National Institute on Aging

Annual Report

October 1, 1982 through September 30, 1983

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Office of the Director

The NIA, now in its eighth year of operation, continues to make progress in conducting, supporting, and encouraging research and training on all facets of the aging process--biological, behavioral, and social; the diseases of old age; and the special problems and needs of older people. Within the broadly based attack on aging, the Institute has identified several areas of programmatic emphasis:

Understanding aging - research which defines and/or significantly influences major manifestations of aging or conditions associated with old age.

Alzheimer's disease - research on the pathophysiological, behavioral, and social changes associated with this important dementing disease, as well as diagnosis and treatment.

Strengthening training for academic leadership - activities which can help provide a sufficient number of new and established scientific investigators and other resources for research on aging.

Maintaining independence - including not only selected interventions with a high probability of beneficial effects for the elderly but also a number of age-related conditions which may be prevented or controlled.

In addition to extramural support, there is continuing development of the intramural research program - including, specifically, the Laboratory of Neurosciences (LNS) which last year opened a small unit in the Warren G. Magnuson Clinical Center; the Baltimore Longitudinal Study of Aging (BLSA); and several major epidemiological studies of established populations of older people. The LNS has activities focusing primarily on the dementias and related neuropathologic diseases of the elderly. The BLSA, a study of normal aging, celebrated its 25th year in September, 1983. The epidemiological study samples permit special attention to the characteristics of dementia in the elderly.

Dr. T. Franklin Williams was sworn in as the second Director of the National Institute on Aging on July 5, 1983. Dr. Robert L. Ringler, interim Acting Director, NIA, retired on July 3. During the initial months in his position, Dr. Williams has been actively reviewing programs with NIA staff, as well as meeting with key NIH staff, agency heads, and congressional staff.

Activities Related to Research on Alzheimer's Disease

The Institute continues to provide major support for research on Alzheimer's disease, and in FY 1983 expended a total of $11,695,000 for extramural and intramural activities in this area. The teaching

nursing home grant awards, which support research programs in nursing home settings in conjunction with university medical schools or medical centers and which have been increased to five in number this fiscal year, have major commitments to research on Alzheimer's disease.

On May 16, the NIA Acting Director, Dr. Robert L. Ringler, participated in a panel of government scientists at a hearing on "Progress Made in the Treatment of Alzheimer's Disease" sponsred by the Senate Labor and Human Resources Subcommittee and chaired by Senator Charles E. Grassley. The initial panel speaker was Secretary of HHS, Margaret Heckler.

The new NIA Director, Dr. Williams, served on a scientific panel with the Directors of NINCDS and NIMH at the hearing on "Understanding, Treating, and Coping with Alzheimer's Disease," held by the House Energy and Commerce Subcommittee on Health and the Environment and the House Select Committee on Aging Subcommittee on Health and Long-Term Care.

An HHS Departmental Task Force on Alzheimer's Disease has been established under the chairmanship of Dr. Edward L. Brandt, Assistant Secretary for Health, with representation from the NIA, NINCDS, NIMH, VA, HCFA, and the AoA. The following work groups have been organized:

Etiology and Diagnosis
Epidemiology and Treatment
Clinical Course and Family
Systems of Care and Training
Public Education, Professional Education, and Information Dissemination

The Task Force is preparing an interim report organized along the above work group structure which is due October 31. A final report including recommendations of the Task Force is to be completed by December 1984. For the above areas the report includes information on the current state-of-the-art, gaps in knowledge, promising research directions, recommendations for the enhancement and development of research, and mechanisms for implementing the recommendations. Overarching issues of training, education, research dissemination, and financing and reimbursement will also be addressed.

Technology Assessment Conference

An NIA Technology Assessment Conference on "Evaluating the Elderly Patient: The Case for Assessment Technology" was conducted on June 29-30, 1983, at NIH. The conference was co-sponsored with the Office of Medical Applications of Research, NIH, the American Medical Association, and the National Center for Health Services Research (NCHSR). Its principal goal was to share with a multidisciplinary,

primary care-oriented audience the actual experience of both developers and users of assessment technology as a means of making systematic, comprehensive appraisals of the health status of elderly persons.

The conference was very well attended by over 600 health care providers, administrators, planners, and researchers. Proceedings of the conference will be published in the November and December (1983) issues of the Journal of the American Geriatrics Society.

The next phase of this effort will take place on October 18, 1983, when a small group of assessment technology experts invited by NIA will meet with key staff representing the Veterans Administration and the NCHSR to plan for the development of a research agenda on assessment technology.

Interagency Committee on Research on Aging

The ad hoc Interagency Committee on Research on Aging (IACRA) met twice in FY 1983 (November 1982, April 1983) to review ongoing programs and plan for future activities. IACRA is a long-standing ad hoc committee chaired by the NIA, which meets three times per year and is consistent with the Institute's statutory mandate to coordinate aging research activities throughout the Federal government. IACRA serves an important communication function involving representatives from seven Federal departments and agencies (of which DHHS is one) and 18 DHHS organizational components, all with a stake in aging research. Among the most important products of IACRA has been the contributions its members have made to the development of the first Inventory of Federally Supported Research on Aging (NIA, March 1982).

The NIA also has been significantly involved during FY 1983 in the PHS/Administration on Aging (AoA) collaboration on health promotion efforts involving the elderly. Specifically, an NIA staff member has chaired one of the four work groups (injury prevention and control) which comprise the collaborative venture between PHS and AoA and submitted its report on that area to the Surgeon General on June 7, 1983. In addition, the Office of the Director has contributed financial support, along with other agencies, to enable the convening of a special consultant group on a hip fracture reduction project, an outgrowth of the injury control initiative. The NIA is currently involved in preparing for the National Media Campaign on Health Promotion and Disease Prevention for the elderly to be initiated in May 1984 and retains responsiblity for implementing recommendations of the injury prevention initiative.

The Information Office (IO) of the National Institute on Aging is responsible for planning, organizing, and carrying out a congressionally mandated public information and health education program that is responsive to the interests of the general public, mass media, special interest groups, government agencies, and local organizations--all of whom have an important bearing on the accomplishment of the NIA's mission.

Organization

The Office is organized in two clusters--Public Affairs and Publications and Reports. The Public Affairs cluster plans and executes multi-media health education and publicity campaigns which support the NIA's congressionally mandated responsibilities in this area. The campaigns are accomplished through the production, promotion, and dissemination of materials to the general public and special interest groups, using all types of media (i.e., radio, television, newspapers, magazines, professional and scientific journals, still and motion pictures, posters, exhibits, and advertisements). The group initiates press activities--such as news releases, briefings, and science writers seminars--and works with the Publications and Reports cluster to produce materials that support these activities. The group is responsible for liaison with constituency organizations as well as special interest groups in an effort to support the development and continuation of NIA research programs. They design and produce special events--sometimes in collaboration with the private sector--which present the Institute's programs and policies to the public. The cluster responds to press and general inquiries as well as to Freedom of Information inquiries. They promote and assist in the dissemination of Institute resources and brochures--using announcements, press releases, and radio and television. They assist Institute staff in public presentations and provide suitable accompanying material. They serve as consultants to outside organizations and provide guidance on planning, managing, and executing health education campaigns.

The Publications and Reports cluster is responsible for the development of written materials such as feature articles; annual reports and bibliographies; research highlights; special projects for Congress; brochures; and fact sheets intended to communicate accurate and useful information about the NIA--its programs, interests, and achievements. The cluster develops, writes, and manages the production of individual pamphlets; publication series; technical manuscripts; and bulletins for science writers, researchers, and academic audiences. Where necessary, this cluster provides material which support campaigns by the Public Affairs cluster--in particular, material addressing a professional audience. The cluster provides direct editorial consultation to other NIA offices and advises Institute staff on the intricacies of clearance and of book and manuscript production. The cluster monitors existing publication inventories and supervises contracts that relate to storage and mailing for NIA publications. The group maintains liaison with the private sector in an effort to provide mutual support for the creation and dissemination of NIA materials as well as general information that would be of value to the elderly and to individuals working in the field.

Summary of IO Activities for May 1982 through April 1983

Although the official publications moratorium has been lifted by the Department, the imposition of ceilings on printing costs for the PHS and NIH

continues to limit some of the IO's activities. The rigorous review being given to concept clearances and the frequency with which they are being returned unapproved or with substantial budget cuts also serve to dampen information dissemination activities. One of the biggest losses suffered this year is the future prohibition against printing the Special Report series. This will be effective in 1984. The prohibition is based on the Department's perception that the Special Report is a self-serving document, yet it has been one of our most useful communications with those in the field of aging research and others who are interested in entering this area.

All kinds of inquiries--press, public, grants, and freedom of information---continue to occupy a great deal of the staff's time. Over the past several years the number of inquiries has continued to rise--from 50,000 letters and 24,000 phone calls in 1980 to 72,176 letters and 32,630 phone calls this year. The continuing development of new publications and Age Pages and the projected new fact sheet series will reduce the amount of time needed by staff to respond to these inquiries. The IO has responded to requests for over one-half million publications this year. In addition, we have distributed almost three million Age Pages, most of them in bulk shipments or in cooperation with other agencies or organizations.

Past plans to reduce distribution expenses by having a Senior Volunteer Distribution Center were dropped after it was established that the use of certain types of volunteers is specifically prohibited by the Public Health Service Authorization Act.

With the departure of the Institute's first Director, there was some sense that demand by the press for information would decrease. But this has not been the case. The established reputation of the IO as a source of quick, accurate replies has continued to bring an influx of inquiries from the press on every topic imaginable. Members of the IO are frequently called on to provide everything from statistical information to story ideas to ideas for whole series. Both GRC and BRCM staff have also been an enormous help to the IO on the many occasions when they have served as spokespersons for the Institute. The Associate Director of BRCM has been particularly accommodating and has become very popular with the press. Trends in public and press inquiries often follow each other since the public hears from the media about certain aspects of aging research and then, in turn, calls this office. Alzheimer's disease and life extension have been the big stories in the press this year. Interest in life extension has been prompted by the appearance of best sellers on longevity.

In an effort to give some congruity to the appearance of NIA publications, the IO has established a design "grid-system." The grid establishes a standard for type, publication size, and the location of certain key information on the cover; however, it allows flexibility in graphics. Our early sense is that the system, while not overly restrictive, will contribute to an "identity" for NIA publications without our having to resort to the use of a logo.

A user evaluation of the Age Page was conducted this year by a University of Missouri graduate student who was working as a public affairs intern at the NIA. Questionnaires were sent to three groups of individuals: those among the general public who regularly receive copies of the Age Page, organizations concerned with the elderly, and selected journalists. The evaluation concluded that public response has been almost exclusively positive.

A special expert hired by this office to edit a book on the Baltimore Longitudinal Study of Aging, Ed Watkins, now works almost exclusively in Baltimore. In addition, two other IO staff members provided assistance to GRC in helping to prepare for the GRC's 25th Anniversary Celebration.

The events marking the departure of the first Director made last summer busier than usual. In addition to planning several parties and celebrations and preparing bound volumes of articles by Dr. Butler, the staff sponsored a press coffee in the executive board room of the Ambulatory Care Research facility. The response to the press coffee was far greater than anticipated, with some 20 reporters coming out for an opportunity to chat with Dr. Butler. A number of reporters followed with appointments for in-depth interviews later that month. As a result, stories about the NIA appeared in Science, The Washington Post, Newsweek, and other publications.

The IO activities relating to Alzheimer's disease illustrate the range of activities engaged in by this office, as well as the expertise of staff members. During the past year, IO staff have answered several thousand inquiries from the press, public, and Congress on the subject of senility and Alzheimer's disease; we have researched, written, and--in some cases--published both lay and technical discussions of Institute-supported research on the subject; we have generated publicity for the Institute's basic and clinical research programs on Alzheimer's disease; and we have cooperated with private interest groups who sought to increase public awareness of this disorder. To highlight a few specific events, the IO staff disseminated a press release on the research of NIA-supported investigators at the University of Vermont; arranged local press coverage for the opening of an NIA dementia clinic at the NIH Clinical Center; cooperated with the Alzheimer's Disease and Related Disorders Association in generating national publicity for Alzheimer's Disease Awareness week--November 21 through 27; prepared a progress report on Alzheimer's research for the Senate Committee on Appropriations; and assisted in the planning and preparation of materials for the House Select Committee on Aging hearings on "Senility: The Last Stereotype."

Ongoing Activities

The IO staff routinely complete the following activities:

Respond to inquiries from older people and their families, members of Congress, physicians and other health care providers, media representatives, scientists, students, faculty, social workers, and staff of other Federal agencies.

Fill requests for NIA publications and refer those individuals asking for non-NIA materials to the appropriate source.

Maintain complex inventory of publications.

Provide information on NIA research interests, the grants process, and grant application procedures.

Provide guidance to NIA staff regarding concept clearance and publication procedures.

Coordinate NIA responses to Freedom of Information requests.

Handle requests for speaking engagements and interviews with staff; also, recommend additional speakers as appropriate.

Represent the NIA at meetings and speak before various (mostly local) groups.

Maintain mailing list and press call list.

Screen and clip articles that relate to aging from The Washington Post, The New York Times, The Wall Street Journal, The Christian Science Monitor, Time, Newsweek, U.S. News & World Report, Science, New England Journal of Medicine, Journal of the American Medical Association, and Medical World News.

Maintain extensive subject files of press clippings.

Stock 5th floor hall exhibit.

Publications

Press Releases:
- NIH Makes First Teaching Nursing Home Awards
- HHS Secretary Welcomes New NIA Director

Aging Updates:
- Dr. Williams Appointed New NIA Director
- Hypothermia: A Cold Weather Warning
- Possible Role of Aluminum in Alzheimer's Disease
- Warm Weather Watch for the Elderly
- Four New Members Join the National Advisory Council on Aging
- Elderly Require More of Six Vitamins and Minerals

Publication Announcements:
- White House Conference on Aging Summaries (11 summaries available)
- What is Aging Research?
- What is Geriatric Medicine?
- Special Report on Aging 1982
- Biological Mechanisms of Aging

Annual and Other Reports:
- NIA Annual Report FY 1982
- Scientific Directory 1983/Annual Bibliography 1982--NIA section
- NIH Publications List--NIA section
- Public Affairs Plans and Budgets
- Publications of Robert N. Butler, M.D., 1976-1982; 2 volumes--special collection
- NIA Information Programs
- Age Page User Survey and Evaluation
- Special Report on Aging 1983
- Developments in Aging 1983
- Diabetes Special Report 1983--NIA section
- Arthritis Special Report 1983--NIA section
- Genetics Special Report 1983--NIA section
- Special Report on Minority Aging 1983

Congressional Testimony:

NIA Statement for Alzheimer's disease hearings, May 1983
SDAT summary sheet for Waxman hearings

Brochures, Fact Sheets, and Books:

(Completed)
NIA Awards Ceremony program and announcement (with Awards Committee)
Progress Report on Geriatric Medicine (reprint)
Progress Report on Senile Dementia of the Alzheimer's Type (SDAT)
What Is Aging Research? and What is Geriatric Medicine? (reprints)
The Menopause Time of Life
NIA Publications list (revision)
NIA brochure (revision)
White House Conference lay summaries (reprint):
- Aging and the Family
- Sex Differences and Aging
- Aging and Digestion
- Aging and the Circumstances of Death
- Learning, Memory, and Aging
- Aging and Drugs
- Aging and Minorities
- Nursing and the Aging
- Osteoporosis and Aging
- Aging and Alcohol
- Sleep and Aging

(In Process)
Welcome to the NIA (revision)
Baltimore Longitudinal Study on Aging
Special Report on Aging 1984
Age Words: A Glossary on Health and Aging
Progress Report on Alzheimer's Disease, Volume II
Women over 65
Skin Care
Theories of Aging
Safe Drug Use in Old Age
Age Page compilation brochure
Self-Care and Self-Help brochure (revision)

Assisted the NIA Extramural Programs to Publish and Distribute:

(Completed)
Biological Markers of Aging
Inventory of Federally-Supported Research on Aging
Profiles of National Research Institutes and Programs on Aging: An Analysis by the NIA on behalf of the World Health Organization
Toward an Independent Old Age: A National Plan for Research on Aging
Report of the National Advisory Council on Aging for a National Plan of Research on Aging
International Directory of Organizations Concerned with Aging

(In Process)
The NIA Macroeconomic-Demographic Model

Age Pages:

(Completed)
Constipation
Who's Who in Health Care
Considering Surgery?
Arthritis Advice (reprint sponsored by Pfizer Pharmaceuticals)
Safe Use of Medicines (revision, with FDA support)
Crime and the Elderly
Don't Take it Easy--Exercise!
Urinary Incontinence
Dealing with Diabetes
Hearing and the Elderly
Aging and Your Eyes
Dietary Supplements: More is not Always Better
Osteoporosis: The Bone Thinner
Aging and Alcohol Abuse
Prostate Problems
Cancer Facts for People Over 50 (with NCI support)
Safety Belt Sense (with Department of Transportation support)

(In Process)
Alzheimer's Disease
How to Choose a Nursing Home
Salt

Spanish Translations of Age Pages:

(Completed)
Arthritis
Heat, Cold and Being Old
Skin Care

(In Process)
Crime and the Elderly
Foot Care for Older People

Articles and Speeches:

"Care of the Elderly, Research, and Technology" for joint WHO/Serono Symposium

"Staying Healthy" column for National Council of Senior Citizens newsletter

"Senility: The Epidemic of the Century" for Scientific American

"Health of the Elderly"

"Essay on Geriatric Medicine" for The Oxford Companion to Medicine

"Foreword" to Conference on SDAT: Ethical and Legal Issues Related to Informed Consent

"Triumph of Age: Science, Gerontology and Ageism" for Bulletin of the New York Academy of Medicine

"Aging and the Elderly"

"Hospitals Face the Graying of America"

"Care of the Aged in the U.S." for textbook, Geriatric Medicine and Technology

"Dr. Butler's Departure" for Uptown Citizen

"Alzheimer's Disease: An Examination" for TWA Ambassador magazine

Search For Health Columns Completed:
Urinary Incontinence
Hospice Care
Alzheimer's Disease

Surgeon General's Columns: Nutrition in Later Years

"Aging: A Special Issue" in News and Features

"Health Promotion Among the Minority Elderly" for Public Health Reports

NIH Record Stories:
Radcliffe Alumnae Honor Dr. M. White Riley
Dr. Gibbs, Visiting Scientist
NIA Designated as World Health Collaborating Center
Clinical Center Volunteers Needed for Alzheimer's Studies
New Publication Available on Urinary Incontinence
Dr. Butler Leaves NIA
Dr. Leavitt Dies
Employee at GRC Honored
Spanish Age Pages Available
First Teaching Nursing Home Awards Made
Eminent GRC Sex Researcher Retires
Dr. Williams Appointed NIA Director
Four New Members Join NACA
New Publication Offers Advice to Elderly
NIA Supports Seminar on Museums and the Elderly
Daniel Cowell Receives 1982 Leonard Covello Award
Smithsonian Hosts Lecture Series on Aging

Special Projects

Age Page User Evaluation:

During April and May 1983 an evaluation was carried out on the Age Page health information series. Questionnaires were mailed to 250 persons who regularly receive copies of the Age Page, 200 organizations (including universities, nursing homes, and national associations), and 50 journalists with medical or aging orientations.

The objectives of the evaluation were the following: to determine whether or not the information given in the Age Page is appropriate and adequately meets the needs of its audience; to discern preferences concerning content, appearance, and format; and to gather ideas for future topics or changes.

Out of the possible 250 responses by the general public, 114 questionnaires (46%) were completed and returned to NIA. Of the 200 questionnaires sent to organizations, 92 (46%) were completed and returned. Out of the 50 questionnaires mailed to journalists, 20 (40%) were completed and returned.

The questionnaires from the general public and organizations revealed that the majority found the series to be useful, easy-to-read, interesting, believable, clear, and reassuring. Journalists indicated that they found the Age Page to be useful in their work, for either excerpted material or as background information.

Suggested changes included a greater frequency in publication, the addition of a bibliography or other references, and more in-depth information on specific topics. Suggestions for future topics were directed toward the psychosocial areas of aging and Alzheimer's disease.

Audiovisuals and Still Photography:

The IO has developed several exhibits, public service announcements for radio and television, and slide/tape/photographic files in response to the growing demand for the Institute to provide audiovisual materials to the press, organizations holding conferences or meetings, and the general public.

A three-part videotape series entitled "Understanding Aging" was started this past year. The tapes were designed to be used in the classroom by 5th and 6th grade students. Part one has been completed; it discusses age-related stereotypes and provides a general introduction to the topic of aging. Parts two and three will focus on specific aspects of aging, including how young people can prepare for their own old age. When the three parts are completed, the pilot tapes will be reviewed by teachers in Montgomery, Prince George's, Frederick, and Baltimore Counties. Teachers' guides for activities will accompany the tapes.

Radio spots for accidental hypothermia and hyperthermia were produced and distributed to 3,000 radio stations across the country. Several radio scripts have been outlined for a new radio series called "Age Waves," based on the Age Page. Each program will feature medical experts who will talk about a particular area of concern for older people.

The NIA's large exhibit is used at conferences, seminars, and other relevant gatherings. It includes graphics highlighting demographic and health trends among the older population and videotapes about the NIA and its goals--with an "introduction to aging" using Rembrandt's self-portraits to illustrate one person's aging process, a 30-second animated cartoon, and various public service announcements.

Last year the IO provided black and white and color photographs (still photography) for several large projects, including the new NIA brochure. The photographs were taken at the GRC laboratories, the NIH Clinical Center, senior citizens' residences, nursing homes, shopping centers, and parks.

Report of the Behavioral Sciences Research Program

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BEHAVIORAL SCIENCES RESEARCH PROGRAM (BSR)

The long-range goal of the BSR program is to strengthen the scientific basis for professional practice and public policy that can enhance the health and well-being of older people and can contain the personal and social costs of health care and dependency. Within the National Institute on Aging's legislative mandate "for the conduct and support of biomedical, social, and behavioral research and training related to the aging process and the diseases and other special problems and needs of the aged," the BSR program defines a large, complex, and newly developing area. This area draws upon many social and behavioral science disciplines and requires convergence with the biomedical sciences as well. Established as a separate program late in FY 1979, BSR's official functions and authority include two components: (1) planning, directing, and setting policies for the BSR grant program; and (2) contributing to the broad scientific development of aging research by working with the relevant diverse programs conducted by other governmental and nongovernmental agencies both in the United States and in other countries. Each of these components--BSR grant program and broad scientific development--has special goals and requires distinct types of activity.

The BSR Program of Grants and Contracts

The program of grants for research and research training is now well underway. It has followed two lines of development, emphasizing both (i) a broad program of social and behavioral research on aging and (ii) a more specific "biopsychosocial" focus on the relation of social and behavioral factors to physical health and biomedical aging.

(i) The broad grant program, described in the major program announcement on "Social and Behavioral Research on Aging" (first issued in 1979), is concerned "with the social, cultural, economic, and psychological factors that affect both the process of growing old and the place of older people in society." Periodic re-issuance (and revision) of this broad announcement has several purposes:

- It provides a blueprint for long-range planning, defining the three "clusters" shown in the Organization Chart and the "Program Structure".
- It gives coherence and direction to the diverse research and training activities in many disciplines and institutions.
- It serves to raise the level of excellence of BSR's contributions to the complex mosaic of accumulating knowledge in this large area (priority scores have been improving markedly).
- Perhaps most important, it makes room for applications from already distinguished investigators whose work, though they may not previously have recognized the fact, is relevant to aging. (In FY 1981, for example, nearly half of the approximately 100 projects funded in BSR were from investigators either new to the field of aging or not previously supported by the NIA.)

(ii) Reflecting the more specific biopsychosocial focus is the second major BSR program announcement (first issued in 1981) on "Health and Effective Functioning in the Middle and Later Years." This announcement, which cross-cuts all the research and training areas of the broad program, aims to stimulate such developments as:

- The formulation of new research topics as to how, for example, particular social stresses--feeding through the brain to neural, endocrine, or immune systems--can influence the aging process.

- The adaptation to natural settings of biomedical measurements as, for example, while human subjects at various ages are being tested for responses to nursing home regimens or to socially stressful situations at work, small samples of blood can be taken continuously and concentrations of neuroregulators measured while the subjects move freely about.

- The formation of interdisciplinary research teams who are learning to understand one another's approaches.

Broad Scientific Development

BSR's mission transcends the development of specific grants and contracts that can be funded by a single agency. Its second charge is to foster the broad scientific background that is essential for its own continuing development, and that highlights NIA's potential as the leading center of social and behavioral research on aging. Each BSR finding is seen as one piece in an emerging mosaic of understanding, to which diverse agencies in many disciplines and in many parts of the world are contributing. These pieces, if they are to meet society's needs for this understanding, must be integrated and communicated. As longevity increases and the numbers of older people continue to mount, the entire scientific community must be apprised of the strategic importance of social and psychological factors in influencing the aging process and in maintaining health and well-being in the middle and later years. Thus BSR staff is engaged in such activities as the following:

(i) Coordination with other programs--in line with its mandate to evolve coherent directions from the diverse programs in aging research, BSR maintains working relationships with many national and international organizations, both public and private, including other Institutes at NIH and ADAMHA, the Institute of Medicine, the National Academy of Sciences, the multidisciplinary Committee on Life-Course Development of the Social Science Research Council, the Carnegie Corporation Aging Project, the American Council of Life Insurance, the World Health Organization, and so on. For example, BSR takes leadership in the NIH Working Group on Health and Behavior and in the proposed PHS Initiative on Health and Behavior.

(ii) Resource development--Efforts are made to identify, make available for use by investigators, and contribute to support of existing human study populations and data archives. Such study populations and data archives reduce the cost of longitudinal and cohort research based on large representative samples that are essential for making valid generalizations, telling how many people have particular problems or needs, or understanding

how people change as they grow older. BSR contributes to support, for example, of the National Archive for Computerized Data on Aging and to the Panel Study of Income Dynamics. Such efforts in turn nourish the BSR program as large numbers of funded projects make use of such resources.

(iii) Further development and application of research methods--While the social and behavioral sciences have been pioneers in making strategic methodological advances, BSR has only begun to stimulate their further development and wider application to research on aging. Moreover, many measures of human performance, designed for use with the very young, require revision for use across the lifespan. Methods are needed for analyzing continuous life histories; for linking social change to the aging of individuals; and for causal analyses of interrelated medical, behavioral, biological, and social variables.

(iv) Integration and dissemination of knowledge -- To allow the widely scattered research to evolve into a cumulative body of knowledge, inventories of carefully evaluated and codified research findings are sorely needed. Short of such needed inventories, BSR is stimulating preparation and publication of bibliographies and state-of-the-art symposia such as Aging in Society (published this year) and Age and the Life Course in Anthropological Theory, by Kertzer and Keith, (in press).

These are large objectives. Though important beginnings have been made, much remains to be done. Now is the opportune time and many plans for the future are in the making.

PROGRAM STRUCTURE

Coordinate with its goals, BSR has organized its structure and functions and deployed its personnel as indicated in Chart A. BSR is now divided into three major clusters of program areas, as follows:

I. COGNITIVE AND BIOPSYCHOLOGICAL AGING (CBA)--concerned with the identification and specification of cognitive, intellectual, and perceptual changes and stabilities that, interacting with biological processes, occur with aging.

a) Cognitive Aging--supports research and training on constancy and change in the processes of sensation, perception, attention, memory, learning, cognition and intelligence, motivation, creativity, wisdom, and psychomotor performance.

b) Biopsychological Aging--supports research and training on the relationships between behavioral aging on the one hand, and neurological, immunological, and endocrine aging, on the other. It includes studies in neuropsychological assessment, behavioral genetics, and nutrition.

II. SOCIAL PSYCHOLOGICAL AGING (SPA)--concerned with the changes and stabilities that occur with aging in the health, behavior, personality, and attitudes of people as related to the social environment.

CHART A
BEHAVIORAL SCIENCES RESEARCH PROGRAM
ORGANIZATION

Associate Director--Riley
Secretaries--Vacant 1/
Henderson

Deputy Associate
Director--Abeles

Grants Assistant--Angel

BSR-5

COGNITIVE AND BIOPSYCHOLOGICAL AGING (CBA) CLUSTER

Chief: Jakubczak

Areas

a) Cognitive Aging
Jakubczak
b) Biopsychological Aging
Jakubczak (acting)2/

SOCIAL PSYCHOLOGICAL AGING (SPA) CLUSTER

Chief: Abeles

Areas

a) Psychosocial Aging
Abeles
b) Biosocial Aging & Health
Ory

OLDER PEOPLE AND SOCIETY (OPS) CLUSTER

Acting Chief: Abeles

Areas

a) Age in the Population
Suzman
b) Age and Societal Structures
Bond (acting)
c) Older People and Social Institutions
Bond

Information Specialist (part time)--Darby

Clerical Staff (none)

Clerical Staff--Danzik

Clerical Staff--Redmond

1/ Being recruited

2/ Position requested

During FY 1983, BSR has addressed its large and compelling dual tasks of (1) developing and managing its grant program, and (2) stimulating broad scientific development in the behavioral and social science communities concerned with aging research and training.

Scientific Development

Toward its broad scientific goal, BSR has pursued during the current year all four lines of major staff activity: coordination with other programs, resource development, further development and application of methodology, and interpretation and dissemination of knowledge. Itemized in this report are seminars held, conferences in which BSR has participated, publications stimulated by BSR activities as well as those authored by staff members, and papers presented and sessions organized by staff. Noteworthy among these scientific activities are the issuance of Aging in Society, the continuing associations with the Institute of Medicine, the National Academy of Science, the Social Science Research Council, the World Health Organization, the Carnegie Corporation Aging Project, and many others. As lead agency for the NIH Working Group on Health and Behavior, NIA has been active in holding meetings and in joint planning with ADAMHA of a major interagency, interdisciplinary initiative on Health and Behavior for FY 1985. BSR has organized a conference on Aging, Health and Behavior, sponsored jointly by NIA and the Academy for Behavioral Medicine Research. In respect to resource development, BSR has continued to make small, but symbolically significant contributions to the unique annual surveys of the Michigan Panel Study of Income Dynamics; the set-aside colony of aging nonhuman primates; and to the National Archive of Computerized Data on Aging.

Workshops and Program Announcements

As one cost-effective strategy for guiding the formulation of significant research objectives, for challenging outstanding scientists, and for insuring the excellence of proposals, BSR has initiated a series of small workshops and task group meetings. These workshops are designed to implement specific aspects of the program announcement on Health and Effective Functioning in the Middle and Later Years, and to lead to subsequent program announcements, journal publications, and other inexpensive means of communication with potential grant applicants.

Emanating from these workshops and related BSR activities, four program announcements are scheduled for publication and will be widely publicized. Of the four, one focuses on visual aging; a second on social environmental influences in everyday life that can enhance the aging process. Of special current interest are two others on "Behavioral Geriatrics Research", concerned with health-related behaviors and attitudes of old people, their family, friends, and health care providers, as these affect health, functioning, and longevity. One of these announcements calls for research and training on "informal care" and on the health behaviors and attitudes of older people themselves; the other is a research career award designed to provide behavioral scientists with the background needed for interdisciplinary research in this area.

BSR Program of Grants and Contracts

These recent efforts to stimulate broad scientific development, and in particular to sharpen specific foci through workshops and program announcements, contribute incrementally to growth of the grant program. Each of the three program clusters in BSR, as described in detail below, has taken shape during FY 1983 with the completion of significant projects on which preliminary results have been previously reported and with the funding of new and promising projects that can add to the mosaic of accumulating knowledge of aging processes and the place of older people in the society. In comparison with FY 1982, notable advances have taken place in Social Psychological Aging (SPA), especially in the newly developed area on Biosocial Aging and Health. Cognitive and Biopsychological Aging (CBA) shows some declines. Older People and Society (OPS), despite the loss for most of the year of one of its two HSAs, has retained its strength through the extra effort of other HSAs in the program.

Table 1

BEHAVIORAL SCIENCES RESEARCH

EXTRAMURAL BUDGET ESTIMATE*
Fiscal Year 1983

Activity	Cognitive and Biopsychological Aging (CBA) Cluster	Social Psychological Aging (SPA) Cluster	Older People and Society (OPS) Cluster	FY 1983 Total Estimated Support
Research Projects (R01,P01,R23)	$ 3,657,587 38	$ 5,814,298 46	$ 3,799,702 33	$12,716,429 130
Research Centers (P30,P50)	0 0	234,555 1	0 0	234,555 1
Research Career Awards K01,K04,K07,K08)	55,560 1	51,141 1	164,689 4	271,390 6
Other Research (R03,R09,R13,R21,S06)	57,435 3	84,638 4	100,495 2	242,568 9
Research Training Fellowship Awards (F32,F33)	25,451 1	19,040 1	156,548 3	382,177 5
Training Grants (T32,T35)	834,556 4	250,451 2	355,545 4	1,259,414 10
Research Contracts and Agreements (N01,Y01,Y02)	108,000 2	220,000 3	22,000 1	350,000 6
Total	$ 4,738,589 49	$ 6,674,123 58	$ 4,598,979 47	$15,456,533 154

*Unofficial figures

Table 2

BEHAVIORAL SCIENCES RESEARCH

DEVELOPMENTAL ACTIVITIES
Fiscal Year 1983

TITLE	MECHANISM
Behavioral Geriatrics Research: Health Behaviors and Attitudes of Older People	Program Announcement (new)
Special Emphasis Research Career Award (SERCA) in Behavioral Geriatrics Research for Social and Behavioral Scientists	Program Announcement (new)
Visual Perception and Aging	Program Announcement (new)
Social Environments Influencing Health and Effective Functioning in the Middle and Later Years.	Program Announcement (new)
Social and Behavioral Research on Aging	Program Announcement (revision)
Health and Effective Functioning in the Middle and Later Years	Program Announcement (revision)
Joint with BRCM, Teaching Nursing Home Award	Program Announcement (revision)
Stress at Work: Causes, Effects and Moderators. Robert L. Kahn University of Michigan	Seminar
Psychophysiological Aspects of Social Understimulation in Old Age Bengt Arnetz Korolinska Instituet, Sweden	Seminar
Menopausal Status and Health Correlates in a General Population Sonja McKinlay American Institutes of Research John McKinlay Boston University	Seminar
Aging and Work Martin Kohli Free University of Berlin	Seminar

Table 2
(continued)

TITLE	MECHANISM
Behavioral Treatment and Urinary Incontinence (joint with BRCM)	Workshop
Aging and Cognitive Development: Planning Meeting	Workshop
Task Group on Changing Work Roles and Aging Workers: NIA Research on Work/Retirement	Workshop
Task Group on Independent Living in the Household: Living Alone	Workshop
Task Group on Risk Factors: Status of People Aged 85 and Over, With Special Emphasis on Women	Advisory Session
Health and Effective Functioning in the Life Course American Public Health Association Montreal, Canada	Symposium
Work and the Life Course: Sociological Perspectives Gerontological Society of America Boston, MA	Symposium
Relationship of Values to the Age Stratification of Society Eastern Sociological Society Baltimore, MD	Symposium
Aging, Health, and Behavior Annual Meeting of the Academy for Behavioral Medicine Research Reston, VA	Conference
New Research and Discoveries on Aging Smithsonian Institution	Conference Series
"Microelectronics and Working Women: A Literature Summary," Committee on Women's Employment and Related Social Issues, National Academy of Sciences, Washington, D.C. Diane Werneke	Publication (commissioned paper)

Table 2
(continued)

TITLE	MECHANISM
Aging in Society. Hillsdale, NJ: LEA, 1983. M.W. Riley, B.B. Hess, & K. Bond (Eds.)	Publication (originally commissioned for White House Conference on Aging)
Population Aging, Report of a Conference Sponsored by The American Council of Life Insurance and Health Insurance Association of America in Cooperation with the NIA	Publication
Aging and Human Visual Function. NYC: A.R. Liss, Inc., 1982. R. Sekuler, D. Kline & K. Dismukes (Eds.)	Publication (report of conference sponsored by NAS/NRC Committee on Vision)
"Some Research Needs in Aging and Visual Perception," Vision Research, 1983, 23(3): 213-216. R. Sekuler, D. Kline, K. Dismukes and A.J. Adams,	Publication (NAS/NRC Committee on Vision)
"Aging and Visual Function of Military Pilots: A Review," Aviation Space, and Environmental Medicine, 1982, 53(8): 747-757. R. Sekuler, D. Kline, and K. Dismukes (eds).	Publication (NAS/NRC Committee on Vision)
Resource Development	
NIA/DRR Set-Aside Colony of Aging Nonhuman Primates	Interagency Agreement
NIA/AOA Support of Archive for Computerized Data on Aging	Interagency Agreement

EQUAL EMPLOYMENT OPPORTUNITIES

BSR has worked closely with the Special Assistant for EEO to the NIA Director in furthering the goals of equal employment opportunity within BSR, NIA, and the scientific community. Efforts have been made to identify minority scientists and to encourage their participation in BSR sponsored activities, including research and training. A number of grants by or about minorities have been funded. Communication has been maintained with minority institutions throughout the country and with the minority fellows sponsored by the American Psychological Association and the American Sociological Association. Continuing efforts are made to identify minority professional and support personnel for the few positions that have been available at BSR. BSR is closely informed on EEO policies, plans, and programs; takes care to see that these are disseminated to all staff members; and is committed to the principles of merit promotion, fair treatment, appropriate recognition, career development and full utilization of skills of all employees, irrespective of race, religion, color, sex, national origin, age, or handicap.

EVIDENCE OF PROGRAM PERFORMANCE

Although no neat and easily applicable criteria have ever been developed for evaluating the scientific enterprise, all possible indicators are utilized in subjecting the BSR program to a continual process of critical scrutiny and evaluation. As judged by the available indicators, the program has made significant contributions to the knowledge base; raised the quality of research proposals; attracted many promising younger scholars and several distinguished scientists to the field of aging research and teaching; contributed to both theoretical and methodological development; disseminated new knowledge through specially commissioned volumes, many scientific articles, and professional speeches; added to extant research resources (data archives, longitudinal study populations, non-human primate colonies); and served as an effective liaison to NIH, ADAMHA, National Academy of Sciences Committee on Vision and Hearing, Institute of Medicine, the Social Sciences Research Council Committee on the Life Course, the American Association for the Advancement of Sciences, the World Health Organization, and many other organizations and agencies.

SCIENTIFIC ACCOMPLISHMENTS

While revolutionary discoveries or theoretical breakthroughs rarely occur, scientific knowledge is accumulating in the BSR program and knowledge gaps needing new research continue to be identified. The program is contributing to the already existing massive scientific support for three fundamental, though little recognized, principles:

- o That aging is a psychosocial as well as a biological process. Research shows that the way people age is affected by their life styles, educational levels, nutrition, self-care, economic status, social expectations, family relationships, and many other social and psychological factors. The recognition of the importance of the brain to the endocrine, immune, and other physiological systems - themselves changing with age - leads to new research questions on how accumulating behaviors and social stresses feed through the brain and the mind to affect these systems.

- o That aging is not fixed for all time, but changes as society changes. Research shows that growing old in 1983 is very different from growing old in 1883 or in 2083; and growing old in contemporary Appalachian is very different from growing old in Grosse Pointe, Michigan. Since people do not grow old in laboratories the recognition that people at different times and in different places age in different ways leads to new research questions on how particular social and historical conditions influence aging.

- o Because aging is not immutable, it is subject to a degree of human intervention and control. Research shows that modifications in attitudes, behaviors, social relationships and environments can often prevent, postpone, or reverse disabilities currently associated with old age. The recognition of the mutability of the aging process leads to new research questions on how appropriate interventions can optimize this process.

BSR is gradually adding to the knowledge base which undergirds these principles. The following research highlights are examples of this accumulating knowledge.

Older People in the Society (OPS)

Dramatic changes in longevity, economic status, and geographic distribution of the older population have taken place in the last decade. These changes, documented by research in the OPS cluster, have implications for society-wide planning for the growing older population. For example, longevity, which has been extended far beyond expectations, is creating a little-understood pool of people in the oldest age strata.

- o A major trend in recent years has been an unanticipated decline in mortality especially at the older ages. As earlier reported in a study by Crimmins-Gardner, if the current rate of mortality decline continues, by the year 2000 there will be a substantial increase in life expectancy at age 65 and above and the number of older persons will be far greater than has been projected. An important question arising from the increase in longevity is whether the added years of life will be accompanied by more or less illness. A currently supported study, based on the newly available National Multiple Cause of Death (death certificate) data for 1968-78, confirms the clinical impression that mortality at advanced ages is a result of multiple (and possibly interacting) causes of death. (Manton, R01 AG01159)

- o Since little is yet known about the increasing numbers of people 85 and over, a study underway is beginning to provide detailed information (by five year categories) for this population on such characteristics as population growth trends, geographical distribution, living arrangements, health status and mortality patterns, and international comparisons. The study attempts to resolve discrepancies which exist between census and other data sources such as the Medicare system statistics. (Rosenwaike, R01 AG03120)

- During the past decade older people, in the aggregate, have fared better in the face of inflation than has the U.S. population generally (Maddox, R01 AG02345). The occurrence of poverty among the elderly declined from 25.3% to 14.0% during the 1970s, a greater decline than experienced for the U.S. population as a whole.

- Despite improvements in the economic well-being of the older population as a whole, improvements in income level were much more prevalent in some subgroups than in others, and it is precisely those groups in which income increases are less prevalent that are growing the fastest--women, unrelated individuals, and nonwhites. (Serow, R01 AG01522)

- Elderly persons living in states which had experienced large increases in unemployment between 1973-76 had lower levels of morale, health, and everyday functioning than those living in states not experiencing high levels of unemployment (based on analysis of data from the 1981 Harris Survey of the aging). (Rosow, R01 AG02684, previous PI Suzman)

- In Britain the exponential rate of economic growth and the proportion of governmental expenditures related to health was strongly associated with long term decline of mortality from cardiovascular disease (CVD). Recessions, however, were associated with elevated rates of CVD. Many risk factors, such as alcohol and cigarette consumption, were themselves affected by economic cycles. Business failures were particularly strongly associated with increased CVD rates among older age groups, possibly because many owners and proprietors tend to be elderly. (Brenner, R01 AG00183)

- Home visits by medical personnel are particularly useful in understanding how patients with chronic illness manage on a day-to-day basis, and in identifying factors related to changes in a patient's chronic condition that cannot be identified by conventional case histories or laboratory methods. (Sankar, F32 AG05176)

- Residents of retirement communities are generally highly satisfied with them. In a study of 30 such communities, residents were also found to prefer outside ownership to resident ownership of all land and facilities designated for common use. A result of outside ownership is that developers are in control of many communities and, if they should decide to sell, the residents may face a crisis and may demand that outside agencies intervene to protect them. (Streib, R01 AG02602)

- Adding to the evidence of greater vulnerability of elderly males, especially nonmarried males, as compared with elderly females, a current project shows that married men have higher caloric and nutrient intake levels than men living alone (or with someone other than a spouse). A higher proportion of men who live alone have nutrient intake levels below 2/3 of the Recommended Daily Allowance for calories and major nutrients (calcium, iron, protein, thiamin, riboflavin, and niacin). The men living alone or with someone other

than a spouse also eat fewer servings of food per day and have lower dietary diversity. For women, dietary intake is less closely related to marital status and living arrangements than to income. (Davis, R23 AG02341)

Social Psychological Aging (SPA)

Recent research is beginning to show how social psychological factors affect a person's health and effective functioning. New knowledge of the experience of bereavement, retirement, and the role of life stress in general is explaining illness, coping behavior, and the interaction of health care attitudes and behaviors with health care utilization patterns. Findings from this program cluster are beginning to address issues of major theoretical and practical concern for older people.

- The risk of mortality and morbidity is substantially higher for bereaved men than among non-bereaved men or than among bereaved women. (Thompson, R01 AG01959) This replicates in a different study population a previously reported finding by another NIA-supported researcher. (Helsing, R01 AG00940)

- The causes of excess mortality associated with bereavement differ for men and women. Excess deaths among bereaved women are especially traceable to alcoholism, and among bereaved men, to suicide, accidents, and infectious diseases. This specification has implications for interventions designed to lower the health risks associated with bereavement. (Helsing, R01 AG00940)

- Two studies conclude that the negative attitudes or symptoms of ill health which are frequently reported in connection with the menopause are highly dependent on facilitating cultural norms (Haug, R01 AG02622) and or whether or not the menopause was natural or artifically induced (McKinlay, R01 AG02905).

