen species are generated in cells as a by-product of cellular metabolism. It is a main product of the metabolic processes in each cell, and reactive oxygen species react with proteins, lipids, and DNA. Oxidative DNA damage is thought to be more related to aging than is any other form of DNA damage, and it has been shown to accumulate with aging. Oxidative DNA damage results from various forms of cellular stress, such as through the action of hydrogen peroxide generated intracellularly, and through exposure to acridine orange, X-irradiation, or methylene blue. Oxidative damage is thought to contribute to carcinogenesis, mitochondrial dysfunction, and aging.

Because most reactive oxygen species are generated by the oxidative phosphorylation processes that occur in mitochondria, it is of great interest to understand the oxidative DNA damage processing mechanisms in mitochondria. Mitochondrial DNA is not protected by histones and lies in close proximity to the free radical producing electron transport chain. Mitochondrial DNA contains a higher steady state amount of oxidative DNA damage than nuclear DNA. Oxidative DNA damage that arises in mitochondrial DNA might give rise to the mutations, gene inactivations, or deletions that are commonly found in the mitochondrial genome in association with aging and cancer. Because mitochondrial DNA is subjected to relatively higher amount of oxidative damage, it seems that mitochondria would need DNA repair activity to remove oxidative damage from their DNA. In the absence of DNA repair, mitochondrial DNA mutations would arise at high frequencies. Mitochondria are known to be defective in DNA repair, particularly in nucleotide excision repair. Mitochondrial DNA mutations and deletions accumulate in the elderly and in patients with mitochondrial myopathies. Whether these arise as a result of defective DNA repair remains to be explored.

The early finding of absence of repair of UV-induced pyrimidine dimers in mitochondrial DNA led to the general notion that there is no DNA repair in mitochondria. More recently, DNA repair enzymes have been identified from mitochondria, and gene specific repair experiments have shown efficient repair in mitochondrial DNA. We demonstrated removal of cisplatin interstrand cross-links, N-methylpurines, 8-oxo-G, and some removal of cisplatin intrastrand crosslinks in mitochondrial DNA sequences. These and more recent studies from our group and elsewhere have shown that a number of lesions are efficiently repaired from mitochondrial DNA.

**Oxidative DNA Damage and Repair in Nuclear and Mitochondrial DNA:** When DNA is the target of oxidative stress, a variety of DNA adducts can be formed, of which 8-hydroxyguanine (8-oxo-G) is one of the most abundant lesions generated. 8-oxo-G is thought to be a pre-mutagenic lesion because it can mispair with adenine during DNA replication, and this mispairing results in G-T transversion mutations. Although cells use a combination of base excision repair and nucleotide excision repair to remove lesions generated by oxidative damage, base excision repair is the most important.

We have developed a technique to measure the formation of 8-oxo-G in individual genes. We have shown that this lesion is efficiently repaired both in nuclear and mitochondrial DNA.

We can also measure the repair of oxidative damage *in vitro*. Results based on the use of mutant cell lines and on enzyme inhibitors suggest that nucleotide excision repair is involved in the removal of 8-oxo-G lesions. This is supported by results of experiments with xeroderma pigmentosum (XP) cells. We used XP cells that are completely defective in nucleotide excision repair but are proficient in base excision repair. 8-oxo-G lesions are not removed as efficiently in XPA cells as they are in normal cells. This result suggests that nucleotide excision repair is involved in repair of 8-oxo-G lesions. We are interested in further exploring the interaction between nucleotide excision repair and base excision repair. The repair of oxidative lesions is being studied in various natural human mutant cell lines suspected or known to be deficient in DNA repair.

**DNA Repair in Mitochondria:** Studies on mitochondrial DNA damage and repair have traditionally required the purification of mitochondrial DNA. This purification is laborious, and in addition, it is possible that most purification schemes introduce oxidative lesions in the DNA. As an alternative approach, we modified the gene specific repair assay that we had developed to detect various DNA lesions other than UV-induced pyrimidine dimers. With this approach, we do not need to isolate mitochondrial DNA, and can probe for oxidative lesions in the entire mitochondrial genome or in parts of it. We have established an assay using a repair enzyme that detects 8-oxo-G. This lesion is repaired very efficiently from both mitochondrial and nuclear DNA.

We have partially purified a mitochondrial oxidative damage endonuclease (mt ODE) from rat liver that recognizes and incises 8-oxo-G and abasic sites in duplex DNA. The name reflects that the enzyme incises apurinic/

apyrimidinic (AP) sites. Comparison of mt ODE with other known 8-oxo-G glycosylases/abasic lyases and mitochondrial enzymes reveals that this is a novel protein with similarity to the OGG1 enzyme from yeast, the gene for which was recently cloned. To the best of our knowledge, this is the first characterization of a mammalian mitochondrial enzyme that recognizes oxidative DNA damage.

We have measured mt ODE's incision activity from 6- and 24-month old rat liver. Interestingly, there is no decline, but instead an increase in activity with age. This finding is contrary to current notions of mitochondrial decline and is being pursued further experimentally.

We are also establishing experimental conditions for the study of DNA repair in mitochondrial extracts. For example, we have developed an assay for DNA nicking activity in mitochondrial extracts from rats. The assay can detect nicking activity on plasmids containing different types of DNA damage. We plan to determine which of the different types of lesions are recognized in mitochondria as a way to better understand which DNA repair pathways operate in these organelles. A particular focus is whether there are any nucleotide excision repair or recombinational repair pathways. Mitochondrial repair studies have suffered from a lack of availability of *in vitro* systems for biochemical study. We are now purifying components and antibodies to many proteins involved in nucleotide excision repair and base excision repair, and these will be tested for their effect on mitochondrial DNA incision. We will determine whether the mechanism of mitochondrial DNA repair differs from that of nuclear DNA repair, whether mitochondrial DNA repair declines with age, and whether local DNA repair defects in mitochondria lead to DNA deletions.

**Quantitation of Oxidative DNA Damage**: One of the controversies in the study of oxidative DNA damage concerns the validity of current methods of quantitation of the amount of 8-oxo-G in nuclear and mitochondrial DNA. In general, the amounts measured by various methods (gas chromatography/mass spectroscopy analysis; HPLC; enzymatic analysis) do not agree with one another, and different methods have not been directly compared in the same system.

In collaboration with Dr. Miral Dizdaroglu at National Institute of Standards and Technology, we are using various assays to compare the concentrations of levels of 8-oxo-G in nuclear and mitochondrial DNA. Formation of 8-oxo-G is one of about 100 base changes seen after exposure of cells to oxidative stress, but it is the one for which we have the best analytic tools. Given a sufficiently large DNA sample, however, it

is possible to use gas chromatography/mass spectroscopy analysis to quantitate a variety of oxidative lesions. Preliminary results indicate that the concentration of some lesions decreases with age, whereas the concentration of others increases. Many laboratories reported 10-fold higher steady state concentrations of level 8-oxo-G in mitochondrial DNA than in nuclear DNA. This finding has become one of the cornerstones of the mitochondrial theory of aging, but other observations suggest that it may not be true for cells in culture. We are currently further investigating the formation of 8-oxo-G in mitochondrial DNA. By testing different mitochondrial purification schemes, we are exploring whether 8-oxo-G formation is an artifact of mitochondrial isolation techniques.

**Changes in Mitochondrial Function with Aging**: In Dr. Richard Hansford's section, work on mitochondria isolated from rat heart showed that cardiac mitochondrial cytochrome oxidase (COX) activity decreases 30% with aging. By contrast, the activity of the nuclear-encoded enzyme citrate synthetase does not decline with age, when measured in the same mitochondrial suspensions. This supports the possibility of a specific decline in synthesis of mitochondrial DNA-encoded proteins during aging. All of the COX polypeptides, including the COX I, COX II, and COX III subunits, were markedly reduced in preparations from senescent rats. Western analysis showed a 25% decrease in the concentrations of the COX I polypeptide, but no change in the nuclear-encoded COX IV subunit, supporting the view that there is a specific decrement in the expression of the mitochondrial genome with aging. Northern blot analysis showed a marked decrease in COX I and COX II mRNA isolated from freeze-clamped hearts of old animals compared with young ones. As with protein concentrations, mRNA from nuclear-encoded COX IV and other nuclear-encoded mRNAs showed no age-linked decrease. These results suggest that mitochondrial DNA transcription is less active in the aging rat heart.

**Substrates for DNA Repair Studies**: DNA repair assays are done mostly with UV-damaged DNA, and sometimes with DNA damaged by cisplatin. UV-damaged DNA and cisplatin-damaged DNA can be repaired by nucleotide excision repair. However, for a number of our assays, we needed to have oligodeoxyribonucleotides or plasmid constructs that contain single lesions. We now have single-lesion plasmid constructs containing oxidative-damage sites or pyrimidine dimers, situated either on the transcribed or on the coding strand.

**Premature Aging Syndromes**: A number of rare mutations and disorders in humans are associated with premature aging. The patients prematurely have some signs and symptoms associated with normal aging. However, these syndromes are segmental in that some of the features noted in normal aging are not found in the patients.

We are particularly interested in Cockayne syndrome (CS) and in Werner syndrome (WS), which we believe represent optimal model systems for molecular studies of normal human aging. The *WRN* gene, which causes WS, has been recently cloned. The *WRN* gene, the *CS* gene, and other genes mutated in premature aging syndromes encode putative helicases. Therefore, further understanding of the molecular defects in these disorders is a high and achievable priority in the understanding of normal aging. The functions of the CSB protein, which is associated with CS, and of the WRN protein, which is associated with WS, appear to be at the crossroads of aging, DNA transcription, replication, and repair, thereby nicely affording a combination of our interest in DNA function with our interest in aging.

**Transcription and DNA Repair in Cockayne Syndrome Cells**: Cockayne syndrome (CS) is a rare human disease that is characterized by arrested postnatal growth and other features, resulting in premature aging and death. Cells from CS patients are abnormally sensitive to UV light and to chemicals that mimic the action of UV light. CS cells exhibit delayed recovery of DNA and RNA synthesis after UV irradiation. In normal cells, DNA repair of damaged, transcriptionally active genes occurs faster than DNA repair of inactive parts of the genome. Furthermore, damage in template strands of active genes is normally repaired faster than is damage in the coding strand. CS cells are, however, defective in the preferential repair of active genes, and in strand-specific repair. Complementation studies demonstrated at least two genes, designated *CSA* and *CSB*, are involved in CS.

The complex clinical phenotype of CS, however, suggests that DNA repair may not be the primary defect. Moreover, recent evidence from our laboratory demonstrated that intact or permeabilized CSB cells are defective in RNA polymerase II (Pol II) transcription. Furthermore, we compared Pol II transcription in extracts from CS cells with transcription in extracts prepared from normal cells. We found that Pol II transcription in extracts of CS cells is highly sensitive to minor damages in template DNA arising during purification. This deficiency could be complemented by transfection of a CSB cell line with a normal *CSB* gene. These results support the notion that reduced gene-specific repair in CS is a consequence of a transcription deficiency. Clearly, however, further studies are needed to determine the molecular basis of CS.

