National Institute on Aging
Intramural Research Program

1999 Factbook

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 based at the Gerontology Research Center at Johns Hopkins Bayview Medical Center in Baltimore, Maryland. The Cerebral Physiology and Metabolism Section operates a basic research program at the Clinical Center at the National Institutes of Health. The IRP provides a stimulating, academic setting for a comprehensive effort to understand aging through multidisciplinary 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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Although a longstanding laboratory within the National Institute on Aging Intramural Research Program, the Laboratory of Biological Chemistry (LBC), underwent a change in leadership in 1997 and significant reorganization. The LBC is currently comprised of six independent research programs headed by either a tenured scientist or tenure track investigator. These programs include the Cell Stress and Aging Section, the T Lymphocyte Signaling Unit, the Stress Signaling Unit, the Cell Cycle Control Unit, the Cancer Molecular Genetics Unit and the Molecular Neurobiology Unit.

Major areas of emphasis common to the individual programs include: 1) the elucidation of signal transduction processes and genes involved in regulating cellular responses to environmental signals such as growth factors, cytokines, and stress stimuli; 2) the determination of mechanisms contributing to the maintenance of cellular homeostasis and cell cycle control; and 3) the contribution of dysregulated gene expression, or loss of critical gene functions to the development of cancer. As described below for the individual programs, a wide variety of *in vitro* and *in vivo* models are being employed to approach these issues. These processes have direct relevance to our understanding of critical events associated with various age-related deficits and/or development of age-related diseases including cancer and Alzheimer's disease. The ultimate goal of the programs is to uncover knowledge that can be applied to prevent or delay the onset of age-related disabilities and disease processes, and/or provide new strategies for their diagnosis or treatment.

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. Combined, the programs within the LBC provide extensive and broad expertise in the areas of biochemistry, cellular and molecular biology and genetics. Specialized expertise in a variety of approaches used to analyze or manipulate gene expression is also available within the LBC. The LBC is equipped with state-of-the-art instrumentation and an extensive computer network.

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Recent Publications:

Wolfgang CD, et al. *Mol Cell Biol* 1997; 17: 6700-6707.

Gorospe M, et al. *Mol Cell Biol* 1998; 18: 1400-1407.

Wang X, et al. *Biochem J* 1998; 333: 291-300.

Huang Y, et al. *Oncogene* 1999; 18: 3431-3437.

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

Cell Stress and Aging Section Program: 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, and together these serve to coordinate the cellular response to a given stimulus and ultimately determine the cell's fate. These include, but are not limited to, the tumor suppressor protein p53, the heat shock response, mitogen-activated protein kinase (MAPK) cascades, PI-3 Kinase/Akt pathway and NF*k*B. Recent work has focused on the activation of the various pathways in response to

cell stresses, such as genotoxins, and/or oxidant injury. Our efforts are concentrated on 1) identifying the initiating events and critical mediators involved in the response, 2) examining the crosstalk between different signaling pathways, 3) determining the consequences of activation of particular pathways for cell survival and 4) identifying downstream targets of the signaling cascades that influence cell survival.

Roles of Specific Stress-Induced Gene Products: Numerous stress-regulated 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 or transformation, to growth arrest or programmed cell death. Our research in this area examines specific genes that are believed to mediate these differential effects, the goal being to understand their regulation and determine their function during the stress response. Particular genes of interest include the the transcription factor c-jun and cyclin-dependent kinase inhibitor p21/Waf1/Cip1, both of which are up-regulated during stress, and cyclin D1, which is repressed in response to adverse stimuli. Another long-standing interest of ours is the growth arrest and DNA damage-inducible gene GADD153, a C/EBP-related transcription factor implicated in the induction of both growth arrest and cell death following stress, particularly that involving perturbations of the endoplasmic reticulum. More recently we have initiated investigations using cDNA microarray technology to examine global changes in patterns of gene expression during the cellular response to stress, and identify novel players in the process.

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 demonstrated that aged hepatocytes show reduced activation of ERK in response to both proliferative signals and stress stimuli including hydrogen peroxide, sodium arsenite, and heat shock. This results in reduced induction of ERK-regulated genes and is associated with decreased survival to arsenite treatment. Induction of heat shock proteins in response to heat is also reduced in aged cells. Current studies are addressing other mitogen- and/or stress-activated pathways such as PI3K/Akt that may contribute to the aged cells altered responsiveness to external stimuli. The overall goal is to better understand the general decline in responsiveness of these aged cells so that we might be able to devise strategies to up-regulate these homeostatic responses in aged cells.

Collaborators: Tsonwin Hai, Ohio State University; Dan Mercola , Sidney Kimmel Cancer Center; Thomas Franke, Columbia University; Nicholas Dean and William Gaarde, ISIS Pharmaceuticals; Randal Kaufman, University of Michigan Medical Institute; Vincent Cristafalo, Lankenau Medical Research Center; Tak Yee Aw, Louisiana State University Medical Center; George Roth, Laboratory of Cellular and Molecular Biology, NIA; Patrice Morin, Myriam Gorospe and Yusen Liu, Laboratory of Biological Chemistry, NIA.

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Recent Publications:

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

Gorospe M, et al. *Mol Cell Biol* 1998; 18: 1400-1407.

Gorospe M, et al. *Mol Cell Biol* 1999; 19: 1289-1300.

Biography: Dr. Gorospe received her Ph.D. from the State University of New York at Albany (New York) in 1993. She completed her post-doctoral training at the Section on Gene Expression and Aging, National Institute on Aging, and assumed the position of Investigator in the Spring of 1998. Her research program focuses on post-transcriptional mechanisms serving to modulate gene expression, particularly that of cell cycle regulatory genes.

Cellular Response to Stress and Gene Expression: Aging is characterized by a general decline in the ability of individuals to adequately respond to different stresses, either environmental or endogenously generated. Stressful signals are transduced through various signaling pathways, ultimately resulting in alterations in gene expression. Many such stress-regulated genes have been identified and their expression is believed to play an important role in determining cell fate. While the transcriptional events serving to regulate the expression of these genes have been extensively studied, it is becoming increasingly clear that post-transcriptional regulatory mechanisms also play a critical role in their induction by stress. These post-transcriptional processes, still poorly understood, include mRNA splicing, transport, subcellular localization, stability and translation, as well as post-translational events such as protein processing, transport, phosphorylation and degradation. Our long-term interest is to explore post-transcriptional processes that govern gene expression during the stress response.

Post-transcriptional Control of Cell Cycle Regulatory Genes: We and others have shown that expression of the inhibitor of cyclin-dependent kinases p21 (also known as Cip1, Waf1 and Sdi1) is highly induced by various stresses and this enhances cell survival. In response to short wavelength ultraviolet light (UVC), this induction is due to the stabilization of the p21 mRNA. Our efforts are now focused on searching for RNA regions and proteins involved in regulating the stability of p21 and other stress-response genes, particularly those involved in growth control and cell cycle regulation. This analysis involves both *in vitro* and *in vivo* determinations of RNA binding and RNA degradation, the identification of the RNA-binding proteins involved, and the signaling pathways that modulate these activities.

Functional Analysis of the von Hippel-Lindau (VHL) Tumor Suppressor Gene: Absence of functional von Hippel-Lindau (VHL) tumor suppressor gene leads to the development of neoplasias characteristic of VHL disease, including renal cell carcinomas (RCCs). The VHL protein has been postulated to function in modulating gene expression at the levels of transcription elongation, mRNA stability and protein degradation. Our recent studies showed that various stresses were much more cytotoxic for VHL-deficient than for VHL-expressing RCC cells; at the same time, ubiquitination of cellular proteins was elevated in VHL-deficient cells and the rate of elimination of abnormal proteins was slower in cells lacking VHL. We propose that the toxicity encountered by cells lacking VHL arises from their inability to effectively eliminate abnormal proteins. This part of the program aims at identifying target mRNAs whose expression is altered by VHL using SAGE analysis, and to understand how VHL modulates the stress response.

Collaborators: Dr. Andre Nussenzweig, NIH; Dr. Henry Furneaux, Sloan Kettering Institute for Cancer Research; Dr. Berton Zbar, NCI; Dr. Michael Lerman, NCI; Dr. Nikki Holbrook, NIA; Dr. Josephine Egan, NIA; Dr. Yusen Liu, NIA.

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Keywords:
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glutamate

Recent Publications:

Bai G, et al. *J Biol Chem* 1998; 273: 1086-1091.

Krainc D, et al. *J Biol Chem* 1998; 273: 26218-26224.

Naruse S, et al. *Neuron* 1998; 21: 1213-1221.

Luo J-J, et al. *J Neurosci Res* 1999; 55: 629-642.

Biography: Dr. Kusiak received his Ph.D. in Biochemistry from 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 Macromolecular 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 exhibiting 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 or anti-oxidant drugs suggest a potential ability of these drugs to slow the progression of the disease. One of the hallmarks of AD brains is the presence 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 focuses 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, amyloidogneic C-terminal fragments of APP. This is accompanied by increased apoptotic cell death over several days. Recently, we showed that transient expression of mutated forms of PS-2 also increased the amount of apoptosis in growth factor-dependent PC12 cells.

Taken together, the above results suggest that in AD, the selective neuronal cell loss may be, in part, due to an apoptotic mechanism. This provides a rationale for targeting particular elements of an apoptotic pathway for therapeutic intervention in AD. We have generated 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 possible differential sensitivity of older animals to an increased Aß load.

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 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 their 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 that activation of glutamate receptors plays in initiating a genetic cascade of programmed cell death.

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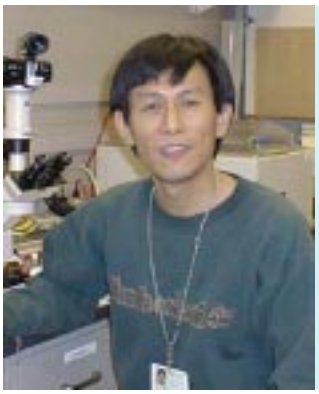

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Keywords:
signal transduction
MAP kinase
stress response
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Recent Publications:

Liu Y, et al. *Exp Cell Res* 1998; 240: 40-48.

Chen W, et al. *Mol Cell Biol* 1998; 18: 5178-5188.

Biography: Dr. Yusen Liu received his Ph.D. degree in 1991 from the Department of Fermentation Technology, Hiroshima University in Japan, and then served as Assistant Professor for a short period of time in the same department. In 1992, he joined the National Institute on Aging as a Visiting Fellow, and in 1995 was promoted to the position of Visiting Associate. At the end of 1996, Dr. Liu was recruited to the position of Investigator. His research has focused on signal transduction pathways involved in the stress response and their implications to the aging process.

Signal Transduction Pathways Involved in the Stress Response: Over the past several years, increasing evidence has emerged from studies in lower eukaryotic organisms that extended longevity is frequently associated with an enhanced resistance to stress. Therefore, investigation of the signaling pathways through which cells detect stressful conditions and activate their defense machinery is of critical importance for understanding the basic mechanisms involved in the aging process. Information gained from the study of the stress response could be exploited for the development of strategies to improve the quality of life for the increasing aged population.

Exposure of eukaryotic cells to harmful environmental conditions evokes alterations in gene expression. Altered gene expression can, at least in part, account for the variable phenotypic changes cells undergo after stress. Almost immediately after exposing cells to genotoxic agents, an increase in the activities of numerous proteins can be detected. Activation of these proteins initiates protein phosphorylation cascades leading to the activation of a group of mitogen-activated protein (MAP) kinases including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase/Stress-activated protein kinase (JNK/SAPK) and the p38 MAP kinase. These MAP kinases are responsible for the phosphorylation of a variety of transcription factors leading to changes in gene expression. While activation of MAP kinases is achieved through phosphorylation by MAP kinase kinases, attenuation of the MAP kinase activities is accomplished through dephosphorylation by a group of MAP kinase phosphatases. Thus, in order to understand the molecular basis for the diversity in gene expression as well as cellular outcomes provoked by stress, it is critical to understand the regulation of the MAP kinase signaling pathways.

We have previously demonstrated that stressful treatments can differentially activate ERK, JNK and p38 MAP kinases. Recent studies have focused on the potential role of growth factor receptors in mediating ERK activation in response to extracellular stresses. Using arsenite as a model stress agent, we have shown that arsenite treatment results in the rapid activation of epidermal growth factor receptor (EGFR), tyrosine phosphorylation of the Shc adaptor protein, and the formation of EGFR-Shc-Grb2 complexes in rat pheochromocytoma PC-12 cells. These events, as well as activation of ERK, were all drastically reduced by treatment of cells with either a selective inhibitor of EGFR, or down-regulation of EGFR expression. These results demonstrate that the EGFR and Shc are critical mediators in the activation of the Ras/ERK signaling cascade and suggest that arsenite acts as a tumor promoter largely by usurping this growth factor signaling pathway.

MAP kinase phosphatases (MKP) are a group of dual specificity protein phosphatases capable of inactivating MAP kinases. Members of the MKP family can display substrate selectivity. For example, MKP-3 is highly specific for the ERK subfamily. MKP-1 was first demonstrated to specifically inactivate ERK, but subsequent reports from several laboratories including ours demonstrated that MPK-1 is effective towards all members of the MAP kinase family. Using molecular approaches, we are studying the substrate specificity of MKP-1. We have found that p38 and JNK, rather than ERK, are preferred substrates of MKP-1.

Furthermore, we have obtained evidence that the carboxyl-terminal portion of MKP-1 has an auto-inhibitory domain that also acts to determine its substrate specificity. Deletion of this domain changes its substrate specificity, enhancing its phosphatase activity towards all MAP kinases.

Age-associated Alterations in Signal Transduction Pathways: Using a number of biological model systems, aging has been shown to be associated with a decline in proliferative capacity. In primary cultured rat hepatocytes, treatment of cells from young adult animals (6 months old) with EGF results in a marked increase in DNA synthesis. This response is significantly attenuated in cells of aged (24 months old) animals, but the molecular mechanisms underlying the age-associated defect(s) are poorly understood. In recent studies we have demonstrated that aging is associated with a decline in the activities of both ERK and p70 S6 kinase. Both of these pathways are essential for G1 to S phase transition of cells. As these two pathways are for the most part distinct, a decline in the activity of both kinases in response to EGF stimulation suggests that aged cells may possess an alteration in an early upstream event common to both pathways, possibly at the level of growth factor receptor. To investigate this possibility, we examined tyrosine phosphorylation of EGFR, Shc, and the formation of EGFR-Shc complexes in young and aged hepatocytes treated with EGF. We have found that both EGFR and Shc become tyrosine-phosphorylated to a similar degree in both young and aged cells. However, EGFR-Shc complexes appear to be less stable in aged cells compared with those in young cells. The reduced stability of the EGFR-Shc complexes will likely impact the later events leading to activation of the ERK pathway. Consistent with this hypothesis, Ras activity in the EGF-stimulated old cells was found to be lower and sustained for a shorter time. Current efforts are focused on determining the causes of the aging-associated instability of the EGFR-Shc complexes.

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ovarian cancer
Wnt pathway
SAGE
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tumor suppressors

Recent Publications:

Morin PJ, et al. *Science* 1997; 275: 1787-1790.

Korinek V, et al. *Science* 1997; 275: 1784-1787.

Lin H, et al. *Cancer Res* 1999; 59: 807-810.

Biography: Dr. Morin received his Ph.D. from Boston University in 1995. He then completed postdoctoral training at the Johns Hopkins Oncology Center before accepting his current position of Investigator at the National Institute on Aging in Baltimore. Dr. Morin also holds an Assistant Professor position at the Johns Hopkins School of Medicine, Department of Pathology.

Research Summary: Our laboratory's interest is twofold: molecular genetics of ovarian cancer and the role of the Wnt pathway in human cancer.

Ovarian Cancer: Ovarian cancer is the fourth leading cause of cancer death among women in the United States. Because of a lack of powerful diagnostic tests, early detection has been difficult. Moreover, the molecular mechanisms involved in the initiation and progression of ovarian cancer remain largely unknown. The two main approaches that we will use to tackle these problems are described below:

SAGE analysis of normal ovary and ovarian cancer. The best hope for identifying tumor markers resides in a detailed understanding of the differences between normal and cancer cells. It is well documented that, in the process of going from normal to malignant, cells reprogram their gene expression. However, consistent changes that could be useful for diagnosis and/or treatment have remained elusive for most tumor types, including the ovary. SAGE, one of the more powerful techniques currently available for the quantitative study of gene expression, is being used to analyze normal ovarian tissue, primary ovarian tumors and ovarian cancer cell lines. This approach has recently been used for colon cancer and has yielded a number of promising tumor marker candidates. Our laboratory is part of a multi-center program sponsored by CGAP (Cancer Genome Anatomy Project, NCI) to perform SAGE on a variety of tissues.

Search for genetic alterations in ovarian cancer. Surprisingly little is known about the molecular alterations in ovarian cancer. We plan to establish a large panel of matched normal tissue and ovarian cancer xenografts and to use this panel, in conjunction with ovarian cancer cell lines and primary tumor tissues, to identify genes important in ovarian tumorigenesis. Techniques used include representational difference analysis (RDA) and LOH studies. Of particular interest are chromosomal regions on Xq, 11p and 6q which frequently lost in ovarian cancers, suggesting the presence of tumor suppressor genes important in ovarian tumorigenesis.

The Wnt Pathway in Human Cancers: The Wnt pathway has recently been shown to be involved in human cancer. APC, a gene mutated in 80% of all colon cancers, is crucial for downregulation of ß-catenin and Tcf-mediated transcription. Moreover, colon tumors containing wild-type APC, frequently contain activating mutations in ß-catenin, emphasizing the importance of this pathway for colon cancer progression. We are studying the regulation of the Wnt pathway in normal and in cancer cells using a number of approaches, including the making of transgenic mice for various components of the pathway, antisense strategies and the construction of stable cell lines. In addition, we are also interested in finding direct transcriptional targets for the pathway using several techniques including SAGE and cDNA RDA.

Collaborators: Ellen Pizer, MD, Ph.D., Johns Hopkins Medical Institution; David Schlessinger, Ph.D. and Reid Huber, Ph.D., Laboratory of Genetics, NIA; Myriam Gorospe, Ph.D., Laboratory of Biological Chemistry, NIA.

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Recent Publications:

Baroja ML, et al. *J Immunol* 1999; 162: 2016-2023.

Griffith CE, et al. *J Biol Chem* 1998; 273: 10771-10776.

Williams BL, et al. *Mol Cell Biol* 1998; 18: 1388-1399.

Zhou YJ, et al. *Proc Natl Acad Sci USA* 1997; 94: 13850-13855.

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: The long term goal of our lab is to gain a better understanding of the mechanisms whereby immunosenescence arises in aging animals. Immunosenescence is characterized by a deterioration of both cellular and humoral immunity, and has been proposed to have its roots in declining T cell function as a consequence of changes in the ability of the T cells in aged animals to respond to mitogenic stimuli. Studies have found no difference between young and old animals with respect to the expression level of the T cell antigen receptor (TCR) or other cell surface receptors involved in responding to mitogenic stimuli. Therefore, we hypothesize that the decline in responsiveness to mitogenic stimuli may reflect changes in intracellular signaling pathways. In fact, many differences have been observed in some of the early TCR-initiated signaling events in T cells isolated from young animals compared to old. However, none of these changes have been demonstrated to account for the age-associated decline in T cell function. Effective investigation of the signaling defects that give rise to declining T cell function with age is hampered by the lack of a complete understanding of the signaling pathways involved in normal (i.e. young) T cell activation. This being so, we are currently attempting to uncover new portions of the signaling pathway that are downstream of engagement of the T cell antigen receptor.