- After their husband's death, it is quite common for widows to live alone. Many social commentators have expressed concern that this pattern leads to the widow's social isolation and lack of health-promoting social support. However, recent findings show that living alone does not necessarily mean social isolation and consequent lack of social supports. Indeed, living alone is found to be associated with having relatively more good friends and above average contact with friends. (Converse, R01 AG02590)

- The widespread notion that retirement has an adverse effect on physical and mental health is being challenged by a major study that examines why, and under what conditions, health may improve or worsen once a person retires from paid work. Despite wide individual differences, retirement on the average is found to have only a minimal effect on health, both as self reported and as objectively rated. Nor are adverse health consequences predicted by circumstances which presumably might make the retirement transition more stressful, such as compulsory retirement, unexpected retirement, retirement from higher prestige occupations, or retirement to a reduced standard of living. And contrary to speculation about vulnerability during certain periods following

retirement, no particular time within 4 years of retirement was found when the health risk was greater. (Bosse, R01 AG02287)

- o The elderly show differences in estimates of their ability to cope with ill health. Although they appear more confident than younger persons that they will successfully deal with a new illness, they are more hesitant when confronted by the most ominous of all diseases--the cancers. The more extensive the personal experience with any kind of illness (the more opportunities one has either to cope oneself and/or to observe effective strategies in others), the more confident one is. Also, if individuals have thus far escaped ill health, they believe they are less likely to have to deal with future illness, or will have to do so for a short time. The greater the illness experience, the greater one's ability to cope with particular diseases appears to be. (Leventhal, R01 AG03501)

- o Major life stressors are most likely to exert long term adverse effects on health when they are experienced in ways which add to day-to-day difficulties and when they serve to increase one's sense of uncontrollability over such stressful events. When stressors do not produce these consequences, the coping resources of most older people appear to be sufficient to buffer them against large, untoward long-term effects. (Rodin, R01 AG02455)

- o A 22 year mortality follow-up of a cohort of 48,000 Seventh Day Adventists (SDAs) suggests that the practice of health promoting behaviors (i.e., not smoking, drinking alcohol or coffee, or eating meats) has a cumulative effect on mortality outcomes. Not only do SDAs have a lower mortality risk than the general population, but lifetime SDA members have lower age-adjusted, cause specific, mortality rates that SDA converts who have adhered to SDA lifestyle practices for fewer years. (Kuzma, R01 AG01532)

- o Many instruments used to measure personality characteristics and attitudes rest on the assumption that they are equally valid at all ages. Typically tests are constructed using people of a particular age (often college students) and then applied to people of all ages in a search for age-differences in attitudes or personality characteristics. However, this practice is under attack as theoretically and methodologically unsound, since the dimensional structures of attitudes and personality may change as people age. For example, a recent finding by an SPA investigator shows that the structure of perceived personal efficacy--an important aspect of the self-concept--changes in adolescence and early adulthood; possible similar changes in later adulthood are now being investigated. (Mortimer, R01 AG03325)

Cognitive and Biopsychological Aging (CBA)

Research in this area tends to be highly specific. Thus new findings are gradually being added to our knowledge of the processes of sensation, perception, attention, memory, learning, cognition and intelligence, motivation, creativity, wisdom, and psychomotor performance. Vision,

hearing, taste, smell, and somesthetic acuity are all known to change with age. The following findings are scattered but they are beginning to add up:

- Contrary to earlier hypotheses that older people tend to be unable to filter out or ignore irrelevant visual information, current research shows that the elderly are capable of organizing small parts of visual signals into meaningful wholes. It is, however, not yet known why older subjects are less able than their younger controls to maintain such organization as the number of parts increases. Improvement may be possible by directing specific attention to the spatial location of small elements in large areas. (Gilmore, R01 AG03209)

- Another study bears upon the well-known fact that elderly people frequently complain about the taste of food. For example, depending upon how citric acid is served, young and old subjects differ in their preferred concentrations. When served in an aqueous base, there are no differences, but when served as a common beverage (e.g., a fruit drink) the elderly prefer a less sour concentration. Thus, as in vision or hearing research, the context of the stimulus rather than age per se determines differences in perception. (Murphy, R01 AG04085)

- With minimum effort older people can make effective use of spatial distinctions as memory cues--especially spatially distinct pictures, objects in environmental designs. Color, however, is not effective as an aid in structuring such materials. (Park, R01 AG02598)

- Education appears to be related to effective use of external mnemonics in compensating for memory lapses. Thus, written notes have been found to be especially helpful to well-educated older persons. (Lovelace, F32 AG05237)

- By pooling the results from a wide variety of studies (thousands of subjects from over 100 studies) based on Wechsler Adult Intelligence Scale (WAIS) on healthy subjects, use of "structural equation modeling" confirms early findings that "fluid" intellectual ability declines steadily and rapidly after age 30 and exhibits small individual variation, while "crystallized" ability (which reflects experience) shows only a small but significant decline which is most apparent after 65, and which exhibits large individual variation. More importantly, when retrieval and speediness factors are statistically controlled, this age pattern in crystallized ability is reversed, resulting in a systematic increase in crystallized ability over the entire age span that exhibits much less individual variation. (McCardle, R01 AG02695)

- The poor "fit" between elderly women and their living environments is currently demonstrated in human factors studies of meal preparation, which show how most kitchens and cooking utensils are poorly designed for the shortening stature and weakening hand grip that tend to accompany old age. (Faletti, R01 AG02263)

- o Only selected areas of linguistic knowledge appear to be vulnerable to the effects of Senile Dementia of the Alzheimer's Type (SDAT): both semantic and pragmatic knowledge are affected earlier and more severely than phonological and syntactical knowledge. (Bayles, R21 AG02154)

- o Depending on the treatment used, maturity onset diabetes may adversely affect cognitive functioning in older people, indicating the necessity for tight controls on compliance. (L. Perlmutter, R01 AG02300)

- o Cognitive processes in older people that require considerable effort appear to be sensitive to adverse effects from centrally acting antihypertensive drugs but not to diuretics. (Wilkie, R01 AG02164)

Piece by piece BSR is adding to the accumulating knowledge about the the changing place of older people in society, the influence of psychosocial factors on the aging processes, and what can be done to optimize health, productivity, effective functioning, and well-being up to the end of life.

PLANNED PROGRAM ACTIVITIES FOR FY 1984

BSR plans for the coming year are fueled by the dramatic societal changes affecting both the aging process and the place of older people in society, as these changes have been highlighted by activities in the BSR program during FY 1983. Societal changes affecting longevity, health, socioeconomic status, and the sheer increases in numbers of the very old--especially very old women-- vitiate many earlier findings and require improved understanding of the changed situation of people who are old today. Oncoming cohorts of people who will be old in the future must be understood as starting life with higher levels of education and new styles of living, as in exercise or diet. The opportunities for all these older people must be examined as they provide or fail to provide work roles, family roles, and access to health care services; and as differential opportunities may create inequities by sex, by race and ethnicity, and by health status. Emerging scientific methods of research design, cohort and longitudinal analysis, and measures of biopsychosocial linkages need to be improved and applied to research on aging. These and many other changes are leading to plans for (A) the developing grant program; (B) workshops and program announcements to develop promising specific areas; and (C) continuing broad scientific development.

The Program of Grants and Contracts

While maintaining both its large compass and its emphasis on Health and Effective Functioning in the Middle and Later years, and while continuing its collaboration with BRCM on TNH and other programs, BSR is looking ahead to three lines of priority development in FY 1984: (1) assessment and integration of the program within each cluster; (2) development of training; and (3) special topics for priority attention.

Internal Integration of Program Clusters. While the accumulating body of knowledge is shedding fresh light on the conditions and mechanisms for

sustaining high levels of cognitive functioning into the later years, several lines of research are needed for further development of the Cognitive Aging area. The highly specialized findings on specific aspects of cognition need to be integrated into a systemic model of information processing. Many laboratory findings need testing for their relevance to real-life situations. Cross-section studies of age differences need to be complemented by longitudinal and cohort studies and new methodologies that examine aging as a process. And performance tests, many of them originally designed for subjects at one particular age, need modification to become applicable across the life course. Meantime, the area of Biopsychological Aging is barely beginning to explore the crucial linkages between psychological age-changes in behavior, cognition, perception, and sensorimotor functioning, and etiology and pathogenesis of such disorders as cancer or SDAT.

Also requiring development is the cluster on Older People and Society (OPS), especially the macro-level areas of (a) Age in the Population and (b) Age and Societal Structures. Not only individuals but also societies are growing older, creating an "Aging Society" which is heavily populated by older women-- with critical implications for understanding both individual aging processes and social changes. Among many pressing new questions now posed by the aging of society are those concerning health:--e.g., will the added category of the very old (85 and over) remain healthy, or will they simply add to the growing pool of disabled or moribund? What economic and other societal changes will affect their health? How can self-care, medical care, or changed social contexts make a difference? Research is needed to address these and many other implications of the Aging Society in respect to: dependency ratios; unemployment problems of older workers; work, retirement, and adult education; morbidity and mortality; health care needs and systems; family relationships and intergenerational tensions; political and economic structures; special problems of minority elderly, older women, and old people in rural areas.

Development of Training. Contingent on the availability of funding, and the possible phasing out of one or two long-standing training grants, BSR is turning major effort toward stimulation of applications for training programs (T32), post-doctoral fellowships (F32, F33), and research career awards (K04 and the new K01). Applications for broad behavioral and social science training in aging will continue to be welcomed wherever strong research programs have developed to support them. In addition, two areas of training will be especially encouraged:

- o Interdisciplinary training (including Behavioral Geriatrics Research training) - to foster research collaboration between social/behavioral scientists and biomedical scientists (e.g., from biochemistry, neurophysiology, anatomy, or endocrinology);

- o Methodological training - to insure wider application in biomedical as well as psychosocial research of the advanced methods which social scientists have pioneered (measurement, population sampling, experimental design, and cohort and longitudinal analysis).

New Initiatives. Implementing BSR's ongoing program in Health and Effective Functioning in the Middle and Later Years, applications for both training and research are expected under the specific program announcements issued in FY 1983. Each of these announcements focuses on selected aspects of the dynamic relationship between aging and three sets of variables, which can be viewed schematically as an X, W, Y chain:

Antecedents (X)	Linkages (W)	Outcomes (Y)
o Behaviors o Attitudes o Social contexts	o Coping, social support o Neural, endocrine, immune changes	o Health o Effective Functioning

This schematic chain (which is in actuality a complex system, with two-way interactions and feed-back loops among the variables, all of which are age-related) is basic to understanding how health and effective functioning can be maintained. Projects stimulated by each 1983 program announcement should add one piece to the emerging mosaic of understanding.

o Special Emphasis Research Career Award (SERCA) for Social and Behavioral Scientists in BEHAVIORAL GERIATRICS RESEARCH

This is the first of several announcements in the emerging NIA initiative on behavioral geriatrics research. Behavioral geriatrics research is undertaking the development and integration of social/behavioral and biomedical science knowledge relevant to health promotion and the prevention and treatment of disease in the middle and later years of life. This announcement aims to develop a much-needed pool of competent investigators by soliciting applications from eligible institutions for interdisciplinary training and research support of social and behavioral scientists seeking careers in behavioral geriatrics research.

o Health Behaviors and Aging: Behavioral Geriatrics Research I

This announcement invites applications for research and research training on those health-related behaviors and attitudes of older people, their families, and significant others, that can affect health and functioning as people grow older. Studies are sought which extend scientific understanding of: how health behaviors and attitudes are learned, how they are affected by the social context, how they change or remain stable with aging, how they interact with physiological and psychological aging processes to influence morbidity and mortality, and how they can be modified as relevant new scientific knowledge is acquired.

This announcement focuses on self-care and informal care, in contrast to formal care on which a later announcement will be developed through the proposed Workshop on Older People and Health Care Systems: Behavioral Geriatrics II.

o Social Environments Influencing Health and Effective Functioning in the Middle and Later Years--program announcement

Research on social and environmental interventions is beginning to identify conditions and strategies for preventing, reversing, or

identify conditions and strategies for preventing, reversing, or alleviating many old age disabilities. For example, jobs can often be modified to improve intellectual functioning and physical as well as mental well-being of workers as they grow older; stoves or bathtubs can be designed to minimize accidents, or special assistance from family and neighbors can be arranged to maintain independent functioning of many frail elderly in their own homes; even in nursing homes, regimens can be adjusted to restore activity, personal control, and well-being of elderly patients. This announcement calls for research to specify how the social environments of older people can be adapted to optimize health and functioning.

- o Visual Perception and Aging--program announcement

  Research has demonstrated many age-related changes in sensation and perception (vision, hearing, taste, touch, smell) and psychomotor skills (e.g., reaction time, coordination, muscular strength). Further intensive study is needed of specific perceptual and neuropsychological and related cognitive processes that are affected by age, and the implications of these processes for older people's productivity and effective functioning in everyday living. Vision (later to be followed by hearing) has been selected for special emphasis as the topic most nearly ready for fruitful development at this time.

Workshops and Program Announcements

As these previously issued program announcements bear fruit during 1984, new topics for future intensive effort will be examined. Through workshops, staff preparation of bibliographies and abstracts, and discussions with outside experts BSR will prepare both to publish and disseminate the accumulating knowledge and to identify gaps needing additional program announcements.

Newly proposed workshops for 1984 will contribute to development of Behavioral Geriatrics Research:

- o A workshop bringing together BSR grantees and other experts to prepare a symposium volume on Health Behaviors and Aging: Behavioral Geriatrics Research I

- o A workshop to lead to a program announcement for research and training on Older People and Health Care Systems: Behavioral Geriatrics Research II

- o A workshop on Care of Dying Older People, (leading to conference publication and a possible program announcement)

In addition to these newly proposed, several previously approved but unfunded workshops, as described in the 1982 Source Book, are still on the 1984 agenda as follows:

o Intellectual and Cognitive Processes in Middle and Later Life--workshop

Following the 1983 planning workshop on this topic, this workshop is designed to encourage conceptualization and operationalization of components of intellectual functioning that are valid and reliable, not only in childhood like most standard measures of "intelligence," but over the full life course. Jointly funded by the Max Planck Institute (Berlin), the focus will be on concepts potentially crucial for later life development, such as creativity, wisdom, and experience-based decision making. This workshop and its published proceedings should lead to a series of program announcements.

o Aging Nonhuman Primate Set-aside Colonies--workshop

BSR will establish--with the cooperation of BRCM--a Task Group to provide NIA with recommendations for the use and management of the NIA/DRR Aging Monkey Set-aside Colonies as an animal research resource for biomedical and behavioral scientists. A panel of experts will develop and recommend guidelines for the utilization and administration of the set-aside colonies.

o Design and Development of Data Bases in Behavioral Sciences Research--workshop

As a resource for NIA biomedical and behavioral researchers, the need for NIA's support of preexisting longitudinal samples of human respondents will be assessed. A Task Group will be convened and given three charges: (1) Establish criteria for selecting for NIA support ongoing or recently terminated longitudinal studies which have the potential for increasing knowledge of the aging process (e.g., full age range, national representative samples, minority samples, special samples of very old people, length of previous longitudinal records). (2) Review studies as candidates for NIA support and make recommendations for how such candidates could be made maximally relevant to NIA's concerns (e.g., adding particular variables). (3) Establish criteria for setting up new longitudinal studies, as these may justify the expense of supplementing existing populations.

o Research on Methods of Longitudinal and Cohort Analysis--workshop

This workshop will encourage development and application of increasingly powerful methods of dynamic analysis of the aging process. These methods are essential to BSR's central concern with cohort and longitudinal methodology, and to the application of this methodology to various aspects of behavioral geriatrics, health and effective functioning in the middle and later years, and aging research in general including biomedical as well as psychosocial research.

Scientific Development

Plans for 1984 involve continuing efforts to coordinate BSR's

work with that of other agencies, to develop and make accessible to the scientific community both resources and methodologies, and to integrate and disseminate the accumulating knowledge. Among the many activities will be included the following:

- o The NIH Working Group on Health and Behavior will be in the process of planning and evaluating interdisciplinary grant programs for the PHS Initiative on Health and Behavior, as proposed by the Assistant Secretary for Health.

- o Participation will continue in committees of the National Academy of Sciences, the Institute of Medicine, the Social Science Research Council, the Carnegie Group on Aging, and many others; and funds are included in the budget to contribute through interagency agreements to support of selected NAS/IOM activities (e.g., on National Statistics and on the Aging Society).

- o Important 1984 book publications, to which BSR has contributed, will include the symposium on Age and the Life Course in Anthropological Theory (edited by Kertzer and Keith) and the NSF Census Monograph on Older People (by J. Siegal).

- o BSR staff will continue to contribute to broad scientific development ("the science of aging") through the exercise of leadership roles in seminars and symposia both here and abroad, through preparation of bibliographies, and through their own scientific papers and writings.

- o Of special importance is the need for continuing inventories of social and behavioral research findings on aging. This project, which would build on the 1968 Aging and Society Vol. I, has long been discussed but never implemented. A continually updated (and computerized) inventory would be the way for NIA to exercise leadership in the social and behavioral sciences.

ADMINISTRATIVE ISSUES

A wide range of issues must be addressed if BSR is to maintain its momentum and maximize its contribution to the overall development of the Institute. During the past three years BSR grants and contracts have remained at less than 15 percent of the Institute budget. Now, however, not only the BSR program but also the social and behavioral science communities have reached a point where, given stronger financial and intellectual support, they are ready to address the Institute's mandated tri-partite "biomedical, social, and behavioral" goals-- goals that concerned the founders and the Council at the inception of the Institute, but have rarely been at the forefront in the interim. The issues involved in capturing this opportunity, as they have currently emerged and as they prompt proposals for the future, relate both to funding and to organization and operation.

Issues of Funding Priorities

Since BSR is a recently re-organized program, with many lines of undeveloped potential, careful consideration must be given to areas for

priority attention and to special grant mechanisms or to "set-asides" of funds for new initiatives. Decisions are necessary (now and in the future) about possible allocation of funds to:

- Efforts to foster training programs in interdisciplinary research, in longitudinal and cohort methods of analysis, and in behavioral geriatrics research (both health services research and research on older people's health behavior and attitudes). Currently BSR has only one or two promising applications in these areas, and only a handful of postdoctoral fellows (F32s and F33s), and funding for new training efforts are urgently needed. Meanwhile, BSR has nine training programs (T32s), some of which are long-standing and could perhaps be phased out in the near future.

- New mechanisms to stimulate research career development to supplement the K04 (the only career award that has been available at NIA to non-MDs). Toward this end, BSR is currently developing for use at NIA a Special Emphasis Research Career Award (SERCA, K01), to be used for training established social/behavioral investigators in biomedical research approaches so that they can conduct interdisciplinary research in behavioral geriatrics. Aimed to develop a few carefully selected interdisciplinary researchers, this mechanism might eventually be given a reverse emphasis - to train biomedical researchers in social science methodologies.

- New initiatives to stimulate research and research training in selected areas (as indicated in program announcements prepared for the future).

- Small grants (R03s), a highly useful special mechanism for BSR.

Issues of Representation and Coordination

As an area unfamiliar to the biomedical establishment at NIH, BSR needs strong representation in Council and review committees and smooth working relationships with other extramural components.

- In view of the NIA Council's important role in advising the Institute, adequate representation of qualified scientists actively engaged in social and behavioral research is essential for the development of the BSR program. A lack of social and behavioral expertise was particularly unfortunate in the past, at a time when the Council developed recommendations based upon the National Research Plan. The recent strengthening of this needed representation is highly appreciated.

- BSR needs broader representation on the Institute's own review committees--the Aging Review Committee and the Teaching Nursing Home review panels. (By contrast, in the precursor NICHD review Committee on Adult Development and Aging, social/behavioral and biomedical sciences were equally represented.) The review of applications generally (not only those to BSR) would be strengthened not only by increased numbers of behavioral scientists, but also by selection of those outstanding social and behavioral scientists who have interdisciplinary backgrounds or with the methodological expertise in which social science excels.

- Grant applications from the fledgling BSR program now compete for funding on an equal footing with other extramural applications, even though NIH study sections are on the whole unfamiliar with the social/behavioral sciences and, where they are familiar, appear to lean over backwards in setting unrealistic standards for approvals. Strong social and behavioral science representation on study sections is urgently needed.

- Continuing care must be taken to strengthen the smooth working relationships between BSR and BRCM as complementary programs, each building respectively upon large but distinct scientific communities which must be drawn into an overall approach to aging. As BSR attempts to bring the power of social/behavioral expertise to bear on understanding physical health and the biological aging process, much collaborative planning, funding recommendations, and negotiations on specific projects occur in many areas (e.g., neuroscience, exercise, nutrition, urinary incontinence, animal models). However, mechanisms are needed for assigning budgetary credit (in dollars or control) to BSR for staff contributions to activities of concern to both programs, such as the Teaching Nursing Home program (TNH) which is a joint BRCM/BSR program to which at least one BSR staff member devotes major attention.

Issues of BSR Structure

Because of its small size, and the need to make optimum use of the limited staff available, BSR currently has an ad hoc structure consisting of three "clusters" or quasi-branches.

- The need here is to work as rapidly as possible to convert these "clusters" into the conventional "branches" which are essential for effective operation in NIH.

Operational Issues

As a newly established program, BSR has special needs for visibility, dignity, prestige, and wherever possible special resources and financial support. Issues here concern the following:

- The staff (which has been disturbed by loss of members, long delays in replacement, illness, and inadequate clerical support) is so small that several of us must often work a 50- or 60- hour week. Moreover, because aging as a serious scientific concern is comparatively new to the basic social and behavioral disciplines, the work load is especially heavy: for every 10 grants actually funded, many dozens of staff communications, correspondence, advice, review, and often site visits are required. Such necessary additions will again in FY 84 be requested: for a biopsychologist to develop that key area, and for a program analyst to release time for the scientific staff to perform their central tasks.

- The space assigned to BSR has been consistently inadequate (crowded, dark, or noisy professional offices; overcrowding of clerical staff)--a situation that is not fully relieved even temporarily by the newly assigned space on the fourth floor.

- Restrictions on BSR travel funds have virtually prevented the direct contacts essential for development of new relationships with the rapidly evolving community of social and behavioral scientists who are potentially interested in aging research.

All such issues, which concern BSR's place within the overall balance of the Institute, require continuing discussion with the Director and the full Executive Staff as well as with Council members and outside advisers.

Report of Biomedical Research and Clinical Medicine Program

Program Description BRCM-1
Program Highlights - Animal Resources Program BRCM-3
Molecular and Cellular Biology Branch BRCM-4
Geriatrics Branch BRCM-14
Physiology of Aging Branch BRCM-17

PROGRAM DESCRIPTION

Program activities in urinary incontinence and infectious diseases highlight the continued efforts to promote research in geriatrics. Specific activities that generated interest in the clinical research community include the issuance of progam announcements in urinary incontinence and infectious diseases, the publication of the summary of the workshop on Infectious Diseases in the Elderly in the Annals of Internal Medicine, and the convening of a workshop on behavioral approaches to urinary incontinence. Tangible evidence of the results of these initiatives include a newly funded program project and two academic awards related to urinary incontinence.

The Teaching Nursing Home program continues to generate considerable enthusiasm from the medical academic community. This year there were 25 letters of intent and 16 applications for Teaching Nursing Home grants. Of these applications, 11 were from institutions that had not previously applied for this award. Prinicipal investigators of component projects in teaching nursing home grants have also submitted regular research grants to the NIA on subjects related to geriatric research. Five of these grants are expected to be funded. They include projects on urinary tract infections and fall injuries in nursing homes.

The Neuroscience Section of PAB has significantly widened its holdings to include several new promising areas of research on senile dementia of the Alzheimer's type. Pathophysiological studies indicate that lower brain centers such as the nucleus basalis may have a significant role in the cortical cholinergic deficits observed in this disease. Evidence is also accumulating which suggests that senile dementia of the Alzheimer's type may be a systemic biochemical disorder involving enzymes of glucose metabolism. Other promising studies are focused on present interventions, such as physostigmine and piracetam, as well as on future interventive techniques such as brain tissue transplantation and modification of neurotransmitter release.

Another major issue is the problem of accurate and early diagnosis of senile dementia of the Alzheimer's type. A workshop will be held on this subject in December, 1983, which will include the leading experts in neurology, neuropsychology, neuropathology, neurochemistry, neuroradiology, and psychiatry. This workshop will discuss research opportunities to improve the differential pre- and post-mortem diagnosis of senile dementias.

The application of new DNA probes to the molecular biology of aging has produced some intriguing preliminary findings. In both *in vivo* and *in vitro* cell populations, an age-related increased amplification of inter-alu sequences has been detected. While the nature of these highly repetitive human DNA sequences needs to be elucidated, these results suggest altered genetic expression with aging. Studies of the mechanisms for diminished enzyme levels in older tissues have also suggested the presence of altered gene expression with aging.

The majority of the recommendations of the Animal Resource Evaluation Panel and the Genetics and Cellular Resources Panel have been implemented. Implementation has included the establishment of *ad hoc* advisory committees for Cell Biology and for Animal Resources. The nine-year aged mouse and rat contracts have been awarded, and independent contracts for pathological and genetic monitoring of these contracts will be awarded this year. Thus, long-term supply of genetically- and pathologically-defined aged animals to NIA-supported investigators has been assured through 1992.

For more detailed descriptions, please refer to the three Branch reports.

BIOMEDICAL RESEARCH AND CLINICAL MEDICINE ORGANIZATION

ASSOCIATE DIRECTOR:
Edward L. Schneider, M.D.
SPECIAL ASSISTANT:
Anne Wilder Zimmer
LEAD SECRETARY:
Loretta M. Alexander
CLERK (STENO):
Lavonne Lela
CLERK TYPIST:
Eleanor Mitchell

OFFICE OF BIOLOGICAL RESOURCES AND RESOURCE DEVELOPMENT (ORD)

HEAD: Richard L. Sprott, Ph.D.
GRANTS CLERK: Jane Soban

GB
GERIATRICS BRANCH

ACTING CHIEF: Evan C. Hadley, M.D.
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George Steinberg, Ph.D.
HSA - (GET) GERIATRIC TRAINING:
Noel List, M.D.
HSA - (GER) GERIATRIC RESEARCH:
Evan C. Hadley, M.D.
HSA - (GER) INFECTIOUS DISEASES:
Evan C. Hadley, M.D.
HSA - (GER) ARTHRITIS:
Evan C. Hadley, M.D.
HSA - Vacant
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SECRETARY: Carolyn Moore

PAB
PHYSIOLOGY OF AGING BRANCH

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HSA - (NUT) NUTRITION:
William A. Kachadorian, Ph.D.
HSA - (EXR) EXERCISE PHYSIOLOGY:
William A. Kachadorian, Ph.D.
HSA - (END) ENDOCRINOLOGY:
Norman S. Braveman, Ph.D.
HSA - (IMM) IMMUNOLOGY:
Norman S. Braveman, Ph.D.
HSA - (IMM) OSTEOPOROSIS:
Norman S. Braveman, Ph.D.
HSA - (SEN) NEUROSCIENCE:
Zaven S. Khachaturian, Ph.D.
GRANTS CLERK: Paula McAlister
CLERK TYPIST: Joan Sheehan
SECRETARY: Dorothy Brown
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MCBB
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HSA - (CBY) CELL BIOLOGY:
DeWitt Hazzard, Ph.D.
HSA - (MBY) MOLECULAR BIOLOGY:
Richard L. Sprott, Ph.D.
HSA - (GEN) GENETICS:
Richard L. Sprott, Ph.D.
HSA - (ANM) ANIMAL MODELS:
Richard L. Sprott, Ph.D.
HSA - (DER) DERMATOLOGY:
Richard L. Sprott, Ph.D.
GRANTS CLERK: Karen Sacks
CLERK TYPIST: Debra Bellamy
CLERK TYPIST: Tracey Palm

PROGRAM HIGHLIGHTS

Animal Resources Program

The objectives of this program are to identify biological resources and animal models essential to the research mission of the NIA, to facilitate the development of resources where they do not currently exist, to characterize existing models, to facilitate the selection of appropriate models in developing research projects, and to make well characterized animals available to the aging research community. The program includes grant-supported studies to identify, evaluate, and characterize vertebrate model systems for the study of normal comparative aging changes, studies of age-associated pathologic lesions that may stimulate or replicate aging processes, studies of environmental effects on survival and aging, and through contracts, provides high quality, well characterized animals to investigators of aging.

FY 1983 saw the culmination of efforts to stabilize the supply of the most heavily used animal models, to bring the quality of animal care in NIA colonies up to the highest current standards, and to evaluate biological resource programs. Completion of these efforts has resulted in improved responses to the needs of the aging community and a significant reduction in the cost of providing basic resources. This reduction in cost should provide the means for the development of new resources which have been identified as priorities for the development of significant research in areas such as neurobiology, behavior, endocrinology, immunology, and nutrition.

The evaluation of the NIA Animal Models Program was completed in August, 1982. Implementation of the recommendations of the evaluation panel is either completed or in progress for almost all of the more than 50 recommendations where NIA staff have the authority to make changes. Among the major recommendations: establishment of an advisory panel is completed, development of a users' manual for recipients of NIA animals should be in progress before the end of the fiscal year, provision of exact information about the health and rearing of NIA animals is provided with every shipment, under-utilized strains of mice and rats have been dropped from NIA colonies, and contracts are being prepared for additional, much needed animal models. Evaluation of hybrid rat models was completed in FY 1983, with the development of a colony of hybrid rats scheduled for FY 1984.

The development of a long-term contract mechanism for the support of NIA mouse and rat colonies was completed with the award of nine-year contracts in February. These contracts will provide a stable supply of animals through FY 1993, will save the NIA an estimated $500,000/year for each of the nine years ($4.5 to 5 million for the period), and will assure NIA grantees of high quality, reasonably priced animals. These contracts are flexible so that the genotypes of animals in the colony can be changed to take advantage of changes in usage or scientific breakthroughs. Additional contracts for genetic and pathophysiological monitoring of these animals are being awarded. These contracts will greatly improve our ability to assure the maintenance of high quality, and to provide animal users with current characterizations of the animals in their research protocols.

As basic resource needs are met, the Biological Resource Program, in cooperation with other NIH offices, is also actively exploring the development of alternative research methods to reduce the numbers of live animals utilized in research to the minimum necessary. While many areas of research cannot be conducted with alternatives such as cell systems and computer simulations, some can, and in others the numbers of animals required can be reduced. Where animals are required, the best available humane care should be provided. Increased attention to animal care issues is being given to NIA grants using

animals, and the Biological Resources Office is working to provide additional advice to all grantees using animals whether from NIA colonies or other sources. NIA, in cooperation with several other NIH institutes, is sponsoring a study of alternative methods of research by the National Academy of Sciences. This study should provide an authoritative assessment of the areas where alternatives are useful and where they are not, and will include recommendations on promising areas for future research.

Molecular and Cellular Biology Branch

The Molecular and Cellular Biology Branch includes three programmatic areas: Cell Biology, Genetics, and Molecular Biology. The objectives of this Branch are to encourage and support basic research on the mechanisms of aging at the molecular and cellular level. It is at this level that the ultimate causes of aging will be found. The basic programming for all life processes resides in DNA. The objectives of the Molecular and Cellular Biology Branch programs are all related to understanding normal DNA functions and the alterations in these functions which can be induced by interaction with the environment and disease processes.

The emphasis in all three programs is increasingly directed toward mammalian research as mammalian processes are more likely to provide information of immediate benefit to the human situation than research with lower organisms. While emphasis on mammalian research is increasing, the very best research from lower organisms related to aging, where very basic processes can be elucidated, continues to be supported. The three programs are being coordinated to provide a concerted attack on the mammalian mechanisms of aging together with the development of a set of mammalian biomarkers of aging which can be used to assess interventions into processes and as a basic research tool.

Cell Biology Program

The broad objectives of the Cell Biology Program are to understand how cells perform their functions within a multicellular organism and how these functions change with age, to study fundamental cellular processes that are likely to be important in aging, and to identify the specific cellular changes that underlie the physiological manifestations of aging. The Cellular Biology Program encourages studies on the mechanisms of cellular aging, utilizing the technologies of cell culture, somatic-cell genetics, cell and tissue transplantation. The study of cells as they "age" in culture and of cells derived from humans and experimental animals of varying ages permits investigation of cellular events consequent to aging, independent of the complexity of the whole organism. The use of differentiated cells in culture is encouraged, as these provide an excellent opportunity to study age-associated alterations in differentiated functions that are expressed by cells in vitro. Such studies could lead to an understanding of mechanisms of age-related functional decline in various tissues and organs. More specific program objectives are: to determine the mechanism for control of cellular proliferation and for the limited replicative life span of normal somatic cells, and to elucidate the significance of this limitation for various processes of aging; to identify specific cellular changes that underlie the physiological manifestations of aging; to determine how cells interact with each other and their environment, as various elements in this interaction undergo changes with age; and to determine the mechanism for expression of differentiated functions and for changes in these functions with age.

In support of research in cell biology of aging, NIA maintains through a contract, a cell-line repository at the Institute for Medical Research, (IMR),

Camden, New Jersey. This repository acquires, develops, characterizes, stores and supplies cultures for gerontological research. The repository contains about 550 cell-lines which include human diploid fetal lung fibroblasts (IMR-90/91), skin fibroblasts from individuals of different ages and fibroblasts from individuals exhibiting features of premature aging, such as progeria and Werner's syndrome. In addition, the repository also maintains several epithelial cell lines cells from primates with different life spans.

The Cell Biology section will continue to support basic research to understand the mechanisms of cellular aging as well as theoretical biology studies which bear upon cellular metabolic age related processes. We are continually evaluating, in consultation with the scientific community, types of studies and various experimental systems best-suited for cellular aging research. Currently, normal human fibroblast-like cells are extensively used in cellular aging research. This system requires further characterization with respect to cell heterogeneity in mass culture, in vivo precursors of in vitro cultures, etc. Through a program announcement (NIH Guide for Grants and Contracts 12(5), 1983) we are encouraging investigators to study various aspects of cellular biology of aging, using differentiated cells in culture. Such studies could provide valuable information on age-associated functional decline of various tissues and organs.

The activities of the Cellular Biology Program center around understanding how cells perform their functions within a multicellular organism and how these functions change with age. This is accomplished by the study of fundamental cellular processes that are likely to be important in aging, and by identifying the specific cellular changes that underlie the physiological manifestations of aging. The following are achievements of the past year which have led us in these directions.

A series of experiments on the finite proliferative life span and the mechanism for cessation of cell proliferation in normal cells when they are senescent, quiescent or anchorage-deprived is of considerable program interest. It has previously been shown that when nonreplicative senescent human diploid cells (HDC) are fused to young HDC, DNA synthesis is not induced in senescent HDC nuclei in heterodikaryons and is inhibited in the young HDC nuclei. Dr. Gretchen Stein, et al. found that young HDC nuclei synthesized DNA in heterodikaryons formed with senescent HDC. However, this DNA synthesis occurred only early (0-10 hours) after fusion, but not at later times (> 10 hours). If young HDC were synchronized so that they were in G1 phase at the time of fusion, none of the young HDC nuclei synthesized DNA after fusion. These data suggest that entry into S phase is inhibited in young HDC fused to senescent HDC. The amount of DNA synthesized in heterodikaryons by young HDC nuclei that were in S phase at time of fusion suggest that they were able to complete the ongoing round of DNA replication. Taken together, these data suggest that senescent HDC contain an inhibitor of entry into S phase.

In a series of studies using transformed cells these investigators reported that cells transformed by DNA tumor viruses acquire the ability to override the inhibitory effect of senescent HDC, whereas cells transformed by carcinogens and by Rous sarcoma virus do not. They have also shown that the ability to induce DNA synthesis in senescent HDC does not correlate with transformation to immortality, lack of the normal quiescence phenomenon, anchorage independence, or tumorigenicity. They suggest that the ability of DNA-virus transformed cells to induce DNA synthesis in senescent HDC may be a function of the viral large T antigen.

Results of studies in which quiescent serum-deprived young HDC were fused to replicative young HDC suggested that serum-deprived quiescent HDC appear to contain an inhibitor of entry into S phase, and that this inhibitor does not disappear immediately after the cells are stimulated with serum. When serum-deprived quiescent HDC were fused to transformed cells of various types, the data indicated that serum-deprived quiescent HDC and senescent HDC contain the same inhibitor of entry into S phase. Dr. Stein concluded that her work indicates that the basic aging change that takes place in HDC is a decrease in their ability to recognize or respond to serum.

In a quantitative morphological analysis by Dr. Thomas Pool, of proliferating and nonproliferating subpopulations of IMR-90 fibroblasts during aging in vitro, early-, mid-, and late-passage cultures (PDL 12, 35, and 51 respectively) were exposed to ^{3}H-thymidine for 48 hours prior to fixation in situ in order to determine quantitatively what ultrastructural changes accompany the loss of proliferative capacity. Analysis of autoradiographs demonstrated that a significant increase in relative cell area, an indicator of cell size, was characteristic of cells unable to incorporate ^{3}H-TdR at both mid- and late-passage, but not at early-passage levels. Nuclear size also increased significantly with progressive passage level but was not related to proliferative capacity. No significant difference in the area fraction of nucleoli per unit area of nucleus or of mitochondria, Golgi, or lysosomes was seen in either subpopulation at any passage level. Dilated asternae of rough endoplasmic reticulum in early-passage cells were seen if cells were harvested with trypsin and fixed either before or after centrifugation, but were not seen in labeled or unlabeled cells from any passage level when cultures were fixed in situ. They conclude that a significant increase in cell size is the only significant morphological change associated with the loss of proliferative capacity of IMR-90 fibroblasts. Their data further indicated that there was no accumulation of secondary lysosomes in HDF during aging in vitro, and therefore does not support any hypothesis of aging or proliferative decline based on lysosome accumulation.

Yet in another study by Dr. James Smith, a long-lived HDF has been isolated from fetal lung with an in vitro life span of about 100 population doublings. The culture grows very well at clonal densities and long-lived clones can be isolated for use in cellular aging studies. The long life span of the culture has allowed isolation from it of a clone, containing a dominant and recessive mutation, having significant remaining proliferative potential. The nature of the mutations will allow for hybrid selection, after fusion of the mutant clone with wild type human cells. The mass culture and clones derived from it provide a valuable new resource for cell aging studies.

Genetics Program

The overall objective of this program is to understand the genetic basis of aging processes and genetic contributions to age-related disease. The Genetics Program includes research using a wide variety of organisms ranging from insects to human populations. Special emphasis is given to mammalian genetics research since mammalian organisms are less remote from man than lower organisms and mammalian systems often offer direct evidence relevant to human genetic influences. The Program administers research grants, program project grants and training grants relevant to its objectives.

The evaluation of the Caenorhabditis elegans (nematode) genetic resource was completed in late FY 1982. This evaluation was highly positive regarding the value of the resource to investigators from many disciplines, not just aging. A major recommendation of the evaluation panel was that this resource be made available to grantees from all NIH BID's, not just those from NIA. To this end, program staff initiated discussions with other BID's to encourage co-funding for this genetics resource so that it will be available to a larger segment of the NIH research community.

At an early stage in the development of the NIA Genetics Program, efforts were made to attract good scientists, working with lower organisms such as Volvox and Drosophila melanogaster to aging research. These efforts were quite successful in the sense that good scientists were attracted to NIA funding and began research programs under NIA sponsorship. However, only a few of these investigators became significantly involved in aging research, while most continued their previous interests in early developmental events. Program staff has reduced the number of non-aging related grants using lower organisms by working with appropriate staff in the National Institute of General Medical Sciences to move those grants which have no significant aging component as they come up for renewal. The result is that the best grants are still being funded, and only those relevant to aging are funded by NIA. Elimination of non-aging related grants from the program provides more funds for research using the exciting new mammalian models and approaches being developed in the Animal Models and Molecular Biology Programs.

Program staff concentrated efforts on the encouragement of geneticists active in the development of new genetic resources to turn their attention to aging research. Excellent applications from a few of these investigators have now been received, and the level of this activity should continue to increase during the remainder of FY 1983 and FY 1984.

In a series of genetic studies, conducted by Dr. Merle Jacobs, utilizing normal and mutant Drosophila melanogaster to study the functional status of muscle cell components (myofibrils, microtubules, and desmosomes) throughout aging, an important correlation between body color and longevity has appeared. The body color mutations (ebony and black) produce abnormalities in the muscle tissue of the flies, but most interesting is the observation that hybrids between the mutant flies and normal flies, which show slight body darkening, show markedly increased longevity. The relationship between microtubule function and longevity is currently being studied.

Genetic selection for longevity often produces increased longevity in the subject populations. This sort of experiment is the first step in the search for genes which control aging processes. However, most such experiments appear to effect longevity by means of effects on nutritional status or by lengthening the period of early development rather than the period of maturity. Experiments by Dr. Leo Luckinbill aimed at isolating these factors in Drosophila melanogaster have produced interesting results. First, it is clear that larval competition for food and space effects longevity with flies coming from an environment densely packed with other flies having reduced longevity, as one might expect. However, the result of selection for early reproduction, and therefore a short maturation period, was to increase longevity, contrary to expectation. This result does not appear to derive from nutritional or density effects, and offers the promise of interesting new genes to study.

Turbatrix aceti, a free living nematode, is a useful model system for aging studies. This lower eukaryote hatches with the same complement of cells it will have for the remainder of its life. There is a slow turnover of 15% of the cells in the gut and reproductive tract. Therefore, an "old" animal consists of "old" cells. This avoids many of the questions associated with tissue culture models for aging. This system can be used to study the mechanisms of DNA repair in young and old cells. Recent results by Dr. Harold Box show that the repair mechanisms used to repair damage caused by UV light are comparable to those in human cells. The major difference between the functioning of young and old cells is that old cells repair damage more slowly. Faster repair mechanisms in young cells also appear in other types of damage repair situations.

The liver enzyme phospoglycerate kinase (PGK) loses efficiency in older organisms, including nematodes and rats. Recent results in Sprague-Dawley rats (Morton Rothstein) show clearly that this change is not the result of post-translational modification but rather is the product of altered gene expression. Thus, the situation may be analogous to that of fetal vs. adult hemoglobin, except that for PGK, the change occurs later in life. It is not yet known whether PGK is unique, or if other losses of enzymic activity also result from changes in gene expression rather than from altered conformation. Research in the coming year by this investigator will pursue this exciting lead. Specifically, research will focus on whether liver PGK enzymes for "young" and "old" are products of different liver genes or are the products of muscle isoenzymes which are converted to liver isoenzymes after synthesis, and on whether other enzymes which are altered with age show the same genetic pattern as liver enzymes. These steps are needed to confirm the existence of separate genes for "young" and "old" enzymes.

Molecular Biology Program

The overall objective of this program is to elucidate the biochemical and molecular events which lead from gene action to phenotypic expression. This program includes investigation of the gradual or programmed alterations of structure and function which characterize normal aging and investigations of the abnormal changes which characterize age-related disease states at the molecular level. Specific objectives include determination of the molecular processes involved in age-related pathology and provision of test systems for molecular theories of aging.

While the ultimate explanation of species-specific maximum life spans lies within the genome, understanding of aging processes can be achieved by biochemical and molecular investigations of the proteins, protein co-factors, and macromolecular protein interactions which vary with age. The Molecular Biology Program is encouraging research on the basic functions of the molecular components of living organisms including: cell membrane structure, composition, and function, including membrane transport mechanisms and receptor mechanisms; applications of new molecular techniques to examine aging theories, such as the oxygen-mediated, free radical theory; intracellular information transfer, including DNA repair, transcription and replication; cloning of genes responsible for free radical transformation from long-lived organisms for study in short-lived model systems; and extracellular components including collagen, elastin, fibronectin, and glycoproteins, where age-related changes can be demonstrated.

In a series of studies of cellular enzyme activities and cell function (Dr. David Gershon) aimed at understanding degradation of cell system function, two exciting findings have emerged. In studies using a novel method developed to permit the elaboration of the molecular state of inactive molecules in the liver and erythrocytes of aging rats, inactive superoxide dismutase (SOD) molecules in liver were shown to have the same molecular weight as that of intact, functioning molecules. This finding indicates that inactivation of this important molecule is not the result of intracellular partial proteolysis, and final proof that the inactive molecules are identical in primary structure to active molecules. Similar results have been observed in SOD in erythrocytes, and with aldolase B in liver and aldolase C in lens. These findings pave the way to the identification of the proteases involved in intracellular protein degradation. The type of modifications which render these proteases less efficient in cells of aging animals should soon be amenable to analysis due to this breakthrough. This research should facilitate the search for the post-translational modifiers (e.g., glycolases and sulfatases) responsible for enzyme inactivation in senescent tissue.

Additional studies of the activity of erythrocyte enzymes which repair peroxidative damage (e.g., SOD, catalase, and glutathione peroxidase) show that the activities of these enzymes are reduced in old animals. Further, young erythrocytes in old animals already possess reduced enzyme levels which are comparable to those found in older erythrocytes of young animals. These findings on erythrocyte aging may mean that young cells of old animals are already damaged at the time of their emergence in the circulation. This damage must be the result of events happening during hemopoiesis or in the erythropoietic stem cells of old animals. The consequence of this is that the erythropoietic system of the old animals must be producing red cells at an enhanced rate. This process may exert a considerable stress on the system and should probably cause detrimental effects in old animals (David Gershon, Technion-Israel Institute of Technology, 5 R01-AG-00459-09). These findings are exciting, but controversial, as other investigators do not find SOD changes in senescent tissue.

Another major theory of aging assumes that biological aging is a process similar to cellular differentiation and is therefore accompanied by alterations in genetic organization and/or expression. Dr. Samuel Goldstein, in a series of studies using the cultured human fibroblast as a model system to test this theory has shown the following:

A. studies of mitochondrial DNA and mitochondrial energy metabolism indicate no serious decrement in mitochondrial functional capacity during aging, rendering it unlikely that mitochondrial failure is responsible for biological aging.

B. human and monkey permanent cell lines have been reported to contain small DNA circles, at least some of which include Alu interspersed repeat sequences. These investigators have confirmed this finding and have found a remarkable association between inter-Alu circular forms and senescence, both in vitro and in vivo. This discovery may provide an important new model for senescence studies of DNA transcription. The fact that the association between specific DNA circles and senescence occurs both in vivo and in vitro is extremely important since it suggests that a common molecular process underlies the change

in both systems. If this proves to be the case, then both the in vivo (lymphocyte) and in vitro (cultured fibroblast) systems can be used as models for a single molecular event. Further, the inter-Alu sequence is structurally similar to several prokaryotic DNA transpositions. Thus, this system offers the possibility of a model of considerable generality. Whether the development of inter-Alu changes is a causative factor in senescence or a secondary indication of DNA instability, has yet to be determined, but in either case the model is of great interest.

The search for biomarkers of aging is an important part of the mission of the Molecular Biology Program. Racemization of amino acids are currently under active investigation as such a biomarker by Dr. Jeffrey Bada. The amino acids in living organisms are optically active. However, once biologically synthesized amino acids are isolated from the biochemical processes which maintain their optical activity, racemization reactions gradually convert the amino acids into an optically active mixture. Racemization in metabolically stable proteins in living animals may induce structural changes which in turn affect the functionality of the protein. Therefore, racemization may not only be a biomarker of aging, but may also be a part of an aging process in mammals. Studies are being done of racemization in several tissue types including teeth of primates and birds, lens tissue in human normal and abnormal eyes, and mammalian and bird tendons. Racemization appears to be a relatively good biomarker in each of these tissue types,and a good model for the study of biochemical alterations.