**Werner Syndrome**: Werner syndrome (WS) is a homozygous recessive disease characterized by early onset of many characteristics of normal aging, such as wrinkling of the skin, graying of the hair, cataracts, diabetes, and osteoporosis. A hallmark defect in WS is genomic instability characterized by karyotypic abnormalities including inversions, translocations, and chromosome losses.

The molecular basis of genomic instability in WS remains to be defined. Our laboratory is using several approaches to identify and characterize the molecular defect in WS cells. One approach is to compare the DNA metabolic activities of WS and normal cells. WS cells are not more sensitive to treatment with DNA damaging chemicals, and do not have defective DNA repair after treatment with these chemicals. However, some WS cells appear to have a subtle defect in TCR and they may also have a lower transcription rate than normal cells. This question is currently under active investigation. Experiments with cell extracts suggest that the decrease in transcription rate is due to the presence of an inhibitor of RNA polymerase II (Pol II) in WS cells which could also explain the defect in TCR.

Cells from WS patients grow more slowly and become senescent at an earlier population doubling than age-matched normal cells, possibly because the WS cells appear to have accelerated losses of the telomeric ends of their chromosomes. Telomeric shortening is an established marker of cellular senescence.

We are also examining the slow growth characteristics of WS cells, emphasizing on determining whether this truly reflects accelerated cellular senescence. Established markers of cellular senescence, such as telomere length and ß-galactosidase activity, are being compared in cultured WS cells and normal cells. We also plan to determine if the cell cycle is disrupted in WS cells.

We are also purifying WRN protein for use in a number of basic and complex biochemical assays. Once purified to homogeneity, the WRN protein will be examined for its activities and potential interactions with other proteins.

**Immunology and DNA Repair**: After antigenic stimulation, genes encoding the variable regions of antibodies undergo somatic hypermutation, contributing to antibody diversity. Dr. Patricia Gearhart's unit is investigating whether DNA repair processes play a role in somatic hypermutation.

We examined a number of knockout mice for DNA repair genes. The results convincingly showed a change in the pattern of somatic hypermutation in mismatch repair-deficient mouse strains. These results suggest that DNA repair processes and DNA polymerase fidelity both play a role in somatic hypermutation. We are also identifying DNA sequences in the variable region that focus the hypermutation on to the gene. To study the mechanism itself, we are looking at the expression of various errorprone DNA polymerases in B lymphocytes..

**Breast Cancer:** Dr. Michele Evans is exploring the efficiency of DNA repair in various forms of breast cancer. Some previous studies have indicated a possible DNA repair defect in individuals with breast cancer and their relatives. Our current explorations involve gene specific DNA repair measurements and also the levels of expressions of repair genes in hormone dependent and independent breast cancer cell lines. We plan to also investigate the DNA repair in old and young breast cancer patients using the cohort in the Baltimore Longitudinal Study of Aging.

**Cell Cycle Progression and DNA Damage Processing**: We have been interested in the relationship between DNA damage processing and cell cycle regulation. We analyzed the effect of DNA damage on the progression of the cell cycle in synchronized Chinese hamster ovary (CHO) cells. We also measured the extent of TCR during different phases of the cell cycle. The results showed that TCR does not vary substantially during different phases of the cell cycle. More recent work involves measuring of cell cycle progression and apoptosis (programmed cell death) after UV exposure of mutant CHO cells that are deficient in different DNA repair pathways. This work showed that accumulation of DNA damage leads to G2 arrest. An important question regards the nature of the signal induced by the DNA damage.

**p21 and DNA Repair**: p21 is a protein that inhibits cyclin-dependent kinases, which in turn regulate the cell-cycle transitions from G1 to S and from G2 to M. p21 appears to be up-regulated in senescent cells. By inhibiting the action of cyclin-dependent kinases, p21 blocks progression through the cell cycle. Transcription of the gene encoding p21 is induced by DNA damage. This induction by DNA damage occurs by mechanisms that are dependent or independent of tumor suppressor p53. Furthermore, p21 is also directly involved in replication via its binding to proliferating cell nuclear antigen (PCNA), a subunit of DNA polymerase. PCNA is involved both in DNA replication and in DNA repair. We are therefore interested in the question of whether p21 plays a role in DNA repair.

**Collaborators:** E.C. Friedberg, University of Texas, Dept. of Pathology, Southwestern Medical Center, Dallas, Texas; J. Hoeijmakers, Erasmus University Rotterdam, Rotterdam, The Netherlands; C.C. Harris, Laboratory of Human Carcinogenesis, NCI; K.H. Kraamer, NCI; A.P. Grollman, State University of New York at Stony Brook; R. Wood, Imperial Cancer Research Fund, Herts, United Kingdom, George Martin, University of Washington, Seattle, Washington.


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**Recent Publications:**


Webb DK, et al. *Exper Cell Res* 1996; 224: 272-278.

Evans MK, et al. *Oncogene* 1996; 12: 651-658.


**Biography:** Dr. Michele K. Evans received her medical degree from the University of Medicine and Dentistry of New Jersey-The Robert Wood Johnson Medical School. She is board certified in both Internal Medicine and Medical Oncology having trained in Medicine at Emory University Affiliated Hospitals and in the Clinical Oncology Program of the National Cancer Institute (NCI). Interest in human cancer-prone disorders and DNA repair has led her to study the role of DNA repair in cancer susceptibility and aging as Senior Clinical Investigator in the Laboratory of Molecular Pharmacology, NCI and currently as a tenure track investigator at the Laboratory of Molecular Genetics, NIA.

**Research**: Tumorigenesis is facilitated by loss of fidelity in the replicative mechanism, accumulation of genetic lesions, and faulty DNA repair mechanisms. Similarly, aging or cellular senescence is characterized by random accumulation of damage or mutation in DNA, RNA, or proteins and perhaps a diminished ability to repair DNA. The increased incidence of cancer as a function of age underscores the mechanistic relatedness of these two cellular processes. The diminished ability to repair DNA appears to be the crucial and convergent factor. However, the consequences are distinct in cancer and aging. The overall thrust of our work has been to understand the role of DNA repair in cellular senescence and cancer susceptibility using heritable human syndromes and tumor cell lines as model systems.

**DNA Repair and Cancer**: Our study of the cancer prone disorder, Xeroderma Pigmentosum (XP), described DNA repair phenotypes for four complementation groups of the syndrome based on analysis of gene and strand-specific DNA repair capacity. Clinical manifestations were also assessed in terms of gene-specific repair capacity resulting in identification of a link between defective transcription coupled repair and acute sun sensitivity. We also confirmed a repair phenotype in XP complementation group C (XPC) in which active genes are repaired despite low levels of bulk DNA repair.

Because mutations in tumor suppressor genes have been implicated in many cancers, we investigated DNA repair characteristics in highly skin cancer prone XPC patients. We found that efficient repair of the *p53* gene is important in UV-induced skin tumorigenesis in XP because defective DNA repair at the gene level directly contributes to cellular transformation in the skin of these patients. These findings also led us to conclude that *p53* may be directly involved in nucleotide excision repair and that heritable syndromes associated with mutations in *p53* may have defective DNA repair pathways. This was explored by study of the Li-Fraumeni Syndrome that is characterized by germ line mutations of *p53* (in most patients), and cancer susceptibility. We were the first to report that nucleotide excision repair is defective in this syndrome. This study also showed that *p53* interacts with XPD, XPB, and CSB; proteins whose association with the transcription factor IIH complex (TFIIH) is indispensable for nucleotide excision repair activity. Our work suggests that p53 may play a direct role in nucleotide excision repair and that mutations in the p53 gene may result in defects of DNA repair that are clinically manifested by cancer susceptibility.

**DNA Repair and Aging:** The accumulation of unrepaired damage to DNA contributes to cellular senescence. DNA repair efficiency may decline in normal human aging. We are studying DNA repair pathways and transcription in cells from patients with segmental progeroid disorders (Werner's syndrome, Hutchinson Gilford syndrome, Rothmund Thomson syndrome) to identify which specific repair pathway may be defective and how alterations in repair and transcription can lead to premature cellular senescence. We studied the ability of Werner's syndrome (WS) lymphoblasts to repair UV-induced DNA damage of transcriptionally active and inactive genes. Gene-specific and strand-specific repair were found to be deficient in the WS lymphoblasts compared with normal controls. However, we found that repair is normal in primary fibroblast cultures from another WS patient. These results do suggest that there is abnormal gene repair in some WS lines. The phenotype is characterized by a milder defect in repair of active genomic regions than seen in another

segmental progeroid disorder which we have also studied, Cockayne's syndrome. Because the repair phenotype differs slightly among the WS patients we studied, it is important to determine the mutational status of the Werner's syndrome gene (*WRN*) in these individuals. Different mutations in this gene could affect the function of the gene product a putative helicase in different ways. We are correlating the currently available information about the *WRN* mutations present in the studied patients with their DNA repair characteristics.

**Breast Cancer and DNA Repair**: Since breast cancer is predominately a disease of older women, if DNA repair declines as a function of age in all tissues, this would result in the accumulation of both environmental and endogenous DNA damage. Data suggesting that DNA damaging agents produce mutagenic lesions in exposed breast tissue implies that defective DNA repair of these lesions may be an early step in breast tumorigenesis. This idea is further supported by our own work and that of others suggesting that diminished DNA repair capacity may be an important risk factor in heritable and sporadic breast cancer. There is currently little available knowledge concerning the role of specific forms of DNA damage or the proficiency of specific repair pathways in breast cancer susceptibility and progression. We have begun to characterize the proficiency of DNA repair mechanisms required to remove mutagenic lesions from human breast tissue.

**Collaborators:** David Orren, Ph.D., Adabalayma Balajee, Laboratory of Molecular Genetics, NIA; and Colette ApRhys, Ph.D., Johns Hopkins University.

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Winter DB, et al. *Molecular Immunol* 1997; 34: 359-366.

Winter DB, et al. *Current Topics Microbiol Immunol* 1998; 229: 1-10.

**Biography:** Dr. Patricia Gearhart received her Ph.D. from the University of Pennsylvania in 1974. She performed postdoctoral training at the Johns Hopkins University and was a staff associate at the Carnegie Institution of Washington until 1982. She then became a faculty member at the Johns Hopkins University until 1995 when she moved to her present position in the Laboratory of Molecular Genetics, NIA.

**Somatic Hypermutation of Immunoglobulin Variable Genes:** Somatic hypermutation of variable (V) genes, which encode a portion of immunoglobulin molecules, occurs at a frequency that is a million times greater than mutation in other genes. The molecular mechanism that introduces these mutations is unknown. Our project has three aims.