Tyrosine Kinases in 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. Therefore, the laboratory is involved in identifying and studying the molecules involved in TCR signaling pathways. Certain tyrosine kinases have been found to be required for effective TCR signaling. Two of these kinases, ZAP-70 (zeta-chain associated protein) and Itk (Inducible T cell kinase), are currently under investigation in the lab. ZAP-70 is required for all distal TCR signaling events, while Itk apparently plays a more limited role in modulating the activity of phospholipase C. Our studies focus on understanding the mechanisms regulating the activity of these kinases, as well as identifying the precise signaling partners that these molecules interact with. Recently we have begun to investigate the possibility that ZAP-70 may play a role in regulating Itk activity, and have, indeed, found such a role for ZAP-70. The lab is currently testing various models for how ZAP-70 could be regulating Itk activity. We are also continuing to explore whether or not there is a strict requirement for ZAP-70 in signaling MAPK (mitogen-activated protein kinase) activation in response to TCR and/or co-stimulatory receptor engagement. These studies are following up our surprising observations of an apparent dispensability of ZAP-70 in the process of Erk activation following *a*-CD3 cross-linking in Jurkat T cells (Griffith CE, et al. *J Biol Chem* 1998; 273: 10771-10776).

Conjoint Re-engineering of ATP and Kinase ATP-binding Sites: A major frustration in studying signaling cascades that include protein kinases is the general inability to determine what the true *in vivo* substrates of a given kinase are. This stems largely from the very general nature of the catalyzed reaction, which precludes the generation of truly specific inhibitors. Even so, partially selective inhibitors or the expression of dominant-negative kinase mutants can only indicate that a particular protein is downstream of a given kinase, not that it is a direct substrate. To overcome this difficulty the lab has initiated a project to make complimentary changes to the structure of ATP and to the structure of the ATP-binding site of protein kinases important in TCR signaling. The approach requires the synthesis of a radiolabeled ATP ortholog that cannot be used as a substrate by any natural kinase, but which can be used by the re-engineered kinase. Expression of the mutant kinase in cultured cells or in the whole-animal then allows the determination of which proteins are being phosphorylated by the kinase in response to any given stimulus. Using this approach in combination with knock-in transgenic techniques it will also be possible to measure how the substrate repertoire and sites of phosphorylation change with development and with age. This then should provide a new and potent tool for discovering differences in signal transduction pathways that occur with age. The initial kinases under investigation are ZAP-70, Itk and Lck, but in principle could be extended to any protein kinase.

Collaborators: Robert Abraham, Ph.D., Duke University; Ezio Bonvini, M.D., Food and Drug Administration; Joaquin Madrenas, M.D., University of Western Ontario; John O'Shea, M.D., National Institute on Arthritis and Musculoskeletal and Skin Diseases; Pamela Schwartzberg, M.D., Ph.D., National Human Genome Research Institute.

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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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Keywords:
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vascular cell chemotaxis

Recent Publications:

Li Z, et al. *Hypertension* 1999; 33(1): 116-123.

Long X, et al. *J Clin Invest* 1998; 101: 1453-1461.

Xiao RP, et al. *Circ Res* 1999; 84(1): 43-52.

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

Biography: Dr. Lakatta received his M.D. from Georgetown University School of Medicine, Washington, DC 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 Science.

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 and their prognostic significance. 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 aging in the heart. Many features of the age-associated changes in heart cells resemble those found during fetal development. For this reason, emphasis has been placed both on studies of development and on that of aging. The focus on early cardiac gene expression has relied greatly on the use of an embryonic stem (ES) cell differentiation model system. In these studies, potential early cardiac gene transcription factors will be identified and the proteins responsible for activating expression are being targeted using standard molecular biological techniques. For aging, a number of model systems are being developed so that specific genes can be targeted during senescence to examine their functional consequences. Each project area has multiple components, and it is hoped that through integration of developmental with aging studies, we will be able to obtain a global view of cardiac gene expression and how alterations in individual gene expression patterns lead to physiological and pathophysiological consequences.

Excitation-Contraction Coupling Unit: This unit's main research focus is on the control of cardiac cell calcium 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 signal transduction mechanisms for G protein-coupled- receptors, e.g., "a and ß-adrenergic and opioid receptors and their subtypes in the heart. The interaction of signals emanating from stimulation of these with other

receptor-mediated signaling pathways are also investigated. Studies are designed to integrate information gleaned from genetic manipulations, including gene transfer by adenoviruses, transgenic and gene targeted animal models, in conjunction with electrophysiologic, confocal imaging and cell biological techniques to probe novel intracellular regulatory mechanisms. In addition, considerable efforts have been put on cardiac aging and heart failure associated changes in G-protein coupled receptor signaling to understand the pathogenic mechanisms and develop new therapeutic strategies for the treatment of human heart failure.

Gene Therapy Unit: Investigators in the unit used constructs of endothelial growth factor (VEGF) with different vectors such as adenoviruses or plasmid/liposome complexes in experiments to deliver genes to promote angiogenesis. The major efforts are directed to develop the optimal methods of delivery of appropriate genetic constructs to targeted tissue *in vivo* and to assess their therapeutic effectiveness. 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 characterization of vascular smooth muscle cells (VSMC), VSMC properties (migration, secretion, invasion) *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. 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 also responsible for molecular biology studies on apoptosis in the cardiovascular system, focusing at this time on the regulation of cardiomyocytes death and survival. The 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.

Collaborators: Jerome L. Fleg, M.D., Rui-Ping Xiao, M.D., Ph.D., Kenneth R. Boheler, Ph.D., Michael Crow, Ph.D., IRP, LCS; George Krause, Ph.D., Max Delbruck Centre for Molecular Medicine; Steven Houser, Ph.D., Temple University School of Medicine; Brian Kobilka, M.D., Stanford University; Robert Lefkowitz, M.D., and Walter Koch, Ph.D., Duke University Medical Center; Remesh Gopal, MBBS, Northwestern University; Ajay Shah, M.D., University of Wales College of Medicine; Konstantin Bogdanov, M.D., Russian Academy of Medical Sciences; Gary Gerstenblith, M.D., Edward Shapiro, M.D., Frank Yin, M.D., and Peter Vaitkevicius, M.D., Johns Hopkins Medical School; Ruth Altschuld, Ph.D., Ohio State University; W. Jonathan Lederer, Ph.D., University of Maryland School of Medicine; Maurizio C. Capogrossi, M.D., IDI-IRCCS; Oscar Bing, M.D., Boston VA Medical Center; David Kass, M.D., Johns Hopkins Hospital.

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Publications:

Bagrov AY, et al. *J Hypertens* 1998; 16: 1953-1958.

Bagrov AY, et al. *Hypertension* 1998; 31: 1097-1103.

Fedorova OV, et al. *Am J Hypertens* 1997; 10: 929-935.

Bagrov AY, et al. *Cardiovasc Res* 1996; 31(2): 296-305.

Biography: Dr. Bagrov received his M.D. and Ph.D. at Ivan Pavlov Medical University, Leningrad, USSR. He subsequently completed his cardiology training and held clinical and academic appointments in St. Petersburg, Russia. In 1992-1994, he worked at the NIA as a Visiting Associate.

We are studying regulation of the activity of Na,K ATPase by endogenous digitalis glycoside-like ligands. The overall objective of our work is to clarify the role of endogenous digitalis-like ligands of the sodium pump (LSP) in the development of hypertension. We have shown that mammalian tissues contain a sodium pump inhibitor, similar to amphibian bufodienolide hormone, marinobufagenin (MBG) MBG and another endogenous inhibitor, ouabain-like compound (OLC) have different sites of origin and different effective stimuli, different kinetics in salt/stress induced hypertension, and interact with different subunits of the Na/K ATPase (NKA).

Our work has three major goals: (i) To define cause and effect relationships between LSP and hypertensive phenotype, (ii) To investigate, in Dahl hypertension, how LSP synergistically with the other vasoconstrictors contribute to cardiovascular remodeling, and whether this synergism involves protein kinase dependent mechanisms, and (iii) To study signaling pathways, which underlie the effects of LSP, and test the hypothesis that protein kinases potentiate the effects of LSP via isoform-specific phosphorylation of the sodium pump. Substantiation of these hypotheses may provide new approaches towards understanding the pathogenesis of NaCl sensitive hypertension and potentially provide new methods of early detection of the risk and prevention of pressor responses to high salt intake.

Goal 1. The studies of MBG and OLC in pathogenesis of Dahl hypertension include the experiments in which central and peripheral effects of MBG and OLC in rats with NaCl induced hypertension are blocked by

MBG and ouabain antibodies. That will allow to assess whether each LSP causes hypertension. These experiments are paralleled by a study investigating (i) whether doses of MBG/OLC administered to rats which are sufficient to promote hypertensinongenic effects are (a) comparable to in vivo plasma levels of CS, and (b) associated with the changes in activity of the NKA and expression of NKA isoforms in cardiovascular tissues.

Goal 2. In Dahl hypertension, the development of compensatory cardiac hypertrophy is followed by the development of heart failure. We investigate interactions of LSP with other vasoconstrictor systems (endothelins, angiotensins) during the development of left ventricular hypertrophy and congestive heart failure in Dahl rats. Plasma levels of LSP, endothelin, angiotensin II and atrial natriuretic peptide will be monitored in Dahl rats on high NaCl diet. In parallel, the combined action of LSP, endothelin, angiotensin II and atrial natriuretic peptide on Na/K ATPase activity from cardiovascular tissues is studied. We expect, that co-regulation of NKA by LSP and other cardiovascular hormones occurs via protein kinase C dependent mechanism. Studies of this mechanism may provide important data on both hypertensinogenic and growth promoting properties of LSP (**Goal 3**).

Goal 3. The studies of mechanisms of action of LSP focus on (i) NKA isoform specificity, and (ii) on the co-regulation of NKA by CS and protein kinases. These experiments will utilize NKA activity and receptor binding assays. The major questions to be answered are: (i) Do protein kinases unmask the effect of LSP via phosphorylation of the sodium pump?, (ii) Do protein kinases affect Na/K pumping and receptor properties of NKA?, and (iii) Is there an isoform specificity in the co-regulation of NKA by LSP and protein kinases? This Project is a continuation of ongoing studies testing the hypothesis that isoforms of the sodium pump represent receptor sites specific for different LSP, OLC and MBG in particular. The expression of a-1 and a-3 isoforms of the sodium pump in membrane fractions (sarcolemma and nerve ending plasmalemma) from rat heart and human mesenteric arteries will be studied. Concentration response curves of the inhibition of NKA in membrane fractions by LSP, including MBG and ouabain, will be determined in the absence and in the presence of activators of protein kinase C.

Collaborators: Peter A. Doris, Ph.D., Institute of Molecular Medicine, Texas University, Houston TX; Ricardo Garay, M.D., Ph.D. INSERM Unite 600 Creteil, France; Natalia Dorofeeva, Ph.D., Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia; Marie-Therese Droy-Lefaix, Sc.D. IPSEN Institute, Paris, France.

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aging
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silent ischemia
heart failure

Recent Publications:

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

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

Rywik TM, et al. *Circulation* 1998; 97: 2117-2122.

Nagai Y, et al. *Circulation* 1998; 98: 1504-1509.

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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Keywords:
heart
vascular smooth muscle
cell migration
apoptosis
adrenergic receptors

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.

We study the behavior of vascular smooth muscle cells (VSMCs) and cardiomyocytes directed toward the goal of identifying the molecular mechanisms through which these cells contribute to the pathogensis of cardiovascular diseases.

Recent Publications:

Bilato CB, et al. *J Clin Invest* 1997; 100: 693-704.

Long X, et al. *J Clin Invest* 1998; 101: 1453-1461.

Crow MT, et al. *Heart Failure Rev* 1998; 3: 45-61.

Blystone SD, et al. *J Cell Biol* 1999; 145: 889-897.

Vascular Smooth Muscle Cell Biology

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 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 engagement of ß3 integrins along with occupancy of the associated protein known as IAP (integrin-associated protein) are required for CamKII activation in response to chemoattractant recognition. Activated CamKII inhibits nonmuscle myosin light chain kinase (MLCK), altering VSMC myosin light chain (MLC) phosphorylation to attenuate stress fiber formation and promote migration. CamKII regulation of MLCK activity is also involved in ß3 integrin signaling, not only in VSMCs, but numerous other cell types, including macrophages, the erythroleukemic cell line K562, and HEK 293 cells. Interestingly, calcineurin, which is activated in the cell by the same signals that activate CamKII (i.e., calcium and calmodulin), can have the opposite effect of MLC phosphorylation resulting in inhibition of cell migration. These studies have what is likely to be general concept regarding migration, i.e., that the relationship between migration and MLC phosphorylation is bell-shaped, with low or no migration occurring at very low or very high levels of phosphorylation. The practical consequence of this is that some cells may require increased phosphorylation for migration, while others decreased phosphorylation so that CamKII (and possibly calcineurin) may play different roles in promoting or inhibiting migration in different cell types.

Another potentially important target of CamKII regulation is TIAM, a protein first identified as a promoter of migration/invasion in T cell lymphomas. TIAM has subsequently been shown to be a guanine nucleotide exchange factor (GEF) for rac1 and to be expressed in many differnt cell types, including VSMCs. Phosphorylation of purified TIAM by CamKII leads to increased GEF and rac activity, promoting membrane ruffling and inhibiting stress fiber formation. Current studies are directed at developing dominant negative inhibitors of TIAM to test the signficance of this CamKII target in migrating cells. Our studies have identified a unique intracellular signaling network in VSMCs that is triggered by chemoattractant recognition and modulated by growth status, secretion of growth factors and extracellular matrix (ECM) components, and ECM-VSMC interactions with ramifications for other cell types in other settings.

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 and the recruitment of inflammatory cells to atherosclerotic lesions. 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 intra-cellular 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 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 (CM) cell death by apoptosis that involves exposing cultured neonatal cardiomyocytes to apoptosis-inducing stimuli. Our previous studies suggested that activation of the tumor suppressor protein, p53, plays a role in cardiomyocyte apoptosis. Using recombinant adenoviruses which we constructed to express dominant negative mutants of p53, we have shown that some, but not all, types of CM apoptosis are p53-mediated. In particular, apoptosis induced by hypoxia apparently is not p53-dependent. In fact, hypoxia-induced CM apoptosis does not rely on activation of the more common

caspase activation pathways, involving caspase 3 and cleavage of its substrates, but appears to be caspase 2-dependent. Caspase 2 is a proximal or initiating caspase and is regulated through protein-protein interactions at its N-terminal domain. In a search for proteins that might regulate this caspase, we and others have identified a cardiac-specific protein known as ARC (Apoptosis Repressor with CARD). Our studies show that ARC inhibits DNA fragmentation associated with apoptosis and that its expression is downregulated in response to hypoxia. Current studies are focused on the characterization of ARC expression in various cardiovascular disorders, determining whether forced expression of ARC can block apoptosis, and the generation of transgenic mice with either constitutive cardiac-specific expression of ARC in the heart or a conditional knock-out of ARC gene expression.

Another major direction of research is the identification that the roles of ß-adrenergic receptor subtypes play in CM apoptosis. Heart failure is often associated with CM cell loss along with alterations in adrenergic signaling. We have shown that ß2-AR signaling protects neonatal CMs from apoptosis induced by hypoxia or exposure to an inhibitor of vacuolar ATPases that causes intracellular acidification. ß2-ARs signal through both G_s and G_i proteins and the protective effects of ß2-AR stimulation are blocked by inhibiting G_i signaling. Remarkably, under these circumstances, ß2-AR signaling is not only no longer protective but becomes pro-apoptotic synergizing with other signals to accelerate cell death by apoptosis. Aside from direct pharmacological manipulations, disruption of G_i signaling may occur through mutations of the receptor that affect G_i docking (ß2-AR alleles have been identified that are linked to cardiovascular dysfunction following bypass or transplant surgery) or through increased expression of one of many regulators of G protein signaling known as RGS proteins. Current work in this area is, therefore, to identify the downstream signaling events responsible for G_i -mediated ß2-AR rescue, to analyze the anti-apoptotic properties of various human alleles of the ß2-AR in a test system of apoptosis, and to characterize the expression of RGS proteins in cardiovascular disease states.

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Stern MD, et al. *J Gen Physiol* 1999; 113: 469-489.

Ribadeau-Dumas A, et al. *Cardiovasc Res* 1999; (in press).

Santalucía T, et al. *J Biol Chem* 1999; 274: 17626-17634.

Biography: Dr. Boheler received his B.Sc. from Duke University and his Ph.D. in Physiology and Pharmacology from the University of California, San Diego. After completing a post-doctoral fellowship and working as a Researcher in Molecular Biology at Unit 127 of the National Institutes of Health and Medical Research (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 in Baltimore to head the Molecular Cardiology Unit of the Cardiac Function Section of the Laboratory of Cardiovascular Science.

The focus of our research program 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), the Na/Ca exchanger (NCX1) and the sarcoplasmic reticulum calcium release channel (Ryanodine Receptor). The work has involved examination of the spatial and temporal 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.

Recent work is focused on use of an *in vitro* differentiation model of mouse 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. Additionally, research in the laboratory has been strongly directed towards the development of mouse models having temporal and spatial control of gene expression. This system is currently being tested and plans are underway to actively apply this system to mouse transgenic models and to differentiating ES cells.

Spatial and Temporal Analyses: Previous studies were performed in 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 differentiation had been addressed through the use of *in situ* hybridization. We 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 NCX1 decreases at or around birth in a compartment dependent manner. NCX1 expression is, however, increased with aging. We have currently prepared a number of transgenic mice containing the promoters for rat NCX upstream of the b-galactosidase gene. The aim of this work is to identify sequences important for regulating cardiac restriction of this gene's products. Secondly, SERCA2 promoter constructs are being used similarly to understand how this promoter can regulate gradients of expression throughout the developing and adult myocardium.

Signal Transduction Pathways Mediating SERCA2 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 mRNA accumulations. Activation by alpha adrenergic agonists and protein kinase C isoforms reduces both SERCA2 mRNA 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 however does not appear to be primarily transcriptional. Transfection into neonatal rat cardiomyoctyes of the 2.8 kb human SERCA2 promoter constructs linked to reporter sequences indicate a lack of response with any of the adrenergic agonists. Recent studies with Nuclear run-on assays have also indicated that transcriptional control of SERCA2 gene expression is not the primary mechanism responsible for increased mRNA, protein and function of SERCA2 seen perinatally. Studies are underway, to elucidate the mechanisms responsible for the post-transcriptional regulation, one possibility of which may relate to an alternatively spliced isoform of SERCA2 seen in the fetal myocardium, whose expression is greatly reduced late in gestation.

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 five 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. The work is now focused on the *in vitro* examination of this promoter. A number of putative GATA binding sites and Nkx binding sites have been identified. In transfection studies, GATA 4, 5, and 6 isoforms have been shown to be sufficient to transactivate this sequence. Constructs lacking these cis-binding elements or mutants of these sequences have been prepared and are being examined both *in vitro* and in the transgenic models described above.