SUMMARY OF RESOURCE CONTRACTS

Biological Resources and Resource Development

Contract Number and Title	Current Annual Level	FY 1984	Course
AG-7-2128-21 Multigenotypic Mouse Charles River Breeding Laboratories	$ 12,000	$150,000	Termination in FY 1986
AG-3-2103-00 Multigenotypic Mouse, nine-year Charles River Breeding Laboratories	$417,024	$802,795	Termination in FY 1992
AG-3-2104-00 Fischer 344 nine-year Rat Harlan Industries	$366,600	$152,400	Termination in FY 1986

SUMMARY OF RESOURCE CONTRACTS

Cell Biology

Contract Number and Title	Current Annual Level	FY 1984	Course
AG-0-2100-05 Genetically Marked Cells Institute for Medical Research	$257,878	$275,895	Recompete in FY 1984

SUMMARY OF RESOURCE CONTRACTS

Genetics

Contract Number and Title	Current Annual Level	FY 1984	Course
AG-9-2113-06 Caenorhabditis Genetics Center University of Missouri	$51,739	$55,000	Recompete in FY 1984

The Geriatrics Branch

The objectives of the Geriatrics Branch (GB) are:

- To support research on clinical problems which occur predominantly among the elderly, or which are associated with increased morbidity and mortality in the elderly. (Geriatic Research Program)

- To promote research on clinical problems associated with nursing homes and other sites of long-term care for the elderly. (Geriatric Research Program)

- To encourage and support the development of researchers in geriatrics and other areas of clinical aging research through development of geriatric curricula and research support for junior clinical investigators and established investigators entering the field of aging. (All GB Programs)

- To identify alterations in pharmacological response (including adverse reactions) that occur with aging, the mechanisms responsible for these alterations, and consequent alterations in dosage requirements. (Pharmacology Program)

- To evaluate the effectiveness of new and current drugs for clinical problems of the elderly. (Pharmacology Program)

GERIATRIC RESEARCH PROGRAM ACHIEVEMENTS:

Urinary Incontinence in the Elderly

NIA-supported research on urinary incontinence in the elderly expanded to eight projects in FY 1983. New projects include studies on the relative prevalence of various pathophysiologic categories of incontinence in the elderly, potential changes with age in receptors for neurotransmitters which control bladder function, effects of estrogen on the female urethra and their implications for urine control, and a clinical trial of "bladder training" for incontinent nursing home residents. Continuing projects include studies comparing pelvic floor and other exercises vs. drug therapies for incontinence in older women, and a study to validate bedside diagnostic techniques in comparison with urodynamic studies in elderly incontinent patients.

Infectious Diseases in the Elderly

NIA-supported research on this problem grew to six projects in 1983. New projects include studies on the immune response to pneumococcal vaccine in the elderly, and on the pathogenesis and epidemiology of catheter-related and other urinary tract infections in nursing homes. Ongoing studies address the role of age-associated immunologic changes in the pathogenesis of herpes zoster, and improvements in laboratory diagnostic techniques for influenza.

Osteoporosis and Osteoporosis-Related Problems

Geriatrics Branch support for research in this area increased to nine projects in FY 1983. New projects include a study to establish normal histomorphometric values for bone in elderly women, a study of the effects of aging on cutaneous vitamin D synthesis in humans, and an epidemiologic study of fall-related injuries. Ongoing projects focus on the potential role in osteoporosis of age-related changes in estrogen and androgen metabolism and of

the relative inability of the elderly to activate vitamin D in response to restricted calcium intake. Two epidemiologic studies are examining the role of physical activity, endocrine, and dietary factors in osteoporosis.

Teaching Nursing Home. NIA's Teaching Nursing Home (TNH) award program was initiated in FY 1982. It supports research by academic centers and nursing homes on health problems, therapies and health maintenance strategies for older persons in nursing homes as well as other institutional and community settings.

The scope of appropriate research for TNH projects includes:

- Epidemiology, pathophysiology, diagnosis, and therapy of specific disorders causing significant morbidity and mortality in the elderly, such as: dementia, incontinence, sleep apnea, musculoskeletal disorders, and infections.

- Behavioral, psychological, social, environmental, and organizational influences on health and health care of the elderly, such as: effects of social supports, stress, and coping patterns on health and well-being; and the impact of alternative long-term care strategies on functioning of aged patients and their families.

- The role of nutrition, exercise, and other factors in preventing diseases and functional disability in the elderly.

- Rehabilitative and prosthetic techniques for ameliorating chronic musculoskeletal, neurologic, and other health problems of the elderly.

NIA's first two Teaching Nursing Home projects were funded at the end of FY 1982. The TNH project awarded to Albert Einstein College of Medicine in New York supports research on the effectiveness of physostigmine for cognitive deficits in dementia, genetic factors in Alzheimer's disease, pathogenesis of osteoarthrits, and neurologic factors in balance and gait disorders.

The TNH project awarded to the Philadelphia Geriatric Center in collaboration with the Medical College of Pennsylvania and the University of Pennsylvania supports research on the pathogenesis and epidemiology of urinary tract infections in nursing home patients, the effects of an exercise program for nursing home residents, cognitive therapy for stroke rehabilitation, the prevalence and etiology of sleep apnea, and the application of positron emission tomography to the study of dementia.

NIA funded three additional TNH projects in FY 1983. Research in these projects includes studies on syncope (fainting) in the elderly, vitamin D requirements in older subjects, and effects of exercise on cardiovascular function and obesity in the elderly.

PHARMACOLOGY PROGRAM ACHIEVEMENTS:

Cardiovascular Research

The cardiovascular system is one of three organ systems singled out for particular emphasis at the NIA's 1981 workshop on Pharmacology and Aging. Current projects include studies of age-related alterations in heart function, response to cardiac glycosides, and Na/K ATPase function; relationships between

cardiovascular disease and regulation of prostaglandin I_2; age-related alterations at adrenergic neuroeffector junctions in cardiac tissue; the effects of anesthetics and surgical stress on the aging heart, and a feasibility study (cosponsored by NHLBI) for a clinical trial of the effects of treating systolic hypertension in the elderly.

Catecholaminergic Pharmacology

Considerable evidence suggests that age-related changes in catecholamine systems are involved in neurologic, cardiovascular, and metabolic disorders of the elderly. Current pharmacology program support includes projects on autonomic regulation of the cardiovascular system, sympathetic responsiveness of the elderly during anesthesia, and effects of neuroleptic drugs on dopaminergic receptors.

Geriatrics Branch: Research Highlights

- In diagnostic studies performed as part of a clinical trial for therapies for urinary incontinence in older women, investigators found a much higher percentage of severe incontinence due to sphincter insufficiency in older women than had been reported in previous studies, which had found bladder abnormalities as the predominant pathology in severe incontinence in older women. If confirmed, this finding should focus additional attention on therapies for sphincteric insufficiency in older women. (Wells, R01 AG03542)

- A study to develop and validate a bedside diagnostic algorithm for urinary incontinence in the elderly has found that diagnoses using the algorithm were identical to those derived from more invasive urodynamic procedures in over 95 per cent of subjects. (Resnick, K08 AG00188)

- A longitudinal study of postmenopausal women confirms that estrogen therapy is associated with delayed and decelerated postmenopausal bone loss. (Hui, R23 AG03423)

- A study of elderly patients with osteoporosis has found that forty percent have elevated levels of parathyroid hormone (PTH). This is a much higher percentage than found in previous studies and, if confirmed, suggests a greater role for PTH in the pathophysiology of osteoporosis than previously believed. (Neer, R01 AG03418)

- A study of elderly persons who have experienced syncope following eating suggests that their loss of consciousness may be due to an abnormal drop in blood pressure following meals. This abnormal drop may be related to a decline with age in the ability to elevate circulating catecholamine levels to counter decreases in blood pressure after eating. (Rowe, P01 AG00599)

- Tolerance to digitalis decreases with advanced age in both humans and experimental animals. It has been found that Na/K ATPase activity of ventricular muscle and the number of ouabain binding sites declines with age in rats. These changes may account for the increased sensitivity of the aged to digitalis-induced arrhythmias. (Akera, R01 AG/HL 02398).

- In aged but not in younger rats, cocaine (an inhibitor of neuronal norepinephrine reuptake) augments heart rate response to norepinephrine. The mechanism for this alteration in response is under study. (Roberts, R01 AG03326).

- It has been found that intraoperative oliguria does not, as has been previously assumed, herald postoperative renal insufficiency. This finding has important clinical applications since currently much effort is spent with aged patients in treating intraoperative oliguria; a treatment which may provide more harm than benefit. (Eger, P01 AG 3104).

Physiology of Aging Branch

The mission of the Physiology of Aging Branch (PAB) is to foster the development of scientific knowledge and to facilitate the translation of basic medical and scientific information into practical applications for the treatment and care of elderly patients. The PAB has responsibility for five programs: Neuroscience, Immunology, Endocrinology, Nutrition, and Exercise Physiology. Since these programs have a complementary scientific interrelationship, they are integrated into a single branch of the Biomedical Research and Clinical Medicine Program (BRCM) of the National Institute on Aging (NIA). The primary thrust of the PAB program is to encourage the development of integrated basic and clinical research in the biomedical sciences represented within the Branch. The PAB, as a unit within BRCM, through its component programs fosters research on age-related physiological processes at the tissue, organ, organ system, and organism levels. Questions concerning age-related changes in the structure and function of various types of membranes is one of the emerging scientific items which potentially ties together the five programs of this Branch. Questions concerning age-related changes in receptor function and transport mechanisms across membranes are some of the most crucial cross-cutting issues facing all of the programs of PAB. During the next fiscal year the PAB plans to solicit position papers on these issues from eminent investigators. These papers will form the basis for developing program plans and new initiatives for PAB.

Neuroscience:

The Neuroscience of Aging Program (NAP) supports a broad spectrum of research which focuses on the relationship between aging and the associated changes in the structure and function of the nervous system. In particular, the NAP encourages both basic and clinical studies on Alzheimer's disease (AD) and other dementias of old age; learning and memory disorders of the aged; age-related changes or impairments in sensory functions; sleep disorders, insomnia and sleep apnea in the aged; function, structure, physiology, biochemistry, pharmacology and biophysics of the aging nervous system; ultrastructure, transport, and permeability at the blood-brain barrier. At present, the NIA Neuroscience Program supports perhaps the most comprehensive portfolio of biomedical research related to senile dementia within the Federal government. Among the topics listed, research related to AD is of particular interest to NAP. AD is a chronic, degenerative disease of the nervous system seen predominantly in the elderly. It is characterized by distinct neuropathologic alterations consisting of prominent senile plaque and neurofibrillary tangle production in the neurons of the frontal and temporal cortex and the hippocampus. Through the achievements of NIA grantees, it is now

possible to start formulating a coherent scientific explanation of the pathogenesis of senile dementia and to identify some exciting scientific opportunities that are ready to be exploited. What is more important is that it is now possible to propose testable hypotheses which can provide a framework for interrelating a number of discrete observations reported by NIA grantees and other scientists. This is truly remarkable because only five years ago very little was known about senility and Alzheimer's disease.

The issue of whether brain changes in dementia represent an accelerated form of normal aging has not been resolved, but evidence suggests that in most cases the morphological changes in old age differ quantitatively, not qualitatively, from those in senile dementia. Therefore, clinical distinctions between normal and pathological brain aging may be difficult because of the many common structural and biochemical features. Of the many brain impairments in the aged, decreased cognitive functioning is generally accepted to be the most consistent and serious problem. In its most severe form, cognitive impairment is the hallmark of senile dementia. Therefore, brain phenomena related to cognitive impairment or brain changes related to memory decline has been the principal focus of NIA's extramural Neuroscience Program.

Two biochemical abnormalities - reduced neurotransmitter synthesis and/or release, and decreased cerebral metabolic rate - occur in aging and dementing disorders, especially in senile dementia. Although the relationship between physiological abnormalities and memory impairment in the aged brain is complex, considerable evidence suggests that one of the most common metabolic disorders in aging and dementia occurs in the neurotransmitter systems. Several studies have shown that age-related changes occur in the synthesis and release of many neurotransmitters (Peter Davies-AG 01066).

In recent years, however, the cholinergic system has attracted the most attention. It is now well-established that there is a significant decrease in the activities of marker enzymes for cholinergic neurons in the brains of the aged and in victims of senile dementia of the Alzheimer's Type (SDAT). Several lines of evidence that support a cholinergic hypothesis of memory dysfunction in the aged include the following observations: 1) Marked changes in cholinergic enzymes occur in the brains of aged animals and humans. 2) Loss of cholinergic function at the neuronal level can account for changes in enzyme activity. 3) Loss of memory with aging can be related to changes in the cholinergic system. 4) Disruption of cholinergic mechanisms in young subjects produces memory impairments similar to those that occur in healthy elderly and demented subjects. When normal human subjects are given anticholinergic drugs, they develop a reversible cognitive disorder that is improved by physostigmine. 5) Memory impairments can be reduced by cholinomimetics.

Several studies suggest that cholinergic systems are also involved in SDAT. For example: 1) The activity of acetylcholinesterase (AChE) is decreased in the cerebral cortex of SDAT patients. 2) The activity of choline acetyltransferase (CAT) is reduced in the cerebral cortex of SDAT patients. Little change is reported in the number of cortical cholinergic receptors, but there appears to be a reduction in somatostatin-like immunoreactivity (Peter Davies-AG01066, Don Price-AG03359).

Despite the focus on the cholinergic system in aging and dementia, there is some question whether this is the only transmitter system to undergo dramatic changes and whether the patterns of age-related changes in other systems are similar to changes in SDAT. There is a strong possibility that other systems,

particularly the catecholamines, may be involved in important age-related changes in brain function and behavior. One example of this is the high correlation between aging and extrapyramidal symptoms, such as the cognitive loss and depression in Parkinson's disease. Recent evidence of cell loss in the locus coeruleus with normal aging and in some SDAT patients further supports this possibility (Caleb Finch-AG03273).

The enzyme CAT, used as a cholinergic marker, is confined to cholinergic neurons in the brain and is involved with ACh synthesis. Within the neuron, CAT is concentrated in the nerve terminal's synthesis. Within the neuron, CAT is concentrated in the nerve terminal's cytoplasmic fraction. It is synthesized in the cell body and is transported by the slower component of the axonal transport. Although the reduction of neocortical CAT activity seems to be one of the crucial changes in SDAT, it has been suggested that CAT may not be the rate-limiting factor in the synthesis of ACh: the availability of substrates might be a more important variable to study. The actually observed changes in CAT may actually reflect cholinergic cell loss (Dean Smith-AG01572).

The cortical cholinergic abnormality in SDAT has been suggested to be compatible with a decline in age-related cholinergic function. The decline has been attributed to a severe loss of neurons in the nucleus basalis of Meynert (nbM) but it is not clear what factors initiate or are responsible for the specific loss of cholinergic neurons in this nucleus of the basal forebrain. The loss of these neurons may reflect an abnormality of a neuromodulator which specifically affects cholinergic neurons in the brain (Don Price-AG03359).

Only a few of the numerous potential modulators of cholinergic neuronal function have been studied to date but we know that complex interactions exist between the various neurotransmitter systems. For instance, cortical acetylcholine (ACh) level or ACh release is modulated by 5-hydroxytryptamine (5Ht), dopamine (DA) and norepinephrine (NE). It has been suggested that there might be other neuromodulators, including polypeptide hormones, that need to be investigated further. There is evidence suggesting that some neuropeptides may be released from nerve terminals to modulate postsynaptic activity. Polypeptides known to influence the turnover of ACh in the cortex and hippocampus include thyrotropin-releasing hormone, somatostatin, neurotensin, angiotensin, substance p, and B-endorphin. The question of selective vulnerability of cholinergic neurons and/or the possibility of a defect in the neuromodulator system in SDAT is one of the crucial scientific opportunities that needs to be exploited. In this regard, it has been suggested that specific extrinsic factors, such as neutrotrophic hormones, may influence the maintenance and survival of neurons. This is an important hypothesis which needs to be tested (Peter Davies-AG01066).

Since ACh synthesis is tightly coupled to carbohydrate oxidation, age-related decrease in oxidative metabolism might offer a reasonable explanation for the reduction in ACh synthesis. The decline in the release of ACh in the aged animals reported by some scientists might be explained by a decrease in the synthesis of ACh. This possibility can be supported, if it can be demonstrated that there is a constant level of ACh with aging, despite decreased synthesis. To answer these issues, Gibson and Peterson have studied the effects of aging on carbohydrate metabolism and ACh synthesis and release in the mammalian CNS. Their results showed that ACh synthesis decreases in the aged without a corresponding reduction in ACh levels. Only the release of ACh

decreased to the same extent as the inhibition of synthesis. At present, it is not clear whether a cause-and-effect relationship exists between synthesis and release, but it is important to note that both synthesis and release are Ca^{2+}-dependent activities. Therefore, changes in Ca^{2+} concentration and/or transport mechanisms might influence both activities, synthesis and release of neurotransmitters. The functional implications of decreased efficacy in the rapid extrusion of excess free Ca^{2+} entering upon depolarization is not well studied, but there is evidence suggesting that increasing intracellular Ca^{2+} concentrations may be quite toxic to nerve cells (Gary Gibson-AG04171).

The other common neurochemical abnormality that occurs in dementia disorders is a decrease in the cerebral metabolic rate. Cerebral blood flow (CBF) is regulated to meet the requirements of cerebral metabolism and function. Cerebral function is evaluated by measuring CBF, cerebral metabolic rate for oxygen ($CMRO_2$), and regional cerebral metabolic rate for glucose ($rCMR_{glc}$). Glucose is the major substrate for oxidative metabolism in the brain. Measures of $rCMR_{glc}$ reflect the local cerebral metabolic rate and local neuronal activity. It has been reported that decreases in cerebral blood flow, O_2 consumption, and cerebral glucose utilization occur in dementia patients, but these changes do not appear to be specific for any particular type of dementia. It is proposed that the specificity of metabolic dementia lies not in the nature of the metabolic abnormality but in the specific anatomical or neurochemical system and the extent of tissue damage. Therefore, it becomes possible to distinguish between normal aging and clinically diagnosable dementia only when there is enough damage to the brain area subserving the higher cortical functions (John Blass-AG03853, Gary Gibson-AG04171).

A Swedish group of investigators have shown that patients with SDAT have a changed carbohydrate metabolism with decreased blood glucose levels. Since carbohydrate metabolism is dependent on hormonal regulation via such systems as the brain-pituitary-adrenal axis, in which the cholinergic and dopaminergic neurotransmitters play an important role, it would be important to determine the relationship between glucose metabolism and the synthesis of neurotransmitters. It is not clear whether decreased blood glucose level can impair the synthesis of brain ACh, but since ACh synthesis is tightly coupled to carbohydrate metabolism, any reduction in oxidative metabolism (whether age-related or not) should lead to reduced ACh synthesis. There is some evidence that cholinergic neurons may be more susceptible to impaired carbohydrate metabolism than other neurotransmitter systems (Gary Gibson-AG04171).

An important enzyme in brain glucose utilization is pyruvate dehydrogenase complex (PDHC); it occupies a pivotal position in regulating energy metabolism. In most tissues, PDHC flux is dependent on hormonal and nutritional status.

However, PDHC in the brain relies on glucose as the main source of energy and remains relatively active in different metabolic states. For neurobiology of aging it is important to have an understanding of the biochemistry of PDHC, since it has a central role in both the regulation of Ca^{2+} and the cholinergic hypothesis of memory disorders. Some investigators have found a significant decrease of PDHC in autopsy samples of SDAT frontal cortex. It has been noted that low PDHC in brain frontal cortex may be pathophysiologically significant because reduced metabolic rate and cholinergic dysfunction are two well-defined characteristics of SDAT. Abnormalities in both metabolic rate and cholinergic function can be associated with impairment of pyruvate oxidation and PDHC activity (Gary Lynch- AG00538).

An English group has suggested that PDHC activity may control the size of the acetyl-CoA pool available for ACh synthesis. Therefore, decreases in the enzyme activity may exacerbate the cholinergic deficiencies in SDAT patients. Acetyl-CoA is generated in the mitochondria through PDHC, but the transport mechanism for moving it out to the cytoplasm is not known. There is recent evidence that PDH activity is tightly linked to the speed with which mitochondria perform their critical function of Ca^{2+} sequestration. A preponderance of evidence appears to link PDHC activity to Ca^{2+} regulation and to relate Ca^{2+} concentrations to neuronal degeneration. This suggests that disturbances in PDHC activity could result in cell death. It has been shown that PDHC activity is regulated by alpha pyruvate dehydrogenase (PDHa). Since phosphorylation of PDHa inhibits PDHC, while its dephosphorylation activates the complex, it is necessary, in order to understand the regulatory mechanisms for PDHC activity, to study the factors that promote the phosphorylation and dephosphorylation of PDHa (Gary Gibson-AG04171).

In a related clinical study it has been shown that SDAT patients have a severe loss of neurons in the nucleus basalis of Maynert (nbM), a nucleus of the basal forebrain that project directly into the neocortex and provides the major source of cortical cholinergic innervation. Previous studies on autopsy material from SDAT patients have demonstrated a reduction in presynaptic makers for ACh-using neurons in the hippocampus and cerebral cortex. These findings are significant because they have shown that cholinergic neurons in the nbM may selectively degenerate in SDAT and provide an example of loss in a transmitter-specific cell population in a major disorder of higher cortical function (Don Price-AG03359).

Using an animal with a deafferentated hippocampus, which creates a similar situation as loss of cells in the nucleus basalis, a group of investigators has tested the hypothesis that synaptic activity influences the regulation of cytosol Ca^{2+} in the pre- and postsynaptic elements. Their results show that eliminating the major input to the hippocampus significantly reduces Ca^{2+} transport into the mitochrondria when the metabolic pathway is fueled with pyruvate. This suggests that the decreased ability of mitochondria to take up Ca^{2+} from the cytoplasm may have important functional consequences in the denervated dendrites. The reduced capacity of mitochondria to buffer Ca^{2+} levels may cause the calcium concentration to rise, resulting in the activation of various CA-dependent proteolytic processes that could lead to dissolution of axoplasm and disruption of the cytoskeleton. The results further suggest that different forms of plasticity in the hippocampus might be mediated by a common biochemical mechanism. For instance, any one of three events - learning, high-frequency stimulation, or hippocampal denervation - may modify the phosphorylation of PDHa, resulting in decreased ability of mitochondria to rapidly eliminate excess cytosolic CA^{2+} followed by activation of a Ca-sensitive proteolytic process (Gary Lynch-AG00538).

The major morphological abnormalities that are frequently present in the brains of SDAT patients include granulovacuolar degeneration, Hirano bodies, neuronal loss, neuritic (senile) plaques, and neurofibrillary tangles. Neurofibrillary tangles and neuritic plaques are the two most characteristic neuronal lesions in the cerebral cortex of SDAT patients. The concentration of these lesions is correlated both with the severity of dementia and with deficiency in the cholinergic system. The lesions can occur in the hippocampus of most aged individuals without dementia, and their frequency appears to increase with age. Several studies have correlated the severity of SDAT with

the frequency of neuritic plaques and with a reduction in presynaptic cholinergic markers in the cortex, but the functional relationship between the pathogenesis of the plaques and cholinergic cortical innervation is not known. In a study designed to determine whether neurites in the plaques consist of degenerating presynaptic cholinergic axons from nbM, Struble and co-workers analyzed the character and distribution of plaques. They found that as the plaques matured, the amount of amyloid increased and the number of neurites and the activity of AChE decreased (end-stage amyloid-rich plaques were devoid of any AChE). These results suggest that denervation of cortical cholinergic fibers plays an important role in the pathogenesis and evolution of the neuritic plaque (Don Price-AG03359).

The presence of tangles in large numbers is not unique to SDAT. They are also found in adult Down's syndrome, dementia pugilistica, subacute sclerosing panencephalitis, Guam Parkinson dementia complex (PD), and Amyotrophic Lateral Sclerosis (ALS), as well as in a high proportion of neurologically intact individuals at relatively young ages, from Guam. Neurofibrillary tangles occur in the cell body, where they displace other organelles. Sometimes tangled masses of fibrous elements are irregularly dispersed throughout the cytoplasm and extend into the neuritic processes. A normal neuron has two types of neurofiber: neurotubules, which are similar to microtubules; and neurofilaments, which are members of the class of intermediary filaments. The neurofibers that make up the tangles consist of a pair of filaments wound around each other to form a paired helical filament (PHF) with a periodicity of 80 nm. The diameter of each member of the pair is 10 nm, with a maximum width of 25 nm for the pair. Occasionally, some tangles have a mixture of normal-appearing filaments and tubules with the abnormal PHF. Despite great interest in the mechanism by which PHFs are formed in both SDAT and normal aging brain, attempts to characterize their biochemical composition have failed. Some investigators believe that although neurofibrillary tangles are made up of fibers that differ structurally from normal fibers of the nervous system, they have proteins related to a normal constituent of the brain (Dennis Selkoe- AG01307, Shu Yen-AG01136).

The difficulty in studying the molecular nature of neurofibrillary tangles can be partly explained by the recent demonstration that PHFs have unusual solubility characteristics. The filaments apparently are highly insoluble in all of the reagents commonly used to solubilize proteins for gel electrophoresis. This study suggests that the principal PHF polypeptides could not have entered into the gel in previous attempts to characterize PHF proteins. Through a series of experiments, it has been shown that PHFs in SDAT lesions contain a highly rigid large polymer whose molecules are held together by an extremely strong bond. The evidence strongly suggests that covalent bonds cross-link specific amino acids of the individual filaments into a rigid intracellular polymer. Covalent cross-linking of structural proteins to form high molecular weight insoluble polymers occurs in human erythrocytes, skin keratinocytes, and senile cataracts; and the evidence from this study is that it also occurs in nerve cells. It has been suggested that these cross-links are assembled under the catalytic influence of Ca^{2+}-dependent transglutaminase and that this reaction may play a general role in restructuring of cells during aging. An increase in cytoplasmic availability of Ca^{2+} activates transglutaminase, and this leads to cross-linking of proteins. Selkoe has demonstrated that transglutaminase is present in the human brain and can cross-link normal human neurofilament proteins into an insoluble high molecular weight

filamentous polymer. These findings are extremely important and exciting because we now can begin to investigate the exact nature of the cross-linking bond and what causes it to form. The accumulation of these highly rigid and insoluble polymers may physically interfere with the normal axonal transport system and have serious consequence for the viability of the neuron. Although very little is known about the crucial events that initiate the formation of neurofibrillary tangles, it is now possible to manipulate the structural proteins of PHF and the proliferation of neural filaments in model systems. Other investigators are beginning to study the biochemical constituents of PHF using monoclonal antibodies (Shu Yen-AG01136).

Since PHF is one of the principal markers for SDAT, it becomes an important scientific challenge to find out the crucial pathogenic event(s) underlying the cellular changes which lead to PHF formation. A group of Canadian scientists, working on the assumption that the crucial pathogenic event involves either endogenous gene failure or an unidentified infectious agent, has proposed three hypotheses concerning the cellular changes underlying SDAT: 1. The crucial pathogenic event, acting through RNA, nonspecifically reduces protein synthesis. 2. The crucial pathogenic event produces an infectious agent that causes PHF formation, depolymerization of microtubules, and impairment of slow cytoplasmic transport, leading to reduced synaptic activity. 3. The crucial pathogenic event changes the blood-brain barrier, making brain cells vulnerable to potentially toxic environmental agents such as silicon and aluminum (Dennis Selkoe-AG01307).

Circumstantial evidence supports the hypothesis that changes in the blood-brain barrier may make nerve cells vulnerable to the effects of agents like aluminum, a well-recognized neurotoxic element. Some evidence exists which suggests that there is an age-related increase in brain aluminum concentration in humans. There is considerable evidence that aluminum concentrations are elevated in the aged brain in association with neurons containing neurofibrillary tangles and with such degenerative diseases as SDAT, Guam and Kii peninsula ALS, and Parkinson-dementia complex with SDAT neurofibrillary tangles. Aluminum appears to accumulate on intranuclear structures in both SDAT and aluminum-induced experimental encephalopathy in cats. Studies employing sensitive probes for DNA damage have shown that chromatin-bound aluminum interacts functionally with its binding sites and may damage DNA. Since there appears to be a high correlation between the presence of both PHF and aluminum, it is important to measure the precise distribution and concentration of aluminum within the cell. Scanning electron microscopy in conjunction with x-ray spectrometry provides an extremely sensitive analytical tool for identifying and localizing chemical elements within a nerve cell. Using this technique, Perl has shown that aluminum concentrates in the nuclear regions of tangle-bearing neurons from the hippocampus of SDAT patients. Adjacent normal-appearing neurons from both SDAT patients and controls are free of detectable aluminum (Dan Perl-AG01415).

In a more recent study, scientists have analyzed tangle-bearing and tangle-free neurons from the hippocampus of Guamanian Chamorros with ALS and Parkinsonism-dementia (PD) and neurologically normal controls. The results show that aluminum accumulates within the nuclear region and perikaryal cytoplasm of the tangle-bearing hippocampal neurons in the ALS and PD cases. The tangle-bearing neurons from the controls also had a high concentration of aluminum. It is interesting to note that the calcium-related x-ray emissions were higher in the tangle-bearing than in the tangle-free regions. Analysis of

the mineralized walls of the blood vessels within the globus pallidus of the ALS and PD patients showed that the extent of aluminum deposition in the blood vessel walls was especially high and considerably greater than in any other neuropathological conditions studied.

The investigators conducting these studies have suggested that the constellation of ALS, PD, and extensive premature tangle formation encountered among the Chamorros of Guam and the Japanese living in the Kii peninsula may reflect common etiological factors. Gajdusek and Salazar have carefully studied the ecology, culture, and diet of the ALS- and PD-affected people. They found them to be undistinguishable from their unaffected neighbors except for the mineral content of their drinking water, which is extremely low in calcium and magnesium but very high in aluminum, silicon, titanium, chromium, iron, and manganese. The functional significance of the mineral content of the drinking water and the appearance of tangle-bearing neurons or the pathogenesis of ALS, PD, or SDAT is not clear but suggest an important role which needs further study. In summary, there is increasing evidence that brain concentrations of aluminum and Ca^{2+} are elevated in ALS and PD. In these patients, the elevated brain levels of aluminum are positively correlated with increased brain concentrations of Ca^{2+} levels which are even higher than in ALS or PD syndrome. Since there appears to be a high correlation between the occurrence of tangle-bearing neurons and the presence of high concentrations of aluminum and calcium, it would be interesting to study in greater detail the distribution of these elements within the cell and to explore their functional relationship to tangle formation. At present, the precise relationship is not clear and it is not known whether aluminum accumulates in small organelles such as the mitochondria and interferes with its calcium buffering action (Dan Perl-AG01415). The results of this last study are extremely important because they provide a crucial clue for beginning to assemble the pieces of the SDAT puzzle. It is now possible to formulate a testable hypothesis which can provide a common framework for interrelating a number of the discrete results reported. As indicated earlier, an abnormality in the blood-brain barrier could allow neurotoxins such as aluminum to get into the brain and eventually inside the nerve cell. Then the aluminum, as $Al(OH)^{2+}$, could compete with the calcium binding sites thus disrupting the normal homeostatic mechanism for maintaining low calcium levels within the cell. Increasing the net Ca^{2+} concentration in the cytoplasm could have a number of adverse consequences because of the key role Ca^{2+} plays as a second messenger within the cell. One of the most important of these effects is in axonal transport. Several studies have shown that Ca^{2+} is the critical factor for maintaining axonal transport. It has been suggested that some of the effects of metabolic inhibitors might be explained by the release of Ca^{2+}; the resulting high concentrations of free Ca^{2+} might inhibit axonal transport. Axonal transport plays a vital role in normal neuronal functioning. In the perikaryon, functionally important substances are synthesized in the cell nucleus and are transported down the axon. Normal functioning of axonal transport depends on many factors, such as axonal continuity, cell somal integrity, oxidative metabolism, microtubules, neurofilaments, and Ca^{2+} (Raymond Lasek-AG00795).

Changing the intracellular concentrations of Ca^{2+} can also influence some of the other age-related changes reported above. Calcium plays a key role in the following: 1) Glucose metabolism and neurotransmitter synthesis. 2) Regulating the activities of key enzymes. 3) Axonal transport. 4) Disassembly

of microtubules. 5) Cross-linking of neurofibers. 6) Mediating neuronal excitability through K^+. 7) Release of transmitters. 8) Possible interaction with neurotoxins such as $Al(OH)^{2+}$.

The biological activity of a living cell is directed toward one goal maintaining homeostasis - and disruption of this activity leads to death. To achieve homeostasis in complex multicellular systems, it is important for each cell to coordinate its activities with those of other cells within the same system as well as with those outside the system. To meet this need for intercellular communication, cells have developed cell-surface receptors that translate the information provided by a messenger molecule from another cell into chemical action. These signals activate internal biochemical reactions that are controlled by a cellular regulator. Calcium-binding proteins such as calmodulin (CaM) have many of the features needed to play the role of cellular regulator. Among such cellular regulators, hormones, neurotransmitters, cAMP, and Ca^{2+} are the most important. Hormones and neurotransmitters play the role of intercellular regulators, mediating communication between cells, whereas cAMP and Ca^{2+} act as intracellular regulators, mediating messages between intracellular organelles. The response to a message by a hormone can last for many hours, whereas the response time to cAMP and Ca^{2+} is much shorter.

According to the second-messenger concept, some stimuli change the cytosol levels of Ca^{2+}, others change the levels of cAMP and many may affect both. In this manner a single cell, by employing one or two second messengers, can have multiple responses. A first messenger such as a hormone or a neurotransmitter can activate a receptor site, which increases the Ca^{2+} flux within the cytosol. Increased Ca^{2+} concentration initiates a Ca^{2+}-dependent response through proteins that detect and respond to the Ca^{2+} signal. Understanding the mechanisms of cell-to-cell communication and factors that might alter it is vitally important for aging research in general and for the neuroscience of aging in particular. Ultimately, the normal or proper functioning of a biological system depends on efficient communication between its various constituent elements. Any disruption of this system of inter- and intracellular communication, from whatever cause, would compromise the normal function of the organism, causing slower and/or poor response, inappropriate response, or no response to stimuli. In very general terms, these might describe some of the consequences of age-related changes in the brain. In view of the available evidence it is reasonable to propose that any change that seriously disturbs the delicately balanced homeostatic system of transporting Ca^{2+} would have serious consequences for the cell's ability to communicate, or even for its viability. Such a disruption of the system could be a consequence of exposure to a neurotoxin, pathogenic agent, aging, or other insults. Examination of the age-related change in the brain at this level of analysis would allow us to relate changes in the brain to changes in other systems, such as the cardiovascular, immune, and endocrine systems, and develop a better understanding of cellular processes of aging.

Exercise:

The central objective of the Exercise Physiology Program is to develop and support basic and clinical research designed to assess the role of physical activity in a) the promotion of health, which includes the concept of rehabilitation, and b) the prevention of premature physical decline in the elderly. Basic biological mechanisms of adaptational responses of the nervous system, of skeletal muscle, of bones and joints, of the heart, blood and vasculature, and of the respiratory system are of particular interest. Because

of the cross-disciplinary nature of the field of exercise physiology, approaches to this kind of research range from cell biological to the level of the whole organism, including not only classical in vivo research, but statistical studies of populations (i.e., epidemiology) as well.

Representative research questions include the following: What factors influence man's ability to make biological adaptations to exercise and how may these factors be influenced by the aging process? To what extent and under which conditions is the medical recommendation of physical activity for the elderly sound? In the recommendation of physical activity for the promotion of health in the elderly, how (in terms of specific activity, intensity, duration, frequency, etc.) is advice formulated to fulfill the needs of the individual? Can regular physical activity prevent or delay onset of age-related disorders (e.g., hypertension, maturity-onset diabetes mellitus) and, if so, how? Some of the research highlights to the Exercise Physiology Program during FY 1983 have been as follows:

- In healthy Americans, a decrease in physical activity, a gain in weight, and aging changes in the cardiovascular system combine to cause a nine percent decline of aerobic power per decade after age 25. If physical activity and body composition are kept constant, deterioration due to aging per se results in a decline of aerobic power per decade by only about five percent. The major factor in this decline is the unavoidable decrease in maximal heart rate associated with age. Nevertheless, some men in their 60's and 70's are able by means of regular physical activity to maintain aerobic power above that of healthy untrained young men.

- Regularly performed physical activity in rats has been shown to increase the sensitivity and responsiveness to insulin of glucose uptake and glucose oxidation in fat cells. Fat cells from trained animals are smaller than those of age-paired, weight-paired sedentary controls. A larger amount of insulin is bound by fat cells of the trained animal, indicating a training-induced increase in the number of insulin receptors. The enhanced rates of sugar uptake and oxidation probably involve a biological adaptation at step(s) beyond the binding of insulin to its receptors. These findings are pertinent to understanding how regular participation in physical activity may prevent maturity-onset diabetes mellitus.

- Fat cells of sedentary rats were found in a recent study to have a 180% greater volume than those of trained animals despite only a nine percent difference in total body weight. Following the termination of training, fat cell size promptly increased. With this increase, the enhanced levels of glucose uptake and oxidation induced by training, also promptly decreased. These results indicate that the effect of exercise training on the response of fat cells to insulin is quickly lost when physical training is stopped. This study is complementary to the results above and appears to be pertinent to understanding the role of exercise in preventing maturity-onset diabetes.

- Studies in progress, but not yet published, show that compared to free-eating sedentary controls or controls which are food-restricted to

equate caloric intake, rats which exercise on a regular basis show significant gain in longevity. Whether this has any application to human longevity is not clear. Though severe food restriction appears to result in even greater longevity than regular participation in formal exercise, it is not yet clear that this is not partly explained by increased movement activity that probably reflects a food-seeking behavioral response to semi-starvation.

- Data from other studies also in progress show already a) that joint increasing common activities in daily living, particularly walking, can improve physical fitness, lower blood pressure and improve insulin sensitivity in sedentary people in their 60's, and b) that large increases in aerobic power and exercise capacity can be induced in sedentary older people if training is sufficiently prolonged and intense.

- Male pathogen-free rats have been exercised by swimming on a daily basis beginning at age six months, for either three months or 15 months. In these rats, mitochondrial enzymes activities, used as indicators of the capacity for aerobic capacity, were found to increase with exercise (compared to sedentary controls) by the same extent in each age group. The results indicate that for rats, the capacity of skeletal muscle to maintain an exercise-induced adaptive increase in mitochondrial enzymes persists into old age (so long as the animal's cardiovascular system and general health permit).

Nutrition:

The Nutrition Program is oriented toward basic and clinical research of issues in the diverse field of nutrition that relate a) to the promotion of health in the elderly, and b) to the prevention of premature decline commonly associated with advanced age. Specific research issues of current interest include: assessment of nutritional status in the elderly (development of valid methodologies for use in the elderly, and the establishment of age-appropriate norms); effects of aging on nutrient digestion, absorption, and utilization, and the relation between these effects and nutrient requirements; the contribution of nutritional status to the etiology and pathogenesis of disease prevalent in the elderly (e.g., osteoporosis, anemia, digestive disorders, and senile dementia); the role of nutrition in preventative and therapeutic regimens (e.g., studies on the interactions of nutrients and therapeutic agents, as well as the nutritional status on the efficacy of therapeutic agents); dietary patterns among the elderly and the relationship between these and nutritional and health status (e.g., studies on physiological, behavioral, and environmental factors which influence the quality and quantity of food eaten); role of under- and overnutrition in health and longevity (e.g., the role of diet and body composition in modifying immune, endocrine, and metabolic processes); and interactions of nutrition, age, and physical activity with respect to age-associated changes in body composition, including loss of lean body mass, loss of bone mass, and increase in adiposity.
Some of the research highlights to the Nutrition Program during FY 1983 have been as follows:

- Data have been presented to suggest that food restriction can have a marked life-prolonging action in rats without reducing caloric intake

per gram of body weight by life span calculations. Food-restricted animals in fact have greater caloric intakes per gram of body weight during their lifetimes than rats fed ad libitum, yet live longer. Accordingly, food restriction appears to slow the rate of aging in the rat apparently by decreasing the metabolic rate.

- Food restriction by about 30% of total calories ingested by free-eating reference-control rats, increases longevity to a greater extent than does regular participation in physical activity.

- Food restriction in rats has been shown to prevent the loss in glucagon-promoted lipolysis with age in fat cells. This may primarily be the result of either a loss of or a change in the characteristics of glucagon receptors with adenylate cyclase. The modulation of receptor-plasma membrane events may be an important phenomenon in understanding the process of aging and obesity (i.e., an increase in percent body fat).

- Food restriction has been shown not only to prolong life, but to extend life span in barrier-maintained SPF Fischer 344 rats. In addition, compared to data for control rats fed ad libitum, renal lesions occur at a later age and progress more slowly in food-restricted animals. In addition, food restriction apparently delays or prevents interstitial cell tumors of the testes, bile duct hyperplasia, myocardial fibrosis, myocardial degeneration, and a decline of skeletal muscle mass. The mechanisms by which food restriction prolongs life in animals need to be more fully understood so that application to human biology might be planned.

Endocrinology:

The endocrine system is one of the three major interacting homeostatic systems of the body and, therefore, knowledge of the way in which it functions can lead to a fuller understanding of many of the age-related changes that take place in other systems (e.g., immune, nervous, cardiovascular, etc.) as well as in the elderly person's response to environmental change. Thus, the main objective of the Endocrinology Program is to encourage and suport research aimed at providing an understanding of the age-related changes in endocrine function and the pathophysiologic states that may result from age-induced changes in this system.

Investigations supported by the Endocrinology Program span steroid and lipid metabolism, steroid receptor-site physiology, enzymology, and mechanism of hormone action at organ-sites. The Program is divided into four areas which correspond roughly to the major sites of impact of aging processes on endocrine function. These areas include: 1) identifying alterations in the dynamics of hormone production patterns which accompany aging in men and women; 2) quantifying the extent of and the determinants of alterations in hormone production; 3) determining the consequences of these alterations on target tissues and organs; and 4) relating those alterations to the development of certain pathological states.

Research on the aging reproductive system is probably the most active part of the Endocrinology Program. This system, unlike many others, is highly responsive to small perturbations in other endocrine and neuroendocrine

functions. Age-related changes in the reproductive system has an important impact on issues relating to the cost of health care, quality of life, and disease prevention. Studies on the reproductive system of animals who are reaching senescence, as opposed to those who have already reached senescence, allow us to understand the process whereby reproductive senescence occurs. Two grantees at the University of Maryland are, independently, using this model to examine ovarian steriod biosynthesis and neuroendocrine control of the reproductive (estrus) cyclicity, respectively. Results from both investigators show that basal hormonal levels do not differ between reproductively normal (young) and middle-aged animals. These findings are not surprising since other investigators working with the reproductive system, as well as with other endocrine systems, have reported that the basal levels of many hormones in young and old subjects tend not to differ or differ only slightly.

Differences that have been found between the aged and young, however, are striking and interesting. Dr. Eugene Albrecht has found that plasma progesterone concentrations do not differ at comparable stages of gestation in young and aged rats successfully maintaining pregnancy. Moreover, pregnancy failures in aged females do not appear to be associated with major alterations in the serum levels of progesterone. There appears to be, however, a subnormal conversion of ovarian progesterone by the ovaries of aged rats maintaining pregnancy. The circulating levels of progesterone appear to be maintained at normal levels in aged animals; however, as a result of a reduction in the metabolic clearance rate of the steroid. Additional research is currently underway to test this and related ideas.

Dr. Phyllis Wise has been studying changes in proestrus gonadotropin surges in middle-aged rats. Her findings show that there are no differences between young and aged animals in the basal levels of leutinizing hormone (LH). However, LH surge levels are lower in middle-aged than in young animals. She has identified what appears to be the initial events that trigger increasingly frequent irregular cycles and eventual acylicity. Changes in median eminence leutinizing hormone releasing hormone (LHRH) and in plasma LH, follicle stimulating hormone (FSH), estradiol and progesterone during proestrus afternoon samplings precede changes in baseline hormone concentrations observed during morning samplings. She has also reported changes in catecholamine turnover rates in afternoon samplings from selected, sexually differentiated, hypothalamic areas. These findings parallel a decrease in the number of estrogen receptors in these brain areas as well as a suppression of estradiol to induce the LH surge in aging rats.

Dr. Wise's findings have been independently confirmed by Dr. Ralph Cooper at Duke University. In addition, however, Dr. Cooper has found differences in the time of the day at which young and middle-aged rats show peak serum progesterone levels even though the concentration of serum progesterone at peak did not differ between the groups. Moreover, Dr. Richard Walker at the University of Kentucky has reported findings which suggest that the suprachiasmatic nucleus (SCN), an area rich in serotonergic terminals, may be the anatomical locus for the age changes in LH surge. He has shown a parallel between the age-related changes in patterns of LH secretion and serotonin rhythm.

An interesting parallel to these findings has been reported by Dr. Calvin Desjardins at the University of Texas at Austin. His findings offer insights about the temporal domains of LH and testosterone secretion in young and old mice. They show that the testes of aged males reliably respond to endogenous LH as judged by the perfect correlation of LH and testosterone episodes during a nine-hour test period. On the basis of this evidence, his working hypothesis is

that the primary deficit in the reproductive system of aged male mice is due to a change in the frequency of the purported neural oscillator which occasions the periodic activation of neurons discharging gonadotropin releasing hormone (GnRH) into the portal blood reaching gonadotropes, which, in turn, provoke these cells to discharge LH or FSH in episodes. These results provide an example as to why reproductive hormones should be examined on a moment-to-moment basis, particularly in mice. Values based on the average level of a hormone in a group of old males may not only be misleading but could be a major source of error contributing to discrepant findings in the literature.

One topic that has not received a great deal of attention concerns the mechanisms underlying, and the impact of menopause, on various physiologic states. Dr. Jerry Robinson at the Wisconsin Regional Primate Center is studying menopause in 12 female rhesus monkeys. Results of these studies show that, as in humans, there is a reduction in the number of oocytes in menopausal monkeys. They have also uncovered a relationship between the length of time for which an animal had been menopausal and their hypothalamic-pituitary sensitivity to estrogen feedback.