**Mismatch Repair and Hypermutation**: The first goal is to study the mechanism that causes hypermutation of the V region. It is likely that a polymerase introduces substitutions into the DNA strands during replication or repair. If so, then immediately following hypermutation, mismatched base pairs will be present along the sequence. The mismatch repair pathway includes several proteins that recognize mismatched base pairs, excise them, and resynthesize the DNA. Thus, the mismatch repair pathway may attempt to remove some of the hypermutations before they are replicated into both strands. This predicts that the type and distribution of mutations will be different in V genes from mice defective in genes for mismatch repair. By removing the veil of mismatch repair, it may be possible to see what the original pattern of hypermutation looks like before repair acts on the mutations. We studied hypermutation in V genes from mice deficient for one of the repair proteins, PMS2, and found that although the frequency of mutation was similar to wild-type mice, the pattern was altered. In the mutant mice, there was an accumulation of adjacent base substitutions, and less bias for some types of mutation to appear on one of the two DNA strands. The data suggest that (1) tandem mutations are generated at a high frequency by a polymerase during a single event, and (2) mutations are introduced into

both strands of DNA and then preferentially removed from one strand during mismatch repair. We are now examining the pattern of mutation in mice deficient for other proteins in the mismatch repair pathway, which so far has revealed novel findings about the hypermutation mechanism.

**Localization of Hypermutation to the V Gene**: The second goal is to identify DNA sequences around the V gene that activate the hypermutation mechanism. The concentration of mutations in a two-kb region surrounding the rearranged V gene implies that *cis* DNA sequences act as signals to target the mechanism. We propose that hypermutation is localized to the V region because the DNA sequences form stem-loop structures during transcription. Single-stranded DNA in the loops is then cut by a single-stranded endonuclease. The loops resolve back into a duplex, the nicks are made into short gaps by an exonuclease, and a DNA polymerase fills in the gaps. The resynthesis step is error-prone and introduces mutations. Mismatch repair attempts to remove the mismatches but is overwhelmed by the large number of mutations and leaves some behind, which are then replicated into both strands. This model makes the prediction that the DNA sequence around the V gene can form stable cruciforms; we are currently testing for these structures using several biochemical techniques.

**Hypermutation in Old Humans**: The third goal is to analyze hypermutation in V genes from old humans. As described above, we have recently correlated several patterns of hypermutation with different proteins in the mismatch repair pathway. By studying the frequency and pattern of hypermutation in old people, it will be possible to determine if the hypermutation and/or mismatch repair pathways have decreased. Genes are cloned from RNA made from peripheral blood lymphocytes taken from old and young humans, and are sequenced to identify mutations. The results will indicate if there is an age-related decline in the hypermutation mechanism, which will produce a lower number of mutations per gene, and in the mismatch repair mechanism, which will produce a different pattern of mutations.

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Filburn CR, et al. *Mech Age Develop* 1996; 87: 35-46.

Hansford RG, et al. *J Bioenerg Biomembr* 1997; 29: 89-95.

Moyes CD, et al. *Am J Physiol* 1997; 272: C1345-C1351.


**Biography:** Dr. Richard Hansford received his Ph.D. from the University of Bristol, United Kingdom, in 1968. After a postdoctoral fellowship with Dr. Albert Lehninger at Johns Hopkins University and a period as a lecturer at the University of Wales in Cardiff, he joined the NIH in 1973. Dr. Hansford and his group have worked mainly on the control of mitochondrial metabolism. Their move to the Laboratory of Molecular Genetics in 1996 reflected an increased emphasis on changes in mitochondrial DNA occurring in aging and implications for energy transduction.

**Production of Reactive Oxygen Species by Mitochondria:** The reactive oxygen species (ROS) theory of aging maintains that the balance between the rate of formation of ROS and the rate of their removal tips in favor of formation with aging, such that oxidative damage becomes cumulative. As the majority of ROS are generated by mitochondria in most cell types, the mitochondrion becomes a likely target for oxidative damage. Oxidation of mt-DNA has been suggested to be particularly important, as it may lead to mutations which may be transmitted to subsequent generations of mitochondria. Several of us in LMG are beginning to critique different aspects of this hypothesis. We have confirmed literature findings of rates of $H_2O_2$ formation of approximately 1% of total electron transport, when the respiratory substrate is succinate at 5mM or higher. However, physiologically appropriate concentrations of succinate or the alternative substrates pyruvate, glutamate or palmitoylcarnitine give limitingly low rates. Thus we would conclude that literature findings using saturating succinate concentrations should be evaluated with caution. We have

sought to identify the parameters that make high succinate concentrations so peculiarly effective in ROS generation. Evidently, both a very high degree of reduction of Complex 1 (NADH dehydrogenase), which we identified as the radical generator in inhibitor studies, and a very high mitochondrial membrane potential are needed. We could not reproduce the literature finding of increased activity of $H_2O_2$ formation by heart mitochondria in senescence. However, oxidative stress is the result of formation and removal of ROS, either (or neither) of which might be altered in aging. The only way to determine the degree of oxidative damage is to measure it, and we have embarked upon such studies (see below).

**Measurement of Oxidative Damage to mt-DNA and Rates of Repair: also Incidence of Deletions**: The model of aging of post-mitotic tissues hypothesizes that oxidation of the bases of mt-DNA leads to mutations and deletions which in turn limit the ability to maintain cellular adenine nucleotide phosphate potentials. There are several gaps in this model. One is that the only oxidized base product of mt-DNA which has been measured is 8-oxodG, and estimates of its fractional occurrence vary over three orders of magnitude. Another missing piece in the argument is that, although some correlation between 8-oxodG levels and the so-called "common deletion" of mt-DNA has been established, it is not known that there is a cause-and-effect relationship. Finally, it has not been shown in a normal aging tissue that the incidence of deletions of mt-DNA actually rises to the point that energy transduction is compromised. Quantitative arguments become difficult as it has been suggested that deletions show a mosaic distribution amongst cells. This necessitates a cell-by-cell examination of both mt-DNA deletion burden and some index of mitochondrial functioning. This is now being approached, in a project on human T cells. Specifically, we have begun to measure the products of base oxidation in mt-DNA prepared from organs of young adult and senescent rats. We are combining enzymic measurements, using fpg-glycosylase, which cleaves 8-oxodG lesions, with chemical determinations using GC/MS and HPLC/EC methods. Initial results do not substantiate the notion of a generalized increase in base oxidation with aging. In order to study mitochondrial function on a cell-by-cell basis, we are sorting T cells from blood samples of BLSA participants on the basis of mitochondrial membrane potential, as measured with the fluorescent cation JC-1. We then use PCR methods to both quantify the so-called "common 4.8 Kb deletion" and to indicate the presence of other mutations and base damage, using LC PCR. The question is whether increased mt-DNA damage correlates with lower mitochondrial membrane potential.

**Chronic Regulation of Mitochondrial Structure and Function**: The reason for our interest in regulation of mitochondrial activities at the level of gene expression is two-fold. First, there is a decrease in some mitochondrial enzyme activities as a function of aging, though there is by no means a generalized decline. Second, there appears to be a decreased ability to up-regulate the tissue content of mitochondria in response to work-load: this is part of the reason for the frailty of the aged. We have chosen to emphasize the enzyme cytochrome c oxidase, as we have previously shown a decrease in specific activity of this enzyme in both heart and skeletal muscle of aged rats. Cytochrome oxidase (COX) catalyzes the terminal step of electron transfer to $O_2$ and exerts substantial control over oxidative phosphorylation. Further, it consists of three subunits (COX I, II, III) encoded on the mitochondrial genome, as well as several encoded on nuclear DNA. The regulation of the synthesis of the protein complex thus involves the coordinated expression of the two genomes. We have shown a decrease with aging in the COX activity of rat heart mitochondria, which is associated with a decrease in the content of the mitochondrial-synthesized polypeptides COX 1 and 11. Further, we have shown decreased rates of incorporation of radiolabelled methionine into mitochondrial proteins in general, and COX 1 and 11 subunits in particular. There is also a decrease with aging in the overall rate of transcription, as measured with isolated mitochondria and radiolabelled UTP. However, Northern analysis of individual mRNA species revealed no decrease in the message for COX 11, whereas message for COX 1 was decreased. Thus, there are issues of message stability which need to be addressed. It is known that the transcription of mt-DNA is regulated, at least in part, by a factor designated MTF-1. In turn, transcription of the gene for MTF-1 may be regulated by a factor named NRF-1. This is of profound interest, as NRF-1 regulates transcription of a number of mitochondrial enzymes which are nuclear-encoded. The obvious questions are whether NRF-1 levels are lower in the aging heart, and if so, why? We are studying these issues.

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# The Molecular Defect Responsible for Premature Aging of Werner's Syndrome Patients

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Webb DK, et al. *ExperCell Res* 1996; 224: 272-278.

Balajee AS, et al. *Proc Nat Acad Sci* 1997; 94: 4306-4311.

Orren D, et al. *Mol Biol Cell* 1997; 8: 1129-1142.

Gilchrest B, et al. *FASEB J* 1997; 1: 322-330.

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Werner's Syndrome (WS) is a homozygous recessive disease characterized by early onset of many characteristics of normal aging, such as wrinkling of the skin, graying of the hair, cataracts, diabetes, and osteoporosis. The symptoms of WS begin to appear around the age of puberty, and most patients die before age 50. Because of the acceleration of aging in WS, the study of this disease will hopefully shed light on the degenerative processes that occur in normal aging.

Cells from WS patients grow more slowly and senesce after fewer population doublings than age-matched normal cells, possibly because these cells appear to lose the telomeric ends of their chromosomes at an accelerated rate. Telomeric shortening is an established marker for cellular senescence. In general, WS cells have a high level of genomic instability, with increased amounts of DNA deletions, insertions, and rearrangements. These effects could potentially be the result of defects in DNA repair, replication, and/or recombination, although the actual biochemical defect remains unknown. The gene that is defective in WS, the WRN gene, has recently been identified. The amino acid sequence suggests that the WRN gene is a member of a large family of helicases with the putative ability to unwind DNA or RNA duplexes. Helicases play roles in a number of DNA involving processes: transcription, replication, DNA repair and chromatin structural organization.

Our laboratory is using several avenues to identify and characterize the biochemical defect in WS cells. One approach is to compare specific DNA related activities of normal and WS cells. After treatment with certain DNA damaging agents, both the cellular sensitivity and levels of overall DNA repair in WS cells is not elevated. However, WS cells appear to have a subtle defect in transcription-coupled repair, the highly efficient removal of lesions from the transcribed strand of active genes. Moreover, a survey

of overall transcription in cells from premature aging syndromes indicated that WS cells have lower transcription rates than normal cells *in vivo.* Experiments with cell extracts suggest that this decrease in transcription might be due to the presence of an inhibitor of RNA polymerase II in WS cells. Other studies are aimed at determining whether this shortfall in total transcription might represent lower transcription of particular subsets of genes. The observed inhibition of RNA polymerase II transcription might also explain the loss of transcription-coupled repair. Studies regarding the transcription and repair activities of WS cells are ongoing; in particular, the establishment of a WS cell line transfected with the normal WRN gene will allow us to assess whether these repair and transcription problems are manifestations of the primary molecular defect in WS.

Our laboratory is also examining the slow growth characteristics of WS cells, with emphasis on determining whether this truly reflects an accelerated cellular senescence. Established markers of cellular senescence such as telomere length and beta-galactosidase activity are being compared in nontransformed WS and normal cells. We also plan to determine whether and how the cell cycle is disrupted in WS cells, reflecting the earlier observation of an elongated S phase in some WS cell lines. The initiation and elongation of replication are prime targets of study in this area, as is the response of WS cells to DNA damage during ongoing replication.