Embryonic Stem Cells and Myocardial Development: This 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. To identify, atrial versus ventricular like cells, expression vector constructs have been made that link atrial and ventricular markers to the green fluorescence protein (GFP) and other selection markers. These constructs have been introduced into ES cells and positive transformants identified through neomycin resistance selection. 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. Specifically, we are examining the expressed sequences of differentiating P19 cells through a technique called serial analysis of gene expression (SAGE). This technique takes advantage of PCR and type II restriction

enzymes to isolate short sequences sufficient to identify RNA products expressed at any time point. Currently, SAGE analyses have been performed on adult mouse myocardium, 3+3 day *in vitro* differentiating P19 cells and a comparative analysis is underway with 3+0.5 day *in vitro* differentiating P19 cells. Through this technique, we hope to use the information gained about the expressed sequence pattern to target and specifically identify gene products that are important to cardiac differentiation.

Temporal/Spatial Regulation: The aim of this program is to develop conditional and inducible gene targeting models, limited to specific cardiac lineages (e.g. ventricular myocytes) and inducible at a desired developmental stage. The tools chosen to accomplish this program are the *Cre Recombinase-Lox P* recombination system and the tetracycline transactivator system. A number of mice have been prepared that carry the *Cre* recombinase transgene under control of a tetracycline-sensitive promoter. Secondly, a targeting construct containing *LoxP* sites has been prepared such that induction of *Cre Recombinase* expression by withdrawal of tetracycline should cause excision of a critical exon in a targeted gene. This system has been placed under control of a lineage-specific promoter so that a tissue-specific knockout can be made to occur at a specified time. Currently a tetop-Cre Recombinase and MLC2V-tTA construct has been injected into pronuclei of C57BL/6 oocytes and a number of founder lines positive for these transgenes have been identified. These lines are currently being studied for appropriate expression using another reporter mice. To inducibly knockout RyR2 expression, a 15 kb mouse 129/SvJ genomic DNA fragment has been cloned, sequenced and the genomic structure determined. This sequence has been appropriately modified and lox P sites and neomycin resistance cassettes placed appropriately within the sequence. This mutant mouse RyR2 targeting vector has also been successfully introduced into embryonic stem cells, injected into blastocysts, and positive chimeras have been identified. This work is on-going.

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Stern MD, et al. *J Gen Physiol* 1999; 113: 469-489.

Stern MD, et al. *Proc Natl Acad Sci USA* 1999; 96(19): 10746-10751.

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 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, 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 differentiation. 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.

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Recent Publications:

Song L-S, et al. *J Physiol* 1998; 512: 677-691.

Sham JS, et al. *Proc Natl Acad Sci USA* 1998; 95: 15096-15101.

Cheng H, et al. *Biophys J* 1999; 76: 606-617.

Shirokova N, et al. *J Gen Physiol* 1999; 113: 377-384.

Biography: Dr. Cheng studied fluid dynamics, physiology and bioengineering, and then served as a faculty member in Peking University, China. To advance his career in biomedical sciences, he came to the United States in 1989, received Ph.D. (physiology) from University of Maryland and joined the Laboratory of Cardiovascular Sciences in 1995. During Ph.D. research, he discovered "Ca^{2+} sparks", now known as the elementary events of Ca^{2+} signaling in many types of cells. His current research interest focuses on local Ca^{2+} and cyclic AMP (cAMP) signaling in the context of excitation-contraction coupling and receptor-mediated signal transduction in normal and diseased hearts. These studies enlist an array of state-of-the-art techniques (e.g., confocal microscopy, electrophysiology and laser flash photolysis), gene-targeted animal models as well as mathematical modeling.

Ca^{2+} Sparks: Ca^{2+} sparks, extremely limited in space (~2 mm) and time (10-100 ms), are the tiniest packets underlying the sarcoplasmic/endoplasmic reticulum Ca^{2+}release. The detection of sparks was made possible with the advent of confocal microscopy and new generation of Ca^{2+} indicators. On the molecular level, the exquisiteness of cardiac excitation-contraction coupling is manifested by the ability of a single L-type Ca^{2+} channel to activate a Ca^{2+} spark. Thousands ($>10^4$) of Ca^{2+} sparks are ignited synchronously, sum up and give rise to global "Ca^{2+} transients" that contract heart muscle cells. In blood vessels, however, Ca^{2+} sparks have an unexpected, counter-intuitive effect to relax smooth muscle cells. The reason for this spark-induced relaxation is because sparks activate Ca^{2+}-sensitive K^+ channels, hyperpolarize surface membrane and shut off Ca^{2+} influx, resulting in depletion of intracellular Ca^{2+}. This is a classic case that a given signaling molecule in the same cell exerts opposing physiological effects due to subcellular localization. Despite extensive studies over the last five years, the exact nature of Ca^{2+} sparks remains to be perplexing. For instance, whether sparks are originated from single ryanodine receptors

(RyR)/Ca^{2+} release channels? what makes the spark size twice that predicted from computer modeling of spark generation and detection? what mechanism terminates Ca^{2+} sparks ? We undertake experimental and theoretical approaches to answer these and other fundamental questions.

Termination of Ca^{2+}-Induced Ca^{2+} Release: In cardiac myocytes, Ca^{2+} release from RyR is activated by L-type channel Ca^{2+} current via the Ca^{2+}-induced-Ca^{2+} release (CICR) mechanism. CICR, with its inherent positive feedback, is expected to operate in an "all-or-none" fashion. In order to generate Ca^{2+} transients of graded amplitude and robust stability, a regulatory mechanism must exist to counteract the regenerative CICR. Several mechanisms, including inactivation, adaptation, and stochastic closing of RyRs have been proposed, but no conclusive evidence has yet been documented.

Our recent studies showed that FK506-binding protein (FKBP), an accessory protein of RyR and also an immunophillin, modulates CICR through shortening Ca^{2+} spark duration and hastening the Ca^{2+}-dependent desensitization of RyR. Results from gene-targeted knockout of FKBP12, the main FKBP isoform, reinforce the idea that FKBP is not obligatory in terminating CICR.

To elucidate the termination mechanism of CICR, we first developed a novel fluorescent technique. By a combination of a fast, linear Ca^{2+} indicator, Oregon Green BAPTA 5N, and a high concentration of Ca^{2+} buffer, EGTA, Ca^{2+} release is visualized as discrete "Ca^{2+} spikes", which reflect the Ca^{2+} *release flux* rather than the conventionally measured *free* Ca^{2+} *concentration*. FPL64176, an L-type channel agonist increases open duration and promote reopening of the channel, but does not prolong or reactivate Ca^{2+} spikes. Latency analysis revealed that Ca^{2+} spikes coincided with the first openings, but not with the reopenings of the L-type channels. Following a maximal release, even a multi-fold increase in Ca^{2+} current upon repolarization fails to trigger additional Ca^{2+} spikes, indicating an absolute refractoriness of RyRs. If the initial release is submaximal, repolarization evokes Ca^{2+} spikes, but only from those RyRs unfired during depolarization. These results indicate that Ca^{2+} release is terminated by a highly localized, release-linked inactivation of RyRs in intact ventricular myocytes.

Collaborators: James S. K. Sham, Division of Pulmonary and Critical Care Medicine, Johns Hopkins Medical Institutions; Weinian Shou, Department of Molecular Physiology and Biophysics, Baylor College of Medicine; Hector H. Valdivia, Department of Physiology, Univ. of Wisconsin Medical School, Madison, WI; Eduardo Rios, Department of Molecular Physiology and Biophysics, Rush University; Joel E. Keizer, Institute of Theoretical Dynamics, University of California; James T. Russel, Laboratory of Cellular and Molecular Neurophysiology, National Institute of Child Health and Human Development, NIH; Collaborators at LCS: Edward G. Lakatta, Michael D. Stern, Rui-Ping Xiao, Kenneth Boheler.

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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:

(**1**) Signal transduction mechanisms underling 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 signaling 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

Recent Publications:

Xiao R-P, et al. *J Clin Invest* 1998; 101: 1273-1282.

Xiao R-P, et al. *Cir Res* 1999; 84: 43-52.

Kuschel M, et al. *Circulation* 1999; 99: 2458-2465.

Kuschel M, et al. *J Biol Chem* 1999; 274: 22048-22052.

Zhou Y-Y, et al. *Mol Pharmacol* 1999; 56: 485-493.

Ca/calmodulin-dependent kinase or cAMP-dependent kinase).
Her 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 animal 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 rat and human failing cardiomyocytes, the efficacy of $ß_2$AR stimulation is markedly diminished associated with a significant increase in Gi protein amount. Inhibition Gi proteins by pertussis toxin fully rescues the diminished $ß_2$AR contractile response in the failing heart. In light of these recent findings, the coupling of $ß_2$AR to G_i may play a critical role in the development of heart failure. 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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Shah AM, et al. *Circ Res* 1997; 80(5): 688-698.

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

Vila-Petroff MG, et al. *Circ Res* 1999; 84(9): 1020-1031.

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.

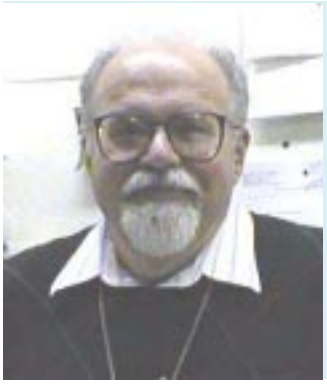
Mechanisms of Perturbed Mitochondrial Function in Cardiac Myocytes: Mitochondria play a central role in the regulation of apoptosis, and contribute to the pathogenesis of human degenerative diseases, aging, and cancer. Mitochondrial perturbations can have this result in a number of ways: by disrupting electron transport and energy metabolism, by releasing and/or activating proteins that mediate apoptosis, and by altering cellular redox potential together with the generation of reactive oxygen species (ROS). Recent research is focusing on the relationship between the mitochondrial electrochemical gradient, ROS production, induction of the permeability transition pore, and functional sequella.

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 (etc.) 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., Jay L. Zweier, M.D., Dilip Kittur, M.D., Sc.D., Johns Hopkins University; Pascal Goldschmidt-Clermont, M.D., Ohio State University; Ajay M, Shah, M.D., University of Cardiff, Wales, UK; Robert S. Danziger, M.D., Columbia University; Antoine Younes, Ph.D., Universite d'Auvergne Clermont, Aubiere, France.



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Recent Publications:

Talan MI, et al. *J Thermal Biology* 1999; (in press).

Poliakova L, et al. *J Thorac Cardiovasc Surg* 1999; 118: 339-347.

Roukoyatkina NI, et al. *Am J Hypertens* 1999; 12: 54-62.

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

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 principal researcher 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 functions evolved into his present interests concerning (1) development and assessment of genetic therapeutic interventions on different models of cardiovascular pathology; and (2) the effects of thermoregulatory responses to environment on the development of cardiovascular risk factors.

Therapeutic Angiogenesis: The broad objective of this program is to perform preclinical experimentation on animal models of myocardial and hindlimb ischemia as well as on different experimental models of heart failure to evaluate the therapeutic potential of gene therapy with angiogenic growth factors. *In vivo* experiments are aimed at characterizing clinically relevant animal models and optimal conditions, vectors, and routes of delivery at which gene transfer of angiogenic growth factors induce therapeutic angiogenesis.

A) Adenovirus-mediated gene transfer of $VEGF_{121}$ stimulates angiogenesis in normoperfused skeletal muscles. Administration of angiogenic factors has been shown to induce angiogenesis in the presence of tissue ischemia and to improve blood perfusion. However, there were no clear evidence that angiogenesis can be induced in normoperfused skeletal muscles. Furthermore, it is also unclear if once induced, the new-formed vessels can preserve blood perfusion upon induction of ischemia. Accordingly, we tested the hypothesis that adenovirus-mediated intramuscular (IM) gene therapy with vascular endothelial growth factor ($AdCMV.VEGF_{121}$) could augment collateral vessel development in nonischemic skeletal muscles and, subsequently, attenuate the hemodynamic deficits related to induced ischemia. Animals received IM injections

of AdCMV.VEGF$_{121}$, AdCMV.Null, or saline in the thigh 4 weeks (rabbits) or 2 weeks (rats) before induction of ischemia in the injected limb. In rabbits, increased tissue perfusion (TP) to the ischemic limb was documented by a superior calf blood pressure ratio for VEGF$_{121}$ group versus controls, improved blood flow in the ischemic gastrocnemius (P<.001) and more angiographically recognizable collateral vessels (angioscore) (P<.0001), at day 1 after surgery. In rats, we found a 29% increase in capillary density for VEGF$_{121}$ (P<.03 vs. saline) and an improvement of the bioenergetic profile of the gastrocnemius muscle obtained through ^{31}P NMR spectroscopy. We concluded that IM administration of VEGF$_{121}$ induces angiogenesis in normoperfused skeletal muscles and the newly formed vessels preserve blood perfusion once ischemia develops. This prophylactic approach could have therapeutic significance as part of an alternative treatment strategy for patients with peripheral vascular disease.

B) Vascular permeability effect of adenovirus mediated vascular endothelial growth factor gene transfer to the rabbit and rat skeletal muscle.
Vascular endothelial growth factor (VEGF) has been used in preclinical and phase 1 and 2 clinical trials as a potent mediator of therapeutic angiogenesis, however, its ability to enhance the vascular permeability might be a source of potential complications. The objective of this work was to evaluate the effects of the intramuscular injection of an adenovirus vector coding for the 121 amino acid form of VEGF (Ad.VEGF$_{121}$) on vascular permeability and edema development in rabbits and rats. Different concentrations of Ad.VEGF$_{121}$ ranging from 10^5 to 10^{10} pfu/ml ($3x10^6$ to $3x10^{11}$ particles/ml) were injected into hindleg or frontleg muscles of Wistar rats or rabbits. The size of scrotum and circumferences of limbs, as well as concentration of VEGF in the serum, was measured daily after injection. The injection of different of Ad.VEGF$_{121}$ into the hindleg muscles of rabbits led to a dose - dependent scrotal edema in rabbits at concentrations higher than 10^7 pfu/ml (p=0.002). The edema developed slowly, reached its maximum level six days after the injection, and spontaneously resolved thereafter. At concentrations higher than 10^9 pfu/ml, the scrotal edema was accompanied by skin necrosis (p=0.001). No scrotal edema was observed in rats. Therefore, excessive increase of vascular permeability after treatment with AdVEGF$_{121}$ is species specific. Results of our animal experimentations suggest that the potential for side effects of VEGF therapy due to increased vascular permeability is not very alarming in generally healthy patients and may not cause a significant clinical problem for treatment of peripheral vascular diseases.

C) Treatment with $VEGF_{165}$ encoded in plasmid/liposome complex stimulates angiogenesis in rabbits hindlimb ischemia model. Liposome-based vectors for gene therapy are considered to have lower transfection rate that adenovirus-based vectors. Nevertheless, comprehensive, *in vivo*, efficacy evaluation of liposome-based endothelial growth factors gene transfer for the treatment of tissue ischemia was not previously conducted. Two days after surgical removal of the femoral artery on one side, the ischemic tissue of different groups of rabbits was injected with different concentrations of plasmid/liposome construct encoded with $VEGF_{165}$, control substance (plasmid/liposome without expression cassette), or saline. Blood pressure distally to removed femoral artery, tissue blood flow, postmortem angiography and capillary density were assessed weekly, for four weeks. Accelerated development of new capillaries and larger vessels was confirmed by all assessment techniques during the first two weeks in $VEGF_{165}$ treated groups. *In vivo* angiogenic efficacy of plasmid/liposome vector encoded with $VEGF_{165}$ was not inferior to that of adenoviral vector.

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 colder seasons. 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 basic experimental model was established as follows:
Adult and aged rats acclimated to thermoneutrality, i.e., temperature that does not requires any metabolic expenditure to maintain body temperature (26°C), were exposed to cold (6°C) for nine weeks followed by five weeks of rewarming (26°C). 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.

Prolonged cold exposure did not result in elevation of systolic blood pressure in experiments involving aged animals or a different experimental design, in which an ambient temperature was gradually reduced, therefore cold acclimation developed slowly. Since shivering component of cold acclimatization was greatly reduced in both experimental models, we concluded that sustained shivering is a pathophysiologic agent responsible for development of hypertension.

We believe that cold-induced elevation of blood pressure in rats represents the first naturalistic experimental model of volume-associated hypertension and the first link between environmental adaptation and cardiovascular remodeling. The detail assessment of underlying mechanisms 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: Richard Spencer, M.D., Nuclear Magnetic Resonance Unit, NIA; Maurizio Capogrossi, M.D., Institute of Dermatology, Rome, Italy; Petro Anversa, M.D., Cardiovascular Research Institute, Valhalva, NY; GenVec Inc., Rockville, MD; Megabios Corp., Burlingame, CA.

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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.

Li Z, et al. *Hypertension* 1999; 33: 116-123.

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. His research program in the active transport field evolved from postgraduate studies, and was subsequently expanded through collaborations established at the NIH, in the U.S., and abroad. In collaboration with Robert Berger (NHLBI, NIH), he developed a rapid mixing, chemical quenched-flow device that was commercially copied and has been extensively used for kinetic characterization of the ion motive ATPases. Application of this technology to Na^+/K^+-ATPase with Wayne Albers (NINDS, NIH) enabled him to identify behavior consistent with the presence of oligomeric homologous subunit interactions in the catalytic mechanism and to establish a conceptual framework for understanding the basic role of oligomeric structure in ATPase energetics. By applying transient state kinetics to the Na^+/H^+ exchanger, he and James Kinsella (NIA, NIH) were first to demonstrate oligomeric behavior in the secondary active transport systems and propose that the functional transport unit in the kidney brush border Na^+/H^+ exchanger is a tetramer. Although internationally recognized for his contributions to the field of active transport in subcellular organelles, he has also investigated Ca^{2+} transport in isolated vascular smooth muscle cells, focusing on the $ß_2$-adrenergic pathways controlling relaxation and the modification of these pathways during aging. More recently, he has begun to examine the vascular smooth muscle response to mechanical injury in a CRADA-supported program aimed at developing a pharmacological approach based on local drug delivery for the prevention and treatment of coronary artery restenosis following balloon angioplasty.

Ion Motive ATPases: The ion motive ATPases form an important class of enzymes that utilize the energy available from ATP hydrolysis to transport ions across membranes against their electrochemical gradients. A major

theme of Dr. Froehlich's research has been the role of quarternary protein (subunit-subunit) interactions in free energy utilization and vectorial cation transport coupled to 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 suggest the presence of oligomeric structure in the functional transport unit. A general feature of oligomeric transport models is out-of-phase coupling of the protomeric reaction cycles so that no two subunits catalyze the same reaction simultaneously. This pattern of intersubunit conformational coupling allows staggered operation of the subunits in carrying out the reactions of the catalytic cycle. A central problem is to understand why out-of-phase coupling is preferred to having all of the subunits turn over in unison. One possibility is that out-of-phase coupling enables chemically and energetically distinct states to interact, creating an opportunity for free energy exchange between homologous subunits. This, in turn, should enhance catalytic efficiency by minimizing the loss of kinetic energy to heat and facilitate unidirectional transport by reducing the probability of pump reversal. Another possibility involves intra-protomeric charge-charge interactions in which binding of a cation to one subunit promotes the release of another cation in a nearby subunit via a local field effect. These and other issues related to the oligomeric behavior of these enzymes are being explored by a variety of special analytic techniques including rapid chemical quenching, the laser flash/lipid bilayer method and time-resolved electron spin resonance. Future studies involving *E. coli* Kdp-ATPase, a prokaryotic ion motive P-type ATPase exhibiting similar kinetic behavior, will be used to test the oligomer hypothesis by application of site-directed mutagenesis.