Menopause is typically considered to be a biologic marker for many pathophysiological conditions. One of these, postmenopausal osteoporosis, is an important health concern by virtue of the number of women afflicted as well as in terms of the pain and deformity associated with the disease. Research on a rat model by Dr. Duke Kalu at the Univeristy of Texas at San Antonio has produced some provocative findings in this area. Studies were carried out to examine the effects of ovarian hormone deficiency on bone and calcitonin levels. The reasons for carrying out this study relate to the observations that bone loss occurs with aging, and the rate of loss is accelerated at menopause. Circulating calcitonin, a bone resorption inhibiting hormone, decreases with aging, a condition which would favor increased bone loss. Estrogen therapy increases calcitonin secretion while oophorectomy decreases thyroidal secretion of calcitonin following hypercalcemic challenge. These findings raise the possibility of an interrelationship between estrogenic hormones, calcitonin, and accelerated postmenopausal bone loss.

The finding that nine months after ovariectomy the thyroidal and plasma calcitonin concentrations were similar to those of controls suggests that loss of ovarian hormones at menopause does not contribute to age-related decreases in circulating calcitonin in postmenopausal women. The attenuation of calcitonin secretion in ovariectomized rats is related to the response of the thyroid to a hypercalcemic challenge rather than to a deficit in the ability of the gland to synthesize calcitonin. The greater percentage of lipid observed in the bones of oophorectomized animals indicates that postmenopausal osteoporotic bones may differ chemically from normal bones. This is in contrast to the current view that in osteoporosis the quality of the bone is unaltered, the only problem being a net decrease in bone mass. More importantly, the greater percentage of lipid in the bones of oophorectemized animals may play an etiologic role in ovarian hormone deficiency bone loss since there are indications that bone lipids are involved in the calcification of bone.

The pituitary is one of the most important CNS structures involved in regulation of various endocrine functions. Research on this structure by Dr. Paul Conn at Duke Unviersity Medical Center suggests that there are major morphological changes in the Golgi complex and in the rough endoplastic reticulum, with age. These structures are associated with protein synthesis and modification and, therefore, may be responsible for the molecular changes that occur in LH with aging.

The research by Dr. Joseph Meites and his colleagues at Michigan State University points to the importance of prolactin secreting microadenomas of the pituitary. These prolactinomas have been found to be common in men and women (incidences of up to 27% in autopsies), and appear to occur with increased frequency with aging. By use of histofluorescent methods, they have found that there is a marked loss of dopaminergic neurons in the arcuate nucleus, which is the major source of the dopamine in the median eminence and pituitary portal blood. The loss of these dopaminergic neurons, together with an increase in hypothalamic prolactin activity, may be the cause for the high incidence of prolactinomas in aging rats.

The somatomedins are a family of low molecular weight, growth hormone-dependent, plasma growth factors with growth promoting actions in a wide variety of tissues. Several peptides have been isolated which satisfy the criteria of a somatomedin: somatomedin A, somatomedin C (SMC), multiplication-stimulating activity (MSA), and two insulin-like growth factors (IGF-I and IGF-II). Originally identified using different assays, IGF I and SMC now appear to be identical peptides and are thus referred to as IGF I/SMC. In her investigations, Dr. Cheryl Conover at Stanford University is using three models: human fibroblasts in culture, the intact rat, and human subjects to examine the role of aging in the action of the somatomedins. Results are preliminary at this point but appear to show that radioimmunoassayable IGF-I and IGF-II levels in humans decreases with age.

Dr. James Florini of Syracuse University has also been examining age-related changes in somatomedins in rats. His results show that there is an age-related decrease in circulating levels of somatomedins in barrier-protected Fischer 344 rats. Data show only a ten percent decrease between young (2-5 months) and middle age (12-17 months) but a 50-60% decrease in older rats (24-28 months). Similar findings have been reported by Dr. Raymond Hintz at Stanford Universty. Dr. Florini also notes that there appeared to be no corresponding drop in GH level leading to the speculation that the decrease in somatomedins is attributable to a decrease in the activity or responsiveness of the tissues (e.g., liver) which secrete somatomedins in response to GH.

Dr. Meites and his colleagues are also examining aging changes in growth hormone (GH). Their results indicate that old male rats have less capacity to increase GH in response to agents that normally enhance GH release. This may be the result of a diminished number of affinity of post-synaptic neurotransmitter receptors, or to an increased release of somatostatin. Since somatostatin antiserum increased GH equally or to a greater extent in old than in young animals, aging rats may release more somatostatin or the pituitary may be more sensitive to the inhibitory effects of this hormone.

GH is the most important protein snythesis stimulator in the body, and there is considerable evidence that protein synthesis is decreased in many tissues during aging. It appears to play a very important role in the transformation of certain classes of T-cells in the immune system to immuno-competent cells. Inasmuch as pulsatile GH secretion is markedly reduced in old rats (and there is now some evidence for this in elderly human subjects), this could account to a significant degree for the overall decrease in protein synthesis in old rats. The decrease in thyroid function that was reported by Dr. Meites in old rats also may contribute to the reduction in protein synthesis, as well as to the reduced kidney, liver, immunologic, and bone function commonly found in old rats.

Many of the age-related changes in endocrine function are not the result of reduced circulating normal or metabolite levels, rather these changes are caused by age-related modifications in the target organ cell or in receptors. One

example of this is the previously noted research on somatomedins by Dr. Florini. One such target system is the hormone-sensitive adenylate cyclases of the liver. Dr. Michael Katz at the University of Texas at San Antonio has been studying this system in relationship to age and dietary history in Fischer 344 rats. Results show that hormone-sensitive adenylate cyclase activities increase with age over the adult life span. Maximal activity of epinephrine-sensitive adenylate cyclase increases throughout the life span (from six through 27 months of age), while activity of glucagon-sensitive enzyme reaches a maximum at 12 months of age and declines thereafter. Unstimulated (basal) activities appear to increase between six and 12 months of age without subsequent change. The stimulatory effects of guanine nucleotides also appear to increase as a function of age, with the greatest increase occurring between six and 12 months of age. Preliminary comparisions of data from rats fed a control diet ad lib after six weeks of life and rats fed the same diet at 60% of the mean ad lib intake after six weeks of life indicate that all of the age-related changes of adenylate cyclase activities are delayed but not abolished by food restriction.

Adrenal cells, similar to the cultured adrenal cells being studied by Dr. James Mrotek at North Texas State Univeristy, are responsible for producing hormones which protect the body against stress. Aged individuals exhibit a reduced tolerance to stressful situations. On continued culture, the adrenal cells in this study lost their ability to respond to stimulation. Several explanations might be offered for this loss of functional capacity by the cultured adrenal cell. Two of these, based on other reports, could involve either changes in the ACTH receptor or changes in the filamentous cytoplasmic cytoskeleton. This project seeks to evaluate these two possibilities by comparing the ability of adrenal cells from two and 27-month rats to produce the intermediate steroids in the corticosterone pathway in response to ACTH. It has been found that there is less cholesterol stored in two-month adrenals than in 27-month adrenals, the two-month old adrenal contains at least twice as much pregnenolone; and the concentration of progesterone, 11-DOC, and corticosterone is greater in the two-month than the 27-month old rat adrenal both at basal levels and following stimulation by ACTH. These findings suggest that older rats are less able to synthesize the final steroids in the corticosterone pathway regardless of whether the animals are a control or are stimulated with ACTH.

One of the most important target organs, from a health standpoint, that has been studied is the prostate in the aging male. Dr. Jean D. Wilson at the University of Texas at Dallas, as part of a program project, has developed the first successful technique for assessing the androgen receptor of the human prostate with natural binding ligands and has shown that the receptor binds dihydrotestosterone approximately ten times as avidly as testosterone. This provides an explanation, at the molecular level, as to why dihydrotestosterone and not testosterone is the effective intracellular androgen for prostatic growth.

A mysterious feature of prostatic hyperplasia is the fact that the condition could be induced in the castrate dog by the administration of dihydrotestosterone but not testosterone, the natural precursor of dihydrotestosterone in the prostate. This raised the possibility that some specific hormone of the testes, such as androstanediol or dihydrotestosterone itself, might be critical in the pathogenesis. Dr. Wilson has been administering long-acting testosterone to castrate dogs and has shown that, if plasma testosterone is sustained at an adequate level, development of prostatic hyperplasia can be induced. It has also been demonstrated that the development of prostatic hyperplasia can be prevented by the simultaneous administration of

an inhibitor of the 5a-reductase enzyme. This important experiment suggests that the reason for the species specificity of prostatic hyperplasia is some metabolic feature of the prostate itself and not the result of a unique hormone of the testes, and that the critical hormonal mediator of the process is dihydrotestosterone.

The issue of glucose intolerance and related matters (e.g., pancreatic functioning, etc.) is of importance as a health issue in the elderly. Research on an animal model, Macaca nigra, by Dr. Howard Charles at the Oregon Regional Primate Center, has shown that these animals spontaneously develop diabetes mellitus with advancing age. They proceed through a series of changes in which there are alterations in hormone concentrations and in the morphology of the islets of Langerhans. Eventually, amyloid is deposited, islet cells deteriorate, and overt diabetes appears.

Cause of the islet lesion in Macaca nigra is not yet known. Amyloid deposition could involve islet proteins, either hormones or other cellular proteins, or it may arise as an antigen-antibody complex. Lymphocyte antigens are being examined with sera raised against Macaca mulatta lymphocytes; there is sufficient cross-reactivity to be useful for identification of Macaca nigra lymphocyte antigens. A similar model using aged rhesus monkeys is being developed by Dr. Jerry Robinson at the Wisconsin Regional Primate Center. Dr. Robinson has found higher fasting plasma glucose levels at 60 minutes after glucose infusion in aged males than in younger males. Similar differences were reported between aged ovariectomized females and aged intact females. He has also found evidence of diabetes in some of the other colony animals.

Immunology:

The main objective of the Immunology Program is to elucidate age-related changes in immune function. The objective is reached by supporting research on the characterization of the aged immune system, the mechanisms that mediate age-related changes, as well as research whose aim it is to provide a clearer understanding of the clinical implications of these age-related changes. Based on knowledge gained from this research, for example, protocols can be designed that are aimed at (a) forestalling the adverse effects of age-related changes in the immune system (e.g., by administration of thymic hormones, by manipulation of dietary factors, or by the design of vaccination protocols for the elderly), and (b) treating diseases that result from age-related changes in the immune system.

Research has established that immune potential declines with age in both humans and animals. Decline in the functional capacities of both T- and B-cells appear to be associated with the reduced capacity of aged animals to respond to mitogenic stimulation. The impaired response to mitogens of lymphocytes from old people has been shown to result from the presence of fewer responsive cells and the failure of these cells to divide normally after stimulation. Dr. Marc Weksler and his colleagues have shown that the successive division of mitogen-responsive lymphocytes from old people are impaired. Sister chromatids were labeled with BrdU and cells that were dividing for the first, second, or third time in culture were identified. It was found that lymphocyte cultures from old people contained only one-half the number of lymphocytes dividing for a second time and less than one-quarter the number of cells dividing for a third time compared with lymphocyte cultures from young people. It was also shown that these changes reflected an absolute decrease in the number of second and third generation cells in cultures from old persons. These results provide additional evidence that mitogen-responsive lymphocytes from

healthy elderly people do not undergo as many divisions in cultures with PHA as do lymphocytes from young persons.

In addition to decreased humoral and cell-mediated immunity, IL-2 levels in the mouse, rat, and human decline with age. Specifically, the capacity of aged animals to produce and respond to IL-2 has been shown to be defective. IL-2 activity in the supernatants of concanavalin A-activated aged spleen cells is five- to ten-fold lower than comparable supernatants prepared using young spleen cells. This lesion in IL-2 activity may limit antibody production to T-dependent antigens, as revealed by the fact that supplementation with purified IL-2 markedly enhances the number of anti-SRBC plaques generated by aged spleen cells and restores the response of aged splenocytes to the level obtained from young adult cells. However, there appears to be a defect in the ability of aged cells to effectively translate the IL-2 signal into B-cell helper activity, in the absence of T-lymphocytes. That is, although young adult, nylon wool purified T-cells can interact with aged T-dependent spleen cells by producing a normal high level anti-SRBC response, IL-2 is incapable of reconstituting the response in aged animals to this level.

Based on the finding that IL-2 can restore in vitro antibody, mixed lymphocyte culture as well as cell-mediated cytolytic responses, Dr. William Weigel and his colleagues postulate that because of low IL-2 levels in aged mice, amplification of activated T-cell clones does not occur. Thus, the addition of IL-2 apparently expands their number to the level of the young adult response. The addition of IL-2 plus antigen to aged spleen cell populations may expand the $Lyt\text{-}1^{+}23^{-}$ cells to express the receptor for Fc subfragments which leads to FcTRF production.

Some of these age-related changes may be linked either directly or indirectly to changes that occur in the thymus, a gland that is important in the differentiation of bone marrow derived precursor cells into T-cells. Research has shown that the age-related decline in the cellular components of the thymus appears to be paralleled by an age-related decline in circulating thymic hormones. In a cell-transfer experiment reported by Weksler and his colleagues, spleen cells from young or old donors are transferred to lethally irradiated young recipients who were then challenged with an antigen.

Results showed that spleen cells from old donors do respond to antigen but the response is characteristically of low affinity antibodies. In fact, other experiments by Dr. Norman Klinman at Scripps Clinic have shown that while there are only small differences between old and young mice in terms of the number of B-cells, on a per cell basis, B-cells from old mice are less responsive than B-cells from young animals. It appears that the specificity of the old B-cells are less well-defined than those for young cells. Moreover, it may be that the remaining nonfunctional B-cells may produce an antibody to its own specificity (i.e., an auto-anti-idiotype).

Adding thymopoietin, a thymic hormone, to the spleen cells from an old donor appears to reconstitute the B-cell response to antigen. This points to the importance of the thymus and its hormones in controlling not only T-cell development but also in the control of humoral immunity. When spleen cells from older animals are transferred together with spleen cells from young animals, the B-cell response of the host was inhibited by as much as 90% relative to animals given spleen cells from young animals only. Thus, the cells from old donors appear to have a net suppressor activity. In fact, other research appears to show that the ratio of suppressor cells to helper cells increases with the age of the animals. However, the shift in the old cells to low affinity antibodies does not appear to be controlled by the T-suppressor subset. The suppressor subpopulation of T-cells appears to down-regulate or suppress the activity of

the entire immune system whereas the change in specificity, as noted earlier, appears to be under the control of auto-anti-idiotypes.

It is known that there is an age-related increase in the incidence of autoantibodies, i.e., antibodies directed against self. This finding has been documented in separate experiments along with the fact that circulating immune complex appears to increase with age. For example, in humans, age-related changes in autoantibodies have been investigated by Dr. Sherman Fong of Scripps Clinic. He has noted that autoantibodies related to rheumatoid factor (IgM anti-IgG) as well as to anti-thyroglobulin (IgM anti-Tg) increase with age. However, the increase appears to occur at different times. Anti-IgG reactive B-cells increase between birth and young adulthood while anti-Tg reactive B-cells increase between young adulthood and old age. Morever, he has shown that the relative avidity of anti-IgG was higher in elderly adults than in young adults. These findings appear to be consistent with findings reported by Dr. Roy Walford of UCLA, who has noted an increase with age in the expression of LPS-induced autoantibody-secreting B-cells. These age-related differences appear to be the result of a decline in the mechanism underlying suppression of the allergic response. Dr. Fong's findings also appear to be consistent with those reported by Dr. James Goodwin of the University of New Mexico, who also notes that rheumatoid factor is higher in healthy elderly individuals. Dr. Goodwin postulates that this increase in rheumatoid factor is the result of an increase in the activity of a specific subset of T-helper cells.

Comparisons between autoimmune prone strains of mice also give clues about the increase in autoantibodies with age. Dr. Edmond Goidl's research with autoimmune prone animals shows that the heterogeneity of affinity of the anti-hapten plaque forming cell responses for T-dependent antigens was relatively restricted in autoimmune prone strains and of high average affinity as compared with non-autoimmune prone strains. For T-independent antigens the heterogeneity of affinity of the anti-hapten plaque forming cellular response was greater for the autoimmune strain than the non-autoimmune strain. Additional research on various genetic variants of the autoimmune and non-autoimmune strains indicated that factors other than those involving the main histocompatibility complex (H-2) are involved in regulating the heterogeneity of affinity of the antibody response.

It has been hypothesized by Dr. Robert Good, of the Oklahoma Medical Research Foundation, that many of the autoimmune diseases occurring among the elderly may be the result of genetically determined age-related deficiencies of stem cells in the bone marrow. He and his colleagues have developed a technique for transplanting bone marrow from long-lived mice to those whose longevity is shortened by the early onset of immunodeficiency. Preliminary findings indicate that the life span of AR mice, a strain that is highly susceptible to leukemia and which tends to die early in life, is almost doubled by transplanting bone marrow from mice that are not susceptible. Morever, techniques have now been developed which allow for transplantation of stem cells across major histocompatibility barriers without encountering problems associated with graft versus host rejection. Not only do cells from the bone marrow appear to have an impact on autoimmunity in the elderly but stem cells produced by the bone marrow also appear to replenish the lymphocyte pool during the individual's lifetime.

Epidemiological studies show an increased prevalence of infectious diseases among the elderly which may be mediated by parallel changes in the immune system. For example, Dr. Osais Stutman, of Sloan Kettering Cancer Institute, has found that the cytotoxic T response to TNP-treated syngeneic cells in aged mice is low, thus providing a possible explanation for the increased incidence of infectious disease in older animals. The decline in cytotoxic ability of

these cells was not due to alterations in the number of cytotoxic lymphocytes nor to a decline in their precursors. Rather, it was the result of the lack of appropriate T-helper cells, and especially the production of interleukin 2 (IL-2) by the Lyt T-helper T-cells. Similarly, Dr. Rita Effros of UCLA, has shown reduced cytotxic T-cell response to Type A influenza virus in old C57Bl/6 mice.

Age-related changes in immunoresponsiveness to Babesia microti has been studied by Dr. Gail Habicht at SUNY-Stoneybrook. She has shown that in human beings this organism causes disease in the elderly and in immuno-compromised individuals. It has also been suggested that its pathogenicity results, in part, from an endotoxin-like effect on the immune system. Mice of different ages were challenged with different doses of B. microti and the development of parasitemia followed. Although 24-month animals developed delayed and lower peak parasitemias, they failed to clear the parasites from the blood and experienced fluctuating parasitemias until death. Babesiosis produced suppression of responses to nonspecific B- and T-cell mitogens. Although aged animals made equivalent antibody titers to B. microti, their cell-mediated immunity failed to develop. It appears that this age-related decline in T-cell responsiveness is responsible for the increased susceptibility to infection. In addition, autoantibodies specific for erythrocyte antigens develop which may be responsible for the development of anemia.

Of particular interest to the NIA are studies aimed at assessing methods to reverse or forestall the age-related decline in immune functioning. The Institute is funding a contract to the University of Alabama for the purpose of assessing the immunologic effects of several thymic peptides or hormones in several strains of mice. The evaluation is both in terms of cross-sectional and longitudinal effects. Preliminary analysis of immunologic measures indicates that, for the most part, the hormones have little or no effect on immune function. However, it does appear that in C57Bl/6J mice, some hormones enhance cytotoxic activity of T-cells while other hormones appear to depress this function. The effect seems to be more pronounced in one-month old than in 24-month old mice, indicating that the effects of these hormones is not particularly important for the aging immune system. A second set of positive results reveals that the survival rate appears to have been increased in CFW mice treated regularly from one-month of age with the hormone TM4. The median survival rate (31.1 months) for treated animals was 27.5% higher than that for untreated animals (24.4 months). At 30 months, 56% of the TM4-treated animals remained alive while only 16% of the untreated were still living. Similar differences were maintained at 36 months.

At the same time, the Institute is supporting several investigator-initiated research grants on the effects of dietary variables on immune functioning. One fruitful area of investigation has involved influence of dietary restriction on the onset of autoimmune disease. In this regard, Drs. Roy Walford and Richard Weindruck have been investigating the influence of mid-life dietary restriction in long-lived mouse strains on immunologic functioning. Restriction beginning at 12, 16, or 19 months of age gives evidence of immunologic regeneration on a battery of five T- and B-cell mitogen tests as well as in response to injected sheep red blood cells as immunogens. Furthermore, mice restricted at mid-life have shown evidence of increased survival in comparison to controls. Finally, mice restricted at mid-life as well as those restricted at the time of weaning show a sharp reduction in incidence of autoantibody production after 24 months of age. This latter finding suggests that dietary restriction, even when it occurs at mid-life, may play an important role in reducing autoimmune disease.

Mechanism believed to be involved in the effects of dietary restriction on immune functioning is the production of prostaglandin E and F. Results from a study by Dr. Gabriel Fernandez indicates that high calorie-high fat diets and high calorie-low fat diets are associated with increased levels of prostaglandin F in the lymph nodes of B/W mice. Only high calorie-high fat diets are associated with increased levels of prostaglandin E in the thymus. Because lymph nodes contain predominantly mature T-cells while the thymus contains immature T-cells, these results may indicate a differential influence of caloric and fat intake on prostaglandin-induced suppressor activity associated with mature or immature T-cells. Finally, Dr. Fernandez has also found that, in short-lived B/W mice, the ability of cells from mesenteric lymph nodes to generate plaque forming cells in response to a challenge by sheep red blood cells was consistently lower than in spleen cells. In contrast, for the long-lived C57Bl/6 strain, mesenteric lymph node cells generated more plaque forming cells than did spleen cells. This finding appears to support the hypothesis that excessive gastrointestinal absorption of foreign antigens, including food antigens which cross-react with autoantigens, may play a role in the development of autoimmunity in older animals.

One area of particular interest to the Immunology Program is research on the role of neuroendocrine factors on the aging immune system. This part of the program is just being developed and thus there are very few holdings; however, the impact of research in this area on an understanding of the aging changes that occur in the immune system can be far-reaching.

One grant in particular is producing some interesting findings. Dr. William Markesbery at the University of Kentucky has been studying the ultrastructure of thymic innervation in the aging rat thymus. He reports that the thymus shows large perivascular nerves composed of myelinated and unmyelinated fibers near the surface of the thymus. In older animals there appears to be an increase in endoneural fibrous tissue. Placement of anterior hypothalamic (AHT) lesions in old animals resulted in an increase in the Con-A and PHA reactivity of splenic lymphocytes four days after lesioning. While the increase in PHA reactivity was significantly different from frontal control lesioned and normal old rats, the Con-A reactivity was not. Analysis of the PHA and Con-A response of splenic lymphocytes at 14 days after AHT lesion placements revealed no significant differences as compared to normal values. Natural killer (NK) cell activity was also examined in young and old animals. Old animals had approximately 75% the NK activity of young animals. Determination of splenic NK activity four days after AHT lesioning of old animals did not result in significant changes as compared to aged control rats. These results are in agreement with the well-known fact that old animals have decreased splenic Con-A reactivity and NK activity as compared to young animals. However, the finding that splenic PHA responsiveness increases is not in agreement with with values in the literature. Moreover, the results indicate that old rats respond differently to AHT lesions than do young animals. Thus, AHT lesioning has no effect on the spleen cell Con-A response or NK activity of old animals whereas it results in decreases in these activities in young animals.

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Overview of Epidemiology, Demography, and Biometry Program

The Epidemiology, Demography, and Biometry (EDB) Program of the National Institute on Aging (NIA) carries out research involving a diversity of problems related to aging and the elderly. This diversity reflects the program's organization around methodologic approaches to such problems rather than around their medical or social substance. Problems are selected for study on the basis of their relevance to the missions of NIH and NIA, and the likelihood that the application of the epidemiologic and population research methodologies in which the program has competence will be fruitful. The EDB is divided into three working areas: epidemiology; demography and economics; and biometry. Although each EDB research project falls under the aegis of one or another of these sections, most studies involve several staff members working cooperatively. The remarkable effectiveness of this teamwork represents one of the program's major strengths.

Research projects are either intramural, contract funded, or accomplished by interagency agreement. At the present time essentially all of the intramural projects represent secondary data analysis, i.e., research using data sets already collected by others at another time and usually for other reasons. Late in FY82 data will begin to become available for analysis from EDB sponsored contract and interagency projects. As a result, a greater proportion of staff time will then be dedicated to analysis and publication of research findings than has previously been possible, i.e., these new data sets will become the basis of a major intramural effort even while many of the contracts and agreements continue.

Overview of Epidemiology Office

As in previous years the FY83 activities of the Epidemiology Office represent the efforts of the entire EDB staff, since such a major part of the achievements were related to the three Established Populations for Epidemiologic Studies of the Elderly (EPESE) (AG-0-2106, AG-0-2105, and AG-0-2107) and the National Health and Nutrition Examination Survey (NHANES I) Epidemiologic Followup Survey--all projects which have involved the total program staff.

Programmatic and scientific achievements related to the three EPESEs include completion of baseline interviews and processing of the related data at all centers. Methodology and instruments have been developed for the interim telephone followup contacts as well as for identifying and processing mortality and morbidity endpoint information. Participation was 80 to 85 percent with nearly all of the baseline interviews conducted in the subjects' households. Plans for coordinated analysis and publication have begun. Initial tabulations are to be presented in a monograph currently being compiled by EDB and study center staffs. Several substudies proposed by study center scientists have been approved by the Project Officer (Dr. Huntley) and are currently being processed for the essential clearances (OMB, USPHS).

FY83 activities and achievements relative to the NHANES Followup population (Interagency Agreement AG-9-0018) also represent the efforts of several EDB staff members. As a result of the cooperation of representatives from NCHS, the following institutes--NCI, NIADDK, NHLBI, NIMH, NIAID, NINCDS, NIAAA, and WESTAT (a primary contractor) the study is progressing even more efficiently and successfully than we had expected. Interviewing is nearing completion in the northeastern United States, is approximately half completed in the south, and is scheduled to begin in May (1983) in the midwest region. Subject location and participation have been excellent. Collection of morbidity and mortality information from hospitals, other institutions, and from state or local offices (death certificates) is now beginning. Preliminary data from the northeast which will be adequate for testing analytic methodologies is expected to be available during FY84.

During FY81 a contract was awarded to Peter Bent Brigham Hospital/East Boston Neighborhood Health Center an affiliate of Harvard University School of Medicine (AG-1-2106) to carry out a longitudinal, prospective study on the Natural History of Senile Dementia. The objective of this study will be to observe and describe the course of cognitive decline and general health in a group of SDAT victims and controls. The dementia cases will have been identified as a result of a community survey and a subsequent neurological evaluation and most cases will not have been previously recognized as suffering from dementia. The objective of this study is to gain a better understanding of the prognosis and course of this disease among individuals first diagnosed while residing in their communities. Their illnesses are expected to be milder, less incapacitating, or earlier than is usual among patients coming to medical attention because of their dementia. During FY83 the contractor formulated his methodologic approach to defining the sample of individuals to be examined for dementia. Based on performance on a memory test included in the baseline EPESE interview, subjects have been

selected for the in-depth neuropsychological and neurologic examination. Each examined person will be judged as to extent of cognitive impairment and (as a separate matter) the likelihood that he is suffering from specific disease conditions which might explain his impaired cognition. The contractor expects to identify at least 100 cases of senile dementia of the Alzheimer's type (SDAT) as a result of examining 300 to 400 persons. An interim appraisal of the screening and case identification method will be carried out as soon as the first 50 persons have received the dementia evaluation, probably by mid-August 1983. Analysis of the results for this part of the sample will guide subsequent activity. Depending on these analyses, the contractor may (1) continue as planned, (2) modify the sampling procedure in a qualitative way, or (3) increase the total number of persons to be evaluated.

The study of dementia in the Framingham Heart Study population (funded by inter-institute transfer of funds to NHLBI; 1-Y01-AG-2-0040-00, initiated during FY82) was continued in FY83. A screening test (the mini-mental status examination) was administered as part of the current (cycle 17) examination. The pass/fail level for this screening examination was defined according to educational attainment, so that approximately 15 percent of persons from each of four education strata would fail (screened positive for possible dementia). Those who fail are being invited back for a thorough neuropsychological and neurologic evaluation in order to identify those who are truly demented. These will comprise the "case" group for a subsequent study aimed at defining risk factors involved in the development of dementia. Most of these same persons received a battery of eight neuropsychological tests during cycle 14 or 15, now approximately 6 years ago. Analyses of these data, previously unutilized, was undertaken by NIA and NHLBI staff members (Drs. White, Farmer, and Kittner), and three manuscripts are currently in draft form. Standardizations and score transformations have been developed which allow an individual's performance on these tests to be compared between tests or averaged over several tests, and which allow for removal of most of the direct effects of education without loss of the effects of age. Based on these analyses, the population (as it was at the time of testing) has been divided into four parts according to average level of performance: the bottom 10 percent (poor performers), 10 to 25 percent, 25 to 75 percent (modal group), and the top 25 percent (best performers). All surviving members of the poor performance group will receive a thorough neuropsychologic and neurologic reevaluation during cycle 17 or 18. One set of matched controls from the modal group and another set of controls from the best performing group will also be reevaluated. These studies are expected to contribute substantially to our understanding of qualitative and quantitative aspects of cognition with advancing age, and to relationships between education, level of functioning, change in level of functioning, survival, dementia, "normal aging," and general health.

During FY83, analyses were completed on a study of race and sex differences in the age-specific incidence of hip fractures in the District of Columbia and nationally, using data from the NCHS Hospital Discharge Survey. This manuscript has been forwarded for NIA clearances and will be submitted to a prominent epidemiologic journal. The results serve to define the effects of race, sex, and age on risk for hip fracture: They show that

among the four main groups (white male, white female, black male, black female), only white females appear to be at increased risk, the relative risk of this group being approximately double that of the other three groups at all ages. In all four groups the risk of fracture was found to increase exponentially with advancing age, doubling approximately every 5 years. These data are interpreted as failing to support the prevalent perceptions of sex and race as simple and direct modifiers of the risk of fracture: This is inferred because no clear sex differences were seen among black persons, and because no clear differences were observed between black males and white males. The unusual susceptibility of white women appears unlikely to be related to the rate of progression of the postmenopausal osteoporotic process alone since the rate of increasing risk with advancing age was similar to that of the other groups. Alternative hypotheses are that the osteoporotic process begins at an earlier age in white females, or that the femurs of white females are already more fragile than those of the three other groups at the time the age-related osteoporotic process begins.

One of the research areas of greatest interest concerns the determinants of good health in life. During FY83 a professional services contract was established to the initiate investigations concerning the precursors and personal characteristics associated with good health among Japanese men participating in the Honolulu Health Study. In this preliminary study, good health was defined as freedom from evidence of disease. In addition to focusing on factors which promote good health, this approach may be useful as an ideal controller compares in the identification of subtle factors associated with specific diseases. Information derived from these preliminary investigations will be applied to examination of similar research questions to be investigated in the other EDB population studies (EPESE and the NHANES followup).

CONTRACT

Name and Number: YALE UNIVERSITY (NO1-AG-0-2105)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: $400,000

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged. Studies are to be completed on problems of pain, vision, hearing, sleep, drug use, constipation, social support and other pertinent areas.

Methods Employed: The project shall include cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population will be included.

Major Findings: Completion of baseline interviews and processing of the related data at all centers has been achieved. Methodology and instruments have been developed for the interim telephone followup contacts as well as for identifying and processing mortality and morbidity endpoint information. Participation was 80 to 90 percent with nearly all of the baseline interviews conducted in the subjects' households. Plans for coordinated analysis and publication have begun. Initial tabulations are to be presented in a monograph currently being compiled by EDB and study center staffs. Several substudies proposed by study center scientists have been approved by the project officer and are currently being processed for the essential clearances.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. It is important to have studies representing existing conditions in a community population. Within obvious logistical contraints populations will be available to the NIA scientific community for specific studies. High priority short term studies will be encouraged.

Proposed Course: A standardized telephone interview to be used at the first interim contact is currently under development with its common components agreed upon. Proposals for the first substudies have been submitted. A publications committee comprised of members from NIA/EDBP and the three EPESE centers has met and arrived at working rules for the analysis and publication of results. These achievements represent the methodologic and administrative foundation for a multi-faceted research effort which will continue over the next several years.

Publication: Cornoni-Huntley J and Brody JA. Correspondence from Washington. J Am Geriatrics Society, 30(10)669-670, 1982

CONTRACT

Name and Number: UNIVERSITY OF IOWA (NO1-AG-0-2106)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: $700,000

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged. Studies are to be completed on problems of pain, vision, hearing, sleep, drug use, constipation, social support and other pertinent areas.

Methods Employed: The project shall include cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population will be included.

Major Findings: Completion of baseline interviews and processing of the related data at all centers has been achieved. Methodology and instruments have been developed for the interim telephone followup contacts as well as for identifying and processing mortality and morbidity endpoint information. Participation was 80 to 90 percent with nearly all of the baseline interviews conducted in the subjects' households. Plans for coordinated analysis and publication have begun. Initial tabulations are to be presented in a monograph currently being compiled by EDB and study center staffs. Several substudies proposed by study center scientists have been approved by the project officer and are currently being processed for the essential clearances.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. It is important to have studies representing existing conditions in a community population. Within obvious logistical contraints populations will be available to the NIA scientific community for specific studies. High priority short term studies will be encouraged.

Proposed Course: A standardized telephone interview to be used at the first interim contact is currently under development with its common components agreed upon. Proposals for the first substudies have been submitted. A publications committee comprised of members from NIA/EDBP and the three EPESE centers has met and arrived at working rules for the analysis and publication of results. These achievements represent the methodologic and administrative foundation for a multi-faceted research effort which will continue over the next several years.

Publication: Cornoni-Huntley J and Brody JA. Correspondence from Washington. J Am Geriatrics Society, 30(10)669-670, 1982

CONTRACT

Name and Number: PETER BENT BRIGHAM HOSPITAL (NO1-AG-0-2107)

Title: Established Populations for Epidemiologic Studies of the Elderly (EPESE)

Date Contract Initiated: June 30, 1980

Current Annual Level: $300,000

Objectives: The purpose of this project is to conduct epidemiologic investigations in a community to develop new knowledge concerning the medical and social factors in health and diseases of the aged. Studies are to be completed on problems of pain, vision, hearing, sleep, drug use, constipation, social support and other pertinent areas.

Methods Employed: The project shall include cross-sectional and prospective studies in a carefully defined and accessible population using standard field and analytical techniques. Yearly surveillance of the population will be included.

Major Findings: Completion of baseline interviews and processing of the related data at all centers has been achieved. Methodology and instruments have been developed for the interim telephone followup contacts as well as for identifying and processing mortality and morbidity endpoint information. Participation was 80 to 90 percent with nearly all of the baseline interviews conducted in the subjects' households. Plans for coordinated analysis and publication have begun. Initial tabulations are to be presented in a monograph currently being compiled by EDB and study center staffs. Several substudies proposed by study center scientists have been approved by the project officer and are currently being processed for the essential clearances.

Significance to Biomedical Research: The population over age 65 has been steadily increasing both in relative and absolute numbers. With this increase has come an awareness of a variety of health and social problems which are creating problems for our social and physical environment. It is important to have studies representing existing conditions in a community population. Within obvious logistical contraints populations will be available to the NIA scientific community for specific studies. High priority short term studies will be encouraged.

Proposed Course: A standardized telephone interview to be used at the first interim contact is currently under development with its common components agreed upon. Proposals for the first substudies have been submitted. A publications committee comprised of members from NIA/EDBP and the three EPESE centers has met and arrived at working rules for the analysis and publication of results. These achievements represent the methodologic and administrative foundation for a multi-faceted research effort which will continue over the next several years.

Publication: Cornoni-Huntley J and Brody JA. Correspondence from Washington. J Am Geriatrics Society, 30(10)669-670, 1982

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 02010 05 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Followup of Health and Nutrition Examination Survey I (HANES I)

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Joan Cornoni-Huntley, Ph.D.
Deputy Associate Director, EDBP, NIA

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Division of Analysis; NCI, Environmental Epidemiology Branch; NHLBI, Epidemiology and Biometry Program; NIADDK; NIMH; and NIAAA.

LAB/BRANCH

Epidemiology Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.20	.20

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

The purpose of this project is to design and complete a followup of persons examined in the HANES I to study how factors previously measured relate to the health conditions that have developed since the survey. The three major areas for prediction of outcome are 1) nutrition 2) risk factors for chronic disease and 3) health care utilization. The survey will have a household interview including self-reporting of health conditions, utilization of health servics and behaviorial and social status plus some physical measurements as blood pressure, height, and weight.

Interviewing is nearing completion in the northeastern U.S., is approximately completed in the south, and is scheduled to begin in May 1983 in the midwest region. Subject location and participation have been excellent. Collection of morbidity and mortality information from hospitals, other institutions, and from state or local offices (death certificates) is now beginning. Preliminary data from the northeast which will be adequate for testing analytic methodologies is expected to be available during FY84.

Publications: Cornoni-Huntley J, Barbano HE, Brody JA, Cohen B, Feldman JJ, Kleinman JC, and Madans J: National Health and Nutrition Examination I--Epidemiologic Followup Survey. Public Health Rep. 98:245-251, 1983.

CONTRACT

Name and Number: PETER BENT BRIGHAM HOSPITAL/
EAST BOSTON NEIGHBORHOOD HEALTH CENTER (NO1-AG-1-2106)

Title: Senile Dementia: Natural History in a Noninstitutionalized Population

Date Contract Initiated: June 16, 1981

Current Annual Level: $416,517.

Objectives: This study is to provide an in-depth assessment of a community-based sample of persons with senile dementia. The study includes a clinical examination plus a surveillance by contacting the subject and a close relative or friend every 9 months. Comparison will be made with uneffected persons to define risk factors and etiological events.

Methods Employed: This is a 5-year study of a noninstitutionalized population. Cases and controls will be selected by a two-stage procedure consisting of an initial screening test (done as part of an independent community survey) followed by a thorough clinical evaluation. Individuals who are thereby registered in the study will be reevaluated at a 9 month interval in order to obtain information on clinical course, demographic and social characteristics, psychological factors, cognitive functioning, drug usage, health service utilization, and other related factors. Information will be gathered from both the subject and a relative or close friend.

Major Findings: Since data collection has not yet begun, there are as yet no findings.

Significance to Biomedical Research: Little is known about the occurrence and course of senile dementia. Etiologic mechanisms and risk factors are essentially unknown; the natural history of this illness is obscure. Senile dementia and depression are apparently the most common disorders which primarily affect mental abilities in the elderly. Moreover, the two disorders may be indistinguishable. The project will provide a better definition of senile dementia and identify associated risk factors.

Proposed Course: The study will continue as planned. It is behind schedule because the community survey which will be used to identify possible cases and controls has itself been delayed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 04003 01 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Dementing Illnesses in the Framingham Heart Study

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Lon R. White, M.D., M.P.H.
Chief, Epidemiology Office, EDBP, NIA

COOPERATING UNITS *(if any)*

NHLBI

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

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

The Framingham Study population is being utilized for studies on dementia in the elderly. The major focus will be on: (a) a search for risk factors for the development of dementia, (b) the clinical course of persons who appear to have been demented when examined 5 years previously, and (c) a search for early evidence of illness in persons who were not demented 5 years ago, but who have become demented in the interim.

All study participants are currently receiving a brief mental status screening test as part of the regular (seventeenth biennial) examination. Participants suspected of intellectual impairment are being brought back for neurological and neuropsychological evaluation in order to identify individuals suffering from either SDAT or from MID. The analysis phase of this part of the study is expected to begin in 1984. In addition, an analysis of the prior neuropsychological test data has been undertaken by NIA and NHLBI staff. These prior results have been used to identify three groups to be the subjects of a followup study: the 212 persons who performed least well (the bottom 10 percent), 212 modal performers (25-75 the percentile range), and 170 of the top (greater than 75th percentile) performers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 04010 04 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Catchment Area Study of Senile Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Joan Cornoni-Huntley, Ph.D.
Deputy Associate Director, EDBP, NIA

COOPERATING UNITS *(if any)*

National Institute of Mental Health
Center for Epidemiologic Studies

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05	.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

In conjunction with NIMH, NIA is completing a study of senile dementia in an adult community population., A contract has been awarded to Yale University to survey a sample of the New Haven, Connecticut adult population for this purpose. NIA is specifically interested in defining the occurrence of senile dementia in persons 65 years of age and older. The study will consist of a household interview and a followup of all subjects. A questionnaire has been developed by the investigators and the survey is currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 04001 03 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Analysis of Functional Disability Data From Framingham Study

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

PI: Mary E. Farmer, M.D., M.P.H.
Medical Officer, EDBP, NIA

COOPERATING UNITS *(if any)*

NHLBI

LAB/BRANCH

Epidemiology Branch

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Disability status of approximately 2800 surviving Framingham Heart Study participants was assertained. First description of the population is in press. Additional data analyses are currently in progress. The relationship between cognitive functioning and physical disability is being studied. Associations between disability, age, and work status are also being studied.

Other professional personnel:

Jacob A. Brody, M.D	Associate Director,EDBP, NIA
Joan Cornoni-Huntley, Ph.D.	Deputy Associate Director, EDBP, NIA
Lon R. White, M.D., M.P.H.	Chief, Epidemiology Office, EDBP, NIA
Manning Feinleib, M.D. DrPh	Associate Director for E&B, EBP,DHVD,NHLBI
Robert J. Garrison	Chief, Biometrics Research Branch, EBP,DHVD,NHLBI
A. Jette, M.D.	Boston University, School of Medicine
L. Branch, M.D.	Boston University, School of Medicine

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 02090 05 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

General Analysis of National Center for Health Statistics Data Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Joan Cornoni-Huntley, Ph.D.
Deputy Associate Director, EDBP, NIA

COOPERATING UNITS *(if any)*

Division of Analysis, NCHS

LAB/BRANCH

Epidemiology Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.30	.10	.20

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This interagency agreement allowed NIA to work cooperatively with NCHS in conducting analyses of appropriate data sets. The research topics included: 1) Major chronic conditions of the elderly and (2) the relationship between osteoporosis and the existence of fluoride in drinking water.

Publications: Madans J, Kleinman JC, and Cornoni-Huntley J: The relationship between hip fracture and water fluoridation: An analysis of national data. Am J of Public Health, 73(3)296-98, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 07050 01 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Race and Sex Differences in the Age-Specific Incidence of Hip Fractures

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Lon R. White, M.D., M.P.H. and Mary E. Farmer, M.D., M.P.H., Epidemiology Office, EDBP, NIA

COOPERATING UNITS *(if any)*

NHLBI

LAB/BRANCH

Epidemiology Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Phase I of this study was carried out by Dr. Mary Farmer. It involved the analysis of data from the Hospital Discharge Survey (NCHS) and the District of Columbia Council of Governments. These two sources were used to estimate age-specific incidence rates according to sex and race. In all 4 groups (F, W; F, NW; M, W; M, NW) the incidence increased at the same exponential rate, doubling every 6 years. White women differed from the other 3 groups in intercept but not in slope of the curve, so that the incidence among white women was twice that of the other groups at every age. These results are now being prepared for publication.

Phase II is currently being planned. It will involve a 1 to 2 year surveillance of the District of Columbia for all hip fractures among D.C. residents age 40 or older. The purpose of this study is to compare incidence rates among black vs. white persons of equivalent socioeconomic status in order to gain a better understanding of the relative contributions of race, sex, age, and environmental factors to the risk of hip fracture. A contract award date of September, 1983 is expected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 04004-01 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Healthy Aged--Honolulu Heart Study

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Lon R. White, M.D., M.P.H.
Chief, Epidemiology Office, EDBP, NIA

COOPERATING UNITS *(if any)*

Honolulu Heart Program, NHLBI

LAB/BRANCH

Epidemiology Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A professional services contract was awarded to Dr. Benfante to carry out research using the data and facilities of the Honolulu Heart Study. This study will provide important clues for future research in the area of health promotion, longevity, and preventive medicine in the aging. The results of these analyses will be tabulated for final publication.

Overview of Demography and Economics Office

The Demography and Economics Office has emphasized scientific work in population aging and macroeconomics as well as household studies of the well-being of the elderly. This effort has involved the completion of major research contracts on the Macroeconomic-Demographic Model (MDM) (AG-0-0024) as well as the initiation of new research in health expenditures. Inter-disciplinary work has been initiated in the area of age, work, and disability, utilizing the Disability Study in the Framingham population which had been partially supported by EDBP, NIA. The wealth and income of households are being studied across age cohorts with particular emphasis on the valuation of pension and social security benefits. These efforts will lead to various publications in the coming fiscal years.

The Demography and Economics Office is editing for publication, two important NIA publications that have been exempted from the moratorium on publications by the Assistant Secretary for Health. The first publication shall be entitled A NIA Macroeconomic-Demographic Model: U.S. Retirement Income System and Simulation Results. The second publication is entitled Projecting Alternative Futures for the Retirement Income System. The combination of these two reports represents a significant scientific achievement in providing information on NIA's research on the population aging. Underlying these reports is a complete, operational computer model that embodies a "base case" view of the U.S. economy and retirement income system for the next 75 years. The base case is consistent with all written reports as verified by our office. In addition, a User's Guide was updated which explains the computer operation of the model. In addition, a memorandum was written to document the stand alone population model. As a measure of quality control, all aspects of the model have been saved on a tape that may be provided to other interested agencies upon request. So far, we have delivered the model to the Social Security Administration and the Health Care Financing Administration.

A major program achievement has been the award of a contract (AG-2-2138) to ICF, Incorporated for "Aging and Health Modeling." This effort involved briefing and coordination with such agencies as Health Care Financing Administration, Social Security Administration, Assistant Secretary for Planning and Evaluation, National Center for Health Statistics, and the National Center for Health Statistics Research to ensure duplication and waste would not be created by our own effort. Our office has successfully negotiated with the National Center for Health Statistics to receive a preliminary version of the National Medical Care Utilization and Expenditure data for use in the model.