With the recent cloning of the WRN gene, our laboratory has also set our sights on obtaining purified WRN protein for use in a number of basic and complex biochemical assays. Thus far, the WRN gene has been inserted into a baculovirus vector that has been transfected into insect cells, which will putatively allow overproduction and subsequent purification of significant quantities of WRN protein. Once purified to homogeneity, WRN protein will be examined for its biochemical activities and potential interactions with other proteins.

Although progress is being made, the true nature of the biochemical defect(s) in WS is still a mystery, as is the nature of the processes that occur in cellular senescence and normal human aging. Our ongoing and future studies will be directed towards elucidation of the causes of the accelerated aging phenotype in WS, with hope that this knowledge can also be applied to our current understanding of both the aging of cells and organisms in general.

**Collaborators:** Drs. George Martin and Junko Oshima, University of Washington, Seattle, Washington; Anni Andersen, University of Aarhus, Aarhus, Denmark, Brian Clark, University of Aarhus, Denmark.

## Oxidative DNA Damage Processing

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One theory of aging holds that oxidative damage to cellular components, such as proteins, lipids and DNA, accumulates with age, leading to the cellular dysregulation that result in the process of aging experienced by the organism. We are interested in understanding how exogenous and endogenous sources of reactive species produce oxidative damage in DNA, how that damage is processed in human cells, and the effects of unrepaired damage. Reactive oxygen species produce a wide variety of products in DNA. Differences in how these lesions are processed have made the repair of oxidative damage in DNA difficult to understand. In addition to the complex chemistry of the reactions of these reactive species with DNA and the multiple pathways involved in their repair, at least two of these species also act as intracellular messengers affecting the control of cellular processes. We seek to tease apart these complexities by introducing well defined oxidative lesions into DNA in cells *in vivo* or by studying the reactions of cell extracts or purified proteins with DNA containing well defined lesions *in vitro.*

**Repair of DNA Damage Induced by Photoactivated Methylene Blue By Human Whole Cell Extracts**: The damage produced in double-stranded DNA by exposure to visible light in the presence of methylene blue consists almost exclusively of the lesion, 8-oxodeoxyguanosine. This lesion is also produced in significant amounts in DNA, along with many other products, by exposure to gamma irradiation or hydrogen peroxide. By examining the repair of the methylene blue-damaged DNA by proteins extracted from human cells, we have identified two pathways of repair. The kinetics of the two pathways are distinctly different, suggesting different mechanisms involved in repair. In addition, the proportion of the lesions processed by each pathway differs markedly in cell lines derived from patients with the genetic diseases, xeroderma pigmentosum-Group A

and Cockayne syndrome-Group B, in comparison with a cell line from an unaffected individual. Both xeroderma pigmentosum and Cockayne syndrome cells have characteristic defects in the repair of damage induced by ultraviolet light and in the processing of other types of oxidative damage.

**Age-Associated Effects in the Repair of Oxidative Damage By Human Cell Extracts:** Previously, 8-oxodeoxyguanosine has been studied as the prototypical oxidative lesion in association with aging. To test whether the two pathways for the repair of oxidative damage identified in the above study are differentially affected by the process of aging, we are examining the repair capacity of extracts derived from subjects with a range of ages. From this study, we expect to determine the variation in the repair of one type of oxidative damage in the normal population and discern any age-associated effect on these pathways.

**DNA Repair Defect in Alzheimer Disease:** Recent work in other laboratories, using an indirect technique reflective of DNA repair capability, has suggested that cells from Alzheimer's disease patients are defective in the processing of DNA lesions induced by irradiation with fluorescent light. We are using more traditional measures of DNA repair to assess the relative repair capacity of cells from normal and Alzheimer disease patients for various types of oxidative damage.

**Collaborators:** M. Dizdaroglu, National Institute of Standards and Technology, Gaithersburg, MD; J.M. Egly, Centre National Research, Strausbourg, France; C. Cullinane, LaTrobe University, Australia; Don Jerina, NIDDK, NIH; Jane Sayre, NIDDK, NIH; C.C. Harris, NCI, NIH; E. Appella, NCI, NIH.

# Transcription and DNA Repair in Cockayne Syndrome Cells

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Balajee AS, et al. *Proc Natl Acad Sci* 1997; 94: 4306-4311.

Orren D, et al. *Nucleic Acids Res* 1996; 24(17): 3317-3322.

Dianov GL, et al. *Nucleic Acids Res* 1997; 25: 3636-3642.

**Group Members:** Grigory L. Dianov, Ph.D., A.S. Balajee, Ph.D., Morten Sunesen, Alfred May, David K. Orren, Ph.D., Robert Brosh, Ph.D., and Vilhelm A. Bohr, M.D., Ph.D.

Cockayne syndrome (CS) is a rare human disease characterized by arrested post-natal growth and resulted in premature aging and death. Cells from CS individuals are abnormally sensitive to killing by ultraviolet radiation as well as certain so-called UV-mimetic chemicals, such as 4-nitroquinoline-1-oxide and N-acetoxy-2-acetylaminofluorene. This cellular phenotype prompted extensive studies on the ability of CS cells to carry out nucleotide excision repair both in intact cells and in cell-free systems. Most conventional assays, including the use of a cell-free system that supports transcription-independent nucleotide excision repair, indicate no defect in CS cells. However, CS cells are defective in the enhanced rate of repair of the template (transcribed) strand relative to the coding (non-transcribed) strand of transcriptionally active genes. In recent experiments from this laboratory, we have demonstrated that mutations in the CSB gene are the cause of the transcription coupled repair defect. In hamster cells homologous to CSB, we can transfect a normal CSB gene and complement the repair defect. The mechanism of TCR in eukaryotes remains to be elucidated, and the CSB protein appears to play an important role in this process.

These observations and the discovery of the dual function of transcription factor II H (TFIIH) in transcription and DNA repair, led to the "transcriptional hypothesis" which postulates that transcription defects are the underlying basis for the pathology in some human diseases including Cockayne's Syndrome. In this laboratory, we were interested in testing this hypothesis experimentally, and we have demonstrated a reduced level of RNA polymerase II (Pol II) transcription in intact and permeabilized CS-B cells. The molecular mechanism responsible for this deficiency was further investigated in a cell-free system. We utilized an *in vitro* transcription assay and determined Pol II transcription activity in extracts

prepared from different CS cell lines in comparison to extracts prepared from normal cells. We found that *in vitro* transcription in CS extracts is highly sensitive to minor damage in template DNA arising during purification. This deficiency may be complemented by transfection of a CS-B cell line with a normal CSB gene.

Studies of transcription *in vitro* in a plasmid based system demonstrate a significant transcription defect in CSB cells. This defect may be related to oxidative damage or structural changes in the DNA which somehow affect the transcription in CSB cells but not in normal cells. Experiments in intact cells also demonstrate a defect in basal transcription which can be complemented by transfection with the normal CSB gene. Further, these experiments suggest that CSB cells may have a defect in the assembly of the higher order chromatin structural organization in conjunction with transcription and DNA repair. This is supported by the observation that CSB chromatin is much more sensitive to detergent than normal chromatin.

**Future Directions:** Our data suggest that a defect in CS cells may be due to increased sensitivity of RNA polymerase II transcription to DNA damage or/and accumulation of some unidentified DNA damage in CS cells. The future aim is to identify a possible DNA repair deficiency and to understand the mechanism of increased RNA polymerase II transcription sensitivity to DNA damage in CS cells.

**Collaborators:** E.C. Friedberg, University of Texas Southwestern Medical Center at Dallas.

**Stanley I. Rapoport, M.D., Chief**

# Laboratory of Neurosciences

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The Laboratory of Neurosciences (LNS) was established in 1978 at the Gerontology Research Center in Baltimore. In 1984, the LNS moved to the Clinical Center on the Bethesda Campus, where clinical research could be performed using outpatient and inpatient facilities and available brain imaging capabilities. The LNS currently is divided into two sections that were formed in 1982: a basic research Section on Cerebral Physiology and Metabolism (Stanley I. Rapoport, M.D.) and a clinical research Section on Brain Aging and Dementia (Mark B. Schapiro, M.D., Chief).

The basic and clinical sections of the LNS interact to study brain function, metabolism and structure with regard to aging and disease, including Alzheimer's disease, Down's syndrome (which inevitably leads to Alzheimer-like neurodegeneration) and hypertension. Clinical protocols use positron emission tomography (PET) to measure regional brain metabolism and blood flow, functional (fMRI) and structural magnetic resonance imaging to measure brain blood flow and volumes of brain structures, magnetic resonance spectroscopy to measure brain metabolites such as *myo*-inositol and phosphorus compounds, and neuropsychological and behavioral assessment to evaluate normal cognition and dementia. Protocols are designed to quantify reorganization of brain functional networks, brain atrophy and changes in brain metabolites in relation to aging and disease, to identify and characterize the pre-clinical and clinical phases of Alzheimer's disease, and to evaluate pharmacotherapeutic efficacy in aging and Alzheimer's disease. Activation studies involving PET and fMRI use cognitively task or passive stimulation that are varied parametrically with regard to stimulus intensity and/or difficulty, as "stress tests" of brain function. The effects of physostigmine (cholinergic antagonist) and other modulators of neurotransmission on performance and brain responses are evaluated in tests. Studies are related to genetic markers (apolipoprotein E alleles) and post-mortem brain studies. An hypothesis being tested is that Alzheimer's degeneration reflects progressive synaptic and related metabolic-flow dysfunction, particularly in brain association areas, and that the first stage of this dysfunction is potentially reversible whereas the later stage is not. We currently are

examining central cholinergic mechanisms using PET in relation to this hypothesis, using labeled ligands of brain cholinergic receptors, muscarinic agonists and antagonists (arecoline and physostigmine), and labeled arachidonic acid to study cholinergic signal transduction.

In the basic section, post-mortem brain from clinical control and Alzheimer's patients is subjected to histochemical, molecular, immunocytochemical and enzymatic techniques to examine the basis of reduced glucose metabolism and blood flow in life. Evidence for reduced expression of genes coding for subunits of mitochondrial oxidative-phosphorylation enzymes supports staging of metabolic dysfunction into potentially reversible and irreversible steps. Additionally, animal models for Down syndrome (trisomy 21)-- trisomy 16 and trisomy 65DN mice (the 16th mouse chromosome corresponds to human chromosome 21)-- are developed to understand mental retardation in that disorder. Abnormal ionic currents and ionic channel densities in cultured neurons from these models, and accumulation of *myo*-inositol in their brain, suggest specific defects in signal transduction which may contribute to mental retardation. Thirdly, an experimental *in vivo* model has been developed in the LNS to quantify brain membrane remodeling and signal transduction involving phospholipids, using radiolabeled long chain fatty acids. The model is elucidating changes in phospholipid metabolism during recovery from cerebral ischemia, in an animal model for Alzheimer's disease (nucleus basalis lesion), with regard to the mechanism of action of lithium, and in relation to central drug action. It recently has been extended to clinical PET studies (see above). Finally, an analytical laboratory quantifies concentrations of drugs and their metabolites in clinical studies and related basic experiments.