Vascular Smooth Muscle Relaxation Mechanism: Older individuals manifest an increase in systolic blood pressure together with increased arterial stiffening and diminished 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 dye and ratiometric fluorescence imaging. The $ß_2$-adrenergic agonist, isoproterenol (ISO), was observed 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. Cells from older animals exhibited larger SR Ca^{2+} stores following ISO, which reduced the cytoplasmic Ca^{2+} buffering capacity of SR and increased the probability of enhanced vascular tone. An increased arterial tonus might explain arterial stiffening and the 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 vascular smooth muscle pharmacomechanical coupling.

Local Drug Delivery and Restenosis: A complication facing 40-50% of patients undergoing percutaneous transluminal coronary angioplasty (PTCA) for symptomatic coronary artery disease is restenosis, a condition associated with neointimal hyperplasia and late vascular remodeling. Efforts to develop a pharmacological approach for the prevention of restenosis have focused on the use of paclitaxel, an antineoplastic drug with proven efficacy in preventing vascular smooth muscle cell migration and proliferation. In a 4 week (short-term) study employing minipigs, intracoronary metallic stents coated with paclitaxel were shown to produce a significant dose-dependent reduction in coronary artery neointimal hyperplasia and luminal encroachment without thrombotic complications. Safety concerns related to local drug toxicity observed at the highest paclitaxel dose were addressed in a 6 month (long-term) study which showed no evidence of aneurysm or lethal complications (e.g., rupture) from prolonged exposure. However, efficacy was reduced at the highest drug dose because of neoinitimal regrowth which served to repair the weakened vessel wall. The optimal dose suggested by the 6 month study was in the intermediate range of paclitaxel concentrations where therapeutic and toxic effects were more evenly balanced.

Future studies will concentrate on developing improved ways of delivering a reproducible drug dose to the tissue on coronary stents and on establishing the time course of re-endothelialization of the vessel wall.

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

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Recent Publications:

Fedorova OV, et al. *Am J Hypertens* 1997; 10: 929-935.

Anderson DE, et al. *J Hypertens* 1998; 16: 1015-1022.

Anderson DE, et al. *Biol Psychol* 1998; 49: 151-163.

Anderson DE, et al. *J Hypertension* 1999; 17: 1073-1080.

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 this Section is to investigate physiological and biochemical mechanisms that mediate the development of sodium sensitive forms of hypertension, and to develop and apply nonpharmacological methods to prevent hypertension in older humans. Experimental studies are based on an animal model of experimental hypertension involving high dietary sodium intake and intermittent behavioral stress that induces renal sodium retention. A guiding hypothesis of the work is that the mechanism involves a conditioned suppression of breathing that increases pCO_2 within the normal range, resulting in changes in acid-base balance and renal sodium-hydrogen exchange, such as is observed in some forms of human hypertension. It is also hypothesized that behavioral stress that suppresses respiration increases circulating concentrations of endogenous digitalis-like factors (EDLF) that can inhibit sodium pump activity in vascular smooth muscle and increase vascular tone.

Behavioral Influence on pCO_2 and Blood Pressure Regulation: Experimental studies with human subjects have shown that high end tidal CO_2 is a marker for blood pressure sensitivity to high sodium intake. In studies with both older persons in whom the incidence of sodium sensitivity was

high and younger persons in whom it was lower, positive associations were observed between resting $PetCO_2$ and magnitude of blood pressure increase. Large individuals differences are observed in resting $PetCO_2$ in humans. $PetCO_2$ of men falls progressively across the life span, but remains stable in women. High resting $PetCO_2$ was found to be associated with a greater tendency to worry and to experience negative affect.

Humans can be trained to increase $PetCO_2$ via a respiratory gas monitor and feedback system. Voluntary increases in $PetCO_2$ are associated with a transient decrease in plasma pH and increase in plasma bicarbonate concentrations. Voluntary increases in $PetCO_2$ are also associated with decreases in urinary excretion of volume and sodium, and with increases in urinary excretion of EDLF. These findings extend the results of previous studies with micropigs showing that acute behavioral stress increases in pCO_2, decreases plasma pH, and increases plasma EDLF. Thus, evidence accumulates that chronic suppression of behavior is associated with expansion of plasma volume which may increase susceptibility of blood pressure to high sodium diet.

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 levels of circulating carbon dioxide in long-term regulation of blood pressure in men and women, in African and Caucasian Americans, and in normotensive and hypertensive humans. Studies in progress with participants in the Baltimore Longitudinal Study on Aging are confirming the hypotheses that the levels of end

tidal carbon dioxide and the relationship of end tidal carbon dioxide to systolic blood pressure are greater in older women than in older men, and in African Americans compared with Caucasian Americans. These studies may clarify why rates of hypertension increase more sharply with age in women than men, and why African Americans are slower to excrete a salt load and have 50% more hypertension than Caucasian Americans. In addition, studies are underway to clarify the role of blood gases and sodium regulation in experimental hypertension in genetically normotensive rats which is created by the combination of high sodium intake and intermittent behavioral stress.

Collaborators: Alexei Y. Bagrov, M.D., Ph.D., Sechenov Institute of Evolutionary Physiology, Russian Academy of Sciences, St. Petersburg, Russia; Olga V. Fedorova, Ph.D., Laboratory of Cardiovascular Sciences, NIA; Angelo Scuteri, M.D., Ph.D. Laboratory of Cardiovascular Sciences, NIA.

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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. Between 1994 and 1998, Dr. Roth served as Acting Chief, LCMB. Since 1998, Dr. Donald Ingram has been Acting Chief.

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 regulation of physiological and behavioral functions utilizing whole animal and cellular models of hormone and neurotransmitter 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. Collaborations 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.

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Long JM, et al. *Neurobiol Aging* 1998; 19: 497-503.

Meyer RC, et al. *Ann NY Acad Sci* 1998; 854: 307-317.

Patel N, et al. *Neuroreport* 1998; 9: 171-176.

Umegaki H, et al. *Neuroreport* 1997; 8: 3553-3558.

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. In collaboration with Drs. N-P. Weng and Y-Q. Luo of the Laboratory of Immunology, we have also begun to use microarray technology to identify genes involved in memory formation and possible age-related changes in gene expression that occurs.

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

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

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Luo Y, et al. *J Biol Chem* 1998; 273: 3756-3764.

Kitano S, et al. *Am J Physiol* 1998; 275: C146-C154.

Luo Y, et al. *J Neurochem* 1998; 71: 980-990.

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 approximate 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 change 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 $G\alpha q/11$ 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 G*a*q/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, Ph.D., Loyola University, Chicago; Mark Mattson, Ph.D., University of Kentucky.

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

Lane MA, et al. *J Anti-Aging Med* 1999; 1(4): 327-337.

Roth GS, et al. *J Am Ger Soc* 1999; 47(7): 896-903.

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 as a Senior Staff Fellow and is currently a tenure-track investigator in the Laboratory of Cellular and Molecular Biology at the GRC. His work at the GRC is focused on basic mechanisms of aging and their possible modulation by caloric restriction and other interventions using both rodent and nonhuman primate animal models. Particular emphasis is placed on primate models of human aging process, development of biomarkers of aging, and the effects of nutritional intervention on aging and age-related disease. An additional aspect of his work involves elucidation of the biological mechanism(s) that underlie the diverse beneficial effects of caloric restriction on aging. This work focuses on the possible role of insulin signal transduction in aging and caloric restriction (CR) and the discovery of CR mimetic interventions with beneficial effects on aging processes that are not dependent on reduced food intake. He is a member of the Gerontological Society of America and was recently recognized as that Society's 1998 Nathan Shock New Investigator. He is also a member and sits on the Scientific Board of the American Aging Association.

Calorie Restriction in Primates: Among gerontologists calorie restriction (CR) is widely recognized as the only intervention proven to consistently extend both median and maximal 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 defined. 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 and Donald Ingram, Chief, 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 underlie 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. Furthermore, long-term follow-up of the primates is planned to test whether CR prolongs their life span.

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. Initial studies have shown that reductions in metabolic rate, body temperature, and insulin responsiveness are among the earliest changes to

occur during CR. Interestingly, these metabolic adaptations precede any CR-induced changes in body composition suggesting that to some extent, the metabolic effects of CR are independent of reductions in body weight or fat.

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. Studies in rodent models are focused on the possible relationship of glucose and insulin metabolism to the underlying mechanisms of CR. In a recently published study, we showed that administration of a glucose analogue to rats induced several physiological effects known to occur during CR. Specifically, administration of 2 deoxy-D-glucose in the diet 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 the 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 arterial stiffness and other risk factors. Preliminary studies in older (> 18 yr.) monkeys suggest that even when initiated in older animals, CR may have beneficial effects on several risk factors such as hyperinsulinemia, hypertriglyceridemia, and obesity/central obesity.

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 associated with 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 selected 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 albumin levels. Markers identified in skin fibroblasts include the accumulation of pentosidine (a collagen cross-link product) and the age-related increase in low pH ß-galactosidase positive cells. 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. In collaboration with several primate research centers, the NIA Biology of Aging Program and the National Center for Research Resources, a primate aging database for evaluating candidate markers is being developed. Although still under development, the database contains data on several species and has yielded several candidate markers that are under evaluation.

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, Ph.D., University of Texas Health Science Ctr. San Antonio; Richard Feures, Ph.D., National Center for Toxicological Research; Vincent Monnier, Ph.D. and David Sell, Ph.D., Case Western Reserve University.

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Recent publications:

Yu QS, et al. *J Med Chem* 1998; 41: 2371-2379.

Yu QS, et al. *J Med Chem* 1999; 42: 1855-1861.

Greig NH, et al. *Diabetologia* 1999; 42: 45-50.

Shaw KT, et al. *Neuroreport* 1999; 10: 53-56.

Biography: Nigel Greig was trained as a pharmacologist with a background in medicinal 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 technology for the delivery of neuropeptides, antisense oligonucleotides and proteins to the brain. Leaving NIA in 1989, Dr. Greig was involved in the initiation of the successful California biotechnology company, Athena Neurosciences, now Elan Pharmaceuticals. Returning to NIA as a tenured scientist in 1991, his research has evolved into his present interest, the design and development of drugs and diagnostics for the treatment of Alzheimer's disease and of type 2 diabetes. This has resulted in the development of several agents from concept in the laboratory, through the required U.S. Government regulatory requirements to the bedside.

Design of Drugs and Diagnostics: The goal of the Drug Design and 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 ß-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 correlate best with 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.

One of our efforts has 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 crystallography, biochemistry and pharmacology resulted in our 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 time duration for the potential treatment of a variety of diseases, including AD, age-associated memory impairment and other dementias as well as of 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 reason for this is that formation of reversible drug/ enzyme complexes allows selective enzyme inhibition over a long time duration, which 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, drug/receptor stimulation requires the continued presence of drug, and its time-dependent maintenance at the target receptor for continued activity. Our use of the former method, targeted enzyme inhibition, enhances specificity and reduces toxicity, and has resulted in several novel compounds with dramatic sustained cognitive action for once daily dosing with wide therapeutic windows and minimal toxicity. For example, the novel drug, phenserine, a long-acting and brain-directed, selective AChE inhibitor has completed FDA required preclinical toxicological assessment, and an IND then was prepared and filed to support assessment of its clinical utility in patients with AD. Other novel agents from SCIT are presently being developed as the first available reversible, nontoxic and brain-directed selective inhibitors of the enzyme BChE. BChE, unlike AChE and most other enzymes in the AD brain, has been found elevated early in the disease process, particularly in brain regions associated with AD. The association of BChE with the AD neurotoxic peptide, ß-amyloid, has been shown to dramatically amplify the toxicity of the peptide. In addition, a mutant variant of BChE, the K form, when found together with the ApoE 4 allele, is associated with an increased susceptibility of sporadic AD. Hence, inappropriate BChE activity can increase the risk of AD and accelerate the disease process. Our novel selective inhibitors of BChE will test the new hypothesis that central nervous system BChE inhibition is of value in the treatment of AD, and a representative of this novel class of compounds will be ready for clinical assessment within 2 years.

Another of our focuses to develop therapeutics for treating AD relates to reducing the production and secretion of ß-amyloid, a toxic peptide that derives from the misprocessing of the normal endogenous protein, ß-amyloid precursor protein (ß-APP), that is found in brain and throughout the body. In this regard, we have developed and are presently optimizing a pharmacophore that binds to and regulates the production of ß-APP (in collaboration with Jack Rogers, Ph.D., Harvard, MA) both in tissue culture and in the brain of rodents. Recent collaborative studies (Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Mayo Clinic, FL) have demonstrated that these reductions lead to reduced synthesis and secretion of ß-amyloid peptide. 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 ß-amyloid and gp120. In this regard, novel subtype-selective nicotinic receptor channel modulators are being developed in collaborative studies with John Daly, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases (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 targets for 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 reuptake 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 2 diabetes, a disease prevalent in the elderly that is caused by a relative refractoriness of the insulin receptor to its ligand and a deficiency in its normal release. The focus of these studies has been to

optimize the performance of pancreatic islet cells both in vitro and in rodent diabetic models with peptides that stimulate insulin release to develop novel therapeutics. Extensive studies have been undertaken on the peptide, exendin-4 (Ex-4), which bears a 52% homology to the endogenous insulinotropic peptide, glucagon like peptide-1 (GLP-1). GLP-1 is released from the gastrointestinal tract during eating to stimulate pancreatic insulin release to lower blood glucose levels. Like other endogenous hormones, it is short acting. In contrast, Ex-4 has a duration of action of some 16 hours, is more potent than GLP-1 and maintains blood glucose levels chronically without toxicity. Our studies have supported its transition from the laboratory and into phase I and II clinical trials as an experimental therapeutic for type 2 diabetes.

Collaborators: Arnold Brossi, Ph.D., University of North Carolina, Chapel Hill, NC; Donald Ingram, Ph.D., LCMB, NIA, NIH; Debomoy Lahiri, Ph.D., University of Indiana, IN; Kumar Sambamurti, Ph.D., Mayo Clinic Jacksonville, FL; Jack Rogers, Ph.D., Harvard, Boston, MA; Judith Flippen-Anderson, Ph.D., Naval Research Center, Washington DC; John Daly, Ph.D., NIDDK, NIH; Amy Newman, Ph.D., and Charles Schindler, Ph.D., NIDA, NIH; and Josephine Egan, M.D., Diabetes Unit, NIA, NIH.

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Horská A, et al. *Am J Physiol* 1999; 276: E766-E773.

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Spencer RGS, et al. *Am J Physiol* 1997, 272: H409-H417.

Potter K, et al. *Matrix Biology* 1998, 17: 513-523.

Biography: Richard Spencer obtained his Ph.D. in Medical Physics from the Massachusetts Institute of Technology (MIT) in 1987, working with Professor Joanne Ingwall at the NMR Laboratory for Physiological Chemistry of Harvard Medical School, and his M.D. from Harvard Medical School in 1988. He was a postdoctoral fellow with Professor Robert Griffin at the Francis Bitter National Magnet Laboratory of MIT before joining the NIH. Dr. Spencer joined the National Institute on Aging in 1991, as Chief of the Nuclear Magnetic Resonance Unit. He completed medical residency training at Johns Hopkins Bayview Medical Center in Baltimore. He is a Diplomate of the American Board of Internal Medicine and an Associate Professor of Medicine at Johns Hopkins Medical School in Baltimore, Maryland.

Nuclear Magnetic Resonance Unit: The interests of the Nuclear Magnetic Resonance (NMR) Unit are primarily in imaging (NMRI) and metabolic studies of three-dimensional cartilage grown from chondrocytes in culture, and spectroscopic studies of cardiac and 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: Osteoarthritis is the leading cause of joint pathology in the older population. One 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 which 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 addition, our work may aid in the development of tissue engineering protocols for cartilage tissue suitable for transplantation.

In cartilage developing from whole chick sterna, we have investigated the correlation 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 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: Atherosclerosis is a critical factor in the development of both peripheral vascular disease and cardiac ischemia. One approach to treatment of ischemic vascular disease is the 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 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) 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. These results were consistent with concomitant studies of blood flow using contrast angiography and blood pressure measurements.

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: Maurizio Capogrossi, M.D., Mark Talan M.D., Ph.D., and Ed Lakatta, 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; Eric McFarland, M.D., Ph.D., University of California at Santa Barbara; Walter Horton, Ph.D., Northeast Ohio University College of Medicine.

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Nagababu E, et al. *Bichem Biophys Res Commun* 1998; 247(3): 592-596.

Rifkind JM, et al. *Life Sci* 1999; 64(4): 237-247.

Ramadas N, et al. *Biophys J* 1999; 76(4): 1796-1811.

Ajmani RS, et al. *Gerontology* 1998; 44(2): 111-120.

Risby TH, et al. *J Appl Physiol* 1999; 86(2): 617-622.

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 (NICHD) 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 which 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 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. In collaboration with the LCI, we are 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, Ph.D., Indian Institute of Technology, Madras, India; Avraham Mayevsky, Ph.D., Bar Ilan University, Israel; Victor McDonald, Ph.D., Walter Reed Army Institute of Research; David Danon, M.D., Weissman Institute, Rechovot, Israel; Paul Costa, Ph.D., Laboratory of Personality and Cognition, NIA; Jerome Fleg, M.D., Laboratory of Cardiovascular Science, NIA; Jeffrey Metter, M.D., Longitudinal Studies Section, Laboratory of Clinical Investigation, NIA.

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The Laboratory of Clinical Investigation (LCI) chiefly focuses on clinical research issues of importance in gerontology. Clinical work includes the activity with volunteers on the Baltimore Longitudinal Study of Aging (BLSA), and cross-sectional studies in a variety of age-related disease areas including diabetes, metabolism, cardiovascular disease, neurologic disease, and cancer.

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.

The **Molecular and Clinical Pharmacology Section** studies the role of age- and disease-related changes in calcium signaling in vascular smooth muscle on vascular responses in aging, hypertension, and atherosclerosis and seeks to understand how such changes affect drug responses. The high prevalence of hypertension and atherosclerotic disease in the elderly and their contribution to morbidity and mortality make understanding therapeutic responses and development of new therapies a priority.

In the laboratory study of calcium channel variants is allowing improved understanding of how changes in cellular calcium homeostasis change cellular function. In addition such studies give insight into mechanisms of drug action and provide possible new targets for drug action. Clinical studies of forearm vascular responsiveness allow testing of the cellular and molecular findings as well as evaluation of proposed new therapeutic targets. The applied goal of these studies is the development of new approaches to reverse impairments in vascular response in hypertension and atherosclerosis. In addition these studies often provide insight into mechanism of effect of currently used therapies.

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Recent Publications:

Soldatov N, et al, *J Biol Chem* 1998; 273: 957-963.

Oz M, et al, *Mol Pharmacol* 1998; 54: 1106-1112.

Trapnell CB, et al, *Clin Pharmacol Ther* 1998; 64: 597-602.

Jones DS, et al, *Clin Pharmacol Ther* 1999; 65: 408-412.