An interagency agreement (1-Y01-AG-2-0041-00) continued with the Assistant Secretary for Planning and Evaluation to support grant research with the MDM. This research focuses on pension acceptance and the integration of the model with another large scale micro-simulation model of the Urban Institute. Projections of the retirement income system from both models will be compared for consistency. A further extension was necessary because of the untimely death of a key member of the research team.

The Demography and Economics Office has completed a professional services contract with The Johns Hopkins University (Dr. Karen Davis) to receive a chartbook and graphic computer display on population aging and long-term care. This gave us baseline information on the problem related to long-term care issues. The population model of the MDM was used to produce the demographic scenarios that were required for the computer. The information was also presented to the World Health Organization Conference on Health Planning for the Elderly in Budapest, Hungary in November 1982 and at the meeting of the WHO Scientific Group on Epidemiology and Aging in Geneva, in January 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 01050 04 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Age Structure in a Macroeconomic Model of the U.S. Economy

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: William S. Cartwright, Ph.D., Chief, Demography and Economics Office, EDBP, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Demography and Economics Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A Demographic-Macroeconomic Model of the U.S has been developed. The impact of population aging will be examined on the nation's economy, retirement income system, labor force, and the welfare of the aged. The Demographic Macroeconomic Model represents the integration of eight models - Hudson-Jorgenson Growth Model, Anderson Labor Model, ICF Inc. Population Model, Social Security Model, Private and Public Employees Pension Model, Supplemental Security Income Model, and Medicare Model.

A model has been statistically estimated and an operating simulation program developed. The President's Commission on Pension Policy has used the current model for analytical purposes. Final Reports, Volume 1 and 2, entitled, "A Macroeconomic-Demographic Model of the U.S. Retirement Income System" have been delivered as well as a Research Report. Permission to publish has been obtained from the Assistant Secretary of Health and editing of the final reports is being completed under a professional services contract.

Publication: Woodruff, TC. Development of a demographic macroeconomic model of the U.S. economy. Chapter 37; and Findings on the impact of pension policy on the economy, Chapter 38 in The Final Report of the President's Commission on Pension Policy, April 1981.

PHS 6040 (Rev. 1/83)

GPO 895-100

CONTRACT

Name and Number: ICF, Incorporated (NO1-AG-2-2138)

Title: Health Expenditures and the Aging Population--A Computer Modeling

Date Contract Initiated: September 30, 1982

Current Annual Level: $228,542

Objectives: The objective is to develop enhancements to the NIA Macroeconomic-Demographic Model (MDM) of the U.S. economy and retirement income system. These enhancements will be in the area of health care expenditures and demography and their relationship to the U.S. economy. Total aggregate consumption will be disaggregated into components of which health care expenditures will be one important component. Within health care expenditures, a model will determine the expenditures and costs of the components of the health care system. Special attention will be paid to the age and sex distribution of health care expenditures, with particular concern for unique patterns of health care expenditures incurred by the aged. In addition, health care expenditures will be modeled to integrate both governmental policies and private financial arrangements.

Methods Employed: The health expenditures model shall be developed using economic theory. Behavioral relationships shall be statistically estimated from health data. Both time series and cross-sectional data on health expenditures shall be used. The behavioral relationships shall be placed in a computer simulation model that is suitable for integrating with the NIA MDM.

Major Findings: A contract was awarded in September 1982 to ICF Incorporated. A draft report has been delivered entitled "Background for the Development of a Macroeconomic-Demographic Model of Health Expenditures: Literature Review and Model Specification."

Significance to Biomedical Research: The consequences of demographic changes as well as government and private institutional policy changes are a critical issue affecting both the level of national expenditure on health care and the future well-being of all Americans. In particular, as population shifts to an aging structure, health care delivery systems will have increased demands placed upon them. The enhancement of the NIA MDM will permit rigorous investigation into health care expenditures and its links to the U.S. economy so that total health care expenditures per capita and the ratio of health care expenditures to Gross National Product may have their determinants fully developed. Included in this effort will be information on health care expenditures by the aged as well as other demographic groups. Both enhancements to the current NIA model and a research report are envisioned as outputs from this work.

Proposed Course: Upon receipt of the National Medical Care Utilization and Expenditure survey data, the contractor shall begin preparing this data for modeling. The population model shall be revised upon receipt of the new Census projections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 01055 02 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Retirement Income System Research with MDM

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: William S. Cartwright, Ph.D., Chief, Demography and Economics Office, EDBP, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Demography and Economics Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The NIA enters into an agreement with the Assistant Secretary for Planning and Evaluation (ASPE) for the purpose of research on pensions, retirement, and labor force as well as issues involved in integration of micro and macroeconomic models. In FY83 we will support the revision and reestimation of the labor market model and simulation studies of the labor supply of the elderly.

We have received a detailed grant proposal specifying the work to be undertaken. A new version of the Pension Model has been prepared and delivered to NIA's MDM.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 01057 02 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Planning Long-Term Care for the Elderly

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: William S. Cartwright, Ph.D., Chief, Demography and Economics Office, EDBP, NIA

COOPERATING UNITS *(if any)*

The Johns Hopkins University School of Hygiene and Public Health

LAB/BRANCH

Demography and Economics Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10	.10	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

NIA will be provided with data from a computerized, interactive graphical display of long-term care in the health sector. The information on the future health needs of the elderly, resource requirements, cost, and economic implications will be particularly helpful to the Institute in its long-term care research planning.

Overview of Biometry Office

The FY83 activities and accomplishments of the Biometry Office encompass the initiation and maintenance of major intramural and contract supported research projects as well as continued methodological consultation and statistical support for research programs conducted by the other offices in EDB, other programs in the Institute, and other agencies in Government and the private sector. With the recent addition to the staff of Ms. Mary Lafferty, a senior computer programmer/analyst, the capabilities and expertise of the Office have been expanded to include the areas of statistical computing and data management. This expansion has occurred at a very appropriate time, since it follows closely the arrival of the baseline survey data tapes from the three Established Populations for Epidemiologic Studies of the Elderly (EPESE).

Office activity in the three EPESE projects remains at the high level maintained throughout the past year. Mr. Foley is continuing to oversee the planning, preparation, and submission to the Office of Management and Budget (OMB) of supporting statements for instruments developed in connection with the various portions of the studies, including now the telephone followup surveys and the numerous substudies which are being directed at specific hypotheses to be addressed in the three populations. Dr. Brock chaired a meeting of the EPESE Documentation Committee at which final decisions were made on the documents necessary to be submitted to NIA for complete descriptions of the procedures followed by the three centers in conducting the baseline survey. Future meetings of the committee will address documentation requirements for the telephone followup surveys, mortality and morbidity surveillance, substudies, and all data processing activities.

Data analysis of the EPESE baseline survey is now underway, with writing committees established for a Resource Data Book of descriptive measures to be produced by EDB in collaboration with the three centers. Biometry staff serving as writing committee chairmen include Mr. Cosmatos, Mr. Foley, Ms. Grigson, and Dr. Brock. A large portion of the work necessary to produce this publication involves management, processing and tabulation of the large body of data generated by the surveys. This work is currently being conducted by Biometry staff under the supervision of Ms. Lafferty, including Ms. Grigson and Ms. Cruz. The preliminary data tapes have been checked for errors, and the three centers have been consulted regarding errors in the data, and tape documentation for the tapes. At the present time, tables for the publication are being produced and planning and documentation are underway for creation of a data file of variables common to all three centers.

Developmental work on the Survey of the Last Days of Life has continued during FY83. In September 1982, a contract (NO1-AG-2-2137) was awarded to DMH Associates, Inc., a small business located in New York City, to develop field instruments and procedures for conducting the survey and collecting the data. DMH in turn wrote a subcontract to the Hebrew Rehabilitation Center for Aged in Boston to assist in instrument and methodology development. In the time since the award, the contractor has set up a steering committee and has established relationships with the medical community in Fairfield County, Connecticut, where the study will take place. Analysis of patterns of mortality among the elderly in the community has

been completed and documented, leading to a proposed sampling design for the study. Biometry Office staff have reviewed the sample design and suggested modifications for final implementation of the plan.

A concept clearance document prepared by the contractor has been submitted to the OMB for approval. Meanwhile, a draft questionnaire for the study is currently being revised to be submitted to OMB along with a supporting statement for the survey once the concept clearance is obtained. Plans call for a pretest of questionnaires and procedures to be conducted in late fall and, after revision, full fieldwork to begin after the first of the year 1984. The study will document, for a sample of elderly decedents, events and conditions present at the time of death, such as location and time of death, state of consciousness of the decedent just prior to death, prior knowledge of impending death, presence of pain and use of pain medication, and prior health conditions of the decedent. In addition, the lifetime history of certain events and condition will be studied. Examples include dementia, deafness, blindness, hip fracture, Parkinson's disease, paralytic stroke, and admissions to nursing homes. Use of devices such as pacemakers and hearing aids will also be studied.

Last year Drs. Brody and Brock analyzed mortality data from 1900 to 1975 by 5-year intervals, noting that recent declines in mortality among the elderly may be the continuation of declines that began earlier in the century. In 1900, approximately 25 percent of all deaths occurred in people 65 years of age and older, while today the age at death has been pushed back so that by 1980, 30 percent of deaths occurred in those over age 80. At the same time a disquieting set of data from the National Center for Health Statistics has shown that rates of illness and disability are increasing. Thus, a focal issue is whether in increasing life expectancy we are also improving health status and the quality of life. We are continuing our efforts in analysis of mortality and morbidity data to increase our knowledge in these areas.

The study of the effects of climatic extremes on mortality and morbidity is continuing. A professional services contract with M/A-COM Sigma Data Inc. has been completed and a sophisticated computer program to link the climatic data base to mortality records has been devised. The program allows selection of various factors in determining the mortality data/climatic link. Specific work data sets can then be generated to address specific hypotheses. Methodological development of appropriate summary statistics necessary for the analyses of these types of data is presently in the final stages. These statistical procedures will allow analyses of the data with consideration for the geographic and political variations in each climatic data reporting area.

Mr. Foley and Dr. Cartwright have completed an analysis of data from the Framingham Disability Study concerning age, work, and disability. The analysis involved the application of multiple logistic regression to develop a prediction equation for disability as a function of several independent variables.

CONTRACT

Name and Number: DMH ASSOCIATES, INC. (NO1-AG-2-2137)

Title: Survey of the Last Days of Life

Date Contract Initiated: September 30, 1982

Current Annual Level: $150,000

Objectives: The purpose of this project is to collect descriptive data on the last days of life for a community sample of persons age 65 and older whose deaths occurred in a one-year period. In addition to providing specific data on basic events and circumstances surrounding death, the study will provide lifetime prevalence data for a set of conditions related to, but not necessarily causing death. The new knowledge gained from this study will be extremely valuable in relieving the burden of anxiety on family, friends of the dying person, and to providers of care.

Methods Employed: A sample of death certificates will be selected over a period of one year in the community chosen for study. Retrospective information concerning the decedent's last days of life will be obtained in a face-to-face interview with an informant identified from the information contained on the death certificate. Followup information will be obtained from medical sources identified by the informant for those cases in which it is appropriate.

Major Findings: A contract was awarded in September 1982 to DMH Associates, a small business in New York city with a subcontract to the Hebrew Rehabilitation Center for Aged, in Boston, Massachusetts. Extensive developmental work on the survey is now underway. This includes instrument design, sampling design, and planning for a pretest to be conducted in late fall. Full fieldwork is expected to begin in January 1984.

Significance to Biomedical Research: A considerable body of literature exists in the geriatric and psychological fields as well as in the lay press about dying. Yet specific data about basic events associated with dying are lacking --such as who dies peacefully in his/her sleep, who dies in great pain, what persons are present at the time of death, who dies after a long illness with full awareness of his impending demise, and who dies suddenly with no warning. Further, the proportion of the dying who need and actually receive pain medication is unknown. The study will provide an opportunity to obtain epidemiological data on the numbers of persons affected by the major health conditions that confront the dying elderly as well as the lifetime likelihood of certain events and conditions such as blindness, deafness, dementia, hip fracture, and others.

Proposed Course: Upon approval of the questionnaire by the Office of Management and Budget (OMB), the contractor will conduct a pretest of the survey instruments and field procedures to determine the most appropriate time interval after the death to approach the informant for the interview and to assess the reliability of data gathered in this manner. After the pretest, revisions will be made in the procedures, if necessary, and full fieldwork will begin. Data collection will take place for approximately 15 months, at which time data cleaning, editing, and processing will begin. When this is completed, the data will then be available for analysis by NIA staff, in the fall of 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 06000 04 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Climatic Effects on Morbidity/Mortality in the Elderly

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) PI: Dennis Cosmatos, M.S., Statistician (Health) EDBP, NIA

COOPERATING UNITS *(if any)*

National Climatic Center, NOAA

LAB/BRANCH

Biometry Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.40	.40	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Data on daily climatic conditions as reported from approximately 300 weather stations will be obtained from the National Climatic Center, and linked to daily mortality data for the period 1972 through 1977. An age-specific analysis of average daily mortality at various levels of climatic variables will be performed in order to ascertain relationships between age and climatic conditions. Climatic variables will include temperature, relative humidity, rapid changes in temperature, departures from normal temperatures, and sustained heat waves and cold fronts. Levels at which hypothermia and hyperthermia are more likely will be investigated in greater detail. Concomitant information on socioeconomic status and geographic location of the cases will also be considered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 06020 03 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Health Effects of Environmental Pollutants on the Elderly (UPGRADE Project)

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) P.I.: Dennis Cosmatos, M.S.
Statistician (Health) EDBP, NIA

COOPERATING UNITS *(if any)*

M/A-COM Sigma Data Inc.

LAB/BRANCH

Biometry Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.10 (NIA)	.10 (NIA)	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Investigations will be made into the effects of environment on the health of the elderly. Interactions of environmental pollutants with climate variables will be investigated with respect to their effects on a variety of specific chronic conditions and mortality. Computer programs have been developed to allow linkage of mortality and climatic data sets. Final reports and documentation on these programs have been received.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 03010 01 EDBP

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Analysis of Trends and Differentials in Mortality and Morbidity Among the Elderly

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Dwight B. Brock, Ph.D., Chief, Biometry Office, EDBP, NIA

Jacob A. Brody, M.D., Associate Director EDBP, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Biometry Office

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Analysis of our extensive data base of mortality rates for the U.S. elderly population has been conducted, for the years 1900 to 1975. The data base is being expanded to include data from later years as they become available and possibly data from other countries for comparison. In addition, morbidity data is being sought to complement the mortality data which have already been analyzed.

Summary of Direct Operations

As we seriously contemplate our future plans in terms of scientific opportunities and future directions we are faced with several oppressive realities. Most acute is the fact that in the formation of the EDB we were instructed to hire a few highly trained professionals to develop a large program on a par with other NIA Programs. It was the intention of everyone at the time to supplement EDB with the necessary personnel to carry out the program as intramural research after it was developed and put in operation. We, therefore, have committed major resources of NIA in a series of very large studies costing 20 to 25 million dollars. In essence, we defined several broad parameters and bought data and its collection. The data are coming in and during the next 1 to 2 years will form a monument of information concerning numerous aspects of health and disease among the elderly. Our staff, however, has not grown concommitantly because of the freezes on hiring which have existed almost since the establishment of NIA. We would surely need no fewer than eight more scientists and support staff to be in a position to properly analyze the data we have already paid for. We would, of course, welcome having the authority, funds, and space to hire the additional personnel. Lacking that, we are seeking other solutions, and are really in need of help. One possibility is that some of our contract money could be reprogrammed for professional service contracts and various purchase orders to have specific tasks such as cleaning computer data tapes or conducting the first stage of analysis for given studies. We would prefer that individual awards be made in order to keep very tight control over the development of the data so that when we turn it over for public use we will be assured of its accuracy and completeness and cognizant of its potential value. However, even this mechanism would require additional EDB personnel.

All of our recent research contracts have been awarded to small businesses through the Small Business Administration (SBA). This is proving to be far more time consuming to EDB staff than anticipated since we find that it is necessary to be more vigilent in order to ensure quality research. We expect no change in Government policy regarding the SBA and, hence, we will probably have to accept a compensatory reduction in the number of new research contracts we can award and manage.

Summary of the Associate Director for Epidemiology,
Demography, and Biometry Program
National Institute on Aging

Major activities of the office of the Associate Director are to provide leadership in the melding, maintaining, and developing of the component EDB programs and to serve as a focal point for data and concepts relating to epidemiology and aging for the Institute and other Federal, national, and international groups. Major contributions have been made to ongoing studies, the content of which are discussed by the three Offices in the Program. The Established Populations for Epidemiologic Studies of the Elderly (EPESE) now has in place a viable management mechanism whereby work and territory are discussed and allocated within the various committees established for the execution of these studies. The major, initial documentation is being assembled by EDB in the Resource Data Book. This will form the keystone for all subsequent analysis of baseline information. The NHANES followup is proceeding ahead of schedule and a critical meeting of the cooperating Institutes and the NCHS was conducted this year to formalize the various studies, substudies, and special interests being explored by the contributing organizations. We now have in place a mechanism for analysis and publication of data. The flow of data on senile dementia proceeds. Our initial effort was the conducting of a prevalence survey in New Haven, Connecticut involving 2,500 people 65 and over. The final interviews and analysis of these data are nearing completion. Our second effort to describe the natural history of noninstitutionalized patients with senile dementias is in full operation with cases being identified in preparation for a 5-year followup period. A major effort is to utilize the Framingham Heart Study cohort which now consists of about 3,000 men and women age 65 to 90. We will be able to relate mental symtoms to disabilities and outcomes. Further, having complete medical histories we will evaluate possible affects of environment and prior health status on dementias.

The orchestration of the macroeconomic-demographic model and the attendant health expenditures and aging health and aggregate consumption sectors are becoming an increasingly viable portion of the overall program. We are slowly succeeding in the merging of physicians, epidemiologists, biostatisticians, and economists in utilization of this and perhaps other data models. We hope over the next few years to make significant contributions utilizing this broad approach to complex issues with potential policy implications.

This year the biometry program was able to commence the absorption, integration, and documentation of data from our largest studies (EPESE and NHANES followup). This is gratifying since the material we are collecting is unique and we are closer to being assured of adequate documentation, availability, and utilization.

During the year, through numerous consultations, presentations, and publications in research and problem areas relating to epidemiology, health, and aging we interacted with a large sector of the health and aging community. Our mortality analyses during the 20th century and projections into the next century are gaining acceptance. We have emphasized that the elderly have been increasing in numbers as the result of decreased

age-specific mortality over age 65 since at least 1900. At present, the elderly are the fastest growing segment of the adult population and projections indicate that this trend will persist. By the year 2025, there will be approximately 60 million people over age 65 and they will comprise 20 percent of the entire population. We have pointed out that the life expectancy by 2025 will not yet have reached 80 years of age, thus, falling far short of the idealized projections by Fries and other commentators. We caution that there is no indication that morbidity is declining and our predictions for the immediate future are the very rapid accumulation of a physically-compromised elderly population. We are holding discussions with various groups trying to improve on the availability and usefulness of morbidity data. Unless we understand the epidemiology of morbid disease and the secondary disabilities, we have no good means of gaging progress in health as we prolong life. During the course of these studies we have made the observation that cancer becomes a less prominent cause of death after age 65 or 70, declining from about 30 percent of all deaths at age 65 to about 10 percent of all deaths at age 85. Because of the demographic shifts with more people dying at older ages (at present, 30 percent of all deaths occur in the 2.3 percent of the population over age 80), there will be an absolute and relative decline in mortality from cancer.

We have raised serious questions about the effectiveness of our health promotion policies in the elderly. We point out that many of our recommendations are based on soft and unconfirmed data such as that relating to exercise, weight control, special diets, and salt restrictions in the absence of hypertension. We further question the amount of reduction in morbidity and disability we can expect if we were able to produce a population of paragons who did not smoke, or drink and controlled their blood pressures etc. We point out that by overstatement we lose credibility in implementing proper health promotion efforts. Further, we have become intrigued with the accumulating data that behavior modification works only rarely. Further, it appears that education (and, of course, its concomitants, all of which are harder to measure) exerts a disproportionately powerful affect on life expectancy. Thus, we are trying to modify the behavior of the least educated population and have not directed our efforts to cope with this high risk but difficult to reach subgroup.

We are attempting difficult studies in hopes of defining health rather than disease with age, and also the affects of nutrition on longevity. Currently we are exploring possibilities of analyzing data from the two most longevous populations on earth, Sweden and Japan. The two countries had very different dietary experiences and age-specific mortality patterns during this century. The fact that they have arrived at approximately the same point in life expectancy from apparently quite different directions suggests that important leads to the major factors involved in health and age may be detected through proper analysis of the Japanese and Swedish experience as well as our own population studies. It should be noted that while the U.S. and Sweden claim gains in health and longevity by restricting the typical Western high fat diet, even greater gains are occurring in Japan where for the past 25 to 30 years they have been increasingly adopting Western diets.

REPORT OF THE INTRAMURAL RESEARCH PROGRAM

(Gerontology Research Center)

Office of the Scientific Director	GRC/OSD-1
Laboratory of Behavioral Sciences	GRC/LBS-13
Laboratory of Cellular and Molecular Biology	GRC/LCMB-79
Laboratory of Molecular Aging	GRC/LMA-101
Laboratory of Neurosciences	GRC/LN-132
Clinical Physiology Branch	GRC/CPB-217

Intramural Research Program

Office of the Scientific Director

The major NIA intramural research program is located at the Gerontology Research Center (GRC) in Baltimore, Maryland. In addition to the basic and clinical research conducted by NIA investigators, the intramural research program is the major setting for post-doctoral training in biomedical and behavioral science related to gerontology and geriatrics.

Until the current fiscal year, all activities relating to NIA intramural research were conducted at the GRC. Consequently, historical tradition has tended to make intramural research programs (IRP) synonymous with GRC. However, with the recent emergence of the Laboratory of Neurosciences programs at the Clinical Center in Bethesda, it is necessary to point out the reality that the Gerontology Research Center should no longer be used to identify all intramural research activities.

The research accomplishments of the intramural program are highlighted in the following branch and laboratory summaries. However, during 1983 perhaps the most challenging opportunity facing the NIA IRP was to develop a focus for research and training in geriatric medicine in concert with the Johns Hopkins University Medical Institutions and the Baltimore City Hospitals. Historically the clinical research activities at the GRC have been confined almost entirely to the study of normal individuals. Nevertheless, the interaction of age and disease raises such important questions that the IRP staff was convinced there was a need to develop a greater degree of involvement in geriatric medicine.

A close professional and personal relationship with Dr. William Hazzard enabled the IRP to work out an agreement to provide funding for a three-year clinical fellowship in geriatric medicine. The first year of the fellowship will be undertaken at Hopkins (BCH) and the latter two years in a research activity within the framework of the NIA intramural research program. The first clinical fellow started work in July, 1983. Additional resources have been committed to this collaboration so that ultimately three such fellows will be supported by NIA.

Administratively, the Scientific Director, NIA is directly responsible to the NIA Director in all matters concerning the content, quality, substance, and direction of research undertaken by the intramural program. In addition, the OSD embraces several administrative and support functions which are central to the orderly operation of the overall intramural research program. These include Administration, Photography and Arts, Library, Procurement, Public Information (GRC), Personnel (GRC), Animal Resources, central data acquisition and processing, and shops which design, fabricate and maintain special research equipment. Immediate responsibility for the operation of all central ADP functions, as well as the shops, lies with the Chief of the Technical Development Section, Mr. Thorne, who reports directly to the Scientific Director. Finally, the OSD contains two small research units, the Comparative Nutrition Section and the Experimental Morphology Section. The purpose and recent accomplishments of the constituent units of the Office of the Scientific Director follow.

Administration

The range of services carried out by the Administrative Office encompass Budget, Travel, Payroll, Timekeeping, Building Operation, Space, Property, Personnel Budget/Ceilings, Safety, Disbursing Activities, Service Contracts, Training, and participation on many of the intramural committees which play an instrumental role in the internal governance of the GRC. Highlights of FY 1983 activities follow along with summary reports of the three units that report to the Administrative Officer:

- Retrofit of cold and constant temperature rooms has been completed;
- A contract to balance building air is in progress and will be completed within the next few months;
- A system for control and forecasting of FTE ceilings has been developed;
- As part of the continuing effort to up-grade equipment, CPT word processors and Digital VT100 terminals to connect to the GRC Vax 11/780 have been rented; and
- Interaction with DES/NIN continues on a periodic basis to maintain the GRC facility.

Library

The GRC Library holdings include books and periodicals covering all of the scientific disciplines represented at the Center. In addition, the library serves as a specialty library in gerontology and geriatrics housing a collection of thousands of books, journals and reprints covering the entire gamut of literature on aging.

The Research Librarian position was filled in May, 1983. Major duties include not only the management of the GRC Library, but service as a liaison between the Library and other research or hospital libraries in the Baltimore and Washington, D.C. areas. Prior to the position being filled, the former Library Technician and two part-time clerk-typists provided a range of library services to the intramural community. In part, this was attributable to the continued availability and augumentation of on-line reference capabilities. Both the BRS and OCLC data base systems have facilitated referencing and interlibrary loan functions with speed and accuracy.

Several special projects are planned and partially initiated. Collection development, including updating books, reference materials and weeding unwanted items, will bring the Library's holdings up-to-date. Automation of library operation is being planned. A small computer system has been considered and hopefully will be implemented soon. The conversion to an automated system will eliminate the repetitive, error-prone procedure of typing and filing numerous catalog cards and circulation cards; furnish accurate data of the entire collection on file; provide a better control mechanism of material circulation; and allow users easy and prompt access to review the online catalog. Implementation of an automated computer system should prove effective over the long-term and significantly enhance the utility of this important resource.

Photography and Arts Section

The Photography and Arts Section provides all photographic and illustration services for the intramural research program. These services range from simple graphic representation of data to complex medical illustration and from simple copy to technically complex macro-photographic procedures. The products of the Section are used by investigators for slide and poster presentations, scientific publications, and support of research projects.

This year, the Section has added a new computerized Phototypesetter. With this machine, text can be inputted directly from various word processors throughout GRC, thereby speeding up the process of making slides or posters.

Procurement Office

The Procurement Office continues to provide exceptional service to intramural staff in procuring items ranging from general office/laboratory supplies to unusual or less readily obtained materials. During the year, additional training was successfully completed by staff members to ensure familiarity with the latest procedures governing procurement policy and to augment their capabilities.

Animal Resources Facility

The Animal Resources Facility (ARF) is responsible for the supply of experimental laboratory animals to support research conducted at the Gerontology Research Center. These animals are supplied through inhouse production and contracts.

Production and quality control programs are currently concerned with ten species of inbred, outbred, mutant, and random bred mice, up to six strains of rats, rabbits, dogs, monkeys, and chickens. All animals are maintained under strictly controlled enviromental conditions. In addition to the production and procurement of these animals, the majority of them are aged to the near limits of their life expectancy within the Center. Approximately 25,000 animals, supporting 50% of the research projects at the GRC, are issued and maintained by the Animal Resources Facility.

The Animal Resources Facility was first fully accredited by the American Association for Accreditation of Laboratory Animal Care on June 21, 1977 and has maintained accreditation since. The GRC-ARF is an institutional member of the American Association for Laboratory Animal Science (AALAS), as well as of the National Capitol Area Branch of AALAS. A contract with Johns Hopkins University School of Medicine, Division of Comparative Medicine, provides veterinary coverage.

The ARF staff of 26 range in experience from 1-20 years. All animal caretakers are currently enrolled in the NCAB, AALAS's Assistant Laboratory Animal Technician Course, conducted by ARF Supervisory staff. Animal caretakers have successfully completed the Purina Training Course.

The Animal Resources Facility is comprised of two sections totalling 30,500 sq. ft. The Conventional Colony is made up of the C57 Aging Mouse Colony, beagles, primates, rabbits, experimental rats, mice and chickens (16,200 sq. ft.). The second section is the Closed Wistar Rat Colony, which is maintained in the basement of the GRC, totalling 14,300 sq. ft. Essentially, 100% of all GRC program requirements for animals of all ages are fulfilled by the ARF Aging rodent colonies. Approximately 10,000 animals from the aging colonies were issued during Fiscal 1983.

Technical Development Section

This Section is responsible for providing technical support to the intramural research staff. This support includes maintaining the GRC computer system at a level which meets expanding needs; supporting the installation of laboratory-based systems with lab-interface design and construction, system level software development, assisting with the procurement and by configuring system hardware; development of specialized laboratory instrumentation; fabrication of mechanical assemblies; and various building maintenance responsibilities.

Recent accomplishments include the following:

- In the past year, major improvements to the GRC computer system have been initiated. These include expansion of disc space and memory, the purchase of several software packages and the addition of a graphics capability.

- The Cardiovascular Section's laboratory computer system is being replaced. Data acquired by the new system will be processed by both itself and the central system, via a network connection.

- The obsolete computer used by the Psychophysiology Section to maintain several monkeys on operant conditioning schedules has been replaced by a multi-microprocessor system, developed and fabricated by the Technical Development Section.

- The path taken by a rat in a very large 50-station maze is detected by optical sensors and recorded by a microprocessor in equipment recently developed for the Learning and Problem Solving Section.

Information Office

The Information Office at the Gerontology Research Center, IRP, NIA, functions as the communications vehicle for the Institute's inhouse research programs to the public-at-large, other government and private agencies and the numerous electronic and print media covering science and medical news.

Responsibilities involve internal and external dissemination of information on research programs conducted by intramural and extramural supported investigators. The office also conducts extensive community outreach programs dealing with students, senior adult groups, minority interest organizations, and service-oriented hospitals and agencies.

Further, the Office has responsibility for certain employee relations activities and educational programs, along with carrying out special projects important to the mission and goals of the NIA.

Information Office activities this year focused heavily on coordinating the landmark 25th anniversary of the Baltimore Longitudinal Study of Aging. This celebration of the dedication and contributions made by the study's 1,000 men and women volunteers has been two years in planning. Final arrangements for an excellent program and activities for the September 9-11, 1983 event were completed by late summer.

Another major project is the editing and processing of a scientific manuscript detailing the organization and conduct of the longitudinal study of aging since its inception in 1958. One full time and one part time employee have been continuously engaged in this monumental effort for the past year. The book is expected to be ready for publication within the next few months.

The Office has carried out educational efforts with a wide variety of groups and individuals, addressing nearly 600 people via lectures, briefings or tours since last summer. Visitors included foreign scientists and service providers, Federal and state health officials, minority students from high schools and colleges, health curriculum teachers and students, and older adults participating in senior center programs, or retiree organizations.

Writers prepared articles on Center investigations for publication in the Journal of the American Medical Association, Public Health Reports, and NIH News and Features, as well as press summaries for the Gerontological Society of America's annual meeting. The IO staff handled all pre-anniversary publicity for the BLSA celebration, prepared text for the invitation package, program, other background material, and ran a full working Press Room for the actual event.

Over the year, staff handled 190 media inquiries from a variety of media, ranging from a Baltimore summer job corps newsletter to the Baltimore Sun, Newhouse News Service, Parade Magazine, Encyclopaedia Britannica, and Dynamic Maturity. On the air, stories and interviews on the NIA and GRC have been broadcast by NBC-TV, AP Radio, Cable News Network, Group W stations, WBZ-TV (Boston), KYW (Philadelphia), Japanese TV, Canadian TV and Radio, BBC Radio.

Fiscal Year 1983 highlights include:

- Held nine planning meetings for the BLSA Silver Anniversary. Program, logistics and hotel arrangements were completed; invitation and program copy prepared; publicity mailed to volunteers; a slide/tape on the first volunteers and their community was developed by Marsha Love of the Bethesda NIA, IO; and an update on the study for insertion in "To Understant the Aging Process," was completed by Esther Solomon of the NIA IO with input from GRC.

- Stories on the geriatric continence clinic, thallium scanning for undetected heart disease, and on inappropriate use of digoxin were prepared.

- Five stories were prepared for the NIH RECORD and one for the DRG Director's newsletter, two press summaries for the annual GSA meeting, and an advance media notice on the BLSA Anniversary.

- Communication Officer interviewed on aging research and the BLSA by the Canadian radio program, "Discovery" and by Baltimore public radio station, WBJC-FM.

- Major aging series, published in five parts by the Baltimore Evening Sun, featured a BLSA subject, and considerable information was provided by NIA staff. The IO staff provided extensive background to the Pulitzer prize-winning science writer, Jon Franklin.

- Redesigned internal GERON NEWS newsletter and periodical sent to volunteers, "Six S's News." Twelve issues of the former and two of the latter written and published by the Information Office during the year.

- Conducted briefings, tours, or outreach lectures for 600 individuals and groups and held a special briefing for American Indian high school youth this summer. Held 6th annual laboratory demonstration program for Maryland high school science honor students attending Science and Humanities Symposium in Baltimore.

- Coordinated and conducted a number of special programs or projects such as two Red Cross Blood Drives, an IRS tax workshop, employee orientations, a Black History display, Asian/Pacific American Week events, and preparation of copy for the NIH Handicapped Advisory Committee display for the Bethesda campus.

- Helped set up GRC filming for programs on aging either broadcast or in the works for NBC-TV, WCBS-TV, CBC-TV, CNN, BBC-Radio, BBC-TV, Gannett TV, WBZ-TV, Sniper's TV (Japan), AP Radio, RAI-TV (Italy) and CBN-TV.

Comparative Nutrition Secion

The Section on Comparative Nutrition investigates the relationship between nutrition and aging, specifically 1) the effect of age on nutritional needs; and 2) the basic biological mechanism by which dietary restriction increases life span.

The possibility that vitamin supplementation of the aged would be advantageous was pursued this year. The Section examined three groups of C57BL/6J male mice, ages 30 days, 14 months, and 20 months, which were fed diets containing either an amount of vitamins recommended by the National Academy of Sciences-National Research Council, one-half this amount, four times this amount, or an amount of vitamins recommended by the American Institute of Nutrition. Mice, regardless of age, offered one-half the RDA of vitamins began to die within two months, and all were dead within eight months from the initiation of the study. At present, the data do not indicate any beneficial effects from increasing the vitamins in the diet to four times the RDA. Therefore, these data suggest that efforts must be made to maintain vitamin intakes equal to RDA levels.

Recent Section experiments clearly demonstrate a reduced total cellular protein in dietarily restricted animals (those fed low protein diets and those fed intermittently) and suggest that a common mechanism accounting for the increased life span may be a lower level of cellular protein synthesis. However, measurements of individual enzyme levels show that they are not necessarily low following dietary restriction, and may indeed be two- or three-fold higher than those of control animals. Thus, the common mechanism is not a delay in development such that restricted animals mimic chronologically younger ones, but rather they are biochemically different and distinct from normal animals.

In order to establish the validity of the concept of dietary restriction in human populations, it will be necessary to develop reliable methods for studying the phenomenon in adult animal models. At present, subjecting adult animals to methods successfully used in young, growing animals results in precipitous mortality. This group's past animal experiments, carried out in late adulthood, indicate that dietary protein cannot be decreased lower that 12%, and caloric restriction cannot exceed an amount that results in a 25% loss in body weight. Therefore, future experimental manipulations will attempt to define successful conditions within these limits.

The lack of reliable biomarkers of aging makes difficult the strategy of relating the effect of dietary restriction on the rate of aging. Therefore, a new attack will be attempted. In general, survival is an expression of a successful response to stress. By comparing known responses to selective stresses or challenges, differences in characteristics of normal and dietarily restricted animals may be established. It may be possible to relate these differences to the increased life span of dietarily restricted animals. The animals will be maintained in a steady state with minimal loss in body weight but a reduction in hepatic protein of approximately 25%. The latter condition is found in young-growing animals whose life spans are increased by dietary restriction.

The only consistent biochemical change found in biochemical variables in animals subjected to various dietary regimens in our and other laboratories has been a reduced hepatic protein. This finding suggests a reduced protein synthesis in all dietarily restricted animals. Efforts will be made to confirm this suggestion.

Experimental Morphology Section

This Section examines morphologic parameters important to aging and basic cell biology with research emphasizing microscopy. As a service facility, the Section provides advice and technical support for research projects conducted by other intramural components. Projects which originate from within the Section include study of embryonic programmed cell death as a model for aging, nuclear matrix organization, cellular adhesion, structural changes of the knee joint in exercise and aging, and functional morphology of peripheral nerves.

Research Activities over the past year follow:

Programmed Cell Death. Programmed cell death in gonadal cells of the nematode Panagrellus redivivus occurs in normal development and is genetically controlled. Regulation of programmed cell death may be a model for regulation of aging in organisms. With Nomarski optics, we have followed the nuclear changes which take place prior to death, and with fluorescence optics, we are now determining which of the shape and contrast changes we see with Nomarski optics are reflections of DNA, RNA, or nuclear matrix changes. Each stage is also being examined by electron microscopy for a finer description. The changes are similar to those of necrotic cancer cells but do not include the irregular nuclear shape characteristic of both cancer cells and many cells from old organisms.

Nuclear Matrix. The apparent changes in nuclear matrix during programmed cell death lead to questions about how it is organized. Methods of labeling nuclei with antibody probes, rapidly freezing, fracturing, etching and freeze substitution are being developed to discern the three dimensional organization of the matrix in order to understand how the nuclear components interact to regulate.

Cell Adhesion.

- Sponge aggregation factor, a large glycoprotein complex, containing multiple side arms, is involved in species specific aggregation. It is the first such factor to be visualized on the surface of aggregating cells. It provides a most promising model for analysis of specificity in cellular interactions. It apparently functions by a single macromolecule bridging between two cells with some arms extending to one cell and some to another. Many such linkages ligate adhering cells. Studies designed to achieve visualization of the complex at higher resolution are now being conducted collaboratively with scientists at NINCDS.

- Pallidin, a cell surface lectin implicated in mediating intercellular adhesion in the cellular slime mold, is inhibited by an endogenous factor which binds to it. the inhibiting factor, apparently a receptor, appears as a hemisphere in the electron microscope. Pallidin binds all over the surface of the receptor like a fringe. This is the second aggregating factor to be visualized.

Functional Morphology of Peripheral Nerves. The vertebrate nerve is an organized structure which has very directed growth during development and regeneration. Once established with it target organ, the normal nerve and its specific environment for the neuronal axons must be maintained throughout the lifespan of the animal. The objective of this new project is to examine the cellular functions and interactions for nerve growth and maintenance. The morphological and functional characteristics of two major nerve compartments--the nerve sheath and endoneurium--will be studied in vivo and in culture. One specific aim of these studies will be to define functions of these cells that can be further examined in aging tissues in culture an in vivo. An immediate objective is to develop methods to label and define the different cellular and tissue components of peripheral nerves during development and aging. Results include:

- Morphology of embryonic, young chick and adult chicken peripheral nerve sheaths was examined with light electron microscopes. Light junctions are prevalent in the 18-day embryonic perineurium. Vesicular pils become characteristic in these cells as do the large amounts of intermediate-sized filaments which increase greatly after hatching. Connective tissue components also show large increases during development.

- Indirect immunofluorescence staining with rabbit anti-chick fibroblast vimentin of whole nerve and cultured explants revealed that colecid-sensitive, vimentin-type intermediate filaments exist in the predominant cell types in both the endoneurium and nerve sheaths of chicken peripheral nerves and contribute to the large numbers of filaments observed in the perineurial cells.

- Mammalian peripheral nerve of different ages is being examined in vivo morphologically and in culture. Monoclonal antibody methodology has been learned and is being carried out for the purposes of obtaining monoclonal antibodies to components of the peripheral nerve sheath.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00101-07-OSD

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Relation between Nutritional State and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Charles H. Barrows, Jr., Chief, SCN,OSD,NIA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Gerontology Research Center, Office of the Scientific Director

SECTION

Section on Comparative Nutrition

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Slopes of the regressions of specific enzymes and age were calculated for normal and dietarily restricted animals. The data support the concept that the rate of aging of most biochemical variables is less in dietarily restricted animals. The final results of the experiment subjecting 25 month old mice to various degrees of dietary restrictions are now complete. The mean life span of the animals fed the 24% protein diet ad libitum was 29.2 ± 0.4 months. Dietary restriction did not significantly increase the life span of any of the animals. Three month old mice fed a high level of cellulose in the diet (50%) for one month consumed approximately 30% less calories and experienced a 5% loss in body weight while the control animals' body weights increased 16%. Thus in spite of increased intake, dietary restriction may be achieved by feeding cellulose in the diet. Data indicate that offering a diet containing 50% of the RDA of vitamins to old mice aged 17 - 19.5 months decreased their life span expectancy by 47 and 30% respectively. Thus, at least in mice, longevity may be increased by ensuring the ingestion of the RDA daily.

Other Investigators: Gertrude C. Kokkonen, Chemist, SCN,OSD,NIA

Objectives: Attempts were made to describe the biochemical characteristics of animals of different ages subjected to various dietary regimens known to increase life span. In addition efforts were made to determine the effect of vitamin intake on longevity. Finally adult animals were subjected to various levels of dietary intake and dietary protein to determine the most efficient method of increasing life span.

Methods Employed: In a series of studies, concerning biochemical characteristics and dietary restriction, weanling male $B_6D_2F_1/J$ mice were fed either: 1) a 24 percent protein diet ad libitum; 2) a 4 percent protein diet ad libitum; or 3) a 24 percent protein diet intermittently (diet offered 24 hrs. on Monday and Thursday and eight hours on Friday). Those animals referred to as intermittent-fed were sacrificed either on Tuesdays or Thursdays, i.e. following a 24 hour feeding period. Those referred to as intermittent-fasted were sacrificed either on Wednesdays or Fridays, i.e. following a 24 hour fasting period. The animals were sacrificed at 12, 27, and 32 months of age. In the first study the homogenates of liver and kidney were analyzed for the activities of malic dehydrogenase, cholinesterase, succinic dehydrogenase and cathepsin and for the concentrations of DNA and protein. In the second study the activities of aldolase of the cytosol and NADPH-cytochrome c reductase and ethylmorphine N-demethylase of microsomes isolated from livers and the concentrations of DNA and protein of liver homogenates were measured.

Four hundred and fifty 25-month-old C57BL/6J male mice were divided equally into seven groups. Five of the groups were offered one of the following dietary protein levels ad libitum: 24, 12, 8, 6, or 4 percent. The two remaining groups were fed the 24 percent protein diet either for 24 hrs. on Monday and Thursday and 8 hrs. on Friday, or for 24 hrs. on Monday, Tuesday, and Thursday and 8 hrs. on Friday. The life span of these animals were determined.

Three groups of C57BL/6J male mice, ages 30 days, 14 months, and 20 months were fed ad libitum diets containing either an amount of vitamins recommended by the National Academy of Sciences-National Research Council, one half this amount, four times this amount, or an amount of vitamins recommended by the American Institute of Nutrition. Each dietary age group contained 50 animals. The body weights and life span of these animals were determined.

Cellulose was added to the 24 percent protein control diet which has been routinely used in this laboratory. The amounts added were such that the Kcal./gr. diet varied from 1.8 to 3.8 and the protein/gr. diet from 0.12 to 0.24. Three month old male C57BL/6J mice were fed these diets ad libitum and food intakes and body weights determined.

Major Findings: Slopes of the regression of specific enzymes and age were calculated for normal and dietarily restricted animals. More than 75% of the various measurements in control animals were found to exhibit statistically significant age regressions. Approximately 90% of the slopes of the age regressions of dietarily restricted animals were lower than those of control animals. Over 50% of the age regressions in dietarily restricted animals were significantly different from those of the control animals. Therefore these data

support the concept that the rate of aging of most biological variables is less in dietarily restricted animals. In some instances the age regressions were the same among all animals. Since dietary restriction is associated with an increased life span, the longevity of an animal and its rate of aging need not be related.

The final results of the experiment subjecting 25 month old mice to various degrees of dietary restriction are now complete. The mean life span of the animals fed the 24% protein diet ad libitum was 29.2 ± 0.4 mos. Dietary restriction did not significantly increase the life span of any of the animals.

Final results indicate that animals at all ages fed 50% of the daily recommended allowance (RDA) of vitamins for mice experienced a life shortening as compared to those animals fed the RDA. Increasing the vitamin levels to 4 RDA did not result in an increased life span.

In these preliminary studies the feeding of diets containing 33 or 50% cellulose to three month old mice for one month did not result in large losses of body weight. The animals compensated for the cellulose in the diet by increasing their food intake. Indeed, animals fed the low level of cellulose increased their intake such that their caloric intake was approximately the same as the control animals. However animals fed the high level of cellulose consumed approximately 30% less calories and experienced a 5% loss in body weight during this feeding period; while the control animals' body weight increased 16%. Thus, in spite of increased intake, dietary restriction may be achieved by feeding cellulose in the diet.

Significance to Biomedical Research and the Program of the Institute: Decreased nutrient intake among the elderly has been a consistent finding. Unfortunately the question regarding whether remedial procedures should be initiated has never been vigorously pursued. However the data presented in this report indicate that offering a diet containing 50% of the RDA of vitamins to old mice aged 17 - 19.5 months decreased their life expectancy by 47 and 30% respectively. Thus, as least in mice, longevity may be increased by ensuring the ingestion of the RDA daily.

Intermittent feeding which has been shown to increase life span results in some problems in terms of interpretation of nutritional data as well as its application to man. The large variations observed in enzymatic activities during the period of starvation and refeeding make difficult a definition of the biochemical characteristics of the animals. Furthermore it seems unlikely that human subjects can tolerate the long period of inanition necessary to bring about the beneficial results of this dietary manipulation. Therefore the feeding of diets containing cellulose has been initiated to bring about beneficial dietary restriction with ad libitum feeding.

Proposed Course:

1) Investigate the possibility of bringing about dietary restriction by feeding ad libitum diets containing cellulose.
2) To establish the relationship between caloric and protein requirements and longevity in adult animals.

Publications: None.