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energy metabolism

**Recent Publications:**

Hatanpää K, et al. *Ann Neurol* 1996; 40: 411-420.

Rapoport SI, *Lipids* 1996; 31: S97-S101.

Rapoport SI, et al. *Neurodegeneration* 1996; 5: 473-476.

Stoll J, et al. *Int J Dev Neurosci* 1996; 14: 749-760.

**Biography:** Dr. Rapoport received his M.D. from Harvard Medical School in 1959, interned in Medicine at Bellevue Hospital, New York, from 1959-1960, and received post-doctoral research training at the Department of Physiology, University of Uppsala, Sweden, and at the Laboratory of Neurophysiology, National Institute of Mental Health (NIMH). He was appointed as a tenured scientist at NIMH in 1968, and in 1978 Chief of the Laboratory of Neurosciences, NIA. He is a Fellow of the American College of Neuropsychopharmacology, the American Academy of Neurology and the Gerontological Society of America.

The Section on Cerebral Physiology and Metabolism studies basic aspects of brain function and metabolism, particularly in the following areas:

**Markers of Oxidative Phosphorylation (OXPHOS) Within Brain Mitochondria Suggest Two Stages of Functional Failure in Alzheimer Disease:** *In vivo* brain imaging in Alzheimer's disease (AD) patients using positron emission tomography (PET) demonstrated reductions in cerebral glucose consumption and blood flow, progressing with dementia severity. Post-mortem brain studies showed that the reductions corresponded to reduced regional brain activity of cytochrome oxidase (rate limiting enzyme for mitochondrial oxidative phosphorylation, OXPHOS). Furthermore, mRNA levels for mitochondria (mt)-encoded subunits COX I-III and for nuclear-encoded subunit COXIV were reduced, whereas mt-encoded 12S rRNA and total mtDNA were unchanged. A similar pattern of reduction (down regulation) has been reported in monkey lateral geniculate nucleus following visual deprivation, and can be reversed following restoration of vision. We then quantified COX III mRNA by *in situ* hybridization in individual pyramidal AD neurons in relation to cytoplasmic density of neurofibrillary tangles. Compared with tangle-free neurons from control brains, tangle-free AD neurons showed reduced COX III mRNA but no change in mt-encoded 12s rRNA or polyadenylated mRNA. COX III mRNA fell in relation to tangle content until tangles filled more than 50% of the neuronal cytoplasm, when

non-OXPHOS RNA levels also were reduced. We hypothesize that an early event in AD is dysfunction of synapses (where most energy is consumed), leading to reduced neuronal energy demand and potentially reversible down-regulation of OXPHOS. This is consistent with our evidence that the AD brain can be fully activated early in disease. In later stages of disease, interference with mitochondrial delivery to distant dendrites by cytoplasmic neurofibrillary tangles likely causes irreversible neuronal loss. In the next year, we intend to evaluate our hypothesis by identifying the factors that couple neuronal activity to expression of OXPHOS in the brain and how they may be affected in AD, and by quantitatively relating markers of early synaptic dysfunction in AD to OXPHOS gene expression.

**Mouse Models for Mental Retardation in Down Syndrome:** Down syndrome (DS, trisomy 21) is the major known genetic cause of mental retardation (1/1000 births) and leads inevitably to AD neuropathology after age 35. Our section uses molecular and electrophysiological techniques to examine functional and metabolic deficits in animal models of DS: mouse trisomy 16 (which dies in utero) and partial trisomy (65DN) (which survives and shows learning deficits), where the 16th mouse chromosome contains genes found on human chromosome 21. Hippocampal fetal Ts16 neurons in primary culture showed abnormal action potentials and abnormal ionic currents. A reduced inward Na current corresponded to reduced membrane Na channel density. Normal levels of mRNA's coding for Na channel subunits suggested post-transcriptional defects in Na channel expression. Additionally, *myo*-inositol was elevated by 50% in the Ts 65DN mouse brain and in cerebrospinal fluid from DS subjects, possibly reflecting localization of a myoinositol transporter gene on mouse chromosome 16 and human chromosome 21. Excess *myo*-inositol may interfere with the phosphatidylinositide cycle involving phospholipase C. Thus, retardation in DS may arise from abnormal signal transduction arising from a number of causes. Future research will study the mechanisms of the observed changes.

***In vivo* Imaging of Brain Phospholipid Metabolism:** Phospholipids are major constituents of cell membranes and participate in neuroplastic remodeling and signal transduction. We developed in rats an *in vivo* method and model to localize and quantify brain phospholipid metabolism, and turnover of fatty acids within specific sites of brain phospholipids. A radiolabeled long chain fatty acid (unsaturated arachidonate or docosahexaenoate, saturated palmitate) is injected intravenously and its rate of incorporation into brain is measured using quantitative autoradiography and chemical analysis. With this model, we showed in rats that recovery from the massive release of fatty acids due to

cerebral ischemia is promoted by selective reincorporation of arachidonic acid (precursor for prostaglandins and prostacyclins) into brain phospholipids. Lithium, used clinically to treat manic depressive disorder, reduces arachidonate turnover by some 80% without affecting turnover of docosahexaenoate and palmitate, and thus likely acts at phospholipase A2. Additionally, 11C-labeled fatty acids were synthesized in collaboration with the PET Department at NIH and were used to image phospholipid metabolism of monkey brain with PET (tracer uptake was independent of blood flow) and to initiate a clinical PET protocol on healthy controls and patients with AD. In the coming year, we plan extend this protocol to evaluate cholinergically-mediated signal transduction in AD patients, to develop a fluorescent method to measure regional phospholipase A2 activity in brain slices in relation to local fatty acid metabolism, and to phospholipase A2-mediated signal transduction involving the brain dopaminergic system.

**Collaborators:** Charles Epstein, Department of Pediatrics; University of San Francisco School of Medicine; William Eckelman, PET Department, CC, NIH; Nancy Lane, University of Cambridge, UK; Scott Eleff, Department of Anesthesiology, Johns Hopkins School of Medicine; Greg Gillen, National Institute of Standards and Technology; Jeff Alger, Department Radiological Sciences, UCLA School Medicine; Alfred Yergey, Laboratory of Cellular and Molecular Biophysics, NICHD.


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**Recent Publications:**


Dani A, et al. *NeuroReport* 1996; 7: 2933-2936.

Pietrini P, et al. *Am J Psychiatry* 1997; 154: 1063-1069.

Krasuski JS, et al. *Biological Psychiatry* 1998; 43: 60-68.

Alexander GE, et al. *NeuroReport* 1997; 8: 1835-1840.


**Biography:** Dr. Schapiro received his M.D. from the University of Tennessee in 1976. After completing residencies in Pediatrics and Neurology, he served in the Laboratory of Neurosciences in 1983 as a Medical Staff Fellow and, later, a Senior Staff Fellow before becoming Chief, Section on Brain Aging and Dementia in 1990.

**Neurodevelopmental Disorders: Down Syndrome:** One goal of the Section on Brain Aging and Dementia is to understand the genetic determinants of brain aging using *in vivo* brain imaging in relation to mental retardation and vulnerability to Alzheimer's disease (AD). Our work has focused on Down syndrome (DS), a disorder in which an overexpression of different genes on chromosome 21 leads to mental retardation and dementia. In studies designed to examine the hypothesis that defective signal transduction causes mental retardation in DS, we showed a 50% increase in *myo*-inositol (a molecule involved in signal transduction) that is related to cognition, suggesting a gene dose effect of the extra chromosome 21 on which the human sodium/*myo*-inositol cotransporter gene is located. We will follow up these findings with a study in DS adults who have received lithium, which disrupts *myo*-inositol recycling. Additionally, we reported a 50% increase in choline (a precursor of membrane phospholipids and the neurotransmitter acetylcholine), consistent with a gene dose effect and suggesting that a locus on chromosome 21 is responsible for choline homeostasis.

**Genetics and the Preclinical Stages of Alzheimer's Disease:** Another goal of the Section is to develop techniques for the early diagnosis of AD, since AD may be more reversible in the earlier rather than the later stages of disease. To develop such techniques, we studied older DS adults who develop AD after age 40 years. We showed that the dementia syndrome in DS is phenotypically similar to AD and that the dementia syndrome can occur without the mental retardation, suggesting that only a portion of the

brain cells need to be trisomic for chromosome 21 genes for the development of dementia. We currently are testing this hypothesis with an *in situ* hybridization study of the brain to quantify the location and degree of trisomic cells.

Because there is variation in age of onset of dementia and survival in DS, we studied the influence of other genetic factors. We showed that two genetic factors (amyloid and apolipoprotein E4) can interact to accelerate the development of AD in DS. We further showed that language function, which is relatively preserved in the preclinical stages of AD in DS, is related to APOE genotype. We are continuing these studies to understand how changes in brain metabolism are related to APOE genotype.

Longitudinal evaluation of cognition, brain structure, and resting metabolism showed 2 phases of decline in older DS subjects. There was first a stable phase of at least 7 years preceding onset of dementia with no alteration in brain function or structure. This phase was followed by a linear decline in cognition, structure and function coincident with onset of dementia, suggesting a fundamental change in brain physiology with onset of dementia.

Given the stability of the first phase, we developed methods for the preclinical detection of AD. We showed that subjects in the preclinical phases of AD have medial temporal lobe atrophy which selectively correlates with memory performance, showing that the medial temporal lobe is the earliest site of AD. We plan to apply a discriminant analysis method to medial temporal lobe volumes to identify subjects at risk for AD on the basis of other genetic markers.

We also have identified brain changes in the preclinical stages of AD using PET scanning. By examining brain metabolism while the subjects rest with a discriminant analysis, we were able to identify subjects at risk as having AD. Further, an activation PET scanning procedure showed abnormalities in regions known to be affected in AD in DS subjects in the preclinical stages of AD. These findings show that analysis of PET metabolic data can be used for early detection of AD in individuals at risk. We plan to continue our studies of early diagnosis in DS and in other genetic models of AD using PET scanning with other activation paradigms developed in our Laboratory. We further plan to refine techniques that will quantitate an individual’s risk of AD using resting and activation PET data. Finally, based on these techniques, we plan to initiate therapy early and evaluate the effectiveness of treatment with the above techniques.

**Mechanisms of Disease**: A final goal of the Section is to conduct experiments on the pathophysiology of AD in order to understand the cause of the treatment failure. The implications of loss of synapses (which are necessary for effective neurotransmitter replacement) in AD are being studied with PET scanning. We recently combined PET with psychophysical stimulation and showed that AD patients in the early to middle stages of disease retain the capability to respond to stimulation despite baseline resting hypometabolism. However, in the later stages of disease, there is a loss of the capability to respond to stimulation up to the point where no response occurs. Such findings emphasized that different stages of AD may respond differently to therapies. PET is now being combined with cognitive and pharmacologic stimulation in order to further study the efficacy of neurotransmission and compensatory mechanisms as a function of disease severity. Currently, cognitive stimulation paradigms are being used that require active participation (ie, working memory, and attention) or require only passive viewing (ie, viewing a movie, visual textures, flashing lights). Further, drugs are being used to modulate the degree of activation from cognitive stimulation. Additionally, to understand the postmortem findings of loss of presynaptic M2 cholinergic receptors but not M1 postsynaptic receptors, a new ligand to examine specific muscarinic receptor densities has been developed in collaboration with the Nuclear Medicine Department, Clinical Center.