Biography: Dr. Darrell Abernethy received his M.D. and Ph.D. (Pharmacology) degrees from the University of Kansas School of Medicine in 1976. Training in Internal Medicine through Board Certification was at the University of Miami/Jackson Memorial Hospital, and postdoctoral training in Clinical Pharmacology at Massachusetts General Hospital followed this. He joined the faculty at Tufts-New England Medical Center as an Assistant Professor. Following this he was at Baylor College of Medicine where he became Associate Professor of Medicine. Dr. Abernethy then moved to Brown University School of Medicine as Chief of the Division of Clinical Pharmacology and became Professor of Medicine at that institution. He then moved to Georgetown University School of Medicine as Francis Cabell Brown Professor of Medicine and Pharmacology and Director of the Division of Clinical Pharmacology. Dr. Abernethy became Clinical Director and Chief of the Laboratory of Clinical Investigation in April, 1999. Early in his career Dr. Abernethy made fundamental contributions to understanding of drug tissue distribution and the factors that regulate drug distribution. He then worked in the area of cardiovascular drug responses and their changes in aging and hypertension. This led to his current focus on understanding mechanisms of calcium homeostasis, its changes with age and disease, the effects of calcium antagonist drugs in these systems, and identifying new targets for therapy for hypertension, atherosclerosis, and other diseases of altered calcium homeostasis.

Calcium Channel Variants in Aging and Disease: Alternative splicing generates diversity of the calcium channel alpha subunit, but does not significantly change the overall topology of the protein, which is highly conserved in the regions of calcium antagonist drug binding. Instead regions of diversity appear to regulate function of the calcium channel, in particular with regard to the rate of its inactivation following stimulation. The alternatively spliced variants of the calcium channel have been identified in different tissues, and appear to be expressed differently as a

function of age. We are exploring the molecular correlates of calcium gating in this channel and how gating differs in the various naturally expressed channel variants. In addition we are studying the heterogeneity, distribution patterns and regulation of the splice variants in human cardiac and vascular tissues in relationship to age, hormonal, and pathological stimuli. L-type calcium antagonist drugs have become very important in cardiovascular therapeutics for the treatment of angina pectoris and hypertension. For further improvement of calcium channel targeting drugs, these studies will provide understanding of the molecular bases of regulation of the calcium channel.

Mechanisms of Calcium Antagonist Drug Action: Mechanism of calcium antagonist drug induced arterial vasodilatation is generally assumed to be due to L-type calcium channel blockade on vascular smooth muscle. Interference with other systems has not been well appreciated. We demonstrated in clinical study that calcium antagonist drugs block angiotensin II and endothelin mediated vasoconstriction. It was unclear if this was a specific effect, however we have recently shown that calcium antagonist drugs alter angiotensin II signaling at the molecular level, suggesting that there is specificity to the clinical finding and that this is a further explanation of the mechanism of these drugs. We currently are studying this effect in calcium channel variants and extending these studies to understand the role of the vascular endothelium in calcium antagonist drug effect.

Role of Genetic Variants in Vascular Responses: Recently a number of genetic polymorphisms in systems that have important roles in vascular contraction have been identified. For example 5-10% of the population appear to have an altered endothelial nitric oxide synthase enzyme which has been suggested to be associated with myocardial infarction. The role of such a variant in altered responsiveness to drugs is not well appreciated. We very recently showed that the individuals with the altered nitric oxide synthase gene have markedly diminished ability to relax their blood vessels in response to acetylcholine, which causes relaxation via the activation of this enzyme. A large number of these kinds of genetic variants are being discovered, however many do not have disease and/or drug associated consequences. We are developing strategies to select those variants which we believe will have pathophysiological and pharmacological importance in aging and disease and in clinical studies determining if our strategies are effective. In the longer term we believe these studies will be critical for the development of patient specific therapeutics and in the individualization of drug therapy in a way to minimize drug toxicity.

Collaborators: Nikolai Soldatov, Ph.D., GRC; Martin Morad, Ph.D., Georgetown University; Janice Schwartz, M.D., Northwestern University; Jane Freedman, M.D., Georgetown University; David Flockhart, M.D., Ph.D., Georgetown University; Irving Wainer, Ph.D., Georgetown University; Stephen Donahue, M.D., Bristol-Myers Squibb Research Institute.

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Keywords:
GLP-1
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Recent Publications:

Greig NH, et al. *Diabetologia* 1999; 42: 45-50.

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. *Am J Physiol* 1995; 269: B983-B990.

Biography: Dr. Josephine Egan 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 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 2 diabetes mellitus.

Aging and Type II Diabetes: The goal is to design new drugs to restore glucose sensitivity to the beta cells in Type 2 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 2 diabetes in beta cells and search for agents which can alter these processes. The approach is to take the agents which 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 2 diabetes develops, for the most part, because with increasing age, adiposity and changing lifestyle, insulin becomes less effective at its target tissues. 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 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 which would restore glucose responsivity to the beta cells and which would prevent the decrease in replication that occurs in beta cells of 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 2 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 have completed animal testing of this compound and have begun human testing. When given intravenously to normal and Type 2 diabetic subjects its biological action lasts about twelve hours and it is extremely potent at inducing insulin release. We are just about to begin a long-term study in Type 2 diabetic subjects. 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
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Recent Publications:

Garant MJ, et al. *Biochemistry* 1999; 38: 5896-5904.

Montrose-Rafizadeh C, et al. *Endocrinology* 1999; 140: 1132-1140.

Lee-Kwon W, et al. *Biochemistry* 1998; 37: 15747-15757.

Biography: Dr. Bernier received his Ph.D. from the University of Montreal, Canada, in 1983, and completed two postdoctoral trainings. The first one was held at INSERM U.162 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 joining the NIA in 1990. His research interest encompasses 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 not a single disease but rather a complex of several metabolic diseases all characterized by chronic hyperglycemia. The hyperglycemia may result from defects in insulin secretion, insulin action, or both. Deficient insulin action results from diminished tissue responses to insulin at one or more points along its complex signaling cascade pathways. The main goal of my research program is to provide a coordinated approach designed to bring new insights into insulin action by studying elements that control the activation of the insulin receptor and its downstream signaling functions. The specific aims are as followes:

A. **Insulin Receptor Structure and Function:** Insulin action is initiated by insulin binding to its cell surface receptor, which activates the tyrosine kinase of the ß-subunit of the insulin receptor and triggers a cascade of post-receptor events resulting in an array of metabolic and mitogenic responses. Redox regulation is emerging as an important mechanism by which various cellular activities are modulated. We recently reported a structural change in the extracellular domain of the insulin receptor caused by plasma nonprotein thiols (such as reduced glutathione) concomittant with alteration in the intrinsic catalytic activity of the receptor. Recent evidence supports a role of plasma free radical production in mediating insulin resistance both in the elderly and type 2 diabetic patients. Therefore, we plan to perform studies to elucidate the impact of metabolic

imbalance and antioxidants on insulin receptor structure and function in several cultured cell lines (e.g., from muscle, liver, and fat) and in intact animals.

B. **Insulin Receptor Signaling and Apoptosis:** The number of cells in tissues is determined by the homeostatic balance between proliferation of new cells and death of senescent or damaged cells. Apoptosis, also termed programmed cell death, is an active, genetically controlled process that has been identified as a key phenomenon in the pathogenesis of a wide array of diseases, including diabetes. In recent years, evidence accumulated to indicate that insulin has antiapoptotic properties, in part, by activating transcription factors that are known to be involved in the control of the apoptotic process. However, the signaling pathway involved in this action of insulin is poorly understood. We found that signaling was partly dependent on intact receptor kinase activity because it was prevented in cells expressing kinase-dead insulin receptor mutants. Our analysis also revealed the importance of a farnesylation-dependent pathway in conferring protection against apoptotic cell death mediated by growth factor withdrawal. Work is underway to identify the farnesylated protein(s) responsible for the survival function of insulin receptor. Strategies will also be implemented for extending survival of pancreatic beta cells by a mechanism involving activation of the insulin receptor alone or together with that of the GLP-1 receptor (See section C).

C. **Convergence of Intracellular Signaling Pathways in Ligand-mediated Activation of the Glucagon-like Peptide-1 (GLP-1) and Insulin Receptors:** The GLP-1 receptor is a member of the superfamily of G-protein coupled receptors whose activation leads to enhanced gene expression, and increased insulin synthesis and secretion in pancreatic beta cells. Given the importance of GLP-1 effects and its analogs on the development and maintainance of pancreatic beta cells, we focused on elucidating the signaling pathways that are triggered upon GLP-1 binding to its receptor. We found that heterologous expression of the pancreatic form of the GLP-1 receptor in fibroblasts resulted in the receptor coupling to several G proteins. Addition of GLP-1 lead to increased activation of various members of the mitogen-activated protein (MAP) kinase family (e.g., p38 MAP kinase and ERK1/ERK2) via a cholera toxin-sensitive signaling pathway both in the GLP-1 receptor-transfected cells and in insulin-secreting cells. When insulin receptor kinase was activated by insulin, GLP-1-induced enhancement of MAP kinase activity was significantly increased, suggesting a convergence of signals between these two families of receptors. Interestingly, although G-proteins traditionally associate with seven transmembrane receptors, the insulin receptor is known also to interact with G-proteins. Effort is underway in our

laboratory to further characterize a putative G-protein beta subunit, termed GßL, that we have recently isolated. GßL expression was found to be markedly increased in insulin-treated adipocytes, while being sharply reduced in adipose tissue of diabetic animals.

Collaborators: Buel D. Rodgers, Ph.D., The Johns Hopkins University School of Medicine, Baltimore; Ronald A. Kohanski, Ph.D., The Mount Sinai School of Medicine, New York; Ashok K. Srivastava, Ph.D., University of Montreal, Canada; Josephine M. Egan, M.D., NIA.

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Keywords:
aging
growth hormone
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Recent Publications:

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

O'Connor KG, et al. *J Gerontol* 1998; 53(3): M176-M182.

O'Connor KG, et al. *Metabolism* 1999; (in press).

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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cardiovascular
exercise

Recent Publications:

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

Nagai Y, et al. *Circulation* 1998; 98: 1504-1509.

Lynch NA, et al. *J Appl Physiol* 1999; 86: 188-194.

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Conwit RA, et al. *Muscle & Nerve* 1998; 21: 1338-1349.

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 joined the Laboratory of Cardiovascular Sciences in 1977.He has been Head of the Human Cardiovascular Studies Unit since1992.Dr. Fleg became the Interim Director of the Baltimore Longitudinal Study of Aging and Acting Chief, Longitudinal Studies Section in 1998.

The **Longitudinal Studies Section** is responsible for the operation of the Baltimore Longitudinal Study of Aging (BLSA). Research has focused primarily on the BLSA in the following areas:

(1) Prostate growth and disease: Our work is defining anatomic and physiologic characteristics that distinguish normal prostate growth with age and the development of prostate disease; characterizing the development and normal progression of benign and cancerous prostate disease; identifying hormonal changes important in the diseases; characterizing markers (serum and genetic) that identify high risk groups; and improve diagnostic strategies for prostate cancer detection and prevention.

We plan to continue to use knowledge of the natural history of prostate growth to improve diagnostic acumen, and raise awareness that some men with low PSA levels may not need intensive screening, while other men are at high risk. We are currently examining PSA blood levels as a general risk factor for prostate cancer rather than as a specific diagnostic test. We plan to explore dietary issues that may affect prostate cancer or BPH risk, as well as their impact on PSA levels. Two studies have been developed to examine genetic factors contributing to prostatic disease. The first study examines specific genes associated with prostatic cancer. The initial focus is on four genes: (1) the u-class glutathione S-transferase, gene GSTM1, (2) the _-class GST gene GSTP1, (3) the human

androgen receptor gene hAR, and (4) the inherited prostate cancer susceptibility gene PRCA. The second study will identify genes associated with prostatic growth.

(2) Neuromuscular changes with aging: our goal is to understand the time course of strength loss, factors that contribute to the loss, and the degree the exercise response differs between old and young individuals. Our research has three main components.

1. Descriptive cross-sectional and longitudinal characterization of neuromuscular and functional changes with age. We are examining the relationship between a variety of clinical, physiological and genetic factors and their contribution to age associated changes in muscle strength and muscle mass. Of particular interest are changes that begin during midlife. A better understanding of the contributors can lead to better preventive measures that may allay the marked changes that occur in late life. We are currently looking at the effect of changing serum testosterone, DHEA, and DHT levels on muscle strength in men. In addition, we are exploring whether muscle related genes are associated with age-associated strength changes.

To the study the relationship between strength and functional performance, we have established a collaboration with Dr. William Paloski of NASA to examine balance in the BLSA using the Equitest equipment used to study the effects of space flight on balance in astronauts. As a result of the Glenn flight, NASA wants to know the impact of age on balance performance. The BLSA data will be compared to the astronaut data to (1) examine the implications of increasing age on potential long term space flight, and (2) document the sequence and magnitude of changes in various elements of the balance system during normal aging. In addition, we will examine the relationship between muscle strength, cardiovascular fitness, leisure time physical activity on balance performance, and the association between balance performance, gait and falls.

2. Comparison of exercise response to resistive strength training in young and old subjects. We have recently completed an exercise intervention study with the Department of Kinesiology of the University of Maryland, College Park, comparing the response to resistive training in old and young men and women.Using knowledge gained from the intervention, we are now working with the School of Nursing at Johns Hopkins University to examine the effects of electromyostimulation versus an educational intervention to increase leisure time physical activity in elderly subjects with moderate to severe osteoarthritis of the knees. The

alternative strategies being tested were selected with the hope of overcoming the need for a more comprehensive strength training program, which is usually not well accepted and maintained in older adults.

(3) Examination of the motor unit and its relationship to muscle strength and exercise response.The goal of this project is to understand the changes that occur in motor units with aging, the effects of these changes on muscle strength and how these changes affect the exercise response. We have developed a protocol to examine motor unit size as well as firing characteristics at different levels of muscle exertion in the vastus medialis.The protocol is unique in that we are able to sample the active motor unit pool at specific force levels that span much of the force generating capability in the vastus medialis.We are currently examining the effects of aging and motor neuron disease on motor unit physiology.A protocol has been developed with NASA researchers that will examine the motor unit physiology before and after 17 weeks of absolute bedrest.These problems are of importance to the health and independence of the elderly and to the effects of microgravity during space flight. To further understand the effects of extended bedrest, a protocol has been developed with Dr. Christine Kasper, and Dr. Laura Talbot, School of Nursing, Johns Hopkins University, to examine the recovery in rats following hind limb suspension.

(4) Cerebrovascular changes with aging: This project is studying carotid and intracerebral arteries using Doppler ultrasonographic techniques in BLSA participants. The goal is to determine whether differences in arterial structure and function explain racial and gender differences in cerebrovascular disease (CVD), and whether changes in arterial characteristics are associated with fitness and frailty. These findings and previous LCS findings that stiffness properties of the central arteries are inversely related to cardiovascular fitness (VO_2max) raise new questions about the potential value of arterial properties in characterizing the risk for CVD in apparently healthy subjects.We plan to continue this work by examining the following questions. (1) What is the relationship between intimal medial thickness and arterial stiffness? (2) Are there relationships between carotid intimal medial thickness (IMT), arterial stiffness and cerebrovascular flow characteristics? (3) How do longitudinal changes in IMT affect arterial stiffness? (4) Does IMT, arterial stiffness, or their interactions predict hard endpoints including myocardial infarction, stroke or CVD death?A large body of evidence extols the importance of diet in the prevention of cardiovascular disease. The primary focus has been on alteration of fat intake, but evidence exists for the importance of antioxidants, folate, B12 and other nutrients. Clinical trials have

demonstrated decreased risk through diet, drugs, and exercise. However, the relationship between diet and arterial structure and function is less clear.This leads to (5) Do dietary factors, e.g. lipid intake, antioxidant intake, B12, or folate affect IMT or arterial stiffness?

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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Recent Publications:

Nagai Y, et al. *Circulation* 1998; 98: 1504-1509.

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

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

Lynch NA, et al. *J Appl Physiol* 1999; 86: 188-194.

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 my 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 we began to use 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 that 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, and to optimize forms, records, and protocols.

Prostate Aging and Disease: The BLSA is characterizing normal aging in the prostate and identifying transitions to prostate disease, particularly benign prostatic hyperplasia (BPH) and prostate cancer. In addition, the research is using information about structure and function of the prostate to improve 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. Prospectively, BLSA men aged from 30 to 79 have physiological, clinical and imaging of their prostate. The prospective study will continue through 2003.

Prostate growth To date, the major accomplishments have come from analyses of prostate specific antigen (PSA) which show that PSA increases linearly relatively more 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 goes up exponentially 5-7 years prior to diagnosis. Furthermore, the ratio of free to total PSA is able to distinguish men who develop prostate cancer from and those who do not

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.

Alterations in prostate structure or function are studied in relation to the possible development of prostate disease, particularly BPH. Currently, magnetic resonance imaging of the prosate are performed at each visit. The 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 American Urological Association 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.

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 who, 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 susceptibility gene PRCA. A study of familial genetics in BPH has been planned. 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.

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 an in house constructed equipment that measured isometric strength and power in the upper extremities. The purpose is 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. We are also interested in the contribution of muscle strength to functional performance and the development of disability, balance problems, and falls.

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. The study has been completed, and analyses are currently underway.

3. Examination of the motor unit and its relationship to muscle strength and exercise response. A clinical 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 allow 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.

Age-Associated Race and Gender Differences in the Carotid and Intracerebral Arteries: This project is studying intracerebral blood flow velocity and resistance, carotid blood flow velocity, and carotid wall characteristics using doppler ultransonographic techniques in BLSA participants. The goal is to determine whether differences in either carotid or intracerebral 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 concommitant with dilitation. Greater carotid wall thickness is associated with increasing risk for the development of both overt and silent coronary heart disease after adjusting for age, and that the common carotid wall thickness is thicker in the presence of asymptomatic coronary disease. Carotid doppler ultrasonography is commonly used during evaluation of cerebrovascular disease. Our findings suggest that examining the carotid wall thickness can increase the suspicion for coronary artery disease. In a related analysis, we found that women who use estrogen replacement postmenopausally show less arterial stiffness than women who are not on replacement. Improved arterial function may be another result of hormone replacement therapy that contributes to lower rates of heart disease. We have also observed that age change in flow velocities in the carotid artery is poorly correlated with the flow velocities in the middle cerebral arteries. We have compared different measures of arterial stiffness across age and explored which measures are most related to the development of coronary heart disease.

Body Composition and Bone Aging: This project was formerly in the Applied Physiology Section under the leadership of Dr. Jordan Tobin. With Dr. Tobin's retirement, the section was merged into the LSS. The focus of the work has been on the physiological and pathophysiological changes in bone and body composition that are associated with three of the most common problems of the elderly, osteoporosis, osteoarthritis, and sarcopenia. Most of the research at present is on the BLSA where we are examining longitudinal changes in bone mineral density and body composition. 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 is becoming more important as more men live to an age at which hip fracture is common.

The higher rate of loss of bone in women, with twice the incidence of hip fractures as compared to men, has led to the Perimenopausal Initiative that is examining the changes in the rate of bone loss in women as they traverse the menopause. 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. In addition to the bi-annual BLSA visit, 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. As of the end of 1998, 21 women have completed the protocol, an additional 25 were classified as perimenopausal, and 26 were still premenopausal. Analyses will proceed as more women complete the study.

Collaborators: Jerome Fleg, M.D., S. Mitchell Harman, M.D., NIH; Michele Bellantoni, M.D., Robin Conwit, M.D., Christopher Earley, M.D., Ph.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; Laura Talbot, RN, CS, Ed.D., Ph.D., Johns Hopkins University; William Palosky, Ph.D., NASA.