ANNUAL REPORT OF THE LABORATORY OF BEHAVIORAL SCIENCES

National Institute on Aging, National Institutes of Health

As most people age, they express concern about their health and about their capacity to perform behaviors which they performed when younger. Furthermore, they recognize clearly that these two biological systems, the physiological and the behavioral, interact. It is the goal of the Laboratory of Behavioral Sciences (LBS) to study the mechanisms which determine the interactions between physiological and behavioral processes. In pursuit of this goal, LBS conducts both basic science and clinical investigative programs. Thus, LBS projects include basic studies of cognitive processes such as learning or problem solving in human or animal models, clinical studies of the role of personality variables in normal development or in the expression of disease states, and clinical studies of behavioral interventions in the treatment of age-related, medical disorders. These studies fall into three, overlapping areas of investigation--behavioral medicine, biological science and aging.

Behavioral Medicine. The behavioral medicine programs address a range of clinical issues especially relevant to the elderly. One project, which was recently completed, studied the behavioral features of angina pectoris. Patients referred to the Johns Hopkins Hospital Cardiology Division for assessment for coronary artery bypass surgery were asked to complete a self-report check list of behaviors, symptoms and affects each time they had an anginal episode. Through these self-reports, it was possible to characterize many of the features of anginal episodes and to compare these data with characterizations provided by the referring physicians. Finally, on the basis of psychometric evaluations also available on the patients, it was possible to identify some of the behavioral and characterological features which differentiated patients with coronary artery disease from those whose coronary vessels appeared normal on angiography (about 20% of the patients). Among the many findings were the following: most anginal episodes occurred while the patients were at home, in the presence of another person and during periods when the patients felt content or happy; the referring physicians significantly overestimated the frequency of their patient's anginal episodes and the painfulness of the attacks; the referring physicians usually correctly judged many of the symptoms which the patients reported as characteristic of their chest pains, but incorrectly judged some of the most common symptoms such as sensations of pressure or aching or feelings of weakness; it was possible to develop an index based on the reported symptoms and affects which significantly differentiated between patients who had angiographically clear vessels and those who had measureable coronary artery disease. Patients who had clear vessels showed a pattern of symptoms and affects which also correlated highly with psychometric measures of neuroticism. These findings indicate that behavioral assessments of angina could provide useful supplemental data for assessing patients with chest pain, particularly in enabling physicians to identify personality or behavioral mechanisms as well as vascular mechanisms which mediate complaints of chest pain.

Another study in the behavioral medicine area utilized the data collected in the Baltimore Longitudinal Study of Aging to assess the role of life stress in illness. Stress was measured by means of a questionnaire based on the presence of life events unlikely to be biased by health-specific problems; illness was

measured by physicians' clinical histories of new medical diagnoses. The analyses showed no relationship between the degree of life event stress and the number of newly reported illnesses. This finding adds support to previous findings in this laboratory which suggested that the often-reported relationship between life stress and illness is an artifactual consequence of including illness-related items in the life stress questionnaire.

Two studies of the clinical effectiveness of behavioral interventions are being conducted in this laboratory. One study is looking at the effect of a staged, behavioral treatment program in the management of high blood pressure. The stages include: patient self-monitoring of blood pressure, patient-administered systolic blood pressure biofeedback and patient-administered relaxation. All stages involve professional supervision as well as patient administration. This study is a follow-up to one which was reported earlier in which it was shown that these behavioral interventions were effective in enabling patients with so-called "mild" or "borderline" hypertension to lower their pressures and to maintain these reduced pressures. The present program is assessing these procedures in patients with higher levels of blood pressure who are taking anti-hypertensive medications. Presently, there are too few data to warrant an assessment of this program.

The second clinical program is one designed to evaluate the effectiveness of behavioral treatments in the control of fecal or urinary incontinence in geriatric patients (person 65 yrs. or older). Fecally incontinent patients are being treated with anal sphincter biofeedback, a technique originally developed in this laboratory; urinary incontinent patients are being treated with feedback from the pelvic floor and abdominal muscles (stress incontinence), feedback from the bladder (hyperreflexia), or habit training (urge incontinence). Results to date have been very encouraging; among the 17 fecally incontinent patients studied, 15 show sustained reductions of 50% or more in the number of bowel accidents, and 8 of these patients now have fewer than 1 accident/month; among the 17 patients with histories of stress incontinence, all showed at least 50% reduction in total accidents and 3 have had no accidents since completion of treatment; among the urge incontinent patients all 5 of them have 50% or greater reduction in accidents, and 4 have had no further accidents; finally, among the 10 patients with hyperreflexia, all showed 50% or greater reduction in accidents, and 5 have fewer than one accident/month.

Biological Studies. There are a number of biological studies in this laboratory which look at the interactions among behavioral, physiological and neurochemical events. One program has been studying age differences and age changes in the ability of C57BL mice to cope with cold stress. Among the many findings from this program are the following: body temperature in a normal environment is stable in this strain until about 24 mos. of age after which it falls monotonically; tolerance to cold stress (capacity to maintain body temperature while restricted for 3 hours at an ambient temperature of 10°C) declines with age across the lifespan. However, in the senescent animal (after about 28 months of age) tolerance falls precipitously. Finally, several studies have shown that the intolerance to cold stress can be modified experimentally. Animals which are fed every other day from the age of 6 months showed no evidence of age-related declines in cold tolerance when tested at the age of 28 months or older; old animals which either self-stimulated or were stimulated by the experimenter in so-called positive reward areas of the hypothalamus showed an arrest of the age-related deterioration in cold stress tolerance; finally,

old animals which were allowed to exercise in a run wheel for several weeks prior to cold stress testing were no different from age-matched control animals indicating that none of the effects just cited can be explained on the basis of differential activity levels between groups.

In addition to the cold stress studies just cited, other studies of dietary restriction also have been completed this year. One study involved the use of a comprehensive battery of tests of motor peformance with C57BL mice which had been fed every other day from 6 months of age. Compared to control animals (ad lib fed), the test animals showed a retardation of age-related declines on measures of balance, agility, strength and endurance which could only be explained, in part, by differences in body weight or in motivation after these factors were controlled.

Another study of rats which had been dietarily restricted since weaning looked at the distribution of enzymes involved in neurotransmission in the brain. There were a number of complex differences in the cholinergic and adrenergic systems. Many of these differences showed that restricted animals were more like younger animals. However, there were some exceptions. For example, there were no differences between restricted and ad lib fed control animals in any brain area measured in gamma-amino butyric acid, an important component of the adrenergic, neurotransmitter system. In general, the enzyme studies show that the effects of dietary restriction are selective rather than non-specific, and that the behavioral findings predict the neurotransmitter systems most likely to be affected.

Also included in this series of biological studies are a number of experiments designed to identify some of the physiological correlates of age-differences in memory. One study compared young (6-8 mos.) and old (26-28 mos.), C67BL mice on a test of short-term memory, performance in a radial maze. Interestingly, no age differences were seen even though previous studies in this laboratory had found age differences in this same task in rats. It will be interesting to explore this species difference further.

Another study using rats compared young (8-10 mos.) and old (26-28 mos.) animals both in spatial memory (where success or failure on the task is based on the animal's ability to remember in which direction the T-maze is oriented) and working memory (where success or failure on the task depends upon the ability of the animal to remember what its response was on the previous trial). Previous research in this laboratory had shown that young animals can solve this task, and that young animals with lesions in the pathways connecting the septum and hypothalamus were impaired in the working memory portion of the task, but not in the spatial memory component. The present study showed that old animals were impaired in both components of the task, indicating that age-differences exist in both spatial and working memory.

In an effort to develop pharmacological agents to treat memory deficits in older person, psychopharmacologists have developed a number of animal models of memory. One such model is the passive avoidance task in which an animal is given foot shock on a grid floor when it steps off of a lighted perch into a darkened box (rats are negatively phototropic and will always go from a lighted area to a darkened area). The memory test occurs when the animal is put back into the apparatus 24 hours later, its latency to step off the perch is measured: long latency implies good retention. This animal model frequently

is used to test the effectiveness of drugs which are supposed to enhance memory: if the drug is given immediately after the animal steps off the perch on the first trial, and on retesting animals which received the drug are found to have a longer latency than control animals, it is inferred that the drug enhanced memory. One, major, implicit assumption in this test is that the drug does not affect motivation since, it it does, it becomes difficult to separate the effect of the incentive from pure memory. One drug which has received a large amount of attention from pharmacologists is arginine-vasopressin, because a number of investigators have reported that it can enhance memory in the passive avoidance task. Research completed in this laboratory this year has shown that this drug may have strongly aversive properties which could account for its putative memory enhancing effect. The research showed that animals which were injected with the drug but which did not receive shock in the darkened chamber had a longer latency to step down from the perch. Thus, the drug probably acts like an aversive stimulus, and its affect on performance may be due to it acting additively to the shock rather than to any memory enhancing effect.

Age Processes. A number of researchers have argued that social supports are important moderators of the effects of stress and major life crises on physical health or psychological well-being. This issue has been especially important for gerontologists since, as persons grow older, they are at risk to lose social supports. Therefore, they could be more vulnerable to various stressors. Studies in this laboratory have looked at several indices of social support from the Baltimore Longitudinal Study of Aging to see if there is any evidence that these changes appreciably as subjects grow older. Utilizing a sample of about 1000 men who ranged in age from 18 to 98 yrs, it was possible to define two factors of social support from an activities and attitudes questionnaire. Longitudinal analyses were based on a cohort of about 400 men who were retested at 6 years and a smaller group of about 170 of these men who were tested again after 10 years. The analyses revealed first, that social support was consistent for the men who were retested once or twice; and second, that the mean levels of social support did not change over time. These findings suggest social supports can remain very stable over time, and that loss of social support may not necessarily be an age-specific mediator of life stress.

In addition to the psychometric studies of aging carried out by LBS investigators in the Baltimore Longitudinal Study of Aging, there also are a number of experimental studies of age changes in cognitive performance. Several of these have looked at age changes in problem solving ability. While those studies have consistently shown that problem solving ability declines late in life, the longitudinal changes have been smaller than the estimates of age changes based on comparisons of men born at the same time but tested at different times. This difference usually was attributed to an attrition effect, since within-cohort comparisons are based on everyone available at the time of testing, whereas longitudinal results only can come from persons who are either healthy enough or who have survived long enough to be retested. Thus, longitudinal studies will tend to underestimate maturational effects. A statistical analysis which included only men who were retested revealed that the attrition effect could not account for the discrepancy between longitudinal results and within-cohort estimates of age change. The findings suggested that estimates of age change based on within-cohort regression analyses may be more accurate than longitudinal changes, and suggested further that even when subjects are retested after 7 years, they may still show some benefit from a prior experience with the task

(it should be noted that the specific items were not repeated, only the tasks).

Investigators of memory know that different tests of memory tap different skills and yield different age effects. For example, recognition tasks tend to show small age effects while recall tasks show large age differences. These differences between recall and recognition have been interpreted as evidence for age deficits in retrieval. But there is a problem in comparing the magnitudes of age differences on different tests. a statistical technique was developed this year, using data from the Baltimore Longitudinal Study of Aging, which may enable investigators to surmount this problem. The technique compares the linear regression of recognition on recall and also the regression of recall on recognition among a number of age groups. If there is an age-related deficit in retrieval, then the slope of recognition on recall should be larger for older groups since more material will be recognized per unit item recalled. This model was applied to data from the Baltimore Longitudinal Study on Aging, and the results confirmed the model. It now should be possible to apply this method to results from several other studies.

Most of the research in the literature on complex, cognitive behaviors such as concept problem solving are based on group studies. The findings are useful in describing group differences; however, they are of limited value in characterizing the performance of an individual. Several years ago a model was developed by Herbert Simon and his colleagues to use in the development of computer-simulated models of concept problem solving. This model uses a technique called, "thinking aloud: to help in the analysis of the problem solver's behavior, and in the synthesis of explanatory models to characterize the underlying behavioral mechanisms. To utilize this model most effectively, one must study the performance of a single individual extensively. Studies completed this year in LBS utilized this model to study age differences in problem solving tactics. Three men, aged 17, 63 or 96 years participated in the project. Each subject was studied over several months, while he attempted to solve each of more than 100 logical problems. Clear age differences in strategies became appparent. The 63 year old man and the 17 year old used similar inferential processes which relied heavily on retaining a large amount of information in memory. However, the 63 year old man utilized a number of heuristic strategies which enabled him to accomplish comparable problem solving results while minimizing the number of items he had to remember. The 96 year old subject displayed a significantly reduced capacity to retain information. Consequently, his inferential process was much more simplified than was that of the younger men. The findings indicate that the differences in inferential processes which the three men used to solve the problems could be attributed to their differences in memorial capacities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-AG 00062-10 LBS

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Daydreaming and Aging: Normative and Experimental

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Leonard M. Giambra, Research Psychologist, LBS GRC NIA

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning & Problem Solving Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	.8	.3

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this work is to determine the parameters of spontaneous-thought-intrusions (daydreaming) and related mental activity such as insight, intuition, mindwandering, and curiosity. This is accomplished through the use of retrospective questionnaires and experimental manipulation. Topics of present interest are: (a) the lifespan changes in daydreaming in a longitudinal sample, and (b) the relation of daydreaming incidence to tasks of varying vigilance requirements.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel engaged on the project:

Reginald Quilter	Electronics Tech.	LBS GRC NIA

Project Description:

Objectives: The goals are: (1) to determine the incidence and content of daydreaming in specific subpopulations (e.g., young, middle aged, elderly) from various socio-economic classes, with various origins, etc; (2) to attempt to relate these differences in daydreaming to any underlying mechanisms such as physiological state, education, cultural values and beliefs, differential daily experiences, and (3) to investigate experimentally variables which normative studies have indicated may be potent determiners of daydreaming.

Methods Employed: The normative aspects of daydreaming are determined through the use of a structured self-report. Each participant completes a 21 item biographical questionnaire and a 344 item Imaginal Processes Inventory (IPI) which has both specific and general items concerning daydreams, nightdreams, fantasies, etc. There are 33 factors in the IPI. Each item has five choices which are points on a continuum implying frequency or quantity. The choices were assigned values of 0, 1, 2, 3, or 4.

In a vigilance task, the subject must detect changes in visually or auditorially presented material. For example, the subject may see a stimulus designated "A" flash on a projection screen at a specific rate; sometimes, however, another stimulus designated "B" will occur instead of A. The subject's tasks are to detect and report when B appears and to report, by means of a button press, the occurence of all task-unrelated-thought intrusions. The proportion of B's can be controlled as well as the number of stimuli presented per minute and the interval during which stimuli remain in view. These factors determine the difficulty and tediousness of the vigilance task and are expected to be related to the incidence of daydreaming.

A second example of a vigilance task used is the Mackworth Clock (MC). The MC task is of one hour duration. The participant is told to monitor a clock-like pointer which moves in discrete steps once every second. After relatively long, varying periods of time, the clock's pointer describes a continuous double step in the one second period. The participant is asked to press a push-button, as quickly as possible, as soon as the "double jump" is perceived.

I. Vigilance and daydreaming incidence.
The first vigilance task described above is being used to measure directly the incidence of daydreaming and mindwandering for people across the lifespan. A life-span sample is being used since the self-reports of subjects, based on the Imaginal Processes Inventory, suggest that with increasing age there is an increased ability to concentrate on any task without the intrusion of daydreams. A series of studies investigating the effects of various parameters of the vigilance task and of the subjects themselves on the frequency of daydreaming and mindwandering has begun. The first study varies the length and tedium of the vigilance task. In this study so far, 274 longitudinal have participated. They ranged in age from 19 to 90 years old. A second study replicates the first study in design but involves asking the participants to

indicate after ever 15 seconds if the previous 15 second interval contained a task-unrelated-thought intrusion. At this point 96 individuals 17-90 years of age have participated in this study. Data analysis is anticipated to begin on both of these studies in 1984.

II. Mackworth Clock longitudinal study of vigilance.
The Mackworth Clock vigilance task is being used in a longitudinal study of subjects who participated in the identical study in 1962-1964. Reaction time and skin potential are measured. These same subjects also receive the other vigilance task allowing correlations to be made among the present tasks as well as with tasks performed in 1962-1964. A total of 57 men have participated who participated in 1962-1964; of these 16 participated a third time on a subsequent visit. In addition 157 men aged 17-92 and 79 women 17-84 have participated for the first time; of these, 13 men and 2 women participated a second time 2 years later.

III.A longitudinal study of daydreaming and related mental activity.
Starting in 1979, men in the Baltimore Longitudinal Study of Aging again began completing the IPI. Presently 332 men who took the IPI 6-8 years ago have been retested and also 222 men have been tested for the first time. This data collection will continue until the entire BLSA population of men has been completely tested or retested. In 1980 the women in the BLSA began to take the IPI. Presently 173 women have taken the IPI. A subsample of these women participated in a separate study in 1975 and therefore represent a longitudinal repeat subgroup. A second sample of 130 women tested in 1975 were retested. In addition, 51 males aged 17-28 years and 62 females 17-22 years were tested at Towson State University. Data analysis is anticipated to begin in 1983.

Proposed Course of Project

Since many of the present experiments are of various stages of data collection stage and since available resources in terms of personnel and equipment are being fully utilized, no additional studies are being planned for the next fiscal year.

Publications

Giambra, L. M., & Stone, B. Australian-American differences in daydreaming, attentional processes, and curiosity: First findings based on retrospective reports. Imagination, Cognition, and Personality: The Scientific Study of Consciousness, 1982-83, 2, 23-35.

Giambra, L. M. Daydreaming in 40 to 60 year old women: Menopause, health, values, and sexuality. Journal of Clinical Psychology, 1983, 39, 11-21.

Quilter, R. E., Giambra, L. M., & Benson, P. E. Longitudinal age changes in vigilance over an eighteen year interval, Journal of Gerontology, 1983, 38, 51-55.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00063-16 LBS

PERIOD COVERED

October 1, 1982, to September 30, 1983

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

Learned Modification of Visceral Function in Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Bernard T. Engel, Ph.D., Chief, LBS

COOPERATING UNITS *(if any)*

NIDA, CPB, LNS, Medical University of South Carolina, Johns Hopkins University School of Medicine, Lederle Labs. New Jersey.

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Psychophysiology

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Res. Ctr., Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.9	2.95	7.95

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this project is to investigate the role of the central nervous system in behavior. In some experiments monkeys (Macaca mulatta) are used to examine the extent to which the cardiovascular system can be modified by instrumental conditioning. In other experiments we examined age-related behavioral changes in rats and mice in a variety of tasks, including motor performance, thermoregulation, learning and memory. Biochemical and electrophysiological methods assess neurobiological correlates and mechanisms of age-related behavioral dysfunctions. Studies are also conducted which attempt to modulate behavioral aging through pharmacological, nutritional, and environmental manipulations, including use of cholinergic agonist drugs, dietary restriction, brain stimulation and exercise.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other professional personnel engaged on the project:

J. A. Joseph	Research Psychologist	Lederle Labs. N.J.
G. S. Roth	Research Chemist	CPB, GRC, NIA
D. Ingram	Senior Staff Fellow	LBS, GRC, NIA
M. Talan	Visiting Associate	LBS, GRC, NIA
E. London	Research Pharmacologist	NIDA
S. Waller	Staff Fellow	LNS, GRC, NIA
L. D. Middaugh	Psychologist	Med. Univ. of S.C.
D. Olton	Professor of Psychology	JHU, Dept. of Psychology
N. S. Buckholtz	Pharmacologist	Med. Univ. of S.C.
R. Cutler	Biochemist	CPB, GRC, NIA

Project Description:

Objectives:

A-B. To determine the neural mechanisms involved in cardiovascular conditioning in monkeys.

C-J. To analyze age differences in biochemical and structural characteristics of the brain of the rodent in relation to behavior.

Methods Employed:

A. The present experiments were designed both to examine the interrelationship between the cardiovascular system and behavior during electrical stimulation of the brain (ESB), and to carry out a thorough mapping of the cardiovascular control in the subhuman primate. Monkeys were operantly conditioned to speed and to slow heart rate (HR) in sessions comprised of 256 sec baseline period and 1024 sec testing period. Three types of sessions were examined: "Speeding sessions" (F) during which the animal was operantly trained to speed its HR, "slowing sessions" (S) during which the animal was trained to slow HR, and "no feedback sessions" (NF) in which the animal was not required to speed or to slow HR. It simply sat in closed booth throughout a 256 sec baseline period and 1024 sec "training" period. Information on speeding and slowing was provided by lights. A red light indicated that the animal was to slow HR and the green light indicated the speeding condition. A white light functioned as a reinforcement light to indicate to the animal that it was performing correctly. A 10 ma, .45 sec electric shock was delivered to the tail once/8 sec for incorrect responding. Systolic (SBP) and diastolic (DBP) blood pressure were recorded during these sessions from chronically indwelling catheters in the external iliac arteries of the monkeys. The BP signal was electrically conditioned to permit the measurement of HR. The sessions were divided into 16 segments of 64 sec duration. Following training, an acrylic platform containing holes drilled to pre-specified anterior-posterior and lateral coordinates approximately corresponding to those of several different brain regions was stereomounted on the monkey's skull and cemented in place under aseptic conditions. Following a 1 week recovery period, after .5 mg/kg of phencyclidine injection, electrodes were passed through the skull and lowered by hand until a (see below) cardiovascular effect was obtained. When a suitable area was found, the lowest intensity was chosen that reliably would produce a HR change of 10-20 bpm and

comparable BP change. The electrode was then cemented in place. Intensities of stimulation usually ranged from 350 to 1000 ua depending upon the animal and the area being stimulated. Cardiovascular responses were then examined during S, F, and NF sessions carried out as previously described. Stimulation for each session was delivered on alternate segments. One area was examined under all 3 conditions per day. Data were analyzed by examining changes from baseline during each stimulation and non-stimulation segment. Additional methodological development was carried out in order to enable us to study brain stem sites and to increase the precision of electrode placement. For this purpose the platform mounted on the animal's skull was modified to permit us to attach it to a stereotaxic apparatus as needed.

B. In a related series of experiments we have been examining the constraints on physical exercise produced by instrumental cardiac conditioning. In these experiments a monkey was trained to pull a lever attached to a weight box containing 6 - 8 kg, in order to avoid an electrical shock (10 ua, .45 sec) which was delivered to the tail on a fixed interval schedule (usually every 4 sec). Clicks were delivered every .5 sec starting 2 sec after the last pull to indicate that animal is to pull or is to receive punishment according the fixed ratio being established. HR, SBP, DBP, number of lever pulls, O2 consumption and CO2 production were measured during each session. Once stable exercise rates were achieved, each animal was trained as previously described (A) to slow its HR. Once monkeys were slowing HR reliably, exercise and HR slowing sessions were combined so that the monkey had to pull the lever and slow its HR. The cardiovascular parameters as well as O2 consumption and CO2 production were recorded.

C. The age-related performance of male C57BL/6J mice has been analyzed in a battery of motor tasks including grip strength, tightrope, rotorod, exploratory activity, and runwheel activity measurements. Previous studies have focused on the reliability and construct validity of this battery. Presently longitudinal analysis is being conducted on a large cohort (N=150) of aged mice (24 mo) to determine the predictive validity of the tests, that is, whether the test scores correlate with age-related change in scores across intervals of 6 mo and ultimately with lifespan.

D. Studies were conducted to assess the effects of purported memory-enhancing drugs on the performance of male rats in step-through passive avoidance conditioning. From lifespan representative age groups, animals are placed in one side of a two-sided compartment. Lights are activated on that side and a door is opened to a dimly lighted compartment. When the animal enters the adjacent chamber, the door is closed and electric foot shock is delivered. The animal is removed and tested for retention of this learning at various intervals.

Young (6-8 mo) and aged (26-28 mo) male C57BL/6J mice and female Wistar rats are also being tested in a complex 14-unit T-maze. Some groups have now been tested in an automated version of this maze which detects movement by use of infrared detectors and computer scoring of errors. Animals are first adapted to food or fluid deprivation schedules and trained to run for food or water rewards in a straight alley. The animals are then given one daily trial in the complex maze over a period of 28 days with entries into arms deviating from the path to the goal box counted as errors; and the time to negotiate the maze is recorded.

During each trial a food or water reward is provided in the goal box followed by 2 hr of ad libitum access to food or water in the home cage. At the end of training, the animals are sacrificed, and bio-assays are conducted to determine neurotransmitter synthetic enzyme activities-choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD), and tyrosine hydroxylase (TH) in the hippocampus and several discrete regions of the cerebral cortex.

Young (6-8 mo) and aged (26-28 mo) male C57BL/6J mice have also been tested in radial maze performance. The animals are first adapted to a 22-hr fluid deprivation schedule and then trained to run in a straight alley for water rewards. During 6 daily trials, the animals are placed into the center of the radial maze and allowed to freely explore among eight arms radiating from the center. A water reward (0.5 ml) is placed at the end of each arm; thus, the optimal strategy is to enter all arms of the maze without re-entering arms, which constitutes an error. This paradigm was designed as a test of the mouse's working memory and, as such, employed elements of short-term memory to a much greater degree than in the 14-unit T-maze, which stressed long-term memory. At the end of training, the mice were sacrificed and bioassays were made to determine the activity of TH, CAT, and GAD in the hippocampi and several discrete areas of the cortex.

Young (8-10 mo) and aged (26-28 mo) male ACI rats have been tested in a T-maze that requires components of working memory and spatial memory. Hunger motivation is used in this task by reducing the body weight of rats to 75% of baseline and providing diet at a level to maintain this weight throughout the study. Food rewards are provided in the goal box of the maze. Two T-mazes are used and are located in different areas of the room with different visual cues available. The rat is required to learn a left-right discrimination in the stem of the T-maze, with only one side being correct (not a cul-de-sac), depending upon which maze the animal is being run. This component of the task taxes spatial memory, i.e., remembering always to go right or left based upon the maze's location in the room. Upon reaching the choice-point of the T-maze, the animal is required to alternate between left and right arms of the maze, regardless of the location of the maze. Only one arm is baited with the food reward for the first trial; and on the next trial the other arm is baited and so on. This component taxes working memory, i.e., remembering where the response was made on the previous trial. The rats are run 20 trials per day with runs randomized but balanced across mazes. Previous research has demonstrated impaired performance of rats with lesions in pathways connecting the septum and hippocampus compared to unlesioned controls in the working memory component of this task, but not in the spatial memory component.

E. Animals were subjected to various nutritional and exercise regimens at different stages in their development to determine the effects of these treatments upon lifespan, to find developmental correlates of lifespan, and to assess behavioral vigor at advanced ages. Nutritional manipulations include level of dietary protein (4%, 26%, or 48%) and schedule of 24% protein feeding (ad libitum vs. every-other-day), and restricting feeding to maintain specific weights (60% or 85% of baseline). The exercise regimen involves housing the animals in activity-wheel cages, thus allowing voluntary exercise. Subjects include male and female Wistar rats, several strains of male inbred and hybrid mice, and male obese mutants. Behavioral assessments are made of the effects of the

treatments, including tests of exploratory and runwheel activity, grip strength, tightrope, rotorod, passive avoidance learning, food intake, metabolic rate, and body temperature. In addition, biochemical comparisons are made, including neurotransmitter synthetic enzyme activities, neurotransmitter receptor concentrations, and the rate of lipid peroxidation.

F. This set of studies continues to analyze age differences in exploratory activity in response to drugs. Previous studies assessed the effects of opiate agonists. Current studies are assessing the effects of stimulants, specifically caffeine. Young (6-8 mos) and aged (28-30 mos) C57BL/6J mice were subjects for all experiments. Locomotor activity was assessed in oval runways enclosed in sound and light controlled boxes. A unit of activity was defined as ½ revolution as detected by infrared photodetectors. Mice were habituated to the apparatus and then injected subcutaneously with either saline or caffeine (5, 15, 30 or 45 mg/Kg). Activity was recorded at 5-min intervals over the next 3 hr. Recent evidence suggests that caffeine-induced changes in locomotor activity of rodents is mediated by its antagonist action on adenosine receptors. Therefore, adenosine receptor binding was determined in cortical, hippocampal, striatal, and cerebellar tissue from mice in the above age groups using N^6 -cyclohexy [^{3}H] adenosine ([^{3}H] CHA) as the ligand.

G. Previous studies have indicated that uric acid is a potent antioxidant that may have served as a longevity-determinant process in the evolution of mammals by providing protection against free radicals. Uric acid sparks behavioral interest because it is an end-product of purine metabolism and has been hypothesized to act pharmacologically as a stimulant. We have assessed the possible behavioral effects of uric acid in several ways. First, a review of the literature comparing the performance of primate species in learning tasks was made, and these differences were correlated with literature values and values determined by assay for serum and brain levels of uric acid. Second, we injected oxonic acid and prepared diets of 3% oxonic acid, a compound which retards degradation of uric acid, and examined effects on exploratory and wheel activity of young (8 mo) male C57BL/6J mice. Third, we examined age differences in the plasma levels of uric acid in male C57BL/6J mice (4, 8, 12, 18, 24, 28 mo) and related these to age differences in exploratory activity. Fourth, we examined mouse strain differences (C57BL/6J, A/J, AKR/J, BALB/cJ, 129/J, DBA/2J, and B6D2F_1/J) in plasma urate levels and related these to differences in exploratory activity.

H. It has been reported previously, that old mice are not able to withstand cold exposure as well as adult animals. A cold stress test which demonstrates the differences in thermoregulatory ability between old and adult mice was described in the literature and used in our pilot study. This test consisted of a number of factors in addition to cold exposure, such as preliminary 20-hr food deprivation, repeated insertion of thermoprobes in the rectum and motor restraint. The present study was designated to determine the significance of each of these factors and to develop a convenient test of thermoregulatory ability in mice.

The objective of Experiment 1 was to assess age differences in cold tolerance when gross motor restraint was imposed. Adult (8 mo old) and aged (30 mo old) male C57BL/6J mice were divided to two groups and exposed for 3 hr to 10° C. The animals were placed individually in plastic cages (group 1) or plastic

restrainers with an internal diameter of 3 cm (group 2). The colonic temperature was measured every 30 min. The slope of the temperature over time was calculated for every animal. The cold tolerance of the groups was compared by calculation of ANOVA of individual slopes.

The purpose of Experiment 2 was to assess the significance of preliminary food deprivation on cold tolerance. Each of 8 adult and 8 aged mice was tested in 4 different experimental conditions: (1) 3 hr restraint at room temperature; (2) 3 hr restraint after 20 hr food deprivation at room temperature: (3) 3 hr restraint at 10°C; (4) 3 hr restraint at 10°C after 20 hr food deprivation. The sequences of experimental conditions for each animal was counterbalanced using latin square design. Temperature measure and computation procedure was the same as described in Experiment 1.

In Experiment 3, the method of temperature measurement was evaluated to determine its importance for results of the cold test. Two groups of aged mice were tested twice in the cold stress with a 1-week interval using thermoprobes permanently inserted throughout the test in one group and repeatedly inserted in another group. During the second test the conditions were reversed.

I. In a related study, 80 male C57BL/6J mice of 8, 13, 15, 22, and 30 mos of age were subjected to the cold stress test every 2 weeks. The protocol of the cold stress test and computation procedure was described above.

J. Previously we reported that electrical intracranial self-stimulation (ISS) of so-called "rewarding" areas of the hypothalamus retarded the deterioration of cold tolerance among aging C57BL/6J mice. The objective of this study was to determine whether enhanced motor activity associated with ISS might account for increased cold tolerance as the results depend on electrical activation of specific brain sites. Two groups of 30-mo old animals underwent the implantation of concentric electrodes in the vicinity of the medial forebrain bundle. After one week of recovery the animals were tested on their ability to perform ISS after which the first cold stress test was conducted. After testing one group of animals received 3 weeks of daily 30 min sessions of ISS, while mice from another group served as yoked controls. Every time the animal from the first group pressed the lever receiving ISS, its yoked partner received the same electrical stimulation of the brain. In 3 weeks, all mice were retested in cold stress. in another experiment one group of aged mice was housed singly in standard cages and served as a control while another was housed in singly in standard cages but received forced treadmill exercise (daily 60 min sessions at 5m/min); and another group was housed singly in activity-wheel cages and voluntarily exercised at a daily rate of 1.5 m/min.

MAJOR FINDINGS:

A. The effect of ESB on HR, SBP and DBP during S, F or NF trials was analyzed in one monkey from three brain sites in the anterior hypothalamus (AH). The results which were obtained from one site supported our previously reported findings. The HR increase to electrical stimulation of this site during S (11.8 beats/min) was similar to the HR increase to ESB during NF (9.9 beats/min) and during F (9.6 beats/min). The pattern of changing in BP during S, F, and NF trials was the same. Therefore, the animal was not able to attenuate HR during

ESB from this point of AH. However, the preliminary data from another site suggested, that the monkey may be able to adjust the decelerating effect of electrical stimulation of some sites of AH. The HR decrease to electrical stimulation of this site during speeding sessions (-3.81 beats/min) was significantly less than the HR decrease to ESB during NF (-9.24 beats/min). The special device was developed to attach the stereotaxic apparatus to the monkey chair and to the platform premounted on the monkey's skull. This allowed us to implant the electrodes stereotaxically without deep anesthesia.

B. The results obtained from one monkey showed that under conditions of the combination of the S trial with exercise (C), HR increase was significantly less than during exercise alone (E). Data from 37 E and 37 C sessions revealed the following: During C the monkey made significantly fewer pulls than during E (22 per 64 sec vs 31); HR during C was reliably lower. During E HR increased 40.4 beats/min from baseline while during C HR actually decreased; 0_2 consumption was not different in these two conditions, while double product (HR x SBP) and CO2 production was less during C trials. The slope of HR over number of pulls (work performed) was not different in both conditions. However, the slopes of HR over oxygen consumption and over CO2 production were significantly steeper during E trials. All these findings indicate that the monkey had much more efficient cardiovascular performance during conditions when physical exercise was accompanied with cardiovascular conditioning.

C. Preliminary analysis of two longitudinal studies indicates that the motor behavioral battery can be used to predict lifespan of 24-mo old C57BL/6J mice. In the first study, a combination of behavioral variables applied in a multiple regression could account for over 50% of the variance in lifespan. In the second study of 24-mo old mice, a longitudinal decline in performance was noted in exploratory activity, wheel activity, grip strength, and tightrope scores. The rate of decline across age could be used to predict lifespan. A marked decline in performance was indicative of impending death.

D. Using sc injections of arginine^8-vasopressin (AVP) in young (3 mo) male Fischer-344 rats in doses ranging from 6.8)g/rat to 27)g/rat, we have found a dose-dependent increase in 24-hr latency in a passive avoidance task, indicating that the drug might have some memory-enhancing properties. Further experimentation, however, has indicated that the drug might have aversive qualities that enhance conditioning through a behavioral rather than a purely pharmacological mechanism. Specifically, when rats are injected with a high dose of AVP (27)g/rat) immediately after stepping into the darkened box in the absence of shock, their 24-hr latencies increase significantly over that of vehicle injected controls. Such findings indicate that researchers should exercise precaution when ascribing enhanced performance to purported memory-enhancing drugs.

In collaboration with the laboratory of Dr. David Olton at the Johns Hopkins University, further investigation was made of age differences in performance of complex maze tasks. An earlier study in our laboratory had found that aged Wistar rats exhibited performance deficits in a 12-arm radial maze compared to young counterparts, which suggested an age-related impairment in short-term memory processing that Dr. Olton's laboratory had observed in rats with experimental lesions in septal-hippocampal connections. Our new study found no

significant age differences in the performance of male C57BL/6J mice in an 8-arm radial maze. Whether these negative results reflect species differences or methodological differences is not clear at this time. Correlational analysis revealed that correct responding in the maze was positively related to CAT activity in the cingulate cortical region but negatively related to CAT activity in the sensory-motor cortex. These findings were relevant to two previous observations. Regarding the positive correlation between performance and cingulate CAT activity, previous research at the GRC has identified this brain region as highly sensitive to increased glucose metabolism induced by cholinergic agonist drugs in rats. Regarding the negative correlation between performance and sensory-motor CAT activity, previous research in our laboratory has identified a relationship between motor performance and cortical CAT in aged mice.

Also in collaboration with Dr. Olton, consistent age differences were found in the performance of male ACI rats in the two-component T-maze task. Moreover, the aged rats were equally impaired in both the spatial and working memory components of the task. In contrast to the opposing findings in mice, these findings suggest age-related impairments in more than one type of memory processing in rats but not in mice. Following training, the brains were removed and dissected into the hippocampus (HIP) and the following cortical areas: cingulate (CIN), sensory-motor (SM), occipital (OC), frontal (FRO), auditory (AUD), and pyriform-perirhinal (PYR). The activities of L-glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT) were assayed in these regions, as was muscarinic binding. The pattern of correlation between maze performance and neurochemical parameters differed between age groups. Among young rats, correct responding in the stem and arms was positively related to CAT in the HIP, FRO, SM, and CIN, and to muscarinic binding in the AUD and PYR, but negatively related to muscarinic binding in the OC. Among aged rats, correct responding in the arms was positively related to muscarinic binding in the SM, but negatively related in the FRO.

Pilot studies have been conducted in a new 14-unit automated T-maze which replicate and extend previous observations. The learning rate of rats matches that previously observed. Moreover, the automated maze has permitted assessment of the role of visual cues in maze learning. Groups of young Wistar rats have been run in the dark. Their performance was not different from that of young rats run in lighted conditions. Therefore, it appears that visual cues are not important to efficient learning in this maze, which is an important observation bearing on further behavioral analysis of the age difference in performance observed in this task. The performance of young and aged C57BL/6J mice has been studied in response to water rewards in the automated maze. The age deficit matches that previously observed. Furthermore, preliminary analysis of the pattern of errors suggests it is very congruent to that observed previously in a smaller maze of the same configuration but with very different external and internal cues available. This similarity in results suggests that the mice are using a response strategy, probably an alternation strategy, to learn the maze rather than relying upon specific sensory cues.

E. Male C57BL/6J and $B6AF_1$ mice, maintained on a schedule of intermittent feeding (every-other-day) since 6 mo of age, were tested in a motor behavioral battery, and their performance was compared with groups maintained on ad libitum diets. Compared to the ad lib diet, the restricted diet results in about a 30%

decrease in food intake and body weight. Groups tested in the behavioral battery included young (7 mo) and aged (26-28 mo) animals. Preliminary analysis suggested that aged mice on the restricted diet showed a retardation of the age-related decline in activity in run-wheels and performance in a tight-rope and rotorod tests, requiring strength and coordination to remain suspended from a taut string and to remain on a rod rotating at 3 rpm, respectively. However, these results may be due partially to differences in body weight and/or motivational factors because the differences were partially offset when they were compared with the performance measures of groups of aged animals that had been on the restricted diet for only one month or had been on the restricted diet since 6 mo of age and then shifted to an ad lib diet 1 mo before testing.

In collaboration with Dr. E. London of NIDA and Dr. S. Waller of the LNS, GRC, preliminary analysis of several new neurochemical parameters have been made in 24-mo old male Wistar rats on the intermittent diet since weaning and compared to counterparts on the ad lib diet. In the striatum, choline acetyltransferase (CAT), the synthetic enzyme of acetylcholine, was found to have a significantly higher activity among restricted animals compared to controls, and the concentration of cholinergic receptors, as measured by QNB-binding, was found to be higher in this brain region among the restricted animals as well. CAT was also significantly higher in the hippocampus of restricted animals; however, there was no significant difference in cholinergic receptor concentration in this region, but affinity for the receptors was higher among the restricted animals. In the cerebellum, CAT activity was also higher among restricted animals, but the affinity of the receptors was lower compared to ad lib animals, and there was no significant difference in receptor concentration. In the cerebral cortex, no significant difference between restricted and ad lib groups in CAT activity was observed, although receptor concentration was higher among restricted animals, with no difference in affinity. The activity of tyrosine hydroxylase (TH), the adrenergic synthetic enzyme, was found to be lower in the cerebral cortices of restricted rats compared to controls, but did not differ in other brain regions examined including the striatum. Finally, no differences between restricted and ad lib groups in the activity of glutamic acid decarboxylase, the synthetic enzyme for gamma-amino butyric acid (GABA), were found in any brain region examined. The results indicated that dietary restriction selectively affects parameters of chemical neuro-transmission in aged animals. One brain region in which the effects seem to be very beneficial is in the striatum. In collaboration with Dr. G. Roth of LCP, GRC, and Dr. J. Joseph of Lederle Laboratories, we have replicated and extended our previous observations on the effects of dietary restriction on the age-related loss of striatal dopamine receptors. A regimen of intermittent feeding since weaning in male Wistar rats resulted in higher dopamine receptor concentrations at 12 and 24 mo of age but not at 3 mo compared to ad libitum fed counterparts. Two weeks of intermittent feed in 24-mo rats fed ad libitum throughout life did not produce an effect on receptor concentrations. Thus, the effect on this neurobiological parameter appears to result from a chronic regimen of intermittent feeding. Finally, preliminary analysis of lipid peroxidation in the brains and livers for 24 mo old, Wistar male rats on intermittent feeding suggests that their tissues are susceptible to autoxidation than those of ad libitum-fed counterparts.

Previous reports from this laboratory have indicated that a regimen of intermittent feeding could not increase lifespan when introduced at 10 mo of age in

mice. In fact, in some strains this regimen was detrimental to survival. A new survival study was instituted which applied a different restriction regimen to alleviate what might have been the stressful effects of an intermittent diet observed previously. Over a 6-week period, the food intake of 24-mo old C57BL/6J was reduced gradually to attain a body weight of 85% of baseline weight. This body weight is being maintained, and the survival of these animals (n=30) is being compared to ad libitum fed controls. Thus far, the mortality rate in the restricted group has been 24% versus a rate of 41% in the control group.

F. In collaboration with Drs. L. M. Middaugh and N. Buckholtz of the Medical University of South Carolina, we have found that the exploratory activity of both young and aged C57BL/6J mice was elevated by the 15 mg and 30 mg/Kg doses of caffeine; however, the extent of elevation above pre-drug levels was greater for aged than for young mice. This was particularly evident at later time periods when ratios of post- to pre-injection activity counts ranged from 1.5-2.0 for aged mice compared with 0.8-1.0 for young mice. In spite of the greater degree of activity elevation for the aged group, the time course for elevated activity was similar for both groups. For both age groups, the greatest amount of [^{3}H] CHA binding occurred in hippocampus and cerebellum. Binding did not differ according to age in the cortex, hippocampus, or striatum; however, it was 15% higher in cerebelli of aged than young mice. Although young and aged mice appear to differ in their reaction to caffeine and in [^{3}H] CHA binding, whether or not the two age differences are functionally related remains to be determined.

G. In our literature survey, we have found that serum and brain levels of uric acid are directly correlated with good performance in learning tasks among different primate species. In our mouse studies in collaboration with Dr. R. Cutler, CPB, GRC, we have found no evidence that uric acid acts as a behavioral stimulant. Injections or diets of oxonic acid, which retards degradation of uric acid, have diminished exploratory and wheel activity. We found that the level of plasma uric acid increased with age in C57BL/6J mice and was therefore negatively related to exploratory activity since activity declines with age. Finally, we found mouse strain differences in plasma uric levels with the following rank-order from highest to lowest: BALB/CJ > B6D2F$_1$/J > A/J > C57BL/6J > 129/J > AKR/J > DBA/2; however, again exploratory activity was found to be negatively related to these levels. Thus, we have found no behavioral data indicating that uric acid may be a behavioral stimulant; and, in fact, in mice it might be a depressant.

H. In Experiment 1 an age difference in response to cold stress was observed only in the restrained condition. There was a significant main effect for age ($F(1,34)=22.48$, $p<.001$) and for condition of experiment ($F(1,34)=9.55$, $p<.01$), as well as a significant interaction effect ($F(1,34)= 17.68$, $p<.001$). Analysis of the simple main effects demonstrated that differences between old and adult animals during the cold stress test were significant only for old mice under the restrained condition.

The results of Experiment 2 showed that food deprivation as well as cold exposure, was associated with a greater decrease in colonic temperature among old animals than among adults. However, there was no interaction between these two

conditions ($F(1,14)=1.61$, $p>.1$).

No difference between two groups of animals was found in Experiment 3, which proved that the method of measuring the colonic temperature did not contribute to the results of the cold stress test.

Therefore, the results of these experiments demonstrated that only the cold exposure itself was responsible for the observed age differences. Neither preliminary food deprivation nor the method of temperature measurement was critical for obtaining results. However, physical restraint during the cold test was important. On the basis of these findings, a protocol for the cold stress test was developed: An animal is restrained in a Plexiglas cylinder, 3 cm in internal diameter and 10 cm in length with multiple holes for circulation of air; the mouse is exposed to a temperature 10°C for 3 hr (unless colonic temperature falls below 24°C); colonic temperature is taken initially and at 30 min intervals; after testing all mice are placed under a warming lamp for 1 hr.

I. The analysis of the results of repeated cold stress testing of mice from different age groups indicated that all animals in the study improved their tolerance during the first 2 or 3 tests after which their response did not change with repeated testing. This finding is congruent with data existing in the literature. However, 30-mo old animals are an exception to this rule. Their ability to withstand cold exposure did not improve with repeated testing, but rather deteriorated. It also is noteworthy that the cold tolerance among 15 mo old animals was consistently worse than 22 mo old subjects. This was true for several cohorts of animals tested at different times of the year. The relationship between cold tolerance and age could best be described by linear regression. This linear regression was not significant for the first test, however, starting with the second test the correlation coefficient between age and cold tolerance became significant. A relationship exists also among age of animals, colonic temperature before the test and cold tolerance. The data are presented in the table below:

Test	Correlation coefficient (age - slope of temp.)	p	Multiple correlation coefficient (age - colonic temp. - slope)	p
1	0.12	ns	0.13	ns
2	0.33	<.05	0.44	<.05
3	0.29	<.05	0.30	ns
4	0.28	<.05	0.71	<.05
5	0.21	<.05	0.46	<.05
6	0.45	<.001	0.45	<.05
7	0.40	<.001	0.49	<.05
8	0.43	<.001	0.79	<.05

J. The ANOVA revealed no significant difference in cold tolerance between the animals undergoing ISS and their yoked-controls. Therefore, the retardation of the thermoregulatory ability, demonstrated previously in old animals experiencing ISS probably depends on the effect of electrical stimulation of specific areas of the brain. There were no differences in cold tolerance among groups of aged mice receiving forced, voluntary, or no exercise. Thus, enhanced motor activity could not account for the improvement in cold tolerance.