We are also examining intracellular signal transduction as a result of postmortem findings suggesting that it is compromised in AD and results in failure of neurotransmission. Metabolites of phospholipid metabolism that are involved in signal transduction (i.e., *myo*-inositol, choline) are being measured in AD and older DS adults with $^{1}H$ magnetic resonance spectroscopy. Additionally, an *in vivo* brain imaging method to study receptor mediated stimulation of phospholipid metabolism through activation of phospholipase A2 is being conducted. This method will allow quantitation of the regionally selective turnover of the fatty acid arachidonate in the sn-2 position of phospholipids at rest and during stimulation.

**Collaborators:** Umesha Shetty, LNS; Melvin Ball, M.D. and Geoffrey Murdoch, M.D., Ph.D., Oregon Health Sciences University; Ann Saunders, Ph.D. and Allen Roses, M.D., Duke University Medical Center; Katherine Sanford, Ph.D., NCI; Jay Robbins, M.D., NCI; Neill Graff-Radford, M.D., Mayo Clinic Jacksonville.


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McIntosh AR, et al. *Cerebral Cortex* 1996; 6: 571-584.

Rumsey JM, et al. *Brain* 1997; 120: 739-759.


**Biography:** Dr. Horwitz was trained as a theoretical physicist at the University of Pennslyvania, where he received his Ph.D. He taught physics at King's College (Wilkes-Barre, PA), Vassar College, and Texas Women's University before moving to the Laboratory of Neurosciences as a Senior Staff Fellow in 1982. He became a tenured Research Mathematician in 1988. His major research interests focus on using functional brain imaging data and neural modeling methods to understand how the brain performs specific cognitive tasks, and how these are altered by aging and disease.

**Network Modeling of Neuroimaging Data:** The major goal of the Brain Imaging and Computers Unit is to develop methods to understand how the brain constructs networks of interacting regions (i.e., neural networks) to perform cognitive and sensorimotor tasks. In particular, a major focus concerns how these networks are altered during healthy aging, and in brain diseases such as dementia. These issues are addressed by combining computational neuroscience techniques with functional neuroimaging data, obtained using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). The network analysis methods developed in this laboratory allow us to evaluate how brain operations differ between tasks, and between normal and patient populations. With respect to disease, this research allows us to ascertain which networks are dysfunctional.

A second major area of investigation concerns using statistical techniques applied to neuroimaging data to discern differences between individual patients and healthy subjects. The goals are early detection of disease, assessing therapeutic interventions, and differentiation of patient subgroups. In particular, a major thrust is the development of cognitive "stress" tests that would result in different functional neuroimaging patterns (as evidenced by altered neural network behavior) between patients with very early dementia and healthy controls. Looking for subtle

deviations from normality among the brain regional interrelationships may provide the extra dimension needed to identify abnormalities in the scans of single individuals before the appearance of clinical abnormalities.

The method of structural equation modeling is used for constructing systems-level network models from an experimentally obtained interregional correlation matrix. In the structural equation models, one combines two sets of data: (1) the known neuroanatomical connections between brain areas, and (2) the interregional correlations between these regions. One attempts to calculate the functional strength of each anatomical path (called path coefficients or functional couplings), which represents the magnitude of the influence of each directional path. The best-fit of the combination of the anatomical network and interregional correlations creates a functional network for each group/condition. The functional networks can be compared between tasks or groups to identify task-specific or group-specific functional interactions within the same anatomical network.

Structural equation modeling was applied to data obtained during a PET study of object vision (using face matching as the task) versus spatial vision in young subjects. It was found that the functional network for the right cerebral hemisphere showed dominant path influences that included ventral occipitotemporal, anterior temporal and frontal areas during the object vision task, whereas the dominant influences in the spatial vision task included dorsal occipital, parietal and frontal areas. Recently, we extended the analysis of the face matching task to healthy old subjects and to patients with mild dementia of the Alzheimer type (DAT) and found that the old healthy controls had strong functional linkages in the same ventral network discussed above for young subjects. The functional linkages in the DAT group involving the ventral frontal area with posterior extrastriate regions were markedly reduced. However, frontal areas showed more extensive positive correlations with other frontal areas in the DAT patients than in controls. These results suggest that mildly demented DAT patients, who are able to perform the face matching task with the same accuracy as the controls, are utilizing different neural circuits than do controls, emphasizing the critical role played by neural plasticity in early DAT.

In the last year, this method has been used to delineate the functional networks involved in a delayed match-to-sample object vision task, and in two single word reading tasks. In the latter, in collaboration with Judith Rumsey, we found that the left angular gyrus plays a critical role in single word reading in normal subjects, but is functionally disconnected from other network components in subjects with developmental dyslexia.

In order to understand the relationship between what is observed in functional neuroimaging studies and the underlying neural dynamics, a large-scale computer model of neuronal dynamics that performs an object-matching task similar to those designed for PET studies was implemented. The model is composed of elements that correspond to neuronal assemblies in cerebral cortex, and contain different elements that are based on types identified by electrophysiological recordings from monkeys as they perform similar tasks. It includes an “active” memory network involving the occipitotemporal visual pathway and a frontal circuit, and is capable of performing a match-to-sample task in which a response is made if the second stimulus matches the first. A PET study is simulated by presenting pairs of stimuli to an area of the model that represents the lateral geniculate nucleus. rCBF data are computed from the model as it performs the tasks by integrating synaptic activity within the different areas. Simulated rCBF data similar to that found in PET delayed match to sample visual tasks was obtained, as were the correct neuronal dynamics in each brain region.

In the coming year, we plan to fully delineate the functional networks associated with reading and naming. The effect of cholinergic agonists on the delayed match to sample systems level network also will be investigated. The large-scale neural network will be expanded to include more brain regions so that more complex tasks can be investigated. Because one can include specific types of pathologies in simulated “brains”, explicit tests of hypotheses concerning how neural networks are altered by diseases such as Alzheimer’s dementia can be assessed.

**Collaborators**: Cheryl Grady, Ph.D., Rotman Institute (Toronto); Judith Rumsey, Ph.D., National Institute of Mental Health; Marie-Pierre Deiber, Ph.D., Robert Weeks, M.D. and Mark Hallett, M.D., National Institute of Neurological Diseases and Stroke; Susan Resnick, Ph.D., Laboratory of Personality and Cognition, National Institute on Aging; Malle Tagamets, National Institute of Diabetes and Digestive and Kidney Diseases; Hans Mueller-Gaertner, M.D. and Bernd Krause, M.D., University of Dusseldorf; Adrian Owen, Ph.D. and Trevor Robbins, Ph.D., Cambridge University.

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# Laboratory of Personality and Cognition

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The fundamental scientific paradigm guiding research in the Laboratory of Personality and Cognition (LPC) is the analysis of individual differences. Few phenomena are more basic than the fact that human beings differ—in health, in rates of aging, in cognitive ability, in personality, in happiness, and in life satisfaction.

The Laboratory of Personality and Cognition (1) conducts basic and clinical research on individual differences in cognitive and personality processes and traits; (2) investigates the influence of age on these variables and their reciprocal influence on health, well-being and adaptation; and (3) employs longitudinal, experimental, and epidemiological methods in the analysis of psychological and psychosocial issues of aging, including health and illness, predictors of intellectual competence and decline, models of adult personality, and correlates of disease risk factors.

The Personality, Stress, & Coping Section conducts basic and applied research on personality as it relates to aging individuals including studies of stress and coping, mental and physical health risks and outcomes, adaptation and well-being. Basic research has centered on a taxonomic model of personality traits and its assessment.

The Cognition Section conducts studies that attempt to distinguish pathological from healthy, age-related cognitive changes in a broad range of cognitive tasks including short-term and long-term memory, visuo-spatial rotation, attention and decision tasks. In addition, structural and functional brain changes are examined using MRI and PET. Studies are performed on regional structural brain changes, especially the hippocampus, and their relationship to cognitive performance and dementia. Regional differences in cerebral blood flow derived from PET studies at rest and during cognitive challenge are related to aging and patterns of cognitive change.

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**Recent Publications:**

Costa PT, Jr, et al. *Recognition and initial assessment of Alzheimer's disease and related disorders: Clinical Practice Guidelines, No. 19.* 1996; AHCPR Pub. No. 96-0702.

McCrae RR, et al. *Amer Psychologist* 1997; 52: 509-16.

Costa PT Jr, *Applied Psychol: International Rev* 1996 ;45: 225-41.

Costa PT, Jr, *J Pers Assess* 1997; 68: 86-94.

**Biography:** Dr. Costa received his undergraduate degree in Psychology from Clark University and his doctorate in Human Development from the University of Chicago. After academic positions at Harvard and the University of Massachusetts at Boston, he joined NIA to inaugurate a Stress and Coping Section. Since 1985 he has been Chief of the Laboratory of Personality and Cognition. His research interests include adult development, personality assessment, and Alzheimer's disease.

The Laboratory of Personality and Cognition addresses the psychology of aging through research on individual differences and intraindividual changes in cognitive abilities and personality dispositions. Its two Sections share use of the BLSA population, a focus on psychometrics and construct validation, and an emphasis on longitudinal research. Some research involves data from both Sections—for example, studies relating Openness to Experience to cognitive abilities, and explorations of brain activation patterns associated with personality factors. Both Sections share a developing interest in the molecular genetic basis of psychological characteristics, and both are concerned with applications of findings for health promotion and disease prevention.

**Basic Research in Personality - The Five-Factor Model:** Although many theoretical perspectives (including psychoanalytic, behavioral, and humanistic) have been taken on personality, most empirical research is based on trait models that address individual differences in characteristics of the person. A major obstacle to progress in personality psychology for many decades was the inability of psychologists to agree on a taxonomy of traits that would offer a comprehensive yet manageable set of trait constructs. Since 1983, this Laboratory has contributed to a worldwide consensus that the Five-Factor Model provides such taxonomy. The broad factors of Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness appear to encompass most specific traits, and offer a framework for systematic literature reviews and research designs.

Because the Five-Factor Model arose from the convergence of several independent lines of research, there are several slightly different versions of the model, and a number of distinct operationalizations. Research in this Laboratory has used the Revised NEO Personality Inventory (NEO-PI-R), in which the five factors are each defined by six specific facet scales. This hierarchical feature of the NEO-PI-R means that personality can be described either in the broad terms of the 5 domains or at the detailed level of the 30 facets.