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Recent Publications:

Holmes S, et al. *J Feminist Family Therapy* 1994; 6: 27-48.

O'Neil J, et al. *The New Psychology of Men* 1995; 164-206.

Biography: Dr. Holmes was trained in family studies at the University of Connecticut, where she received her Ph.D. in 1996. Her interests in aging research focused on mother-daughter relationships in later life. She furthered her research training by completing a Post-Doctoral Fellowship in Aging at Boston University. During this time, she served as a Visiting Scholar at Radcliffe College where she developed a measure to assess interpersonal dynamics in the mother-daughter relationship in later life. Dr. Holmes joined the BLSA staff in May, 1998, and is currently involved in project management and research in two areas: subject retention and physical functioning.

Subject Retention in the BLSA: This area of the BLSA activity focuses on upgrading the level of involvement of partially active participants to full participation, and on preventing fully active participants from becoming less involved. Partially active participants are defined as those who are at least one year past their visit due date. Efforts to increase the level of participation and to maintain full level of participation are multi-pronged. They involve data collection through informal conversations and through a survey, in order to ascertain the level of participation satisfaction with various aspects of the program, to elicit participants' suggestions for improvements, and to determine the barriers that may interfere with regular participation. Beginning in April 1997, active participants began completing the survey at the end of their visit to GRC. The results will continue to be analyzed to make recommendations for improvements in the BLSA operations.

One of the frequently cited barriers to participation is the lack of transportation. In response to this problem, the BLSA has developed a transportation program for both local and non-local eligible participants, i.e, those who are 60 or older and have had at least 5 visits. At the present time, the transportation program involves providing ground transportation for those who live within a 150 mile radius. However, a significant number of partially active participants live in distant locations, and the cost of airfare or train transportation has prevented some participants from full participation. Therefore, the transportation program was expanded in the FY99 to include providing airplane or train tickets for eligible participants (60 or older and at least 5 visits). As was the case with local participants, Dr. Holmes will contact the out-of-state partially active eligible participants, inform them of this new transportation option, and will encourage them to return to the GRC for a visit. Dr. Holmes will serve as the project officer for the transportation program on an ongoing basis.

Other strategies for subject retention, such as offering weekend visits or abbreviated visits, may be considered in the future. Those partially active participants who do not come for a visit despite our retention initiatives, will be contacted by telephone by Dr. Holmes and interviewed in depth, in order to update their medical history and determine their current level of physical and mental functioning.

Collaborators: Barbara Hiscock, B.A., National Institute on Aging.

Physical Functioning Inventory (PFI): Dr. Holmes is involved in monitoring the administration of the Physical Functioning Inventory to the BLSA participants, in making revisions in the instrument, and in data analyses involving the PFI. Since 1992, the BLSA participants have been administered the PFI, a 22-item questionnaire exploring physical functioning in four areas: activities of daily living (e.g., bathing), instrumental activities of daily living (e.g., preparing meals), mobility, and moderate to strenuous activities. For each item, there are probes exploring the level of difficulty (if any), modifications made in the activity, changes in frequency of performing the activity, and symptoms and conditions associated with the difficulties in performing tasks. To date, research on age-related changes in physical functioning has focused on older populations and on advanced stages of disability. In contrast, the PFI, which is administered to adults from age 20 and up, provides data on physical functioning across the entire adult lifespan. In addition, the PFI was designed to detect changes across the full spectrum of physical functioning, from optimal physical functioning, to subtle, preclinical changes (when subjects may not recognize difficulty and yet report making compensatory modifications in the way they perform daily activities), to recognized difficulty, to dependency and frank disability.

Current analyses involving the PFI data focus on cross-sectional age comparisons in various aspects of physical functioning and on adaptations made in response to declines in physical functioning. Another set of data analyses involved comparisons of partially active and fully active participants on terms of their physical functioning, with special emphasis on implications for subject retention and recovery, and the inclusion of data from partially active participants in data analyses. The plans for future PFI data analyses include exploring the relationships between physical functioning and gait speed (as a performance measure of physical functioning), physical strength, and physical activity. The PFI data will provide knowledge base for future intervention programs aiming to redesign the physical environment and the daily tasks.

Collaborators: Linda P. Fried, M.D., P.P.H., The Johns Hopkins Medical Institutions; Barbara S Hiscock, B.A. and Jeffrey Metter, M.D., National Institute on Aging; Lauren M. Whetstone, Ph.D., East Carolina University.

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Recent Publications:

Beamer BA, et al. *Diabetes* 1998; 47: 1806-1808.

Andres R, *Obes Res* 1999; 7(4): 417-419.

Sorkin JD, et al. *Epidemiological Reviews* 1999; (in press).

Sorkin JD, et al. *Am J of Epidemiology* 1999; (in press).

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.

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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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, the Developmental Genomics Section under the direction of Minoru S.H. Ko, and a Gene Recovery and Analysis Unit headed by Ramaiah Nagaraja.

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. Five 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. Nuclear organelles that determine large-scale chromatin remodeling events. Such events are involved in chromosome dynamics related to large-scale control of gene expression. The Transcription Remodeling and Regulation Unit is using a combination of approaches to isolate and characterize the critical complexes, including the one that is modified to cause the Werner premature aging syndrome.

4. 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.

5. The genetics of aging-related complex conditions is being approached by interactive studies of the "founder" population in Sardinia. Initial phenotypes to be studied along with epidemiological factors include arterial stiffness, selected psychiatric/psychological traits. For this project investigators from Cardiovascular Sciences (Edward Lakatta and Angelo Scuteri), Personality and Cognition (Paul Costa and Alan Zonderman), and EDB (Tamara Harris and Richard Havlik) are working with Antonio Cao and Guiseppe Pilia, human geneticists at the University of Cagliari, Sardinia.

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, automated sequencing, and chromatin analysis techniques. The Transcription Remodeling and Regulation Unit provides Mass Spectrometric Protein Analysis in a resource headed by Salvatore Sechi. Among specific technological improvements that are being developed are techniques for the recovery of complete genes and YACs in circular, autonomously replicating clones (in the Gene Recovery Unit), and protocols to make and analyze high-quality cDNA libraries from very few cells from subregions of embryos (in the Developmental Genomics and Aging Unit) and in collaboration with the Microarray Laboratory run by Kevin Becker (see Research Resources Branch). The laboratory also benefits from joint 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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ectodermal dysplasia
premature ovarian failure

Recent Publications:

Huber R, et al. *Proc Natl Acad Sci USA* 1999; 96: 616-621.

Mazzarella R, et al. *Genome Res* 1998; 8: 1007-1021.

Pellegrini M, et al. *Dev Dyn* 1998; 213(4): 431-439.

Lin H, et al. *Cancer Res* 1999; 59(4): 807-810.

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 (HUGO) International, and President, HUGO Americas.

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 Simpson-Golabi-Behmel Syndrome (SGBS) or Anhidrotic Ectodermal Dysplasia (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.

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. The genetic potential provided from the Sardinia population provides an increasingly promising resource for genetic risk assessment and the determination of critical genes involved in aging-related conditions.

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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Recent Publications:

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

Zhao K, et al. *Cell* 1998; 95: 625-636.

Xue Y, et al. *Mol Cell* 1998; 2: 851-861.

Huijun Z, et al. *Genomics* 1998; 51: 140-143.

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 characterization of novel ATP-dependent chromatin-remodeling complexes, histone deacetylase complexes, and a helicase complex involved in the Werner premature aging syndrome.

The establishment and maintenance of transcriptionally active and inactive chromatin structure in higher eucaryotes is key for global 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. These complexes can be classified into two different families: one, the histone acetyltransferase or deacetylase complexes which alter the chromatin structure by covalently modifying the tails of histones; the other, the ATP-Dependent Chromatin-Remodeling (ADCR) complexes which use the energy of ATP to disrupt non-covalent DNA-histone contacts. The main focus of our lab is to purify and characterize mammalian ADCR complexes.

Structural and Functional Studies of Mammalian SWI/SNF-Related Chromatin-Remodeling Complexes: The SWI/SNF complex, originally identified in yeast, functions as a chromatin remodeling machine in signaling pathways that lead to activation of gene expression. 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, and in tumor suppression by interacting with RB protein. Mutation of the hSNF5 subunit has been shown as a cause for pediatric rhabdomyosarcoma. We have completely purified several distinct mammalian SWI/SNF-related complexes. By microsequencing, we have cloned all 10 subunits from a major complex of human KB cells. Six of these 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.

NURD, a Novel Complex with both ATP-dependent Chromatin-remodeling and Histone Deacetylase Activities: ADCR complexes are known to facilitate transcriptional activation by opening chromatin structures for activators. We recently identified a new human complex, named NURD, which contains not only ATP-dependent nucleosome disruption activity, but also histone deacetylase activity which is usually associated with transcriptional repression. Our results suggest that ATP-dependent chromatin-remodeling can participate in transcriptional repression by assisting repressors in gaining access to chromatin. One subunit of NURD was identified as MTA1, a metastasis-associated protein with a region similar to the nuclear receptor corepressor, N-CoR; and antibodies against NURD partially relieve transcriptional repression by thyroid hormone receptor.

Purification of a complex containing WRN, the helicase involved in Werner's premature aging disease. Many human helicases discovered to date are related to diseases, which include the Werner's Syndrome gene (WRN), Cockayne's Syndrome (ERCC6), Xermaderma pigmentosum, Bloom's Syndrome and ATR-X (*a*-thalassemia with X-linked mental) Syndrome. Many of the gene products have only been identified recently and their mechanisms of action are not known. We recently found that the gene product encoded by WRN is present in a high molecular weight complex in HeLa cells. We have now purified this complex and identified all of its subunits by microsequencing. We are now studying the functions of the WRN complex. Hopefully, this will lead to our better understanding of the human aging process.

Proteomics: protein identification and analysis by mass spectrometry. HPLC-coupled Mass Spectrometry has become the most powerful tool in protein identification and post-translational modification studies. It requires at least 10-fold less material than previous methods for protein identification. We have used this technique to identify the subunits of NURD and WRN protein complexes. We have recently set up our own Mass Spectrometry facility for GRC. The facility will be used to identify new proteins important in gene regulation and aging.

Collaborators: Dr. Jacques Cote, Laval University Cancer Research Center; Dr. Bradley Cairns, Harvard Medical School; Dr. Jiemin Wong, Baylor College of Medicine; Dr. Xiao-Long Zhang, Smith-Kline Pharmaceuticals.

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mouse gene mapping

cellular immortality

pre- and peri-implantation mouse development

Biography: Dr. Ko received his M.D. degree in 1986 and his Ph.D. in 1991 from Keio University School of Medicine in Tokyo. He held positions as Researcher from 1988 to 1991 and as Group Leader from 1991 to 1992 at the Furusawa MorphoGene Project, ERATO, JST, Japan. In 1992, he moved to the United States as Assistant Professor at the Center for Molecular Medicine and Genetics, Wayne State University in Detroit, Michigan, where he was promoted to Associate Professor and received tenure in 1997. He joined the NIA in Fall of 1998 to establish the Developmental Genomics and Aging Section within the Laboratory of Genetics. In one earlier study, using a steroid hormone inducible gene, he demonstrated a stochastic component in the regulation of expression of individual genes at a single cell level. He has also developed three methods that aid in profiling systematic gene expression in specific cell types. These are: 1) PCR-based amplification of a complex mixture of cDNAs, which allows the analyses of a cohort of genes expressed in the small number of cells; 2) a way to construct a normalized cDNA library in which the abundance of individual cDNA species is equalized; and 3) an efficient PCR-based method for localizing mouse cDNAs or ESTs on the genetic map.

The major goal is to understand the fundamental mechanisms for cellular commitment to mortality. Replicative senescence has been an important focus of aging research for many years, though studies have concentrated on the senescence of cells already committed to mortality. Here we rather concentrate on the critical distinction between immortal early embryonic cells and mortal differentiating derivative cells. Studies will utilize the potential of a systematic genomic approach to analyze early mammalian development. The paradigm for this approach has been established in our efforts over the last five years. The approach includes the construction of cDNA libraries from a small number of cells followed by large-scale cDNA sequencing, cDNA mapping on the mouse and human genomes, in situ hybridization to mouse embryonic and fetal preparations, and

Recent Publications:
Yotsumoto S, et al, *Dev Biol* 1998; 203: 264-275.

Schlessinger D, et al, *Genomics* 1998; 52: 113-118.

Abe K, et al. *Int J Dev Biol* 1998; 42: 1051-1066.

Ko MSH, et al. *Hum Mol Genet* 1998; 7: 1967-1978.

simultaneous gene expression analyses by DNA microarray technologies. This is a powerful route to characterize cells and tissues and their differential functions. Although the approach can be applied to any biological phenomenon, we will focus primarily on two developmental systems in mouse: differentiation of extraembryonic cells and differentiation of germ line cells.

1. **Differentiation of Extraembryonic Cells:** The first differentiation event in mammalian embryos generates two distinct lineages: the trophectoderm (TE) and the inner cell mass (ICM). The ICM will eventually become most of the embryo proper, while the TE will eventually become the extraembryonic tissues such as placenta. The mechanism for this transition of cellular state is not well understood. However, ICM cells of the 3.5-days post coitum (dpc) mouse blastocyst have a feature characteristic of immortal cells: namely, cells from the ICM can be propagated indefinitely in appropriate cell culture conditions as Embryonic Stem (ES) cells. Once cells are differentiated into the TE, however, their life span is set and they cannot be propagated indefinitely. In this sense, TE cells are mortal. Therefore, to look for genes that turn on or off to initiate differentiation of the TE is to look for genes that transform immortal cells into mortal cells.

2. **Differentiation of Germ Line Cells:** Germ line cells are often viewed as immortal, because they provide continuity from generation to generation and do not seem to age. In fact, the embryonic germ (EG) cells established from 8.5-dpc primordial germ cells show the same pluripotent stem cell phenotypes as the ES cells. Therefore, this is another relevant system to look for genetic determinants of cellular immortality.

Collaborators: Dr. Kuniya Abe, Kumamoto University, Japan; Dr. Grant MacGregor, Emory University, Atlanta, GA; Dr. John Schimenti, Jackson Laboratories, Bar Harbor, ME.

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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 disease-associated aging processes. The Laboratory is comprised of two sections – the Clinical Immunology Section (CIS) and the B-Cell Development Section (BCDS). Within the Clinical Immunology Section, there are several research units including the Lymphocyte Differentiation Unit and the Lymphocyte Biology Unit. 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.

Laboratories within the Clinical Immunology Section are currently examining (1) a role for various cytokines, hormones, and chemokines in leukocyte trafficking, cellular activation, and apoptosis; (2) the biological and molecular mechanism of HIV-1 entry and propagation in Th1/Th2 subsets and mononuclear cells obtained from young and elderly individuals; (3) the preclinical and clinical development of immunonologically-based protocols focusing on promoting cellular responses in elderly populations with the ultimate goal of improving the immune function of aged and cancer-bearing individuals; and (4) 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.

The B-Cell Development Section is currently examining (1) the development 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 populations and (2) the forces that shape the development of the B cell repertoire for antigen.

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Recent Publications:

Mikovits JA, et al. *J Virol* 1998; 72: 5231-5238.

Hesselgesser J, et al. *Curr Biol* 1998; 8: 595-598.

Fenton RG, et al. *Immunotherapy* 1998; 21: 95-108.

Hirano A, et al. *Blood* 1999; 93: 2999-3007.

Nilsson G, et al. Blood 1999; 93: 2791-2797.

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 Laboratory of Immunology at the National Institute on Aging as the Section Chief of the Clinical Immunology Section and the Acting Chief of the Laboratory of Immunology.

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. Furthermore, studies examining the differential expression of various cytokines and chemokines post cellular activation via mitogens, hormones, and stress factors are also under investigation. 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, T Cell 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 equally capable of being infected with the various T-cell tropic of HIV-1. However, HIV-1 infection of Th1 but not Th2 clones results in a rapid Fas-and caspase-dependent cell death which may account for the increased numbers of Th2-like cells in AIDS patients. Additional studies have revealed that differentiated human and rodent neuronal cells are also induced to under go active apoptosis upon treatment with HIV-1 as well as a variety of chemokines and HIV proteins. These findings along with our previous findings that chemokines provide activation signals in lymphocytes suggest that signals through chemokine receptors by endogenous ligands or HIV glycoproteins may differentially regulate the cellular growth, differentiation, and activation of human lymphocytes and neuronal cells. 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 and neuronal cells obtained from elderly patients have an increased susceptibility to HIV-1. The Clinical Immunology Section will continue to examine this question. We believe that understanding the immunophysiology of HIV-1 infectivity of young and old T cells, human Th1and Th2 clones, and human neuronal cells and cell lines as well as the various mechanisms of viral propagation and cell death induced post chemokine receptor ligation may provide new insight into HIV-1 pathology.

Additional studies are underway examining the ability of various HIV-1 viral isolates, gp120 proteins, and chemokines to directly induce gene expression in young and old human lymphocytes and neuronal cells. We believe that active transcriptional signals through CD4 and/or chemokine receptor molecules are required for optimal HIV-1 infectivity and propagation as well as for normal lymphocyte adhesion and migration. Using differential display analysis and microarray gene filters and chips,

we are examining the expression of known and unknown genes induced post chemokine receptor ligation or viral infection. We believe that the identification and examination of induced or suppressed genes will not only provide insight into HIV pathogenesis but may also elucidate the molecular mechanisms of inflammation and the various signaling defects observed in aged lymphocytes.

Clinical Monitoring and Preclinical Vaccine/Cancer Therapy 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 and/or elderly patients treated with various human hormones such as growth hormone (GH), prolactin (PRL), and DHEA have been examined for alterations in innate immune function and leukocyte trafficking. In addition, some clinical trials examining the *in vivo* immunoadjuvant effects of PRL and GM-CSF in elderly patients are currently being planned. Preclinical studies from this laboratory have already revealed that GH, PRL, DHEA, or GM-CSF provide costimulatory signals during T cell activation both *in vitro* and *in vivo*. We believe that additional immunological research on cytokine- and 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; Richard Horuk, Ph.D., Berlex Pharmaceuticals; Milan Fialo, M.D., UCLA; Stefan Brocke, Ph.D., NINDS, NIH; Francis Ruscetti, Ph.D., William Murphy, Ph.D.,NCI, NIH, James Lillard, Ph.D., University of Alabama at Birmingham.

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Recent Publications:

Taub DD, et al. *J Immunol* 1997; 158: 2745-2755.

Correa MR, et al. *J Immunol* 1997; 158: 5292-5296.

Srivastava RK, et al. *Mol Cell Biol* 1998; 18: 3509-3517.

Srivastava RK, et al. *Proc Natl Acad Sci USA* 1999; 96: 3775-3780.

Srivastava RK, et al. *Mol Cell Biol* 1999; 19: 5659-5674.

Biography: After completing medical school at the University of Missouri, Columbia and internal medicine training at the Peter Bent Brigham Hospital and Harvard Medical School in Boston, he obtained fellowship and laboratory training at NIH and has been here for 22 years. Prior to his becoming Scientific Director, NIA in 1995, Dr. Longo was the Director, Biological Response Modifiers Program, Associate Director, Division of Cancer Treatment, National Cancer Institute, Frederick, Maryland. He is the author of over 600 articles and book chapters. He is editor of *Harrison's Principles of Internal Medicine*, *Cancer Chemotherapy and Biotherapy*, and *Clinical Oncology Alert*. He is an associate editor of *Blood*, *Journal of the National Cancer Institute*, and *Clinical Cancer Research* and he sits on the editorial boards of six other peer-review journals. He has been cited by *Good Housekeeping* as one of the "Best Cancer Doctors in America" and listed in *Best Doctors in America*.