Proposed Course of the Project:

A. Data collection is continuing on the effects of stimulation of anterior and posterior hypothalamic pressor, depressor, cardioacceleratory and decelatory areas during three procedures, i.e., F, NF, and S. A specific effort will be made to obtain data from AH and PH on the same animal. The areas of mapping will be extended to determine if the effect of stimulation of low brain stem sites or septum could be altered by conditioning. Additionally, efforts will be made to determine the vagal and sympathetic contribution to these changes through the use of various peripheral adrenergic and cholinergic antagonists.

B. Two additional animals are now being trained. Studies of the control mechanisms underlying the learned control of HR during exercise will be implemented. These studies will include detailed analyses of pulmonary function; analyses of cardiovascular control systems--e.g., baroreceptor function; and analyses of the role of various neurotransmitters in the mediation of these effects.

C. Work will continue to improve the behavioral paradigms including reliability testing and predictive validity with respect to age-related behavioral change, lifespan, and neurochemical parameters. On the basis of the outcome of the correlational analyses, pharmacological treatments are being planned, including the use of cholinergic and dopaminergic agonists with specific interest in stimulants, such as caffeine and nicotine.

D. We will continue to refine the shock procedures to assess behavioral variables affecting this type of learning. Further studies will be planned to assess age differences in consolidation of memory in these paradigms and to assess the effects of pharmacological intervention upon the consolidation process with specific interest in cholinergic agonists. Studies will continue that use water deprivation as a motivator in maze tasks. At present, animals continue to be sacrificed to determine possible neurochemical correlates of maze learning. Based upon work to date which suggests cholinergic involvement in maze learning, we will begin a study of the effects of chronic oxotremorine treatment, a cholinergic agonist, on maze performance and upon brain metabolism and neurotransmission in cholinergic systems. Currently underway is a maze study of the chronic effects of Hydergine, which purportedly enhances brain metabolism. Studies will also continue in the radial maze paradigm. We are specifically interested in comparing the types of memory deficits observed in radial maze vs in the 14-unit T-maze. We will begin to determine if the type of brain lesions (transection of the fimbra-fornix) which produces deficits in the performance of rats in the radial maze will produce deficits in the automated 14-unit T-maze. Further behavioral analysis of age differences in performance in the automated T-maze will also be conducted, including a longitudinal study in which 12- and 18-mo old rats will be trained to run the maze and then tested at 6 mo intervals. In addition this year, we will begin to test rats and mice in their ability to learn the maze using shock motivation.

E. Continued assessment will be made to determine behavioral and physiological effects of nutritional manipulations at various stages of development. Collaboration has been maintained to continue to determine effects on cardiac functioning, neurochemical activity and receptor density in the brain and liver, and tissue levels of free radical scavengers.

F. Further work will be conducted to determine the effects of caffeine on motor performance and to determine its actions on adenosine receptors.

G. This project has been terminated.

H-J. Work will continue to attempt to uncover the mechanisms of age-related change in thermoregulation of mice. Special effort will be made to understand the phenomenon of temporary decline in cold tolerance among mice around 15 mo of age. A pilot study will be conducted with an attempt to correlate the level of hormonal activity across the life span with cold tolerance. Special attention will be given to the age-related change of thyroid function in connection with thermoregulation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00064-22 LBS

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Problem Solving and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

David Arenberg Chief, Learning and Problem Solving Section, LBS, GRC, NIA

COOPERATING UNITS *(if any)*

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	.5	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The primary purposes of this project on aging are to: (1) identify reasoning processes; (2) determine how these processes change with age; and (3) develop techniques for reducing age deficits in reasoning performance. Reasoning is studied using problem solving procedures including concept learning and concept identification.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel engaged on this project:

Leonard M. Giambra	Research Psychologist	LBS GRC NIA
Jan D. Sinnott	Towson State University	

Project Description:

Objectives: The general goals are to explore and identify reasoning processes in man, to determine in what ways these processes change with age, and to develop techniques for reducing age deficits in reasoning performance. In this project, reasoning is studied by using problem-solving procedures in which on-going solution behavior can be observed and quantified. Experiments are designed to answer such questions as: (1) Is effectiveness in acquiring relevant information affected by aging? (2) Is effectiveness in synthesizing available information affected by aging? (3) What kinds of solution strategies are used and in what ways are they related to age? (4) How does imposing a memory load affect solution strategies for young and old adults?

Methods Employed: Of the studies in progress, analyses of two have been carried out this FY. Experiment V is a longitudinal study of concept problem solving. Six different types of problems are used, and two problems of each type are administered. In each problem, the task is to identify one or two "poisoned foods." The selection paradigm is used; i.e., subjects select instances ("meals") and each selection is designated as positive ("died") or negative ("lived") by the experimenter. The task is to identify the poisoned food(s) with as few selections as possible. Each selection can be quantified, i.e., information obtained as a proportion of maximum information obtainable at that point in the problem. The mean of the proportions for all selections in a problem is the effectiveness measure. An overall measure of performance is the number of (the twelve) problems solved correctly. Experiment XI is a study of concept learning in which each subject solves about 100 complex problems. By using "thinking aloud" procedures for some of the problems, individual models of problem solving can be constructed. These models are microtheories which specify the memories, inferential processes, and control processes of individuals. When successful, a model can predict quite accurately the step-by-step behavior during the solution of other problems (without thinking aloud).

Major Findings: Data from Experiment V, the longitudinal study of concept problem solving, were analyzed to explore the effects of attrition. Direct measures of change in problem-solving performance were expected to underestimate true age declines especially in the older age groups. Attrition can occur because: (1) some participants leave the Baltimore Longitudinal Study of Aging or continue in the study but cannot participate in a repeat session (e.g., due to development of serious visual impairment); and (2) some participants begin the repeat session but their data are incomplete (e.g., subjects quit or we run out of time). Both of these sources of attrition were assumed to bias the mean changes positively, i.e., reduce the declines, especially in the older groups. In order to estimate the effects of attrition we used regression procedures developed in this Laboratory. Previously we had shown that the within-cohort estimate of age change for men born between 1887 and 1896 with concept-problem-solving measures obtained during the period from

1967 to 1976 was substantially larger than the direct measures of change for men of comparable age. These estimates of age change were derived by regressing first-time measures of performance on calendar time. The men in the 1887-1896 birth cohort were in their seventies in 1967 at the beginning of the study and in their eighties by the end of 1976. The slope of the regression line of performance on calendar time estimates the linear rate of of change per year for this cohort over this period of their lives. When all of the men in that cohort were included in the regression analysis of number of problems solved correctly, it was estimated that a decline of 1.7 problems would occur in seven years. However, longitudinally, the mean decline for men initially measured in their seventies and remeasured seven years later was only 0.4 problems. Some part of this discrepancy was ascribed to attrition. In order to be included in the longitudinal analysis, it was necessary to attempt all 12 problems initially, continue in the study, and attempt all 12 problems again seven years later. In order to be included in the regression analysis, it was necessary only to attempt all 12 problems the first time. It was possible to use the regression analysis with first-time data only for men who were included in the longitudinal analysis. By imposing the same criteria for inclusion in both the longitudinal and the regression analyses, we expected the discrepancy to be resolved; and then we could estimate the impact of attrition on our longitudinal results. The outcome was surprising. The estimate of decline using the regression procedure only with the men who met the criteria for longitudinal analysis was the same as for the unrestricted sample. Apparently attrition does not account for the small mean change in the longitudinal analysis. One possibility is that previous experience with the problems during the first session affected performance seven years later. If so, the estimates of age change based upon regression analyses may be more accurate than the direct measures of change longitudinally. It should be noted that the differences between the longitudinal results and the regression estimates of change are in magnitudes of decline. All of the evidence indicates decline in problem-solving performance late in life.

Models of individual's problem solving were constructed and compared for men 18, 63, and 96 years old. The models were derived from thinking-aloud procedures in Experiment XI. The models of the men 18 and 63 indicated similar inferential processes with the major difference in memory strategies. The 96 year old man had a much smaller memory capacity which could account for his simplified inferential processes. The primary differences in models apparently resulted from vastly different memory capacities of the three men.

Significance to Bio-Medical Research and the Program of the Institute:
Reasoning is among the most prized behaviors of man and among the most elusive for experimental study. In this project, methods have been and will be developed to obtain quantifiable measures of step-by-step performance on reasoning problems. Some of these methods also provide patterns of response which represent strategies in solving such problems.

Measures are obtained in current experiments to study changes in reasoning processes with age. These studies, in addition to identifying basic reasoning processes, should indicate the pervasiveness of reasoning deficits with age,

whether education and cognitive activity mitigate such deficits, and what techniques could be used to minimize decline in reasoning.

Proposed Course of Project: Data collection will continue for Experiment IV, a longitudinal study of logical problem solving (men), and for Experiment V, the longitudinal study of concept problem solving (both men and women). Additional models which describe and predict how an individual solves concept-learning problems will be developed in Experiment XI. Some models will be computer programmed if a fellow can be recruited to do this.

Publications:

Robertson-Tchabo, E. A., & Arenberg, D. Mental functioning and aging. In R. Andres, E. L. Bierman, & W. R. Hazzard (Eds.), Principles of geriatric medicine. New York: McGraw-Hill, in press.

Sinnott, J. D. Adult post-formal reasoning: The relativistic stage. In M. Commons (Ed.), Beyond formal operations: Late adolescent and adult cognitive development. New York: Praeger, 1983.

Sinnott, J. D. Do adults use a postformal "theory of relativity" to solve everyday logical problems? Human Development, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00065-23 LBS

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Verbal Learning and Age

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

David Arenberg Chief, Learning & Problem Solving Section, LBS, GRC, NIA

COOPERATING UNITS *(if any)*

Baltimore City Hospitals
University of Maryland, College Park
Towson State University

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	.4	2.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The primary purposes of this project on aging are to identify measures of learning and memory which change with age, to specify psychological processes and their relationships with age, and to develop procedures for improving learning and memory performance in the elderly.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel engaged on the project:

Elizabeth A. Robertson-Tchabo	University of Maryland, College Park
Jan D. Sinnott	Towson State University

Project Description:

Objectives: Primary objectives are: (1) to identify which aspects of learning and memory change with age (and which do not); (2) to specify psychological processes underlying such age changes; (3) to identify health, nutrition, biochemical, and personality variables which are correlated with performance or with change in performance; and (4) to develop procedures to improve learning and memory performance in the elderly.

Methods Employed: Experiments I, II, and XXXVII are studies in progress. Data collection continues, but no new analyses were carried out this year. Experiment XXXIII is a longitudinal study which includes measures of immediate and delayed memory. Lists of twelve familiar but unrelated words are presented on a screen sequentially; each word is displayed for one second. Immediate memory is measured by the number of words recalled immediately after presentation. Two delayed memory tasks are included. After an interpolated task (digit span), the task is either to remember as many words as possible from the list (delayed recall) or to identify which words were in the list when those words are presented again intermixed with twelve distractor words (delayed recognition). Experiment XXXVIII is a new age study of linguistic integration and memory. It was designed to elucidate an earlier study (Experiment XXXVI) in this area. In that study, it was found that, contrary to some studies in the literature, older individuals not only remembered explicit information less well than young adults, but also inferred implicit information less well than the young. In that study, related sentences were used in which the relation was highly visualizable, e.g., "Ken is taller than Mike." Furthermore, subjects were instructed to learn the hierarchy, i.e., the ordering of the six male names on the dimension of height. In Experiment XXXVIII, a readily visualizable relation ("taller than") is used in one set of sentences, and a less readily visualizable relation ("noisier than") is used in another set of sentences. Furthermore, some subjects are instructed to learn the hierarchy (the ordering of the names), and others are instructed to learn the sentences. A measure of tendency to visualize is obtained using the Richardson Verbalizer/Visualizer Scale to explore whether such a tendency is related to performance measures on the memory/integration tasks and whether these relations are different for young and old adults. Subjects are all students at the University of Maryland, College Park.

Major Findings: A statistical procedure was developed in this laboratory to overcome technical problems involved in comparing recall and recognition memory in order to test the hypothesis that retrieval of information is an age-related process. This new procedure was applied to some data from Experiment XXXIII, the longitudinal study which includes measures of delayed recall and delayed recognition.

Retrieval is one of the important processes which have been implicated in memory declines of the aged. Most of the evidence for retrieval deficits with age is based upon comparisons of recall and recognition performance. Age differences in recognition tasks typically are small (or nil), whereas recall differences are substantial. Inasmuch as retrieval demands are small for recognition tasks and large for recall, these findings support an age-related retrieval deficit. This argument would be more compelling if recall and recognition measures could be compared directly, but an acceptable procedure for achieving this has yet to be devised. The major problem is scaling; measures of recall and recognition are not on the same scale. Furthermore, they are affected differently by response bias.

A procedure to overcome these problems has been developed in this Laboratory. Instead of comparing recall and recognition measures directly, comparisons are made indirectly using regression techniques which avoid the scaling and response-bias problems. In this procedure, for each of several age groups, recognition is regressed on recall and recall is regressed on recognition. If there is an age-related retrieval deficit, then slopes of recognition on recall should be larger for old groups than for young groups. Similarly, slopes of recall on recognition should be larger for the young groups than for the old. When this procedure was applied to delayed recall and delayed recognition in this Laboratory for seven decade groups of men (20-89) and six decade groups of women (20-79), the predictions were confirmed both for men and for women. This procedure also can be applied to data already collected in other laboratories; consequently, the generality of our findings can be determined readily.

Experimental procedures and equipment were prepared for Experiment XXXVIII, the new study of memory and integration of related sentences; and data collection was initiated.

Significance to Bio-Medical Research and the Program of the Institute: Memory and learning are central to experimental psychology, and some of the most striking and consistently reported behavioral age differences in the gerontological literature have been found in such performance. The experiments in this project are designed to identify basic mechanisms of learning and retention and to measure differences and changes in these functions that occur with age. In addition, knowledge about experimental variables which affect age differences will be valuable in developing techniques for optimizing learning and memory of the older person.

Proposed Course of Project: Six- and twelve-year repeat data will continue to be collected for Experiments I and II, longitudinal studies of serial and paired-associate learning. First-time data will continue to be collected for Experiment XXXIII, the longitudinal study of several cognitive tasks involving memory, attention, and response time. Data collection will continue for Experiment XXXVIII, the new study of memory and integration of related sentences.

Publications:

Arenberg, D. Memory and learning do decline late in life. In J. E. Birren, J. M. A. Munnichs, H. Thomae, & M. Marois (Eds.), Aging: A challenge for science and social policy (Vol. 3). New York: Oxford Univ. Press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00066-22 LBS

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Perceptual Retention and Age

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

David Arenberg Chief, Learning and Problem Solving Section, LBS, GRC, NIA

COOPERATING UNITS *(if any)*

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	.3	.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The primary purposes of this project on aging are: (1) to investigate perceptual retention and interference; (2) to determine under what conditions age differences in retention are affected by interference; (3) to investigate processes of interference and perception; and (4) to describe age changes in nonverbal memory and identify correlates and predictors of these changes.

PHS 6040 (Rev. 1/83) GPO 895-100

Project Description:

Objectives: One general objective is to investigate the effects of interference in perceptual retention and in perception: (1) to determine whether aging results in increased susceptibility to interference; (2) to explore conditions which affect age differences in interference; and (3) to develop procedures for testing mechanisms which may account for the empirical findings. Another objective is to study nonverbal memory and the conditions which improve such memory, especially for the old.

Methods Employed: Experiment VII is a longitudinal study of memory for designs in which subjects attempt to reproduce visual designs from memory. The Benton Visual Retention Test is used, and the primary dependent measure is the total number of errors in all ten designs. Each design consists of geometric figures presented for ten seconds and then withdrawn. The task is to reproduce each design from memory. Subjects may take as much time as they need to draw the design.

Major Findings: Previous analyses of time segments of data from Experiment VII, a longitudinal study of memory for geometric designs, have shown clear age effects with substantial mean declines late in life over a six-year interval. As of the end of 1982, data were available for 281 men with three valid measures. The interval between first and third measures was at least twelve years. The groups in their twenties and thirties when first tested showed no mean change; and the groups in their forties and fifties at first test showed small mean declines. These results were similar to the six-year data. The group in their sixties initially showed a small mean decline over the first six years and large mean decline over the second six years. This result is consistent with all the previous analyses which showed substantial mean declines over six years only for the men in their seventies when first tested.

The relationship between blood pressure (B.P.) and performance was explored for these same 281 men. When casual, right-arm, systolic B.P. was correlated with performance at the same visit, all correlations were modest but statistically significant. When the effects of age were removed statistically, however, (and this was done in several ways--partial correlation, multiple correlation, within-age-group correlations) the correlations vanished.

The results for the change data were somewhat different. Change in systolic B.P. was modestly correlated with change in performance from Time 1 to Time 2, and this held even when age was partialled. But from Time 1 to Time 3, that relationship was not found.

Change in performance from Time 1 to Time 3 was also correlated with Time 1 B.P.s and with the mean of an individual's B.P.s at the three times of testing. Systolic B.P. at Time 1 was uncorrelated with change in BVRT. The correlation between mean systolic B.P. and change in performance was statistically significant, but vanished when age was partialled.

Diastolic B.P. was not related to BVRT performance at all.

The conclusion from these data is that B.P. is not an important factor in level of performance or change in performance on memory for geometric designs.

Significance to Bio-Medical Research and the Program of the Institute: The general idea that a person becomes more susceptible to interference as he grows older is well entrenched in gerontological thinking and is often used to "explain" age differences in performance. The evidence for this idea, however, is sparse and not consistent. One purpose of this project is to explore the generality of the age-interference hypothesis for nonverbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. Another purpose of this project is to describe age changes in nonverbal memory, to identify the correlates of those changes to improve our understanding of aging and this aspect of memory, and to study conditions which may improve such memory, especially for the elderly.

Proposed Course of Project: Data collection will continue for Experiment VII, the longitudinal study of memory for designs, for both men and women. The search for predictors and correlates of change in this performance will continue in efforts to understand why some older men decline whereas others do not. In addition, a new research direction is planned. In a recent theoretical development involving automatic and effortful processes in attention and memory, it is hypothesized that aging, which is assumed to reduce attentional capacity, affects only those memory tasks requiring effortful processing. Automatic processes are those requiring minimal attention. Spatial, temporal, and frequency-of-occurrence information are thought to be processed automatically. Previous studies in this Project (Experiments I, II, III, IV, and V) have demonstrated that time estimation is not related to adult age, but that older men are more susceptible to interference in time judgment than young men. The new studies will explore the effects of age on attention and interference in perception within the theoretical framework of automatic and effortful processing.

Publications:

Arenberg, D. Differences and changes with age in memory for geometric designs and correlations with blood pressure. In M. W. Riley, A. S. Baum, & J. D. Mattarazzo (Eds.), Perspectives on behavioral medicine (Vol. IV): Biomedical and psychosocial dimensions of aging. New York: Academic Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00067-16 LBS

PERIOD COVERED

October 1, 1982 through September 30, 1983

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

Learned Modification of Visceral Function in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Bernard T. Engel, Ph.D., Chief, LBS

COOPERATING UNITS *(if any)*

Baltimore City Hospital, Johns Hopkins Hospital

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Psychophysiology

INSTITUTE AND LOCATION

National Institute on Aging, Gerontology Res. Ctr., Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.30	4.05	4.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is concerned with the interaction of behavior and physiology in man. One study investigates behavioral procedures (relaxation and biofeedback) in the control of blood pressure in patients with high blood pressure; another study investigates the physiological mechanisms of heart rate control during exercise in normal man; two studies are designed to learn whether patients with histories of urinary or fecal incontience can be trained to become continent; one study investigates the behavioral and psychological characteristics of patients with angina pectoris who have been scheduled for coronary angiography.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel engaged on the project:

W. Whitehead	Guest Worker	LBS, GRC, NIA
W. F. Baile	Staff Physician	Balto. City Hospital
P. T. Costa	Chief Stress & Coping Sec.	LBS, GRC, NIA
J. Brinker	Dir. Cardiac Catheterization Laboratory	Johns Hopkins Hospital
B. D'Lugoff	Medical Director, Care First	Balto. City Hospital
M. Fredrikson	Visiting Fellow	LBS, GRC, NIA
K. L. Burgio	Staff Fellow	LBS, GRC, NIA
K. McCormick	Research Nurse	LBS.,GRC, NIA
R. Sangal	Medical Staff Fellow	LBS, GRC, NIA
M. Glasgow	Research Physiologist	LBS, GRC, NIA

Project Description:

Objectives:

A. To evaluate the clinical effectiveness of relaxation and biofeedback in the control of blood pressure (BP) in patients with high blood pressure.

B. To identify the mechanisms underlying learned, voluntary control of heart rate (HR) during exercise in patients with high blood pressure.

C. To develop and test behavioral training procedures for treating urinary or fecal incontinence in geriatric patients.

D. To compare the effectiveness of Kegel exercise training and bladder-sphincter biofeedback for the treatment of stress urinary incontinence in women.

E. To determine the relationship between the prevalence of self-recorded episodes of angina pectoris to the prevalence of anginal episodes judged by the patient's physician; to determine the relationship of mood state or personality trait to various characteristics of angina pectoris; to determine whether behavioral or psychological measures of angina pectoris increase the specificity of the diagnosis of patients that can benefit from coronary artery bypass surgery thereby reducing the need for coronary angiography as a diagnostic technique.

Methods Employed:

A. Patients were selected from the outpatient service of the Care First Clinic. All had been diagnosed as suffering from high blood pressure and all were taking some form of antihypertensive medication. Eventually, about 100 patients will be in the program. Patients are classified into 3 groups depending upon medication at the time of entry: Group 1 includes patients taking diuretic medication only; Group 2 includes patients taking beta-blockers with or without diuretics; Group 3 includes patients taking centrally acting antihypertensive medication or peripherally acting vasodilators. Patients receiving reserpine or ganglionic blockers are excluded. All patients participate in a one month baseline phase during which they monitor their blood pressures daily (3 times upon awakening, 3 times in the middle of the day and 3 times before retiring) and also have a professional reading taken once weekly. At the end of

the month, patients are assigned either to a control sub-group or a treatment sub-group. Control patients are followed at 3 month intervals for 18 months. At follow-up times control patients self-monitor their blood pressures daily and also have one professional reading taken. Clinical responsibility for the patient is left to the Care First staff physician. Patients in the treatment sub-group either: 1) Have their medications reduced in a stepped fashion (if their self-determined blood pressure averages during the last 2 weeks of baseline are below 135 (systolic) and 85 (diastolic) mmHg at all times of day. This continues until pressure exceeds or equals the criteria or until all medications are withdrawn. Patients whose medications are totally withdrawn are followed for 1 year using a protocol similar to that for the control sub-groups; 2) If their pressures are above the criteria cited above they are entered into a 3 month treatment program to lower blood pressure using systolic blood pressure biofeedback. At the end of this treatment stage patients who have met the criteria cited above have their medications reduced. Otherwise, the patients are entered into a 3 month relaxation treatment program following which their medications are reduced (if they meet the criteria). Regardless what the medication adjustment procedure is, all patients are followed for one year after completion of last intervention (treatment or drug reduction). Outcome measures include blood pressure responses to treatment and degree of medication change.

B. Two groups of six patients each participated in a study of the role of behavioral conditioning of heart rate in the cardiovascular adjustments to exercise. One group was trained to attenuate their heart rate while exercising; the other group was a control group. All subjects exercised during six, daily sessions. Heart rate, blood pressure and oxygen consumption was measured in all subjects during baseline and during the tasks. The experimental group received continuous feedback on heart rate and were encouraged to maintain their rates as low as possible. The control group subjects exercised comparably to the experimental subjects but received no feedback.

C. Patient flow will be described separately for fecal incontinence and urinary incontinence.

For patients with fecal incontinence a physical evaluation and history is done by a gastroenterologist to identify, for exclusion patients with inflamatory bowel disease, tumors, or fistulas. This examination includes laboratory studies for parasites or occult blood in the stool and a barium enema to evaluate diverticulosis. Physical examination is followed by a rectal motility study to evaluate the following: 1) strength of the external anal sphincter on command, 3) resting tone of the internal anal sphincter, and 4) threshold (minimum volume) of rectal distension which can be subjectively perceived by the patient. Rectal motility testing is done by inserting into the rectum a tube which has attached to it two balloons for independently detecting variations in tonus of the internal and external anal sphincters. A third balloon is used to transiently distend the rectum.

After completing the initial evaluation, the investigator explains to the patient and his family the type of records of incontinence which are to be kept at home, and the bowel training program which the patient is to follow. The bowel training program consists of having the patient sit on the toilet for 10-min immediately after breakfast each day, and giving an enema at the end of

the 10-min period if no bowel movement occurs for two days. The patient is given another appointment for 4 weeks after initial testing, and is advised that a research assistant will arrange a visit at the patient's home. The research assistant may visit one or more times during this 4-week period in order to accomplish the following: 1) ensure that the family understands how to keep the records and does keep them, 2) ensure that the bowel habit training program is being carried out appropriately, and 3) administer the Hopkins mental status exam and the Beck depression inventory.

At the second appointment patients are classified into one of three groups with disposition as follows: 1) if no longer incontinent, patients are scheduled for a follow-up appointment in 6 months and encouraged to continue on the habit training program, 2) if still incontinent but judged too cognitively impaired or depressed to participate in biofeedback training, patients continue in the bowel training program but with the addition of explicit rewards (rewards to be selected in discussions with the family) for periods of time without accidents 3) if patients are still incontinent at the second visit and are able to cooperate in biofeedback training, they are given such training. Biofeedback training is similar to the rectal motility study except that the patient receives visual feedback and verbal praise to help him lean to contract the external anal sphincter with adequate strength when he senses rectal distention. Between biweekly visits during biofeedback training patients are instructed to contract and relax the external anal sphincter 50 times each day to strengthen this muscle.

The active treatment phase lasts 8 weeks during which time patients in the second and third category above (behavior modification and biofeedback) come to the laboratory once every two weeks for evaluation and/or further training. Home records of incontinent episodes continue to be kept. At the end of the treatment phase patients are scheduled for follow-up at 6 and 12 months. At these times the rectal motility study will be repeated.

Patients with urinary incontinence (involuntary loss of urine during waking, occurring at least 2/month are given a physical examination and a medical history is taken by a urologist to identify for exclusion patients with bladder infection, bladder outlet obstruction, detrussor hyporeflexia, and paradoxical (overflow) incontinence. Examination includes urinalysis, cystometry, cystocopy if clinically indicated, and for males, a prostate examination. A urologist carries out a cystometrogram to determine the following: 1) threshold volume of bladder filling which can be detected, 2) threshold volume of filling which produces a sense of urgency, 3) ability to emit and to inhibit bladder contractions at the volume of bladder filling which causes a sense of urgency, 4) maximum volume at which the patient can inhibit bladder contractions, and 5) competency of the bladder neck and distal urethral sphincter mechanisms.

After completing these tests the investigator explains to the patient and his famnily the types of records of incontinence which are to be kept at home and the bladder training program which the patient is to follow. The bladder training program consists in having the patient go to the toilet and attempt urination every four hours. The patient is given another appointment for 4 weeks, and is advised that a research assistant will telephone to arrange a visit to the patient's home. The research assistant visits one or more times

during the 4-week period to accomplish the following: 1) ensure that the family understands how to keep the records and does keep them, 2) ensure that the bladder habit training program is being carried out appropriately, and 3) administer the Hopkins mental status exam and the NIA depression inventory.

At the second laboratory visit patients are grouped into the following categories: 1) If no longer incontinent, patients are scheduled for a follow-up appointment in 6 months. 2) If still incontinent but judged too cognitively impaired or depressed to participate in biofeedback training, patients continue in the habit training program but with the addition of explicit rewards (rewards selected in discussions with the family) for four-hour periods of time without accidents. Additionally, the bladder training routine described by Foxx and Azrin (1973), which involves water loading and rehearsal of appropriate toileting behavior combined with verbal praise and rewards, is carried out in biweekly visits to the clinic. 3) If patients are still incontinent at the second visit and are able to cooperate in biofeedback training, they are given biofeedback training to inhibit bladder contractions during bladder filling and to contract the distal urethral sphincter. This is similar to the cystometrogram except that the patient is given visual feedback and verbal instructions during bladder filling. Between biweekly visits, patients are instructed to contract and relax the pelvic floor muscle several times each day to strengthen these muscles, and they are instructed to practice stopping the stream during every micturation.

The active treatment phase lasts 8 weeks followed by follow-up visits at 6 months and 12 months. Cystometrograms are repeated, and ability to inhibit micturation is retested at these follow-up visits. Home records of the frequency of incontinence are kept throughout the 8 weeks of active treatment.

D. Patients with stress urinary incontinence (involuntary loss of urine occurring after sudden increase in intraabdominal pressure) are given a physical examination and a medical history is taken by a urologist. The purpose of the examination is to differentiate patients with a primary diagnosis of stress incontinence from those with other disorders. The urologist identifies for exclusion patients with bladder or kidney infection or disease, bladder outlet obstruction, detrussor hyporeflexia, or detrussor hyperreflexia. A cystometrogram is performed to determine: 1) the threshold volume of bladder filling which can be detected by the patient, 2) the threshold for the first sensation of urge, 3) bladder capacity, and 4) the competence of the bladder neck and urethral sphincter mechanisms. Also included in the examination is Bonney's test, urinalysis, and cystoscopy if clinically indicated.

After the examination is completed, the investigator explains the bladder training program that the patient is to follow at home and the records which are to be kept. The bladder program establishes a regular voiding schedule by having the patient attempt to void approximately every 2 hours during the waking day. The patient is asked to keep records of each time she voids, of each incontinent episode, and of number of pads or other protective garments used.

After the bladder training program has been followed for 4 weeks, the patient returns for her second visit. If she is no longer incontinent, she is given an appointment for a follow-up visit in 3 months. If she is still incontinent, she

is assigned to one of the two treatment groups: Kegel exercise training or bladder-sphincter biofeedback. Each treatment program consists of 4 biweekly treatment sessions.

In the first and last session of each treatment the severity of incontinence is assessed by catheterizing the bladder, filling it with 350 ml sterile water, and observing for loss of urine while the patient performs several physical activities (coughing, standing, bending, squatting). Then the strength of the external anal sphincter contractions is measured using a small balloon attached to a rectal tube and positioned at the anal opening. The external anal sphincter accurately reflects activity in the external urethral sphincter provided there is no damage to the urethral sphincter.

In the Kegel exercise training, the investigator teaches the patient how to do Kegel exercises. Two gloved fingers are inserted into the vagina and the patient asked to squeeze around them. The patient is given verbal instruction and feedback as she learns. The patient is asked to practice these exercises 50 per day at home. She is also asked to practice stopping the stream of urine whenever she voids.

In the bladder-sphincter biofeedback training, the investigator teaches the patient how to do sphincter exercises. The rectal tube has attached to it 2 small balloons, one positioned at the anal opening to measure the tone of the external anal sphincter, and one inside the rectum for measuring rectal (intraabdominal) pressure. The patient receives visual feedback of these pressures, and in addition, feedback of bladder pressure from the catheter. This feedback plus verbal instruction and feedback are used to teach the patient how to contract and relax the pelvic floor muscles while minimizing intraabdominal and bladder pressures. As in the Kegel group, patients in the biofeedback group are asked to practice 50 exercises daily and to stop the stream of urine during voiding.

After 4 sessions of active treatment, patients are seen in follow-up visits after 3 and 6 months.

E. Eighty-three adult male and female patients were recruited for this study. They had been scheduled for coronary angiography at the Johns Hopkins Hospital cardiovascular laboratory because of chest pain and were contacted by a team member shortly after their scheduling (usually 2 - 3 weeks prior to their catheterization). They were telephoned, the study explained to them, and their participation solicited. A packaged containing a consent form, copies of both the anginal questionnaires and mood sheets, and franked return envelopes were mailed to prospective participants. If a patient returned a consent form, he/she was enrolled in the study. Patients were asked to complete one form for each anginal attack they experienced. Patients were instructed to complete this form retrospectively, after they had treated their angina in their usual fashion (e.g., nitroglycerin, rest) and the episode had subsided. Furthermore, they were instructed to follow any other advice given by their physician even if it prevented the completion of the questionnaire at that time. The questionnaire is designed to delineate the location, intensity, and character of the individual's pain. This information will be compared to the information elicited by the patient's referring physician as well as by the house officer who

does the initial work-up upon admission for angiography. The patient's referring physician was contacted by mail and asked to complete a questionnaire regarding the nature of his patient's angina. Patients also were given copies of the Profile of Mood States and were asked to complete a form once a day usually around dinner time. Patients were asked to continue record keeping after discharge from the hospital for a period of two weeks. Copies of the Cornell Medical Index and the 30-item Guilford-Zimmerman Temperament Survey Emotional Stability Scale from the CZTS were mailed to subjects approximately one week before they entered the hospital for catheterization. Data are being analyzed in the following ways: 1) a behavioral description of the angina will be made, noting ranges of similarities and differences between patients, especially with regard to patients with normal coronary angiograms, 2) emotional correlates of anginal episodes will be analyzed by examining variability on the Profile of Mood States, and scores on the Cornell Medical Index and Emotional Stability Scales, 3) frequency and character of episodes as evidenced by self-report data will be compared to referring physicians' analyses, 4) analyses of anginal patterns post-angiography and pre-angiography will be made.

Major Findings:

A. Since the project is not finished, results will be presented only for all drug groups combined. Twenty-three control patients have been enrolled. After six months blood pressure levels have remained stable. Twenty-two patients have completed the feedback treatment phase. There have been significant declines in systolic pressure (afternoon measurement) and diastolic pressure (afternoon and evening measurements). Nineteen patients have completed the relaxation phase. There have been significant declines in systolic pressure (professionally determined) and diastolic pressure (all times of day and professionally determined). A number of patients in the treatment group have had their medications reduced. However, these data have not yet been analyzed.

B. Patients in the feedback group lowered their heart rates during exercise by about 10 beats/min, while the control patients did not change. Since systolic pressure did not change during retraining, rate pressure product reflected heart rate. Oxygen consumption also was lower in the E group by the end of training. Thus, the findings indicate that neurally mediated changes in cardiovascular responses associated exercise in hypertensive patients can be brought under behavioral control.

C. Eighteen fecally incontinent patients have completed treatment and are in follow-up. Fifteen patients improved by at least 50% and 6 of the 18 regained continence. Follow-up 4 - 27 months later in 13 improved patients revealed that 10 had maintained these treatment gains. Relapse was associated with debilitating illness, depression, and dietary indiscretion. These data suggest that behavioral training can benefit 83% of fecally incontinent geriatric patients.

Forty-nine urinary incontinent patients have been enrolled in the program to date. Forty have completed treatment and are in follow-up; 7 are currently in treatment and 2 are in baseline.

Of 18 patients treated for stress urinary incontinence all decreased the frequency of incontinence by at least 55%; two-thirds improved at least 75%; three

subjects regained complete continence. Of 11 subjects treated for bladder hyperreflexia, all decreased the frequency of incontinence by at least 62%; all but two subjects improved at least 75%; more than half regained complete continence (among these were four men with incontinence secondary to stroke). Of 7 subjects treated for urge incontinence (in absence of bladder hyperreflexia) 5 regained complete continence and 2 reduced the frequency of incontinence by at least 62%; all but two subjects improved at least 75%; more than half regained complete continence (among these were four men with incontinence secondary to stroke). Of 7 subjects treated for urge incontinence (in the absence of bladder hyperreflexia) 5 regained complete continence and 2 reduced the frequency of incontinence by 84% and 88%. Treatment was unsuccessful for 4 subjects with severe cognitive impairment (memory loss and disorientation).

D. This study is now in progress. Eighteen women have been enrolled in the program and 13 have completed treatment. Of 7 patients treated with biofeedback, all achieved a 50% or greater reduction in incontinence. Of 6 treated with Kegel exercises 4 achieved at least a 50% reduction in incontinence, 1 patient regressed, and 1 showed insignificant improvement. Currently, 4 patients are in treatment and 1 is in baseline.

E. Behavioral analyses of the anginal episodes have been completed and these results have been compared with the physicians estimates. The major findings were 1) 65% of anginal episodes occurred at a time when the patient rated his mood just prior to the episode as one of contentment; 2) patients frequently reported several pain locations and several different pain radiations. Thus, there was no consistency either in site of pain or in direction of pain radiation; 3) patients at work usually resumed their on-going activities after the pain subsided. However, patients who were at home when the episode occurred were likely to change activities: 4) physicians tended to overestimate the frequency and severity of their patients' episodes: Severity rating: physicians = 47.4, patients = 32.5; episodes/week: physicians = 11.2, patients = 4.5.

Proposed Course of Project:

A. Data collection will continue until all of the sub-groups are completed and followed for the requisite periods.

B. Research projects designed to assess the ability of subjects to learn to control diaphragmatic breathing are beginning. Non-invasive measurement of pulmonary function and cardiac output will be developed. Studies of various patient groups at rest and during exercise eventually will be implemented.

C. The results of the fecal incontinence training program, when coupled with other results from this laboratory indicate that our procedures are very effective and should be more widely applied. This phase of the study will be completed by September 1983. Results of the urinary incontinence program also indicate that these procedures are very beneficial for most cognitively intact elderly men and women who report urinary incontinence of the stress, urge, or hyperreflexic type. This phase of the study will be completed by September 1984 when 12 month follow-up data will be available on subjects currently in treatment. New projects will be initiated to test the effectiveness of behavioral intervention for post-prostatectomy urinary incontinence in men and for

excessive frequency of micturation in adult women. Currently a retrospective analysis is being conducted on urinary frequency data collected by the Baltimore Longitudinal Study. The analysis will explore frequency of urination and its relationship to age, sex, and medical history.

D. This study will continue until sufficient numbers of patients have been treated in each group.

E. Data analysis of the relationships among psychometric results, behavioral analyses and outcome of coronary angiography will be completed. If these data can separate patients who have occluded vessels from those whose vessels are clear, a prospective study will be planned.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00075-05 LBS

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Stress, Coping, and Personality in Aging Men and Women

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Paul T. Costa, Jr., Chief, Stress & Coping Section, LBS, GRC, NIA

COOPERATING UNITS *(if any)*

Psychophysiology Section, LBS
Cardiovascular Section, CPB
Dept. of Psychiatry, Duke University Medical School

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Stress & Coping Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

This project is concerned with the effects of stresses, coping procedures, and enduring personality dispositions on physiological and health outcomes. One study investigates the effect of stress on medical and psychological outcomes; a second seeks to identify psychologically meaningful dimensions in MMPI responses from CAD patients and to predict from these dimensions the presence and severity of CAD as measured by coronary arteriography, a third measures personality through interviews and projective tests, a fourth utilizes longitudinal data to examine age-related constancy or changes in personality and personal adjustment; the fifth examines the effects of life event stress, personality, and biomedical status on health perceptions and illness complaints.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel engaged on the project:

Robert R. McCrae	Research Psychologist	LBS	GRC	NIA
Marc W. Heft	Senior Staff Fellow	LBS	GRC	NIA
Alan B. Zonderman	IPA Fellow	LBS	GRC	NIA

Project Description

Objectives:
The program of the Section on Stress & Coping, LBS, is devoted to an investigation of the stresses faced by aging adults, the methods and strategies used by them to cope, and the effectiveness of their coping efforts. Stresses are viewed as events or difficulties which the individual encounters. They lead to attempts to cope, and the success of these coping efforts is judged by various measures of adaptational outcomes. Personality and life situations are seen as determiners of all three of these categories of variables. Personality is defined as the organization of mechanisms for adaptation or coping, and the three domains of personality in our model--Neuroticism, Extraversion, and Openness to Experience--each has important implications for the process of coping. The development of life structures--careers, friendships, marriages-- similarly represent global and vital forms of coping with inner and social demands. It is within this broad context of an aging personality in a complex social environment that the study of specific coping and adaptational mechanisms will be made.

Specific Project Objectives:

A. To examine the effect of stress on medical and psychological outcomes.

B. To identify psychologically meaningful construct dimensions in MMPI responses from CAD patients, and to use these dimensions to predict the presence and severity of atherosclerotic heart disease as measured by coronary arteriography.

C. To assess adult personality through a variety of non-self report methods.

D. To describe maturational change or constancy in personality and well-being, and to determine the relations between personality and subjective well-being.

E. To identify the determinants of perceptions of health or medical complaints.

Methods Employed:
A. A previous study examined the widely-accepted belief that life event stress causes poorer physical health and psychological well-being. Support for such a view is based primarily on correlations observed in retrospective self-report studies, which are seriously flawed in four aspects: a) physical health changes are commonly assessed by the independent measure (events) as well as the dependent measure; b) the personality trait of neuroticism leads to the increased occurrence of certain events and to reports of poorer health and well-being; c) retrospective research designs allow respondent's knowledge of the outcome to affect their perception and reporting of prior stressors; and d) the subjective wording of many event items allows individual differen-

ces in neuroticism and response sets to affect (bias) scores on event measures as well as outcome measures. Each of these methodological difficulties involves the independent and/or dependent measures being influenced (contaminated) by one or more variables in such a way as to create a spurious relationship between event stress and its outcome.

The aim of the present study is to evaluate the uncontaminated stress-illness relationship directly employing both a prospective research design and measurement techniques which do not allow the contamination process to occur. Specifically, the event list does not include subjective or health-related events. Illness is measured by reliable, objective external observers (physicians), rather than by self-report, eliminating the neuroticism and distress biases in typical retrospective studies. The illness measure is prospective and independent of the event stress measure. The present study can be viewed as a reasonably unbiased assessment of the life stress event-illness relationship. The specific hypothesis to be tested is that illness is independent of life event stress.

The sample for this study consisted of men and women who were participants in the Baltimore Longitudinal Study of Aging. In order to be included in the analyses, participants were required to have completed the 1979 life events measure administered as part of the Augmented BLSA. They also had to have completed a scheduled visit to the BLSA for physical examination during specified time intervals before and after the life events measurement. A total of 268 men and 74 women met these criteria.

A checklist of 56 life events was administered by mail to the Augmented BLSA participants in October, 1979. Items related to physical health were included in the questionnaire but were not counted in life event scores used for this study. Participants were asked to check and report the date for each event on the list which had occurred to them in the past twelve months. The total score, representing life event stress for the previous year, consisted of the number of events checked. The results of a previous study, in conjunction with the previous literature, indicated little utility for the various weighting schemes which have been proposed, so simple-sum (unweighted) scoring was employed.

Each time BLSA participants visit the GRC, they receive a physical examination and extensive physical/medical testing. Staff physicians record all medical diagnosis which are currently applicable on the basis of the criteria specified in the International Classification of Disease manual (World Health Organization, 1978). Thus a list of current diagnoses is available for each visit a participant makes to the BLSA.

The dependent variable for this study was defined as the number of new diagnoses noted on participants' first visit to the GRC after the stress year, which had not been recorded on their last visit before the stress year. The specific timing of the participants' visit to the Center was determined by the schedule for the larger study (the BLSA) and the convenience of the participants, and could not be controlled for the purposes of this study. In order to be included in the analyses for the present study, participants were required to have had a pre-stress BLSA visit after November 1, 1976, and before the beginning of the stress year (generally about November 1, 1978). Alternatively, if a participant had a BLSA visit during the first four months

of the stress year, and this visit occurred at least one month before any of the events checked on the questionnaire, the visit could be counted as a pre-stress assessment. The post-stress visit was required to have taken place at least one month after the end of the stress year (or one month after the latest event indicated on the event questionnaire and at least twelve months after the pre-stress visit and no more than two months before the end of the stress year) and also no later than December, 1981.

B. As part of a collaborative project with investigators from Duke University Medical Center, psychological data collected there on 2581 individuals scheduled for coronary arteriography are being analyzed. Primary interest is in item responses to the Minnesota Multiphasic Personality Inventory (MMPI), an instrument designed to aid in the diagnosis of various forms of psychopathology. Because of the empirical contrast method of scale construction, the existing scales are not independent, nor do they necessarily represent psychologically meaningful dimensions of behavior or experience. Several hundred research scales have been derived from the MMPI, despite the fact that until recently the size of the item pool precluded an adequate empirical analysis of its item content. In an attempt to define its psychosocial content dimensions and evaluate the comprehensiveness of its items, the present study performed a principle component analysis of the 550 MMPI ideas on a sample of 1576 male and female patients referred for coronary angiography. These medical patients, who were not screened for psychiatric status, were administered Form R of the MMPI as part of a larger study of the psychosocial concomitants of coronary artery disease. Patients completed the MMPI on the morning following coronary catherization and prior to discussing the results of this procedure with their physician. The mean MMPI profile for this sample of psychiatrically unscreened medical patients revealed no signs of psychopathology, and with the exception of scales 1 (HS), 2 (D), and 3 (HY), the mean T-scores were all less than 60. The average age of this sample was 50.9 years (51.4 for men and 50.0 for women) with a range from 18 to 75 years. Ninety-eight percent of the sample were white, 1% were black, and 1% were members of 'other' races. The median yearly income was between $15,000 and $19,999 with a range from less than $5,000 to more than $50,000; 90% of the subjects were married. Forty percent of the subjects had thirteen or more years of education, 24% completed high school, and the remaining 37% did not complete high school. No subjects were eliminated because of test invalidity or missing data. Subjects omitted an average of eleven items (range = 0 to 278). Twenty-four percent of the subjects did not omit any items; 55% omitted between one and ten items; 7% omitted between eleven and twenty items; 7% omitted between twenty-one and thirty items; and 7% omitted between thirty-one and the maximum, 278 items.