One focus of research has been a comparison of the NEO-PI-R system with alternative operationalizations of the Five-Factor Model and alternative taxonomies. One study examined two adjective-based measures of the Five-Factor Model. Although all five factors showed convergent validity, an examination of the particular NEO-PI-R facets with which the adjective measures best correlated showed subtle differences in the conceptualizations. A study comparing the NEO-PI-R to a multi-faceted version of Eysenck's Three-Factor Model demonstrated that the latter is not comprehensive, lacking the Openness factor. Another study examining Tellegen and Waller's Seven-Factor Model showed that the two additional factors of Positive and Negative Valence could be adequately interpreted within the NEO-PI-R system.

**Stability and Change in Personality:** Personality stability and change has been a longstanding interest in PSCS. We have reported longitudinal studies in men for intervals of up to 30 years using the Guilford-Zimmerman Temperament Survey or GZTS. Recently we reported analyses of GZTS scores in women over a 12-year interval that replicated the high levels of stability in individual differences seen in men. Mean levels of personality traits showed little change, although both men and women showed modest declines in General Activity after age 50.

We have also examined possible moderators of stability. In collaboration with Dr. Jeffrey Metter, BLSA Medical Officer, we examined stability in people whose clinically assessed physical health improved, declined, or stayed the same over 6-year periods. The results consistently showed no effects of physical health changes on levels of personality stability. These findings underscore the importance of personality changes when they do occur: They are apparently not normal aging, nor are they due to common physical diseases. They may be most important as early signs of Alzheimer's Disease, as noted in the Clinical Practice Guideline on Early Recognition and Initial Assessment of Alzheimer's Disease.

**Stress, Coping, and Psychopathology**: Personality traits are important determinants of the ways in which people deal with stress. For example,

Extraversion is associated with forms of coping that involve humor, talking about feelings, and seeking support; Agreeableness is associated with stoic and compliant attitudes in the face of stress. Our perspective integrates stress-and-coping research into the broader field of psychology, linked to normal adaptation, psychopathology, and the personality dimensions that affect all these.

Traditionally, normal and abnormal psychology were held to be distinct and qualitatively different. Our research has shown that in many respects they are closely related, and thus that knowledge from one field is relevant to the other. For example, some of our research has focused on depression. We have shown that depressive symptoms are related to the normal personality disposition Neuroticism, can be predicted years in advance from personality traits, and can themselves predict psychiatric diagnoses noted in hospitalization records. Perhaps most important, we have also shown that depressive symptoms and the personality traits that predispose people to depression do not increase as a normal consequence of aging. Most older people are not depressed, and those that are should receive appropriate treatment.

The Five-Factor Model and NEO-PI-R have stimulated a number of studies on the relation between normal personality traits and the personality disorders classified on Axis II of the DSM-IV. These studies led to an edited volume, published by the American Psychological Association, which includes articles on theory, research, and clinical applications of the Five-Factor Model in diagnosing and treating personality disorders. Currently we are extending the scope of this line of research by conducting a collaborative cross-cultural study of personality and personality disorders with colleagues from the Hunan Medical University in the People’s Republic of China.

**Collaborators:** Michael H. Bond, Ph.D., Chinese University of Hong Kong; Sampo V. Paunonen, Ph.D., University of Western Ontario; Gergorio H. del Pilar, Jean-Paul Rolland, Ph.D., University of Paris X Nanterre; Wayne D. Parker, Ph.D., Stephanie V. Stone, Ph.D., Peter Fagan, Ph.D., Johns Hopkins University; Fritz Ostendorf, Ph.D., Alois Angleitner, Ph.D., University of Bielefeld; Margarida P. de Lima, Ph.D., Antoino Simoes, Ph.D., University of Coimbra; Iris Marusic, Ph.D., Denis Bratko, Ph.D., University of Zagreb; Gian Vittorio Caprara, Ph.D., Claudio Barbaranelli, Ph.D., University of Rome; Joon-Ho Chae, Ph.D., Sogang University; Ralph L. Piedmont, Ph.D., Loyola College of Maryland.; D. J. Vandenbergh, J. Wang, and George R. Uhl, NIDA; Gerald Matthews, University of Dundee; Donald H. Saklofske, University of Saskatchewan; Ian Deary, University of Edinburgh; Moshe Zeidner, University of Haifa.

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McCrae RR, et al. *Psychol Bulletin* 1996; 120: 323-37.

McCrae RR, et al. *J Pers Soc Psychol* 1996; 70: 552-66.

**Biography:** Dr. McCrae received a B.A. in Philosophy from Michigan State University, and a Ph.D. in Personality Psychology from Boston University. After three years at the Normative Aging Study in Boston, he joined the NIA to become Research Psychologist and Senior Investigator for Personality, Stress, and Coping Section, Laboratory of Personality and Cognition. His work has been centered on studies of personality structure (the Five-Factor Model) and assessment (the Revised NEO Personality Inventory) and applications in health and aging.

Personality traits are dimensions of individual differences in the tendencies to show consistent patterns of thoughts, feelings, and actions. Traits are important because their influence is pervasive: They affect personal interactions and social support, health habits and somatic complaints, attitudes and values, ways of coping, occupational and recreational interests, and much more. For the past 14 years, research in this laboratory has utilized a particular version of trait structure, the Five-Factor Model, and an instrument developed to assess 30 specific traits that define the five factors, the Revised NEO Personality Inventory (NEO-PI-R). Work in the past year has emphasized basic research on the generalizability of the model, and special attention has been given to Openness to Experience, the least well understood of the five factors.

**Cross-Cultural Studies of the Five-Factor Model**: Cross-cultural studies are of immense importance in personality psychology, because the major variables thought to affect personality development—genetic inheritance, early family environment, and social structural variables such as class, political climate, and religious traditions—cannot feasibly or ethically be manipulated. Personality psychologists must depend on "natural experiments," and many of these are provided by comparing individuals across cultures.

Since the publication of the NEO-PI-R in 1992, researchers outside the U.S. have translated the instrument into over 20 different languages, and many have collected data for their own research purposes. In collaboration with these investigators, we have recently begun cross-cultural studies of personality structure and development. In the first of these we reported an analysis of personality structure in Hong Kong Chinese and Japanese samples. Using statistical methods developed in part in this Laboratory, we showed that the Five-Factor Model is well replicated in both these non-Indo-European languages. Subsequent research has extended this finding to several other languages—in fact, to date no study using an authorized translation, adequate sample size, and appropriate analysis has failed to replicate the five-factor structure of the NEO-PI-R. These data suggest that the Five-Factor Model may be a human universal.

American studies of adult personality development can be summarized by saying that three of the factors (Neuroticism, Extraversion, and Openness) decrease, whereas the other two (Agreeableness and Conscientiousness) increase with age; most of the change occurs between age 18 and age 30. These cross-sectional differences might reflect cohort effects attributable to the historical experience of different generations of Americans. But other nations have had very different histories during the same period, and if age differences are due to cohort effects, it is unlikely that the same kinds of age differences would emerge in cross-sectional studies in those countries. However, reanalysis of data provided by collaborators in five countries (Germany, Italy, Portugal, Croatia, and Korea) show very similar patterns of age differences, suggesting that these may perhaps best be interpreted as effects of intrinsic maturation.

One of the limitations of our research to date is that only relatively modern, industrialized nations have been sampled, and the NEO-PI-R has not been translated into any of the languages native to the Americas or Sub-Saharan Africa. To examine further the generalizability of the Five-Factor Model, we are planning a collaborative study of age and personality structure in Zimbabwe, using a translation of the NEO-PI-R into Shona, a Bantu language.

**The Origins of Personality - Behavior Genetics:** According to Five-Factor Theory, personality traits are endogenous basic tendencies. Genetic factors are expected to play a major role in their origin and development, whereas environmental factors like culture should play a minor role. In collaboration with Swedish researchers, we published one of the first studies on the heritability of Openness to Experience, and we are currently working with John Loehlin to reanalyze the classic National Merit Twin Study data for all five factors. A collaboration with behavior geneticists in

Canada and Germany suggests that the five factors are strongly heritable in both these two cultures. In addition, that study demonstrates that more narrow and specific facet-level traits are also substantially heritable. Thus, it appears that there is a genetic basis for many of the details of personality, as well as the broad outlines.

**Studies of Openness to Experience:** Openness to Experience is the least well understood of the five personality factors. Different versions of the factor have been labeled Culture, Inquiring Intellect, Imagination, and Independence of Judgment. As assessed by the NEO-PI-R, Openness is seen in Fantasy, Aesthetics, Feelings, Actions, Ideas, and Values, and is thus much broader than labels such as Intellect suggest.

Correlational studies in the BLSA have shown that Openness is empirically related to a wide variety of constructs, including Jung's Intuition, Hartmann's Thin Boundaries, Tellegen's Absorption, and Murray's Need for Sentience, as well as to corresponding factors in alternative measures of the Five-Factor Model (e.g., Goldberg's Intellect). It shows smaller, if still significant, correlations with measures of intelligence and divergent thinking ability.

This body of empirical findings has been used to develop a conceptualization of Openness with both motivational and structural aspects. Although Openness is essentially a matter of differences in the internal processing of experience, it has far-reaching consequences in social interactions. A review of the literature showed that Openness or related constructs were important for understanding cultural innovation, political ideology, social attitudes, marital choice, and interpersonal relations.

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Vandenbergh DJ, et al. *Molecular Psychiatry* 1997; 2: 417-419.

Resnick SM, et al. *Neurology* 1997; 49: 1491-1419.


**Biography:** Dr. Zonderman received his undergraduate degree in Behavior Genetics from University of Massachusetts and his doctorate in Psychology from the University of Colorado. After a postdoctoral fellowship in multivariate statistics at the University of California, Berkeley, and academic positions at University of California, Davis and The Johns Hopkins University, he joined NIA as a Senior Staff Fellow in the Stress and Coping Section. Since 1997, he has been Chief of the Cognition Section in the Laboratory of Personality and Cognition. His research interests include individual differences in cognition and personality and their relationship with adult morbidity and mortality, predicting the onset of cognitive impairments and Alzheimer's disease, and the role of genetics in cognitive declines and personality.

**Distinguishing Pathological from Normal Cognitive Aging**: Research in the Cognition Section focuses on distinguishing pathological from normal cognitive aging. The purpose of this research is to identify predictors of cognitive morbidity, and to identify which cognitive processes are preserved with aging and which processes are vulnerable to disease. The primary effort of research in the Cognition Section is focused on longitudinal research in the Baltimore Longitudinal Study of Aging (BLSA). Cognitive tests have been administered to participants in the BLSA since 1960. Some individuals presently in the study have as many as seven repeated assessments beginning in the 1960's.

The cognitive tests administered to participants in the BLSA reflect our primary interest in pathological cognitive impairments, especially Alzheimer's disease (AD). The cognitive testing program is divided into two batteries, one for longitudinal prediction and another for cognitive and neuropsychological outcomes. The longitudinal repetitions of these tests distinguish typical changes in performance associated with aging from changes in performance which may be associated with disease when combined with neurological and neuropsychological outcomes and clinical diagnoses of AD.