The Function of Bcl-2: Bcl-2 is known to prevent apoptosis in response to a variety of stimuli. It is said to work through binding to proapoptotic Bcl-2 family members such as Bax and Bad. However, its precise mechanism of action is poorly defined. We have analyzed Bcl-2 function in the prevention of apoptosis from chemotherapeutic agents and other stimuli. We have found that Bcl-2 function can be altered by phosphorylation. Agents that interfere with microtubule function initiate a signalling cascade that among other things, activates jun N-terminal kinase or JNK, which appears to be involved in Bcl-2 phosphorylation. The site on Bcl-2 that is phosphorylated with attendant inhibition of Bcl-2 function is serine 70. A Bcl-2 mutant that lacks serine 70 or its adjacent loop region is hyperfunctional and cannot be inhibited by phosphorylation. We have also found that apoptosis from paclitaxel and vincristine is mediated substantially through the fas/fas ligand pathway. Bcl-2 interferes with paclitaxel-induced apoptosis by blocking the upregulation of fas ligand expression in the tumor cells. It does this by binding to calcineurin and

preventing its activation. Thus, the transcription factor, NFAT, remains phosphorylated and nonfunctional and is unable to translocate to the nucleus to induce fas ligand expression. Bcl-2 binds to calcineurin through its BH4 domain. Another unexpected finding in this work was that Bcl-2 appears to be capable of preventing the generation of nitric oxide, an important early step in some forms of apoptosis. Future studies are aimed at understanding the mechanism of Bcl-2 inhibition of nitric oxide generation.

The Regulation of Growth Fraction in Tumor Cells: The vast majority of solid tumors have a very low growth fraction at the time they become clinically evident, usually in the range of 3-7%. When the tumor is treated, the growth fraction increases in an effort to maintain the tumor cell mass. This is reminiscent of the organization of most organ systems. Resting bone marrow stem cells are recruited into cycle when under the influence of a myelotoxic stimulus. Surgical removal of a portion of the liver stimulates the recruitment of hepatocytes into the cell cycle to replace the removed tissue. Other examples could also be cited. What is of interest to us is how a tumor cell, with its many genetic abnormalities that tend to promote proliferation, is pulled out of the cell cycle in the first place. Some gene product that is working in the resting tumor cells has managed to antagonize all the oncogene mutations and missing or malfunctioning tumor suppressor gene products and stop the cell from dividing; and it does this reversibly. When the tumor perceives an attack that reduces its volume, cells can be recruited back into the cell cycle. We are separating fresh lymphoma specimens into dividing and nondividing populations, isolating cDNA, and using differential display and microarray techniques, characterizing genes that are expressed in resting cells but not in dividing cells. Such messages will be isolated, their genes identified, and then the message will be introduced into dividing cells to look for growth arrest.

Tumor-induced Immunosuppression: We initially observed, and it has been widely reproduced, that T cells from tumor-bearing hosts are defective in their signalling in response to antigen and in their function. A variety of defects are noted including defective nuclear translocation of the p65 NF-kappa B transcription factor, shortened half-lives for a number of cellular proteins such as TCR-zeta chain and signalling kinases of the src family, among others, and a deviation of the cytokine production profile toward Th2 cytokines (IL-4, IL-10) and away from Th1 cytokines (interferon-gamma, TNF). Evidence of suppression of immune function in mice in which tumor is growing in hollow fibers in the peritoneal cavity without any cell-cell contact in the host suggests that a soluble tumor

factor is responsible for the defect in cellular immunity. We have devised a method of reproducing these tumor-induced changes in normal NK cells in vitro and are in the process of isolating the tumor-derived factor responsible for the changes.

Collaborators: Dennis Taub, Ph.D., National Institute on Aging; Douglas Ferris, Ph.D., National Cancer Institute; William J. Murphy, Ph.D., National Cancer Institute; James J. Kenny, Ph.D., National Institute on Aging.

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Recent Publications:

Liu K, et al. *Proc Natl Acad Sci USA* 1999; 96: 5147-5152.

Weng N, et al. *Immunity* 1998; 9(2): 151-157.

Hathcock KS, et al. *J Immunol* 1998; 160(12): 5702-5706.

Weng N, et al. *Proc Natl Acad Sci USA* 1997; 94: 10827-10832.

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 interests of this laboratory are focused on two areas: 1) the molecular and cellular mechanisms of lymphocyte differentiation and immunological memory; and 2) the molecular basis of learning and memory formation. The function of the immune system is dependent on the ability of lymphocyte division during development, differentiation and activation. It is unknown how naïve lymphocytes differentiate to become memory cells, what is the molecular basis of long-lived memory cells, and how aging influence the immune functions. A large-scale analysis of gene expression in naïve and memory lymphocytes allows us to identify genes that are involved in these processes. Further study of these differentially expressed genes and an understanding of their function will provide a rational basis for developing strategies of experimental and clinical intervention. Another area of interest is the mechanisms of learning and memory formation and aging effects on this process. We use rat stone maze as a model and analyze gene expression dynamics in hippocampus during maze learning. This analysis will allow us to identify and to characterize genes that are involved in the normal maze learning and the changes in aging.

Roles of Telomere and Telomerase in Human Lymphocyte Development, Differentiation, Activation and Aging: Telomere, the terminal structure of chromosomes, has captivated considerable attention recently for its newly discovered function involving the regulation of cellular replicative lifespan. Every telomere consists of an array of tandem hexamer repeats, $(TTAGGG)_n$ and the binding proteins, hTRF1 and hTRF2. The inability of DNA polymerase to completely replicate the ends of chromosomes results in a loss of 50-200 basepair telomere repeats with

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 normal somatic cells. In contrast, germline and malignant cells have infinite lifespan and maintain telomere length due to expression of telomerase. Telomerase is a unique reverse transcriptase consisting of two essential components, telomerase RNA template (hTER) and telomerase reverse transcriptase (hTERT), and functions to synthesize telomere repeats which serve to protect integrity of chromosomes and to prolong replicative lifespan of cells. The selective presence of telomerase 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 previous studies demonstrated that the telomere length is longer in naïve than in memory T cells, reflecting their replicative history *in vivo* and paralleling replicative capacity *in vitro*; and that telomerase is also expressed in lymphocytes in a strictly regulated manner during lymphocyte development, differentiation, activation, and aging. Furthermore, in contrast to the recent findings that transcription of hTERT determines telomerase activity in normal somatic cells, human lymphocytes express hTERT independent of the presence, absence, or quantitative level of detectable telomerase activity. Current ongoing studies are focused on the mechanisms of telomerase regulation, and age influence on the dynamics of telomere length and telomerase activity in subsets of lymphocytes.

Identification and Characterization of Differentially Expressed Eenes in Naïve and Memory Lymphocytes: Immunological memory is one of the defining features of the immune function, yet its underlying mechanisms are not completely understood. Human $CD4^+$ naïve and memory T cells are distinct both phenotypically, naïve T cells express CD45RA and memory T cells express CD45R0, and functionally, naïve T cells require two-signals for activation and memory T cells require only one signal. In an attempt to elucidate the molecular mechanisms of immunological memory, we analyzed difference at the molecular level between $CD4^+$ naïve and memory T cells using cDNA microarray. Approximately half of the estimated total human genes were analyzed and found that naïve and memory $CD4^+$ T cells expressed about 13% of total genes. The levels of expression over 90% of the expressed genes are similar between naïve and memory cells. Using an expression ratio of 2 as criteria of differential expression, we found that about 400 genes that are differentially expressed in naïve $CD4^+$ T cells and close to 300 genes differentially expressed in memory $CD4^+$ T cells. Among these cDNAs,

only 5-10% of these differentially expressed known genes and the majority of them are ESTs. Currently, we are studying the functions of some of the known genes and characterizing the full length of the ESTs.

Analysis of Gene Expression in Rat Hippocampus in Maze Training and Aging: Learning and memory are complex neurological processes that involve in acquisition, storage and/or retrieval of information. Long-term memory formation is involved in several areas of brain including hippocampus, requires de novo RNA and protein synthesis, and declines with increase of age. Although progresses has been made in defining the anatomic areas and elucidating the importance of synaptic plasticity in learning and memory in the past three decades, the molecular mechanisms underlying learning and memory formation as well as the aging influences this process are essentially unknown. In an attempt to dissect the memory process at the molecular level, we used cDNA microarray to analyze the changes of rat hippocampal gene expression before and after training of T-maze and between young- and old-trained rats. After analyzing over mouse 16,000 unique cDNA clones, we found several genes that were up-regulated in T-maze trained hippocampus in both young and old rats and identified differentially expressed genes between young and old rats. Currently, we are characterizing the structure and function of these genes.

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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.

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.

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.

Collaborators: Johanas Reim, M.D., John Hopkins University; Howard Dintzis, Ph.D., John Hopkins University; Howard Young, Ph.D., NC-FCRDC; 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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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 process 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 of DNA repair or transcription coupled component of the DNA repair process.

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Recent Publications:

Cullinane C, et al. *Biochemistry* 1999; 38(19): 6204-6212.

Dianov G, et al. *J Biol Chem* 1999; 274: 13741-13743.

Brosh R, et al. *J Biol Chem* 1999; 274: 18341-18350.

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 from 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.

DNA Repair Processes: Several types of DNA lesions have been observed in mammalian DNA. They are removed by a number of different DNA repair pathways. One 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 of which are 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, BER and mismatch repair. 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. Research questions that are being addressed include: what is the signal for transcription coupled repair ? Which DNA lesions are repaired by this pathway ? Can oxidative DNA damage, thought to accumulate with aging, be repaired by this pathway ? To address these questions, we are taking a number of approaches. DNA damage is induced by exposure of cells or purified DNA to various types of DNA damage or cellular stress. DNA repair is studied in intact cells, in situ, in tissue culture, in cell extracts, or using purified components.

DNA Repair and Aging: DNA damage accumulates with senescence. Defects in DNA repair that might arise during aging could be the cause. The question of whether or not DNA repair declines with aging is central in our research. This decline may be subtle and may reflect changes in the repair of actively transcribed DNA.

Oxidative DNA Damage and Mitochondrial Functions: Reactive oxygen species are generated in cells as by-products of cellular metabolism. They are products of the metabolic processes in each cell, and reactive oxygen species react with proteins, lipids, and DNA to generate oxidative damage. Oxidative DNA damage results from various forms of cellular stress, including exogenous exposures and endogenous metabolic processes. 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 these organelles. 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 inactivation, or the type of deletions that are commonly found in the mitochondrial genome in association with aging and cancer. Because mitochondrial DNA is subjected to high amounts of oxidative damage, it seems that mitochondria would need an efficient DNA repair activity to remove oxidative damage from their DNA. Although the notion has prevailed for many years that mitochondria can not repair DNA damage, there is now evidence to the contrary. Studies from our group and elsewhere have shown that a number of lesions are efficiently repaired from mitochondrial DNA. This includes the highly mutagenic lesion, 8-oxo-G.

Studies on mitochondrial DNA damage and repair have traditionally required the purification of mitochondrial DNA. This purification is laborious, and may introduce oxidative lesions in the DNA. As an alternative approach, we have used a gene specific repair assay that does not require the isolation of mitochondrial DNA. We have established an assay using a repair enzyme that detects 8-oxo-G, and it appears that this lesion is repaired very efficiently from both mitochondrial and nuclear DNA.

We have partially purified and characterized some mitochondrial repair enzymes that recognize specific oxidative DNA lesions, 8-oxo-G and thymine glycols. These appear to be unique but to be homologues of nuclear repair enzymes. Interestingly, the enzyme that recognizes 8-oxo-G is up-regulated with age in rats from 6 to 24 months. Thus, there is no decline, but instead an increase in repair activity with age. This finding is contrary to current notions of mitochondrial decline and is being pursued further experimentally.

We are establishing novel 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 what types of DNA lesions that 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 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 just 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.

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 many signs and symptoms associated with normal aging.

We are particularly interested in Cockayne syndrome (CS) and in Werner syndrome (WS), which we believe represent good model systems for molecular studies of normal human aging. The WRN gene, defective in WS, has been 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 CS protein, which is mutated in CS, and of the WRN protein, which is mutated in 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.

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 occurs by mechanisms that are dependent or independent of the tumor suppressor protein 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.

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Recent Publications:

Kumaravel TS, et al. *Neoplasma* 1999; (in press).

Bohr VA, et al. *NATO Meeting Proceedings* 1998.

Biography: Dr. Michele K. Evans, a board certified internist and medical oncologist, received her medical degree from the University of Medicine and Dentistry of New Jersey-The Robert Wood Johnson Medical School in Piscataway. She received her postgraduate training in internal medicine at Emory University School of Medicine and fellowship training in medical oncology within the Medicine Branch of the Clinical Oncology Program at the National Cancer Institute (NCI). Interest in human cancer prone disorders and DNA repair led her to study the role of DNA repair in cancer susceptibility as a Senior Clinical Investigator in the Laboratory of Molecular Pharmacology, NCI. At NIA, her major research interest centers on the clinical implications of eukaryotic DNA repair in cancer pathogenesis and aging. Dr. Evans also serves as Deputy Scientific Director, NIA.

Research: DNA repair mechanisms are believed to play a vital role in the maintenance of genome integrity. Loss of fidelity in the replicative mechanism, accumulation of genetic lesions, and faulty DNA repair mechanisms facilitate tumorigenesis. 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 highlighting the important clinical manifestations associated with defects in DNA repair mechanisms. The overall thrust of our work has been to understand the role of DNA repair in cellular senescence and tumorigenesis in order to uncover ways to use measured DNA repair capacity as a clinical tool in the diagnosis and treatment of cancer and age-related disease and disability.

Breast Cancer and DNA Repair: Our previous work has shown that nucleotide excision repair is defective in the Li-Fraumeni syndrome, a heritable cancer prone syndrome associated with increased susceptibility to breast cancer. To explore the possible role of DNA repair mechanisms in sporadically occurring breast cancer, nucleotide excision repair of UV-induced dimers has been studied in normal human mammary epithelial tissue and in hormone dependent and independent breast tumor cell lines. Studies of bulk DNA repair reveal a defect in the processing and repair of UV-induced cyclobutane pyrimidine dimers for both estrogen receptor positive and estrogen receptor negative breast cancer lines. Furthermore, results of gene-specific repair experiments performed on both tumor cell lines and normal human mammary epithelial cells, suggest that transcription-coupled repair in the two tumor cell lines is defective. Taken together, these results suggest that defective DNA repair may be important in both heritable and sporadic breast cancer. Our recent work has sought to examine whether this transcription coupled repair defect is associated with alterations in the transcription or protein expression levels of genes or gene products in the NER pathway. Preliminary data suggest that in fact there are alterations of protein expression in at least one important component of this pathway. The clinical relevance of nucleotide excision repair defects in tumor cells may lie in potential use of this DNA repair profiling as a tool in assessing metastatic potential of a specific tumor or in deciding upon appropriate cytotoxic chemotherapy.

Increased sensitivity to endogenous DNA damage and/or defective DNA repair of other lesions may also be important susceptibility factors in the development of sporadically occurring breast neoplasms as well. Increased levels of oxidative DNA lesions have been observed in human breast tumors, suggesting that oxidative DNA damage may play a crucial role in mammary carcinogenesis. Accumulation of oxidative damage may be a result of increased susceptibility to reactive oxygen species. Alternatively, impaired DNA repair mechanisms may fail to eliminate the oxidative lesions thereby resulting in accumulation of DNA damage and subsequent development of mutations and genetic instability. Due to the high levels of oxidative DNA lesions in breast cancer tissue, it is speculated that DNA repair capacity in breast cancer cells may be diminished. Classically, breast tumorigenesis in women has been associated with the hormonal

status and genetic inheritance factors. Traditionally, estrogen has been implicated in breast cancer development. The role of estrogen in DNA damage and repair in human mammary tissue is unknown. New thoughts about its role in breast tumorigenesis center on its possible role on induction of oxidative DNA damage and possibly modulation of DNA repair efficiency. With the recent reports that estrogen may increase DNA damage through its free-radical inducing metabolites, it is even more compelling now to find out whether exposure of human mammary cells to estrogen increases DNA damage or modulates DNA repair mechanisms. Our studies attempt to delineate the role of estrogen on DNA repair in human mammary cells. Thus, the long-term goal of the studies described below is to determine the role of estrogen in DNA repair in human mammary cells. This overall goal will be achieved by testing the following hypotheses: (i) Estrogen modulates DNA repair through transcriptional regulation of DNA repair genes. (ii) Estrogen influences DNA repair at a gene specific level. (iii) Estrogen influences DNA repair through modulation of chromatin architecture. In these studies, H2O2-induced damage will be used to study the effects of estrogen on DNA repair in normal mammary cells and breast cancer cell lines. The efficiency of DNA repair mechanisms as well as hormonal factors may be crucial determinants of mammary carcinogenesis. The results of these experiments may further the understanding of the molecular effects of estrogen on human breast biology in terms of DNA damage and 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.

DNA Repair as a Mechanism of the Adaptive Response: The adaptive response (AR) is a phenomenon whereby the harmful effects of high dose ionizing radiation or other genotoxic agents can be mitigated by prior exposure to a low dose of the same or similar genotoxic stress. The adapted cells show an increased survival, less chromosomal aberration and decreased mutagenesis termed the adaptive response. It is not clear which biologic pathways are involved in the AR; speculation centers on cell

cycle controls, signal transduction, and DNA repair mechanisms. DNA repair mechanisms, once thought to be constitutive, have now been proven to be inducible. Wilson, Mitra, and others have shown that genes and gene products involved in base excision repair are induced after low doses of certain forms of DNA damaging agents. We are working on the hypothesis that induced DNA repair, both base excision and nucleotide excision repair are equally important as underlying mechanisms of AR. The components of the proximal limb of the p53 DNA damage response pathway likely are critical in the initiation and maintenance of the adaptive response. Specifically, we hypothesize that one mechanism of the adaptive response is the induction of DNA repair pathways (nucleotide excision repair and base excision repair) by low doses of ionizing radiation or other genotoxic stress through activation of p53 related genes. There is evidence that PARP and ATM may be required for AR. However, the role of DNA-PK in concert with these two components and possibly c-ABL is unclear. It is also not discerned how other p53 related genes such as interferon regulatory factors 1 and 2 (IRF1, 2), GADD45, p21 are involved. GADD45 known to be induced by low-moderate doses of ionizing radiation and IRF 1 a tumor suppressor protein and transcription factor known to regulate responses to DNA damage by interacting with p53 and p21 have yet to be examined in the AR pathway. We speculate that these genes are critical elements in the AR because they are essential in the DNA damage recognition mechanisms, and cell cycle check points and may activate or induce repair. Our work to date has focused on evaluating the role of DNA-PK in AR. Using SCID mouse models with non-leaky mutations in DNA-PK, we are evaluating AR in terms of biological endpoints that include apoptosis, cell survival, and persistence of γ irradiation induced DNA damage measured by the Single Cell Gel Electrophoresis (COMET Assay). Further studies will examine mutant cell lines with defects in p53 related genes, nucleotide excision and base excision repair related genes will be used as well to dissect the potential interrelationships between the stress induced signal transduction pathways and DNA repair pathways as they relate to the adaptive response.