An increasingly important area of research in the Cognition Section focuses on factors that reduce the risk of cognitive declines. An example of this focus is the finding that nonsteroidal anti-inflammatory drugs reduce the risk of Alzheimer's disease. Another example of this focus is based on recent findings that estrogen replacement therapy reduces the risk for both AD and cognitive declines in post-menopausal women. In an intervention study testing the effects of hormone replacement on cognition, we are examining the effects of estrogen and testosterone in older women and men in conjunction with structural and functional neuroimages.

**Cognitive Declines in Aging Subjects Free of Dementing Diseases:** In people with no signs of dementia, some cognitive abilities resist decline while other abilities show characteristic age-related changes beginning in the 50's or 60's. Research by investigators in the Cognition Section has shown that vocabulary scores generally resist declines, and may increase slowly over time until there are small decreases after the eighth or ninth decades. Immediate visual memory shows a much different pattern of change. We found that errors in immediate visual recall increased exponentially with increased age in both cross-sectional and longitudinal analyses

We also found that there were different rates of change in separate types of errors over time. Distortions, rotations, perseverations and mislocations were the most frequent errors across all ages. Although older participants made significantly greater errors regardless of error type, the greatest age differences were found for distortions and omissions. Men and women showed similar patterns of age-associated increases in errors, but there was a significant interaction between gender and error type indicating that women across all ages made more omissions and rotations, not other types of errors. Longitudinal analyses showed that distortions, omissions and rotations increased with age. Although women made more omission errors, men showed steeper increases with age.

**Long-Term Predictions of Cognitive Impairment and Dementia:** The onset of cognitive impairment is either a discrete event or a gradual process that manifests over time. We asked whether changes in previous test performance predict evidence of cognitive impairment assessed by the Mini-Mental Status Examination (MMSE) over relatively long intervals. We hypothesized that visual memory administered prior to the MMSE would significantly account for cognitive impairment after controlling for age at mental status exam and vocabulary score (a measure highly related to general intelligence). The correlations between visual memory and MMSE over 6-8 and 9-15 years were .36 and .34 ($p < .05$). These results provide preliminary evidence that mental status can be predicted, at least in part, by earlier performance on cognitive tests. Although the present findings are limited to only these cognitive tests, they provide important evidence that early signs of dementia may be detectable as many as 6-15 years prior to noticeable decline on mental status tests.

Six-year changes in immediate visual memory predicted Alzheimer's disease (AD) prior to its onset. Individuals with diagnoses of AD had larger changes in immediate memory performance over the six-year interval prior to the estimated onset of their disease than subjects without AD. Six-year longitudinal change in immediate visual memory performance also predicted subsequent cognitive performance 6-15 and 16-22 years later, even after adjusting for the influences of age, general ability, and initial immediate memory. These results provide evidence that change in immediate visual memory performance has long-term prognostic significance. These results further suggest that change in recent memory performance may be an important precursor of the development of the disease.

Analyses comparing BLSA participants who developed dementing illnesses with nondemented participants also showed that particular errors in visual memory may be more sensitive markers of impairment than others. More than 5 years before the onset of illness, demented individuals made more distortion errors than participants who did not develop dementing illnesses. In addition, individuals with signs of dementia had significantly greater rates of change in perseverations, rotations, and size errors compared with nondemented participants. These findings suggest that immediate visual memory is an important test for distinguishing normal from pathological cognitive decline and that specific types of errors in short-term memory may be important early markers of dementia.

**Risks and Protective Factors for Cognitive Decline:** If cognitive decline is an important predictor of pathological cognitive aging then it seems reasonable to investigate factors that decrease or increase the risk of

cognitive decline. Estrogen replacement therapy (ERT) is increasingly recommended for postmenopausal women due to its potential beneficial effects on physical health in older women. The possibility of a protective effect on cognitive function has also been suggested. In the BLSA, women receiving hormone treatment at the time of testing made significantly fewer errors in immediate visual recall than women who were not on hormone therapy. Less memory change was found in women who started hormone therapy between examinations than women who never received hormone therapy. These findings support the notion that estrogen has a beneficial role on cognitive functioning in aging women.

We continue to extend our present studies on the risks and protective factors for cognitive declines and dementias. In particular, as we gather additional repeat data on which to base reliable measures of cognitive trajectories, we will relate apoE and other genotypic and genomic measures to determine whether there are critical periods of decline. In addition, we will examine the role of modulators of cognitive decline such as hypertension and hormone replacement therapy, particularly in conjunction with MRI anatomical and PET functional assessments. We will also examine chronicity of hypertension, adequacy of blood pressure control, and differential effects and interactions with other known risks such as apoE genotype.

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**Biography:** Dr. Resnick received her Ph.D. in Differential Psychology and Behavioral Genetics from the University of Minnesota and completed a postdoctoral fellowship in Neuropsychology and Neuroimaging at the University of Pennsylvania. She was Research Assistant Professor of Psychology in Psychiatry at the University of Pennsylvania prior to joining the Laboratory of Personality and Cognition, NIA in 1992. She studies brain-behavior associations in health and disease and is currently the principal investigator of the brain imaging component of the Baltimore Longitudinal Study of Aging. This longitudinal neuroimaging study focuses on early structural and physiological brain changes that may be associated with memory and cognitive change in older individuals.

**Brain Changes as Predictors of Cognitive and Memory Decline:** The goal of our research program is to identify brain changes which may predict cognitive and memory decline in older individuals. We use magnetic resonance imaging (MRI) to measure the structure of the brain and positron emission tomography (PET) to measure changes in regional cerebral blood flow (rCBF) during the performance of memory tasks and over time.

**Early Markers of Alzheimer's Disease - Brain Changes in the Baltimore Longitudinal Study of Aging (BLSA)**: We are performing a 9-year neuroimaging study involving annual MRI and PET scans and neuropsychological evaluations in selected BLSA participants aged 55 and older. This longitudinal design provides a sensitive to way to investigate the relationship between changes in brain structure and physiology and decline in memory and cognition. Furthermore, using the wealth of prior psychological and medical information available for BLSA participants, including as many as 8 prior memory assessments over more than 30 years, we are able to examine trajectories of cognitive aging in relation to individual differences in the brain years later. To date, approximately 160 individuals (90 men, 60 women) have enrolled in the brain imaging study, and recruitment of additional women is still ongoing.

The specific goals of this study are: to determine the rate of brain changes with age, including increases in brain atrophy and vascular abnormalities; to determine the association between trajectories of memory and cognitive change and changes in brain structure and function; and to determine whether risk and protective factors, such as hormone replacement therapy, use of non-steroidal anti-inflammatory agents, and vitamins, modulate these relationships. An understanding of the associations between brain and neuropsychological changes, as well as early detection of these changes, will be critical in identifying individuals likely to benefit from new interventions in preventing and treating Alzheimer's Disease and other memory problems in the elderly.

Preliminary results are available for the first 2 years of our longitudinal brain imaging study. MRI data are analyzed using qualitative ratings and quantitative analysis of volumetric images. Results of the qualitative ratings, which are accomplished using the procedures developed and validated as part of the Cardiovascular Health Study, indicate significant effects of age and sex on atrophy ratings, with greater brain atrophy in older compared with younger participants and in men compared with women. Ratings of white matter hyperintensities (WMH), which reflect ischemic and/or demyelinating findings show more extensive WMH in older subjects, but no differences between men and women in this age range (55-85).

A great deal of effort in our laboratory has focused on the development of an image processing approach which provides accurate and valid segmentation and quantification of gray and white matter, and cerebral spinal fluid volumes. Quantitative analysis of regional brain volumes for subjects who have completed 2 evaluations reveals significant effects of age and sex on brain volumes and ventricular volumes. The cross-sectional findings from the Year 1 MRI scans indicate less gray and white matter volume and more ventricular CSF in older compared with younger participants; the magnitude of these findings is different across frontal, parietal, temporal and occipital brain regions. Consistent with previous studies and our atrophy ratings, men have greater ventricular CSF volumes. There are no detectable changes in lobar brain volumes over a one-year period, but there was a small but significant increase in the volume of the ventricles.To determine whether early blood flow changes can be used as predictors of cognitive and memory change, we are performing PET-rCBF studies as part of our BLSA neuroimaging study. PET rCBF scans are obtained under three conditions: during rest and the performance of verbal and figural continuous recognition tasks. This procedure is conceptualized as a cognitive stress test to examine age-associated changes in rCBF during increased demand. We have described

pixel-based maps of the associations between age and resting rCBF (normalized for global CBF). The correlation maps demonstrate significant negative correlations between age and CBF in the insular and superior temporal regions, and in visual association cortex (Areas 18 and 19) bilaterally for both men and women. Significant positive correlations between age and relative rCBF were observed for both men and women in subcortical, sensorimotor regions, and superior frontal gyrus. To our knowledge, this sample represents the largest study of associations between age and regional CBF studied with PET and provides a detailed map of age differences in blood flow during a period of accelerating cognitive and memory decline.

**Effects of Estrogen on Cognitive Decline**: We are also investigating the potential modulatory role of hormone replacement therapy on Alzheimer's Disease and cognitive and memory decline in older women. We have shown that women in the BLSA who had ever used estrogen replacement therapy had a reduced risk of developing Alzheimer's Disease in comparison with women who had never used hormone therapy. We have also shown that nondemented women in the BLSA who were using estrogen replacement therapy performed better on a test of short-term memory for designs compared with never-users. In a small subgroup of women with memory assessments prior to and following initiation of hormone treatment, the estrogen therapy appeared to protect against age-associated decline in memory.

**Future Directions:** Our future work will emphasize continuation of the longitudinal neuroimaging study, including continued acquisition of annual evaluations, further analyses of existing imaging and neuropsychological data, development of new approaches for longitudinal analyses of functional images, and examination of modulating factors on the relationship between brain and neuropsychological changes. In addition, we have begun intervention studies to examine the effects of suggested protective agents, such as sex steroid hormones, on brain structure and function.The data collected over the first 2 years of the study indicate only small changes over one year in regional brain volumes and ventricular CSF. In contrast, the cross-sectional age differences between younger and older participants are 5 to 7% in frontal and temporal volumes and 51% in ventricular volume. It will be critical to continue repeated evaluations of our participants to examine the rate and regional pattern of longitudinal age changes.

Another important area of future research, which has only recently received attention in the brain imaging literature, is the role of modulatory factors on measurement of brain structure and function. We plan to examine suggested risk and protective factors in relation to brain changes,

neuropsychological changes and their association. For example, data on family history for Alzheimer's Disease and related disorders, apolipoprotein E genotype, head trauma, history of hypertension, use of estrogen replacement therapy, and use of non-steroidal anti-inflammatory agents will be examined as potential modulators of the relationship between brain and neuropsychological changes.

Ongoing and future work will include intervention studies to examine suggested protective agents, such as estrogen and testosterone, on brain structure and function. Dr. Pauline Maki, an NRC fellow in our laboratory, is conducting a double-blind placebo-controlled study of estrogen and testosterone effects on cognition and mood in older women and men, respectively. In addition, we will perform MRI and PET studies to investigate concomitant effects on brain structure and regional cerebral glucose metabolism.

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# Keywords Index