Understanding the mechanism of the adaptive response is clinically applicable because it may play a role in modulating the cellular response to chemotherapy and radiation therapy used for malignant disease. In addition, defects in the adaptive response may be correlated with the diminished capacity for senescent cells and elderly individuals to respond appropriately to exogenous environmental stress and genotoxic agents.

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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 at the NIA.

Somatic Hypermutation of Immunoglobulin Variable Genes: Somatic hypermutation of variable 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.

DNA Repair and Hypermutation: The first goal is to determine if DNA repair either introduces or removes hypermutations in variable genes. Nucleotide substitutions are likely inserted by an error-prone DNA polymerase during replication or repair, and the mismatch repair pathway may process the mispairs before they are replicated into both DNA strands. We have studied the frequency and pattern of mutations in mice defective for the mismatch repair proteins PMS2, MSH2, and MLH1. Although the frequency of mutation in the repair-deficient mice was similar to that of wild-type mice, the pattern was altered. The results suggest that the hypermutation pathway frequently introduces tandem mutations which are then corrected by a PMS2-dependent repair process,

Recent Publications:

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Tchorzewski MT, et al. *J Surg Res* 1998; 77: 99-103.

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Phung QH, et al. *J Exp Med* 1998; 187: 1745-1751.

Winter DB, et al. *Nucleic Acids Res* 1998; 26: 4422-4425.

Phung QH, et al. *J Immunol* 1999; 162: 3121-3124.

and frequently mutates G.C basepairs which are then corrected by a MSH2-dependent pathway. The MLH1 protein had no direct effect on the mutational spectrum. We also studied the mutation pattern in mice deficient in nucleotide excision repair ($XPA^{-/-}$) and base excision repair ($OGG1^{-/-}$), and found no effect on hypermutation.

Hypermutation in Old Humans: The second goal is to analyze hypermutation in variable genes from old humans. As described above, we have recently correlated several patterns of hypermutation in mice with 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 were cloned from RNA made from peripheral blood lymphocytes taken from old and young humans, and were sequenced to identify mutations. The results show that antibodies from old people have the same frequency of mutation as those from young people, indicating that old people have high affinity antibodies that can bind to various pathogens. Both the pattern of mutation and increase in microsatellite instability in old humans suggests that mismatch repair declines with age, which may lead to formation of cancers.

Mechanism of Hypermutation: The third goal is to identify genes that are involved in hypermutation. Since mutations are likely introduced by a DNA polymerase, we will eliminate the expression of different DNA polymerases in mutating cells to see if mutation decreases. Other genes involved in the process will be identified on DNA microarrays by comparing RNA from mutating cells against RNA from nonmutating cells. Candidate genes that are unique to mutating cells will then be transfected as antisense cDNA into mutating cells to see if mutation decreases.

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Recent Publications:

Majumdar, et al. *Nat Genet* 1998; 20: 212-214.

Lacroix, et al. *Biochemistry* 1999; 38: 1893-1901.

Biography: Dr. Michael Seidman received his Ph.D. in biochemistry from the University of California, Berkeley, in 1975. He did postdoctoral work at the NIH and at Princeton University. He worked at the NIH and in the biotechnology industry before assuming his present position in the Laboratory of Molecular Genetics, NIA.

Cellular Response to DNA Damage: We are interested in the response of cells to targeted DNA damage and the application of site specific targeting for modulating genomic sequences.

Gene Targeting: Current approaches for manipulating genomic sequences rely on homologous recombination. In these procedures relatively lengthy DNA fragments are introduced into cells and via an enzymologically driven process engage in a search for homologous sequences in the chromosome. After a recombinational intermediate forms the process is completed in a series of additional enzymatic steps. The procedure is inefficient and time consuming. Given the marked increase in sequence data from the genome project there is a clear utility in having a more efficient and less cumbersome process.

We are developing oligonucleotides which can form a three stranded DNA structure called a triple helix. The third strand lies in the major groove of an intact double helix and is stabilized by hydrogen bonds between the bases in the third strand and the purine bases in the duplex. These structures are quite stable and very stringent with respect to sequence. The oligonucleotides can be linked to DNA reactive compounds and site specific modification of DNA with these oligo-reagent conjugates has been demonstrated by many groups. Although these structures have been studied for many years, there have been relatively few accounts of biological applications.

Recently we and our colleagues constructed an oligonucleotide linked to psoralen (a photoactive DNA crosslinker) which was designed to form a triplex with a sequence in the well known cellular housekeeping gene HPRT (hypoxanthine guanine phosphoribosyl transferase). This gene encodes an enzyme engaged in purine salvage. There is a simple selection procedure for cells which lack the enzyme, and, consequently, the gene has become the most commonly used mutation marker gene in mammalian cells. The oligo was introduced in cells in culture and after photoactivation the cells were processed according to a standard mutation selection protocol. Mutations were found at the target site, and sequence analysis demonstrated that the majority were small deletions. This was the first evidence that chromosomal targets are accessible to triplex forming oligonucleotide reagents.

This approach can now be used to deliver additional DNA reactive compounds to specific genomic locations and we are in the process of developing those reagents. We are also looking at the influence of DNA repair deficiencies on the targeted mutation frequencies. This will tell us which DNA repair activities are active in repair of the directed lesions, and lead to the development of strategies designed to inhibit these functions during the time of oligo introduction. Eventually this approach will be used to modulate genomic sequences with targeted gene knockout as a specific application.

Collaborators: Dr. Paul Miller, Johns Hopkins; Dr. Peter Glazer, Yale University; Dr. Gordon Hayer, National Cancer Institute, NIH.

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

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Keywords:
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premature aging

Recent Publications:

Bohr VA, et al. In *The Alfred Benzon Symposium No.44, Molecular Biology of Aging*. T. Munksgaard, pp 262-272, 1999.

Brosh RM, et al. *J Biol Chem* 1999; 274: 18341-18350.

Balajee AS, et al. *Mol Biol Cell* 1999; 10(8): 2655-1668.

Group Members: David K. Orren, Ph.D., A.S. Balajee, Ph.D., Robert M. Brosh, Ph.D., Amrita Machwe, Ph.D, Vilhelm A. Bohr, M.D., Ph.D.

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.

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 hypersensitive to treatment with most DNA damaging chemicals, with the exception of one carcinogen, 4-nitroquinoline. Some WS cells are defective in transcription coupled DNA repair, but no other DNA repair defects have been demonstrated. Experiments with intact cells and cell extracts suggest that WS cells may have a defect in basal transcription. 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.

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.

We have purified the WRN protein for use in a number of basic and complex biochemical assays. The protein has helicases activity and exonuclease function. Interestingly, it interacts with replication protein A (RPA), both physically and functionally. RPA enhances the helicases activity in the unwinding of larger DNA duplex structures.

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, Jan O. Nehlin, Ph.D.

Oxidative DNA Damage Processing

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Keywords:
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Recent Publications:

Dianov GL, et al. *J Biol Chem* 1998; 273: 33811-33816.

Bohr VA, et al. *Biochemie* 1999; 81(1): 155-160.

Dianov G, et al. *Nucleic Acids Res* 1999; 27: 1365-1368.

Croteau DL, et al. *DNA Repair* (in press).

Souza-Pinto N, et al. *Nucleic Acids Res* 1999; 27: 1935-1942.

Dianov G, et al. *J Biol Chem* 1999; 274: 13741-13743.

Lipinski LJ, et al. *Nucleic Acids Res* 1999; 27: 3153-3158.

Group Members: Simon Nyaga, Tanja Thybo Frederiksen, Grigory Dianov, Ph.D., Richard Hansford, Ph.D., Nadja De Souza Pinto, and Vilhelm A. Bohr, M.D., Ph.D.

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*.

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. 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.

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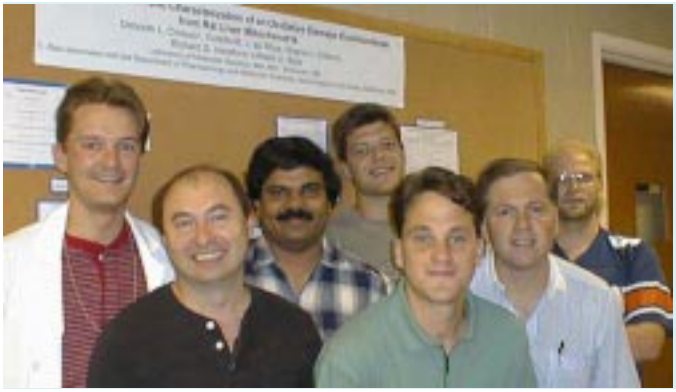

Transcription and DNA Repair in Cockayne Syndrome Cells

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Keywords:
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Recent Publications:

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

Bohr VA, et al. *J Investig Dermatol Symp Proc* 1998; 3(1): 11-13.

Jaiswal M, et al. *Nucleic Acids Res* 1998; 26: 2184-2191.

Dianov G, et al. *Nucleic Acids Res* 1999; 27: 1365-1368.

Group Members: Robert Brosh, Ph.D., Rebecca Selzer, Ph.D., Grigory L. Dianov, Ph.D., A.S. Balajee, Ph.D., Morten Sunesen, Alfred May, 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. Complementation studies demonstrated that two genes, designated CSA and CSB, are involved in CS. 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. 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 complex clinical phenotype of CS, however, suggests that DNA repair may not be the only 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.

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 that somehow affect the transcription in CSB cells but not in normal cells. Experiments in intact CSB 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.

We have generated some stable cell lines with functional domain knockout of different regions of the CSB gene. In one of these cell lines, we have mutated the ATPase/helicases domain of the gene. These cells have a phenotype similar to CSB cells. Thus, this region of the gene is very important for its function. We are analyzing the phenotypes of cell lines with mutations in other regions of the gene.

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

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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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Keywords:
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personality genetics

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.

Costa PT Jr, et al. *Eur J Pers* 1998 ;12: 117-134.

Costa PT Jr, et al. In *Advanced Personality.* Barone, DF, ed. Plenum Press: New York, 1998; 103-118.

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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Keywords:
personality structure
longitudinal studies
openness to experience
cross-cultural research

Recent Publications:

McCrae RR, et al. *Am Psychol* 1997; 52: 509-16.

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

McCrae RR, et al. *Dev Psychol* 1999; 35: 466-477.

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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Keywords:
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risk factors & protective factor for AD
cognitive decline & Alzheimer's disease
behavioral genetics

Recent Publications:

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

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

Maki PM, et al. *Psychol Aging* 1999; 14: 284-294.

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.

Collaborators: Claudia H. Kawas, M.D., Johns Hopkins Bayview Medical Center; R. Nick Bryan, M.D. Ph.D., Johns Hopkins University; David. J. Vandenbergh, Pennsylvania State University.

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Recent Publications:

Resnick S, et al. *Horm Behav* 1998; 34: 171-182.

Davatzikos C, et al. *Cereb Cortex* 1998; 8: 635-640.

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.

Collaborators: R. Nick Bryan, M.D., Ph.D., Johns Hopkins University and the National Institutes of Health; Christos Davatzikos, Ph.D., and Michael Kraut, M.D., Ph.D., Johns Hopkins University; Edythe London, Ph.D., National Institute of Drug Abuse; Barry Horwitz, Ph.D., Laboratory of Neurosciences, NIA; Alan Evans, Ph.D., and Keith Worsley, Ph.D., Brain Imaging Center, McGill University.

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Recent Publications:

Thayer JF, et al. *Biol Psychiatry* 1998; 44: 304-306.

Rossy LA, et al. *Psychosom Med* 1998; 60: 773-781.

Brosschot JF, et al. *Ann Behav Med* 1998; 20: 326-332.

Biography: Dr. Thayer received a B.A. in Psychology from Indiana University, and Master's and Ph.D. degrees from New York University. After academic positions at Penn State University and the University of Missouri, he joined NIA to initiate a program on Emotions and Quantitative Psychophysiology. His research interests concern biological and psychological adaptation and flexibility in the context of dynamical systems models with applications to psychopathology, pathophysiology, and health. This work utilizes indices of autonomic nervous system function derived from cardiac variability measures to probe whole organism systems.

Heart Period Variability as an Index of Neurovisceral Integration: One aspect of our research program is to develop, elaborate, and apply a model of neurovisceral integration in the context of normal and pathological functioning. This model uses heart period variability (HPV) to index the functioning of central-peripheral feedback mechanisms that produce goal-directed behavior. We have related HPV to attentional regulation and affective regulation in humans. These studies suggest that autonomic, attentional, and affective regulation are coordinated in the service of system adaptability and goal-directed behavior.

Autonomic Characteristics of Anxiety and Mood Disorders: Anxiety and depression are disorders associated with somatic symptoms such as tachycardia, rapid breathing, and disturbed sleep. Moreover, anxiety and depression are risk factors for cardiovascular morbidity and mortality. Our research has focused on the autonomic characteristics on these disorders to investigate their physiological and psychological concomitants with an eye toward understanding their development, course, and treatment. Research to date indicates that these disorders are associated with a relative decrease in vagally mediated cardiovascular control. This lack of cardiac vagal control is associated with poor affective and attentional regulation. Importantly, these deficits normalize with therapeutic intervention.

Cardiovascular Variabilities and Health: We are examining the relationship between HPV and cardiovascular system control. This research suggests that HPV and blood pressure variability (BPV) are inversely related in the healthy, intact organism and serves to maintain adequate blood pressure control. In spinal cord injury, the relationship between HPV and BPV can become dysfunctional, leading to poor blood pressure regulation and increased risk for cardiovascular disorders.

Collaborators: Thomas D. Borkovec, Penn State University; Jos F. Brosschot, University of Leiden, The Netherlands; Bruce H. Friedman, Virginia Tech University; Arve Asbjornsen, Kenneth Hugdahl, Bjorn Helge Johnsen, Jon Christian Laberg, University of Bergen, Norway; Richard D. Lane, University of Arizona; Richard A. Tyrrell, Clemson University.

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The Laboratory of Neurosciences (LNS) was formed under the leadership of Stanley I. Rapoport, M.D. in 1978. Dr. Rapoport stepped down as laboratory chief in 1999 and a search for a new laboratory chief is nearly complete. Dr. Rapoport continues as Chief of the Section on Cerebral Physiology and Metabolism. This Section studies brain function, metabolism, signal transduction and structure with regard to aging and disease. Two general areas are studied:

1) **Brain phospholipid metabolism in relation to signal transduction and neuroplasticity:** Radiolabeled long chain fatty acids (arachidonate, docosohexaenoate, palmitate) are injected intravenously into rodents and their rates of entry into brain precursor and phospholipid pools are used to quantify, by compartmental modeling, their turnovers and half-lives in brain phospholipids. Changes in tracer incorporation in response to drugs, demonstrated by *in vivo* imaging, identify sites of phospholipase A_2 mediated signal transduction, and when it is up- or down-regulated. Additionally, *de novo* synthesis of ether lipids is studied using labeled precursors. Studies with fatty acid and ether lipid precursor tracers are directly correlated with altered transcription, post-translational regulation or activity of critical enzymes involved in signaling and brain phospholipid metabolism. Methods include quantitative autoradiography, HPLC, GC-MS, and TLC, mathematical modeling, neuropharmacology, and molecular genetics with Northern and Western blotting and *in situ* hybridization, and positron emission tomography (PET). Rodent models include essential fatty acid deprivation, effect of drugs including chronic lithium therapy, transient cerebral ischemia, inflammation, chronic neurotransmitter dysfunction due to chemical or surgical lesioning, and genetic models — phospholipase A_2 and serotonin-uptake transporter mouse knockouts, transgenic mice with peroxisomal disorders. Non-human primate models include normal and lesioned macaques studied with PET. After animal models of brain dysfunction have been elaborated,

studies are carried out in human subjects using PET and positron emitting isotopes such as [^{11}C]arachidonic acid and [^{11}C]palmitic acid to examine signaling and neuroplasticity in relation to health and disease.

(2) **Coupling between brain synaptic activity and oxidative-phosphorylation (OXPHOS):** Gene and enzyme expression of brain mitochondrial OXPHOS enzymes are related to pre- and post-synaptic markers in rodent and monkey brain and in cell culture. In human control and Alzheimer disease brain tissue, these parameters are correlated with levels and location of neurofibrillary tangles and senile plaques, neuropathological correlates of Alzheimer disease. Reduced expression (mRNA levels) of genes coding for subunits of cytochrome oxidase in single pyramidal cells, in relation to paired helical filament density, supports staging of metabolic dysfunction in Alzheimer disease into reversible and irreversible steps. In normal human aging, reduced synaptic markers occur without reduced mitochondrial enzyme expression, consistent with compensation not involving energy failure.

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Recent Publications:

Chang MCJ, et al. *Neurochem Res* 1999; 24: 399-406.

Chang MCJ, et al. *Brain Res* 1997; 755: 74-83.

Hayakawa T, et al. *Brain Res* 1998; 807: 177-181.

Rapoport SI, et al. *Ann NY Acad Sci* 1997; 620: 56-74.

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.

Brain Phospholipid Metabolism in Relation to Signal Transduction and Neuroplasticity: 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 docosohexaenoate, 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 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 docosohexaenoate and palmitate, and thus likely acts on phospholipase A_2. Additionally, ^{11}C-labeled fatty acids were synthesized, in collaboration with the PET Department at NIH, and are 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 at rest and during activation. We plan to extend this protocol and related animal protocols to image phospholipase A_2-mediated signal transduction involving the brain, cholinergic, serotoninergic and dopaminergic systems.

Coupling Between Brain Synaptic Activity and Oxidative-Phosphorylation (OXPHOS): We demonstrated, by brain imaging with PET, reductions in cerebral glucose consumption and blood flow in patients with Alzheimer's disease, progressing with dementia severity. In post-mortem brain studies, we showed that the reductions likely corresponded to reduced regional brain activity of rate limiting enzyme for mRNA levels for subunits of enzymes involved in mitochondrial OXPHOS, whether the mRNA was derived from mitochondrial or nuclear DNA. In contrast, non-OXPHOS mRNA levels were unchanged. In single pyramidal neurons from affected regions of the post-mortem Alzheimer disease brain, we also showed selective down-regulation of OXPHOS in cells in which neurofibrillary tangles were absent or filled less than 50% of the cytoplasm, whereas cell death and a general reduction in transcription was found when tangles filled more than 50%. Selective down-regulation was related to reduced synaptic markers. We hypothesize that an early event in Alzheimer's disease 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 demonstration that the brain in mildly-demented Alzheimer's disease patients can be fully activated early in disease. In contrast to Alzheimer's disease, brains of healthy subjects do not show reductions in OXPHOS markers with age, whereas markers or pre- and post-synaptic elements fall with age. The net effect appears to be reduced optimal function without compromise of energy metabolism. Such changes likely underlie cognitive declines that have been reported with healthy aging.

Collaborators: William Eckelman, PET Department, Clinical Center, NIH; Norman Salem, Laboratory of Membrane Biochemistry and Biophysics, NIAAA; Pietro Pietrini, Department of Clinical Biochemistry, University of Pisa, Italy; Krish Chandrasekaran, Department of Anesthesiology, University of Maryland; Joseph Deutsch, School of Pharmacy, Hebrew University, Jerusalem, Israel; Kimmo Hatanpää, Department of Neuropathology, Yale University School of Medicine.

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Index of Keywords

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