C. Subjects for this study are participants in the Baltimore Longitudinal Study of Aging (BLSA). In order to supplement and validate the self-report questionnaires used in other studies, a number of alternative methods of assessment are being employed. Interviews, which are video-tape recorded for later rescoring, intensively explore life stresses associated with family, friends, careers, and leisure activities. Inquiries are also made concerning the participant's methods and strategies used to cope with stress. Data from the interview are quantified by the use of the California Q-Sort, which is completed by the interviewer and a second rater who has seen video-tapes of the interview. At a later visit, subjects complete a self-administered version of the Q-sort. Using computer-assisted testing procedures, MMPI data are

now being collected on BLSA participants. On another visit to the GRC, a subset of 93 participants were asked to take a battery of three perceptual-projective tests, including the Holtzman Inkblot Test, a psychometrically improved form of the Rorschach test; the Embedded Figures Test, a measure of the cognitive style of field independence; cards from the Thematic Apperception Test (TAT); and the Projective Assessment of Aging Method (PAAM), a projective test designed for use with older subjects. On their next visit, one to three years later, 44 of these subjects were readministered the Holtzman Inkblot Technique (HIT), to allow assessment of two-year stability of HIT scores. All protocols were scored by a trained technician for 22 variables, and a random sample of 29 first administration protocols were independently scored by a HIT expert to assess reliability of scoring. Retest correlations were computed to estimate stability in individual differences for these variables, and repeated measures analysis of variance were used to determine if there were mean level changes over the two-year period.

D. (1) One implication of the personality stability position is that the point in time at which personality is measured is irrelevant. Once the individual has reached full adulthood, by age 30, a single measurement of personality would suffice for a lifetime. In other words, personality in adulthood and aging might be considered a constant in the lifetime of the individual.

There are, of course, a number of reasons to stop short of so sweeping a stand. It assumes a perfect correlation of tests over time, a condition rarely observed even when corrections for unreliability are made. It also assumes that there are no circumstances under which personality might regularly be expected to change. Evidence to date shows only that the events encountered over the course of a lifetime by volunteer subjects do not systematically produce change. But other circumstances, such as theraputic interventions, cataclysmic events, or severe illnesses, might affect traits. The longitudinal study of personality in adulthood is simply too young a field to rule out these possibilities.

On the other hand, if we turn from the individual to the group, much stronger arguments can be made. On the aggregate level, there appear to be good grounds for claiming that the time at which measurements are made should not affect the results; and in particular, the relations between different tests ought not to depend on the times when they are administered. Data collected in the 1960's might be used to validate tests created in the 1970's, just as if the administration had been contemporaneous.

Participants in the study are members of the Baltimore Longitudinal Study of Aging (BLSA). The criteria of primary interest are scales from the Guilford-Zimmerman Temperament Survey (GZTS; Guilford, Zimmerman, & Guilford, 1976), administered to subjects from 1959 to 1979 on their first or second visit to the Gerontology Research Center. The GZTS was used the "?" response for more than 3 of the 30 items in any scale were considered to have missing data for that scale. Consequently, correlations with GZTS scales are based on a varying number of cases, although all cases for whom data were complete are used in analyses. Although it was subsequently readministered, for purposes of simplicity, only first administration data is analyzed here.

Over the same period of time, data were also collected on the Cornell Medical

Index (CMI). Twelve sections deal with physical symptoms and six with psychiatric complaints. Scores from the WAIS Vocabulary scale and the Army Alpha total collected in the same two-decade period were also used.

All of these measures were used to consider the convergent and discriminant validity of three personality instruments administered in the past four years. Form A of the EPI, with scales for Extrtaversion, Neuroticism, and Lie, was mailed to subjects to be completed at home in September, 1979. The NEO Inventory, was mailed to subjects in February, 1980, and the NEO Rating Form was completed at home in August, 1980, by spouses of subjects whose husbands or wives were also participants in the BLSA. Because some spouses did not participate, rating data are available for only a subset of subjects.

The NEO Inventory measures six facets, or aspects, of three global domains of personality. Neuroticism is represented by anxiety, hostility, depression, self-consciousness, impulsiveness, and vulnerability. Extraversion includes scales for warmth, gregariousness, assertiveness, activity, excitement seeking, and positive emotions. Openness to Experience is measured in the areas of fantasy, aesthetics, feelings, actions, ideas, and values. Total scores for the three domains are obtained by summing the scores of the six facets in each. The present analyses provide evidence of validity against new criteria; in addition, since the construct validity of the NEO scales is already fairly well established, they provide a demonstration of the feasibility of using data collected several years previously in validation studies.

D. (2) The present study was designed to apply a previously developed model of well-being and personality to adult women. Measures of Positive Affect, Negative Affect, Affect Balance, Satisfaction in 14 areas of life and the Delighted-Terrible Scale of Andrews and Withey were correlated with scores on the NEO Inventory in a sample of 256 adult women, aged 24 to 96, participants in the Augmented Baltimore Longitudinal Study. In addition, spouse ratings on the NEO scales for men and women were obtained in order to test the hypotheses that the observed correlation between personality and well-being were not due to artifacts of self-report.

E. (1) Patients referred for coronary angiography because of chest pain complaints usually present with "classical" angina pectoris (AP), a clinical manifestation of coronary artery disease (CAD). However, a significant number of them after cineangiography are found to have minimal or no coronary occlusion and despite persistent reports of chest pain most of these individuals remain free of detectable CAD on continued follow-up. Recent studies report that from 15-20% of patients undergoing coronary arteriography show no evidence whatsoever of CAD. A great deal of confusion exists concerning the distinguishing features, if any, of this group of patients with chest pain of unknown etiology (CPUE), especially in regard to the topographic features or characteristics of clinical symptoms, the role of emotional arousal in the precipitation of anginal pain episodes, and the psychological status or distinctive traits of CPUE patients.

The study was designed to prospectively investigate the incidence and description of patients' anginal attacks or episodes in order to: a) determine the antecedents, concomitants and consequences of chest pain complaints in a group of patients in the two-week period prior to undergoing coronary angiography; b) examine the role of emtional arousal and exertional factors in precipi-

tating anginal episodes; c) determine the relationship bvetween personality dispositions and somatic complaints associated with anginal episodes and relate them to angiographic findings; d) compare self-reports of anginal episodes with physicians' estimates of patients' anginal characteristics.

Fifty-six men (mean age = 55.3, s.d. = 8.8 and 25 women (mean age = 58.3, s.d. = 8.2) completed the following measures: Cornell Medical Index, Emotional Stability Scale from the Guilford-Zimmerman, Temperament Survey (GZTS), Profile of Mood States (POMS), and Anginal Check List after every anginal attack.

The physicians completed a Physicians Questionnaire, a parallel to the Subjects Anginal Check Sheet, where they were asked to describe typical features of patient's angina episode.

E. (2) A review of the relationship between hypertension and personality concluded that the most promising directions for research included (a) determining the kinds of cognitive and behavioral treatments which are most effective, and (b) determining which types of intervention are most effective for individuals characterized by different personality traits. A recently completed study using biofeedback and relaxation on a population of borderline hypertensives demonstrated that a combination of techniques is useful in producing sustained decreases in blood pressure. In another study, personality-by-treatment interactions during electromyographic recordings were examined in a small sample of college students. Qualls and Sheehan reported that individuals open to absorbing experiences may benefit more from relaxation, while closed individuals profit more from a biofeedback approach. The present study attempts to confirm the Qualls and Sheehan findings using blood pressure data from a completed study, and personality measures subsequently administered. The specific hypotheses to be tested are that treatment conditions that require withdrawal from the external environment (relaxation) will be most effective or beneficial for persons with high capacity for absorbed attention, whereas for subjects who are low or limited in their capacity for absorbed attention (closed to experience), conditions (i.e ., biofeedback) that place an external, attentional demand on subjects will be more effective. Subjects were 53 men and women, members of the Columbia Medical Plan aged 28 to 70, who completed the biofeedback and blood pressure study described in Z01 AG 0067, and their spouses, if married. Three questionnaires were mailed to subjects: the Tellegen openness to absorbing experience scale, used in the Qualls and Sheehan study; the NEO inventory, a 144 item personality scale which measures the related concept of openness to experience as well as neuroticism and extraversion; and the NEO rating inventory , which is filled out by the spouses of the subjects and provides an independent non-self-report assessment of personality.

E. (3) To identify the factor structure of Pilowsky's Illness Behavior Questionnaire that might optimally distinguish chronic facial pain patients from non-health care seeking individuals.

Subjects for this study are participants in the Baltimore Longitudinal Study of Aging (BLSA). Facial Pain patients (Myofascial Pain (MPD) and Recurrent Aphthous Stomatitis (RAS)) were seen at the National Institute for Dental Research dental clinic in the NIH Clinical Center. The participants were administered Pilowsky's Illness Behavior Questionnaire, a 62-item self-report

instrument which assesses person's attitudes and feelings about illness, his perceptions of the reactions of significant others (including doctors) to himself and his illness, and the patient's own view of his current psychosocial situation. The responses are scored using the 7 subscales determined by Pilowsky: general hypochondriasis, disease conviction, psychological vs somatic perception of illness, affective inhibition, affective disturbance, denial, and irritability. The measures for the MPD and RAS groups will be compared with the BLSA data.

Major Findings:

A. The independent variable for this study was the number of life events participants reported for the twelve month period, and the dependent variable was the number of new medical diagnoses added to participants' records on their first visit after the stress year. The table below shows descriptive statistics for life events and new medical diagnoses for the total sample and for men and women separately. The participants experienced an average of 2.94 non-health-related events and were diagnosed as having an average of 2.33 new medical diagnoses on their post-stress visits.

Descriptive Statistics for 1978-79 Life Events and New Post-Stress Medical Diagnoses

Measure	Mean	SD	Minimum	Maximum
Life events				
Total sample	2.94	2.51	0	14
Men	2.96	2.51	0	12
Women	2.89	2.68	0	14
Medical diagnoses				
Total sample	2.33	2.57	0	14
Men	2.32	2.38	0	13
Women	2.34	3.17	0	14

Note. The sample consisted of 268 men and 74 women.

The correlation between events and diagnoses is -.17 ($p < .01$), which is in the opposite direction from the hypothesis that stress leads to illness. When one adjusts for age (since the older participants tended to have fewer events and greater illness), the partial correlation is a non-significant -.02. When each sex is analyzed separately, the results are essentially the same as for the overall sample. Because the distribution of both events and diagnoses were moderately skewed to the right, the simple correlations were recomputed using Kendall tau coefficients. The results were highly similar to those obtained with Pearson coefficients.

These results are shown in a different way in the following table which gives the relative risk of illness for participants separated into Holmes-Rahe LCU categories. For this table proxy life change units were calculated in a manner similar to that used in Study One. For each event, the mean stressfulness rating given by the participants who experienced it was calculated. For the events which matched the Holmes-Rahe SRE items the LCU weights were regressed onto the mean weights. The resulting regression equation was then used to estimate LCU weights for the remaining events from their mean stressfulness ratings (the events from the SRE were assigned LCU weights directly from the Holmes-Rahe specifications). As the table indicates, the risk of disease did not increase with increasing life change.

Risk for New Medical Diagnoses as a Function of Life Change Units

Life change units	N	Risk of at least one new disorder	Risk of two or more new disorders
0-100	216	.75	.52
101-150	68	.82	.54
151-200 ("Mid Life Crisis")[a]	26	.65	.46
201-300 ("Moderate Life Crisis")[a]	24	.63	.25
301+ ("Major Life Crisis")[a]	8	.75	.38

Note. The chi-square values are 5.22 ($df = 4$; $p > .10$) for risk of at least one new disorder and 7.65 ($df = 4$; $p > .10$) for risk of two or more new disorders. The new disorders were diagnosed on participants' first visit to the Gerontology Research Center after the year for which event stress was measured.

B. The correlations among the 550 items were computed using several different algorithms for handling missing data: (1) listwise deletion of missing data, in which subjects were eliminated from the analysis if they had missing items; (2) pairwise deletion of missing data, in which each correlation in the matrix was calculated using only the complete data for that pair, thus utilizing slightly different subsets of the total sample; (3) replacing each missing response by the average of the nonmissing data for that item; and (4) recoding false to -1, missing data to zero, and true to 1 (this assumes that missing responses are midway between true and false). The correlation matrices computed by these methods were quite similar. For example, the average difference between the off-diagonal elements in the lower triangles of the matrix computed by listwise deletion and the matrix computed by pairwise deletion were virtually indistinguishable. Similar results were obtained from the analyses of these matrices. The 550 items from the MMPI were subjected to a component analysis, and following varimax rotation, nine factors were interpreted. The first component, Neuroticism, contained 65 items and was characterized by worry, instability, and depression. The second component contained 120 items and was characterized substantively by bizarre thinking and paranoid ideation. However, this component contained many items, rarely endorsed by nonpsychiatric patients, such as those in the Infrequency or F scale. Because infrequently endorsed items artifactually tend to define a separate factor (Gorsuch, 1974), the second factor was interpreted as Psychoticism/Infrequency. The third component, Masculinity vs. Femininity (39 items), contrasted stereotypical masculine interests such as hunting, mechanics, and sports with stereotypical feminine interests in dolls, dressmaking, and nursing, and with fears of the dark, animals, and insects. The fourth component, Extraversion (23 items), involved enjoying parties and crowds, wanting the company of others, and preferring not to be alone. Religious Orthodoxy consisted of 26 items concerning fundamentalist beliefs, strict adherence to religious and moral rules, and attendance at religious services. Complaints of tiredness, stomach trouble, and headaches characterized the 44 items of the sixth component, Somatic Complaints. Inadequacy contained 30

items dealing with shyness and feelings of incompetence when facing adversity. The eigth component, Cynicism (37 items), involved distrusting and disparaging attitudes toward the motives of others, and beliefs in the selfishness of human nature. The ninth component, Intellectual Interests, contained 11 items, and was characterized by interests in poetry, dramatics, and science. This solution used 395 (72%) of the 550 items.

C. Interviews have been given to 113 subjects, including 31 women, and Q-sort ratings have been made by the interviewer and a second scorer for all of them. Self-Q-sorts have been obtained from 209 BLSA participants, including about half of those who had been interviewed. Eighty subjects have completed the computer-assisted administration of the MMPI. The reliability of HIT scoring as judged by correlation with an expert rater ranged from .61 to .98, with a median of .93, suggesting high accuracy of scoring. Retest coefficients were non-significant for two of the 22 scored variables, Space and Form Appropriateness. Other coefficients ranged from .32 for Penetration to .73 for Form Definiteness and .76 for Movement. The median retest correlation was .52. These coefficients suggest that there is moderate stability in response to the HIT after a one- to three-year interval. The number of rejections (cards for which no scorable answer was provided) increased on the second administration, and it was therefore necessary to prorate scores for the number of complete responses. Repeated measures analyses on these corrected scores showed increases in Pathognomic Verbalization, Integration, Hostility, and Popular responses, and decreases in Location, Movement, and Penetration. It is unlikely, however, that these changes represent maturational effects, since cross-sectional analyses on first administration data show no significant age differences for any of these variables except Hostility, which shows a cross-sectional increase.

D. (1) The correlations between GZTS scales administered between 1966 and 1979 and NEO facets administered in 1980, at least one year after the last GZTS are presented in the table below. The average interval between the two tests was 9.1 years. Despite this considerable lapse of time, construct validity is very much in evidence. General activity from the GZTS is most strongly correlated with NEO activity; GZTS assertiveness with NEO assertiveness; GZTS sociability with NEO warmth; GZTS emotional stability and objectivity (negatively) with NEO anxiety; GZTS friendliness (negatively) with NEO hostility; GZTS thoughtfulness with NEO openness to ideas. At a more global level, the GZTS Extraversion scales (G, A, and S) are correlated chiefly with NEO Extraversion scales; GZTS Emotional Health scales (E, O, F, P, and M) show consistent negative correlations with NEO Neuroticism scales. Finally, the magnitude of the correlations requires comment: Almost all exceed .30, and several reach .60. Considering the somewhat different conceptions underlying the two instruments, these are large correlations.

Correlations Between GZTS Scales Administered 1959-1966
And NEO Facets Administered in 1980

NEO Facets	GZTS Scales G	R	A	S	E
Neuroticism:					
Anxiety	-14	-01	-25**	-25***	-67***
Hostility	23**	-24**	07	-12	-47***
Depression	-04	-02	-38***	-38***	-58***
Self-Consciousness	-12	-04	-37***	-33***	-55***
Impulsiveness	12	-35***	16	11	-44***
Vulnerability	-31***	08	-44***	-34***	-39***
Extraversion:					
Warmth	11	-08	31***	56***	18*
Gregariousness	31***	-25***	41***	52***	17
Assertiveness	34***	-14	61***	41***	20*
Activity	61***	-18*	20*	10	03
Excitement Seeking	41***	-34***	42***	21***	-13
Positive Emotions	32***	-29***	27**	30***	10
Openness:					
Fantasy	07	-12	10	-06	-30***
Aesthetics	19	10	26**	26**	19*
Feelings	22*	00	31***	21**	-09
Actions	22**	-10	31***	20*	03
Ideas	12	13	29***	08	07
Values	10	-12	23**	-05	-14

NEO Facets	GZTS Scales O	F	T	P	M
Neuroticism:					
Anxiety	-55***	-31***	17	-31**	-28***
Hostility	-44***	-53***	04	-26**	-24**
Depression	-52***	-26**	11	-29***	-26**
Self-Consciousness	-46***	-25**	02	-22**	-26**
Impulsiveness	-31***	-46***	-06	-27**	-13
Vulnerability	-38***	-03	01	-10	-26***
Extraversion:					
Warmth	15	08	10	12	-03
Gregariousness	16	-05	-23**	16	00
Assertiveness	15	-20	01	01	00
Activity	01	-16	01	-08	06
Excitement Seeking	-08	-33***	-05	01	02
Positive Emotions	07	-06	10	-07	-05
Openness:					
Fantasy	-20*	-22**	22**	-22**	-02
Aesthetics	15	13	31***	06	00
Feelings	-06	-24**	31***	-12	-15
Actions	05	00	09	08	24**
Ideas	08	05	35***	09	11
Values	-13	-11	19*	-11	00

Note: GZTS scales are General Activity (G), Restraint (R), Ascendance (A), Sociability (S), Emotional Stability (E), Objectivity (O), Friendliness (F), Thoughtfulness (T), Personal Relations (P), and Masculinity (M). N = 140 to 152. Decimal points omitted. *p< .05; **p< .01; ***p< .001.

Results of correlations with other measures were clear and in keeping with expectations about the nature of the constructs. Neuroticism was significantly related to (or, to preserve the time sequence, predicted by) GZTS Neuroticism, and CMI physical and psychiatric complaints. It was unrelated to measures of intelligence or education. Extraversion was strongly correlated with GZTS Extraversion, and there was some suggestion that introverts are more intelligent, at least in this sample. Extraversion was unrelated to CMI complaints. Openness to Experience, as measured by the NEO self-reports and spouse ratings, was clearly related to GZTS thoughtfulness, and show a tendency to be associated with Extraversion; it was substantially independent of CMI complaints. Openness was also correlated with measures of intelligence and education (cf. Costa & McCrae, 1978). However, the correlates were not sufficiently large to permit the interpretation that Openness is nothing but intelligence.

For completeness, we have also included the Lie scale of the EPI, which shows small correlations with GZTS Neuroticism and CMI Psychiatric complaints.

Although this might be interpreted as evidence of an artifact of social desirability, other anaylses suggest that the Lie Scale is instead substantively related to Neuroticism (McCrae & Costa, in press).

Correlations with NEO ratings was generally smaller than those with self-reports, and occasionally fail to reach statistical significance, in part because they are based on considerably fewer cases. Considering that the correlations cross instruments, methods of measurement, and 2 to 20 years, however, the pattern of results was convincing. A correlation of .59 between GZTS Extraversion and spouse-rated NEO Extraversion was particularly remarkable.

D. (2) Among this sample of women the relationship between NAS and neuroticism was .54, correlation with E was comparable to those found in men. As in men, depression is the facet contributing most to the prediction of NAS; warmth, assertiveness, and positive emotions are most strongly related to PAS. Openness to experience seems to have similar effects on subjective well-being. Individuals who are open to experience seek variety and novelty, and have an appreciation for the intrinsic value of experience itself. Such people are likely to be more sensitive than others to both positive and negative experiences, and in previous studies openness has been shown to be related to both PAS and NAS, but not to affect balance (McCrae, in press). Examination of results support this hypothesis in men and partially support it among women. Openness to aesthetic experience is particularly associated with PAS; openness to fantasy with NAS. Openness to feelings, appropriately, is positively related to both, as is overall openness.

Using NEO ratings, correlations are somewhat smaller than in self-report studies, but the pattern of results is strikingly similar. All the neuroticism facets are related to well-being, as are warmth, assertiveness, and positive emotions from the extraversion domain. Spouse-rated neuroticism appears to be a much better predictor than rated extraversion. Perhaps the absence of joy is interpreted by external observers as a sign of maladjustment. The openness scales also show the same pattern seen in self-reports, though the correlation of total openness with NAS does not reach significance. Judging from these correlations, facets of extraversion account for about 5% of the variance of well-being, and neuroticism for about 14%.

E. (1) A total of 835 anginal episodes were recorded by 83 patients for an average of 16.5 days prior to catherization.

An Index of Neurotic Anginal Compliants (INAC) was formed by giving each patient one point for each of the following 11 atypical symptoms when reported in 25% or more of the anginal episodes. The symptoms are:

Stabbing	Sighing	Dizziness	Palpitations
Weakness	Anger	Annoyance	Tension
Fear	Worried	Upset	

The internal consistency coefficient (Chronbach's alpha) for INAC was .73. Higher INAC scores were significantly correlated with: a) greater numbers of

psychological (r=.51) and somatic (r=.37) complaints (CMI); b) lower emotional stability scores (GZTS, r=-.42); c) younger age (r=-.25); d) angina experienced while sitting (r=.45) and not walking (r=-.29); and, e) less total coronary occlusion (r=.24), Occlusion measured as maximum % occlusion in each artery summed across all arteries and fewer vessels totally occluded (r=-.26).

Physicians' estimates of patients' INAC scores differed from self-reports in that they were related only to CMI psychiatric complaints.

Correlates of Self-Reported and Physicians' Estimates of Index of Neurotic Anginal Complaint Scores (INAC)

	Referring Physicians' Estimates of INAC scores	Patients' Self-Reports of INAC Scores
Physicians' INAC scores	_____	0.278**
Demographics		
Age	0.008 (65)	-0.250* (76)
Sex	0.105 (65)	0.115 (76)
Marital Status	-0.092 (64)	-0.129 (75)
Education	0.171 (63)	0.160 (73)
Income	0.131 (41)	-0.120 (52)
Personality Traits		
Somatic Complaits Score from CMI	0.065 (53)	0.373*** (63)
Psychiatric Complaints Score from GZTS	0.268 (53)	0.514*** (63)
Emotional Stability (GZTS ES Scale)	0.071 (52)	0.424*** (61)
Anginal Characteristics		
Angina While Sitting	0.206 (61)	0.455*** (81)
Angina While Walking	0.001 (61)	-0.287** (81)
Anginal Frequency Events/Day	0.052 (61)	0.104 (81)

Note: N's in parenthesis. *p<.05; **p<.01; ***p<.001.

In this sample of patients scheduled for coronary catherization: a) so-called "classical" symptoms such as frequency and severity of anginal chest pain complaints were only weakly related to arteriographically-determined coronary

occlusion; b) patients with clean or non-occluded arteries were distinguished from patients with severely occluded arteries by a pattern of atypical symptoms; c) the personality disposition of Neuroticism may be responsible in part for this distinguishing pattern of symptoms of anginal complaints; d) pain, including chest pain complaints, is a complex, multidimensional concept, and patients' reports of symptoms and affects reflect both acute situational reactions and chronic personality dispositions; e) physicians' estimates of their patients' pain reports reflect more the influence of chronic personality dispositions then they do patients' reported anginal symptoms or coronary occlusion results; f) greater attention should be placed by physicians on obtaining detailed behavioral assessments of patients' paun episodes and their characteristic personality dispositions for improved diagnosis and understanding.

E. (2) The interaction of personality and treatment was examined by performing two-way repeated measures analyses of variance on average daily blood pressures from each phase. Separate analyses were performed for each of four dichotomized personality variables, Neuroticism, Extraversion, Openness to Experience, and Absorption. The major hypothesis - that individuals open to experience would benefit differentially from relaxation treatment - was not supported. A significant (triple) interaction was found over time between extraversion and the pattern of succession treatments offered in this cross-over study for diastolic but not systolic blood pressure. This finding suggested that extraverts responded to changes in their protocols differently from introverts. However, these differences failed to replicate for average daily practice over time.

E. (3) Data are currently being collected and there are no findings to date.

Significance to Biomedical Research and the Programs of the Institute:
Psychological stress has long been considered a possible contributor to a number of illnesses, and several interventions have been designed to reduce stress and thus promote health. However, many questions remain about the proposed link. These include the identification of specific illnesses which are stress-related; the length of time between stress and subsequent illness; and the nature and mechanisms by which psychological states influence physical health. In the past, research on these questions has been hampered by the use of unreliable measures and retrospective designs. The program of research of the Stress and Coping Section can make substantial contributions to this area by its use of prospective designs and well-validated measures on a large population with documented medical histories. Research on personality may contribute to an understanding of the mechanisms linking stress and illness; at a minimum, it is essential to an understanding of the measurement of stress and illness. Research on stability and change in personality, and on the determinants of well-being and successful aging contribute directly to the mission of this institute.

Proposed Course of the Project:
A. Investigation of the processes and outcomes of psychological stress will continue. Previous research in this unit has indicated: a) the necessity of using clear, specific measures (with confounds explicitly controlled), and b) the circumscribed nature of stress reactions. Future studies will examine the time course of stress reactions (the hypothesis being that individuals show more distress immediately after events than later) and

the extent to which stresses in certain life areas (e.g., job, marriage) affect satisfaction in those areas versus satisfaction in other life areas (appearance, leisure, and so on). Other directions for research include the examination of forms of stress other than common life events, such as chronic role strain and severe, acute stresses (bereavement, divorce, sudden unemployment). Finally, other research will examine further coping processes which mediate between life stresses and adaptational outcomes (see Z0 1 AG 00076-04 LBS).

B. Further analyses of this data are being planned.

C. Analysis of the Q-sort data in conjunction with age, sex, and self-reported personality scale scores is planned. Data collection by computer-assisted testing will continue, as will self Q-sorts. Neither the interviews nor the projective testing is currently being continued, due in part to the loss of personnel. Four years from now consideration will be given to reinstitution of these procedures for six-year longitudinal studies. In the immediate future, a large-scale peer rating project has been begun in order to determine the correspondence between self-reports and ratings made by friends and colleagues. 268 members of the Augmented BLSA (composed of volunteers from the BLSA and their spouses) nominated a total of 1075 friends, neighbors or co-workers to provide ratings on them. These raters are currently being contacted by mail and asked to provide ratings on a personality inventory, an adjective checklist, and a biographical questionnaire describing their relation to the ratee. Data from these raters will be used to confirm or qualify findings from self-report measures.

D. The longitudinal analysis of change or stability requires the collection of data over a period of many years. A battery of tests has been selected which will be administered by mail to BLSA participants and their spouses on a cyclical basis. Most tests will be re-administered after six years, at which time longitudinal analyses will be performed. The use of a 10% holdout sample, who will receive these tests for the first time after six years, makes possible the use of cross-sequential and time-sequential designs to help in distinguishing generational and times-of-measurement effects from true maturational changes. In the intervening six years, it will be possible to analyze data from new measures in conjunction with existing data from the GZTS, Activities and Attitudes Questionnaire, and other instruments. While true longitudinal designs cannot be used with different instruments, long-term predictive relations can be sought, and certain kinds of quasi-experimental designs can be used.

E. (1) Further analyses of these data are planned.

E. (2) This project was completed.

E. (3) In the coming year, emphasis will focus on the identification of dimensions underlying abnormal illness behavior. Results of previous research have indicated the importance of incorporating such features as perceived disease conviction, illness disruption and vulnerability to illness in a comprehensive understanding of individual's self-perceptions and responses to health and illness. IBQ scales will also be correlated with selected BLSA archival data in an effort to determine their convergent and divergent validity against measures of personality, somatic complaints and health status.

Publications:

Costa, P. T., Jr., McCrae, R. R., & Arenberg, D. Recent longitudinal research on personality and aging. In K. W. Schaie (Ed.), Longitudinal Studies of Aging. New York: Guilford Press, 1983, pp. 222-265.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00076-04 LBS

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Openness to Experience and Coping Styles

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert R. McCrae, Research Psychologist, Stress & Coping, LBS, GRC, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Stress & Coping Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	.6	.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☒ (a2) Interviews

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

This project is concerned with the personality disposition of openness to experience and its relation to coping styles. One study investigates the effects of openness on vocational interests and mid-life career shifts. A second study examines the relative contributions of person, situation, and interaction factors in determining the choice of coping mechanisms, and explores the relations between personality dimensions including openness and specific coping efforts.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other perfessional personnel engaged on the project:

Paul T. Costa, Jr. Chief, Stress & Coping Section LBS GRC NIA

Project Description:

Objectives:

Openness to experience is a broad dimension of personality that comprises a number of traits (including dogmatism, rigidity, openness to feelings, and need for variety) previously studied in isolation. In some personality systems, openness is viewed as the antithesis of defensiveness; and defense, in turn, is seen as the core of all coping processes. This project attempts to measure a broad range of traits in the domain of openness utilizing a variety of assessment techniques. Simultaneously, measures of coping will be employed so that the relation between openness and styles of coping can be determined.

Specific Project Objectives:

A. To investigate the influence of openness on vocational behavior in adulthood by examining the relations between openness and Holland's vocational interest scales, and by contrasting on the dimension of openness those who have and have not recently changed careers.

B. To determine the relative importance of person, situation, and interaction factors in determining specific coping responses, and to identify coping mechanisms used differentially by open and closed individuals.

Methods Employed:

A. Previous work in this Section has shown that openness can be validly measured by both self-reports and spouse ratings, and in joint factor analyses of self-reports and ratings, a factor of openness emerges that is distinct from both neuroticism and extraversion. In the past year, an attempt has been made to trace the influence of openness on other elements of the life structure, particularly vocational behavior. As part of a program of research using subjects from the Augmented Baltimore Longitudinal Study of Aging (BLSA), 217 men and 144 women aged 21-89 were administered the Holland (1979) Self-Directed Search (SDS), a vocational interest inventory yielding scores for six types of vocations: Realistic, Investigative, Artistic, Social, Enterprising, and Conventional. Scores for these six interests were correlated with self reports and spouse ratings of openness to experience that had been obtained previously. Augmented BLSA subjects also complete a checklist of life events on a two year schedule, and one of the items concerns change in occupation. Research conducted elsewhere had shown that individuals who changed occupations during the mid-life period were more open to experience, but it could not be determined whether openness was an antecedent or consequence of job change. Using longitudinal data, 99 subjects under age 65 who reported occupational change before (N = 35) and after (N = 64) administration of openness measures were contrasted with 321 subjects who reported no job change during this period.

B. Previous research conducted in this Section has shown that the nature of the stressful event influences the individual's choice of coping mechanisms. The present study was conducted to assess the relative importance of person, situation, and interaction factors in determining the form of coping employed.

In response to three stressful situations they had recently encountered--a loss, a threat, and a challenge--151 men and women ranging in age from 21 to 90 completed a coping questionnaire from which scores for the use of 27 coping mechanisms were obtained. Statistical techniques adapted from Golding (1975) were used to estimate the proportions of variance accounted for by persons and situations. For the ten coping mechanisms measured by two or more items, Endler's (1966) model was used to estimate proportions of variance for persons, situations, and their interactions. These statistical models have usually been applied to data consisting of hypothetical responses to situations; in the present study, actual recalled coping behavior is analyzed.

Major Findings:

A. For both men and women, openness was significantly correlated with Investigative, Artistic, Social and Enterprising interests. Realistic and Conventional interests were not associated with openness. This pattern of results suggested that open individuals have a wider range of interests than closed individuals and are likely to score high on a variety of scales. When scores were calculated as a proportion of total endorsements, Artistic interests were positively related to openness in men ($r = .41$, $p < .001$) and women ($r = .43$, $p < .001$), and Conventional interests were inversely related to openness in men ($r = -.29$, $p < .001$) and women ($r = -.44$, $p < .001$). Among women only, openness was significantly related to investigative interests ($r = .28$, $p < .001$). The occupations of Author, Anthropologist, Free Lance Writer, Journalist, Playwright, and Independent Research Scientist were particularly appealing to open men and women. These results, based on self-reports of openness, were confirmed when spouse ratings of openness from a subset of 100 men and 93 women were correlated with SDS scales. In the total sample, Investigative ($r = .25$, $p < .001$) and Artistic ($r = .37$, $p < .001$) scores were positively related to rated openness, whereas Conventional ($r = -.17$, $p < .05$) scores were negatively related. Comparison of individuals who reported a change in occupation before and after administration of openness measures with those who reported no job change in this period showed significant differences in levels of openness. Individuals who changed jobs before completing personality measures were significantly higher in openness to experience than were men and women who did not change jobs, thus replicating previous findings. In addition, however, openness scores were also significantly higher for those who subsequently changed jobs in the two-year period following administration of the openness measure, suggesting that openness preceeded and perhaps influenced the decision to change jobs. Individuals who scored above the median in openness were almost twice as likely to change jobs over the interval studied as were those below the median. The association of openness with job change was found in all six facets of openness--fantasy, aesthetics, feelings, actions, ideas, and values--and was particularly marked in women in the present sample, for whom occupational decisions may be less strongly determined by economic necessity, and consequently more subject to the influence of personality dimensions. Job change was not related to neuroticism or extraversion.

B. In the analysis of the 27 mechanisms, persons accounted for an average of 27% of the variance, whereas situations accounted for only about 3%. Person effects were particularly important in the use of Hostile Reaction, Escapist Fantasy, Sedation and Faith. The Endler analysis of ten coping mechanisms produced the following results.

Mechanism	Source of Variance						
	Persons	Situations	Responses	PxS	PxR	SxR	Residual
Hostile Reaction	.170	.009	.045	.114	.092	--	.570
Rational Action	.134	.005	.028	.145	.041	.002	.646
Seeking Help	.066	.004	.019	.337	.070	--	.506
Fatalism	.075	.090	.014	.138	.023	--	.662
Positive Thinking	.061	.018	.082	.077	.098	.040	.623
Escapist Fantasy	.266	.000	.004	.066	.115	--	.549
Intellectual Denial	.094	.000	.097	.029	.143	.015	.622
Restraint	.213	.008	.015	.131	--	--	.668
Self-Adaptation	.121	.046	.029	.092	.092	.024	.595
Passivity	.062	--	.153	--	.122	.033	.644
Mean	.126	.018	.049	.113	.079	.011	.609

Persons accounted for an average of 13% of the variance, situations for about 2%, and the person-by-situation interaction for about 11% of the variance. These results suggest that person factors are extremely important in the determination of coping responses, and personality dispositions, including openness to experience, are likely to be one source of consistency in the individual's coping efforts.

Significance to Biomedical Research and the Programs of the Institute:

Successful adaptation to aging requires successful coping. Medical researchers have been increasingly attentive to the role of individuals' coping efforts in recovery from illness and management of chronic disease. However, no clear framework has emerged for ordering or classifying coping strategies, nor are there theories which would allow the specification of the most appropriate coping mechanisms for a particular individual or situation. This program of research is designed to examine a wide variety of coping and defensive processes in the context of a clearly defined and measured dimension of personality. If general principles relating personality dispositions to coping styles can be discerned, the application to specific medical problems can be greatly enhanced. In addition, the study of openness to experience in aging men and women contributes directly to the mission of this institute by advancing basic knowledge on personality and aging.

Proposed Course of the Project:

A. Research extending the construct of openness to experience will continue. A variety of alternative measures and methods of measurement are currently being collected on BLSA subjects, including Q-sorts, adjective rating forms, and peer ratings. All of these sources of information will be used to further define the concept of openness and trace its influence on the lives of aging men and women.

B. All coping is an effort to minimize distress and/or solve problems. However, not all coping efforts are equally successful. New analyses are planned that will evaluate the effectiveness of different coping mechanisms determined by frequency of use, self-rated effectiveness, and long-range outcomes. Additional analyses will consider whether coping mechanisms are differentially effective for different types of stress or for individuals with different personality characteristics.

Publications:

McCrae, R. R., & Costa, P. T., Jr. Psychological maturity and subjective well-being: Toward a new synthesis. Developmental Psychology, 1983, 19, 243-248.

McCrae, R. R. Situational determinants of coping responses: Loss, threat, and challenge. Journal of Personality and Social Psychology, in press.

McCrae, R. R., & Costa, P. T., Jr. Social desirability scales: More substance than style. Journal of Consulting and Clinical Psychology, in press.

McCrae, R. R. Review of Farrell and Rosenberg's Men at midlife. Journal of Gerontology, 1983, 38, 250.

McCrae, R. R., & Costa, P. T., Jr. Joint factors in self-reports and ratings: Neuroticism, extraversion, and openness to experience. Personality and Individual Differences, 1983, 4, 245-255.

McCrae, R. R., & Costa, P. T., Jr. Personality, stress, and coping processes in aging men and women. In R. Andres, E. Bierman, & W. Hazzard (Eds.), Principles of Geriatric Medicine. New York: McGraw-Hill, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00077-01 LBS

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Oral Physiological Status of Man During Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Marc W. Heft Senior Staff Fellow, Stress & Coping, LBS, GRC, NIA

COOPERATING UNITS *(if any)*
NIDR, NIH

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Stress & Coping

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

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

There has been little systematic investigation of tissues within the aging oral cavity. The purpose of this project is to assess the oral physiolgical status of participants in the Baltimore Longitudinal Study and thus provide baseline information which is lacking.

PHS 6040 (Rev. 1/83) GPO 895-100

Other personnel engaged in the project:

Bruce J. Baum Clinical Director NIDR NIH

Project Description:

Objectives:
This project is designed to describe oral and dental tissues during aging and evaluate physiological and pathological factors likely to influence these tissues. Much of our present knowledge regarding oral health in the aging individual comes from studies using institutionalized persons or persons whose general health status has been incompletely characterized. Participants in this study, as a component program of the Baltimore Longitudinal Study, are well-characterized with respect to a large number of medical, physiological, psychological and sociological factors. The influence of such factors on oral health, as well as the influence of specific oral biological variables, are examined. Participants in this study will be seen once every 8 years. It is hoped; in this way, that we can better define oral health throughout adult life and be able to predict the sequelae of various factors (disease, psychological stress, medications, etc.) on oral functions.

Methods Employed:
Participants in this study are seen by the principal investigator in the dental clinic of Baltimore City Hospitals after signing a statement of informed consent. The examination procedures include: history, clinical oral and dental examinations, radiographic examination, collection of stimulated and unstimulated parotid saliva, and participant's completion of two self-report questionnaires.

Data storage and retrieval systems, and analyses of data with BMDP prepackaged programs, are actively utilized. Update, verification and analyses of selected data on a frequent (usually weekly) basis are performed so that trends in findings, or problem areas, are detected routinely.

Major Findings:
Resumption of data collection began this year and data are being collected. Approximately 125 subjects have been examined.

Significance to Biomedical Research and to the Program of the Institute:
This study provides for a reexamination of many "traditional" stereotypes, associated with old age and oral health. Any alterations in salivary gland functions, because of the critical role of saliva in oral health maintenance, may have broad oral effects. Changes in the dental apparatus can have markedly adverse effects on facial appearance, speech, and dietary patterns. Changes in oral motor and gustatory functions can affect the quality of one's life. By providing basic descriptions of oral physiological functions with age, this study will significantly contribute to a rational assessment of what impact changes in oral function may have on the aging individual. In addition, such information should be quite successful for oral health care planning by helping to define the oral health of the aged.

Proposed Course of the Project:
Data collection will continue.

NIA Annual Report
October 1, 1982 through September 30, 1983
Gerontology Research Center
Laboratory of Cellular and Molecular Biology

The Laboratory of Cellular and Molecular Biology (LCMB) brings together various research projects that are related through a common interest in genetic information transfer, the biological aging process, and molecular mechanisms of physiological phenomena. The Inorganic Biochemistry Section is concerned with fundamental studies at the molecular level of biological molecules involved in genetic information transfer. Age changes are investigated in systems involving these molecules, and particular emphasis is placed on the beneficial as well as harmful effects of metal ions that interact with these molecules. The Macromolecular Chemistry Section is concerned with the design of drugs used in age-related diseases. Beta-adrenergic blockers are modifed with the goal of prolonging their activity and diminishing their side effects, and the solubility of lipid soluble vitamins is increased in order to diminish their toxic effects. The Cellular Aging and Genetics Section consists presently only of Mr. Monticone, who carries out studies at the cellular level in collaboration with the Inorganic Biochemistry Section. Plans to build a new section which would utilize recombinant-DNA technology had to be abandoned because of the loss of positions.

Research Highlights

A. DNA Conformation. One of the exciting recent developments in molecular biology has been the discovery that DNA can be readily interconverted between the right-handed B-conformation and the left-handed Z-conformation. Since this conversion can occur only when the DNA contains alternating GC sequences and reflects DNA sequence, it is believed that the conformational change may be important in gene regulation. We have recently discovered that the metal-complex-induced transition from B to Z DNA does not stop at this first transition, but that Z is then converted to another conformer of whose structure we were uncertain -- we called it X -- and that X is then converted into the well-established ψ-structure. We now have reasonable evidence that X-DNA is A-DNA; it has one ^{31}P NMR peak, as expected for the A conformer or the B conformer, but the latter is ruled out by the CD spectrum. We have now obtained phase diagrams that specify the domains (experimental conditions) in which the four conformers, B, Z, "A", and ψ, are to be found. We can thus predict which conformer is obtained under any conditions of DNA concentration, metal complex inducer concentration, time and temperature. DNA modified by methylation (an important gene regulation device) or bromination is readily transformed into Z, but not into A. Both the Z and A conformers readily lead to the ψ(+) structure (aggregated DNA with an intensely positive CD band).

B. DNA Conformation and RNA Synthesis. We have made significant advances in our understanding of the effect of the B to Z conversion on the ability of the DNA to act as template for RNA synthesis. If this transition is important in genetic regulation, one might expect that RNA synthesis should be affected. The B $\rightarrow$ Z conversion was carried out in three systems: poly(dGdC)·poly(dGdC) using $[Co(NH_3)_6]^{3+}$, methylated poly(dGdC)·poly(dGdC) using the same cobalt complex, and the methylated poly(dGdC)·poly(dGdC) using Mg(II) ions. Even though the transition between conformers occurs at very different concentrations of Co complex with the methylated and unmethylated polymers, in each case the transition

is correlated with a marked decrease in template efficiency of the DNA, and the Mg induced transition produces the same result. Thus, the B to Z transition is shown to be capable of the regulation of gene transcription.

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D. Aging Studies by Nuclear Magnetic Resonance, Metals in Cells. NMR techniques are being applied to a variety of biological materials to study aging at the cellular and organ levels. NMR provides a noninvasive technique for continuously monitoring metabolites. A surface coil probe has been developed to observe the phosphorus metabolism of organs such as brain and kidney in living mice. Using this probe on young (6 months) and old (24 months) mice so far has revealed no age differences in the steady state of ATP and phosphocreatine. ^{31}P NMR is also being used to compare the metabolism in the head and tail sections of the earthworm. No difference in energy consumption has so far been observed under conditions of mild stress such as small changes in the temperature of the environment. These studies are hampered by the fact that some of them require the use of instrumentation in other laboratories to which we have limited access. The ^{31}P NMR spectrum of cultured human fibroblasts has been obtained, thus permitting the study of their metabolism as a function of population doubling level. These NMR studies on in vitro aging are complemented by studies designed to determine the role of metal ions in cellular aging. Using atomic absorption spectrophotometry we have noted higher concentrations of zinc and copper ions with cell passage in IMR 90 cells, as well as a higher rate of uptake of Zn(II) in late passage.

E. Aluminum and Alzheimer's Disease. This laboratory has for some time been engaged in the study of the molecular basis of the apparent correlation between Alzheimer's disease and the accumulation of aluminum in the brain. Although the correlation is controversial, it is clear that Al accumulates in the brain during aging, and the effect of the Al should be investigated. During the past year the increased level of aluminum previously demonstrated in cortical cells undergoing neurofibrillary degeneration in Alzheimer's disease has also been observed in two additional neurological disorders. Since Al has been localized in brain cell nuclei, the significance of our studies on the interaction of Al with DNA and chromatin has become even more apparent. The feature of Al binding to DNA that is most likely to produce deleterious effects is crosslinking of DNA strands by the Al. We have therefore focussed on the crosslinking effect. It occurs in DNA molecules of all base compositions, but the GC rich sequences are preferentially bound. Al crosslinking has been quantitized in synthetic polydeoxy-

nucleotides; 50% of the bases become crosslinked at an Al concentration of 1 Al(II) per 2 base pairs. An extra ^{31}P NMR peak observed with chromatin in the presence of other metals. A comparison of chromatin from Alzheimer brain autopsies and controls has so far led to no definite conclusions: the Al peak may or may not be observed in Alzheimer's but has not been observed in controls.

F. Metal Probes of Nucleic Acid Structure. Some time ago we utilized the copper acetate dimer as a specific reagent to distinguish a ribonucleoside from a deoxynucleoside. The distance between the 2 copper atoms in the reagent exactly matched the distance between the OH groups of the ribose. Since the copper acetate dimer is stable only in non-aqueous solution, thus limiting its suitability for studies under biological conditions, we have attempted a similar reaction with a rhodium dimer. We prepared a hydrated rhodium acetate dimer $[Rh_2(OAc)_2(H_2O)_4]2H_2O$, which did not react with the OH group but rather with the bases, but in a specific manner, so that adenine reacts much more readily than any of the other bases. Such a reaction could be explored to probe nucleic acid structure.

G. ESR Studies on Membranes. It has been shown that the cholesterol content of biological membranes, which generally increases with age, plays an important role in regulating membrane fluidity and function. In order to relate alterations in cholesterol level with cellular function, it would be important not only to know the amount of cholesterol, but to be able to distinguish changes in the distribution and arrangement of cholesterol within cellular systems. We have found that we can address these crucial questions by supplementing chemical analysis with studies on the kinetics of the enzymatic oxidation of cholesterol. We have shown that this reaction is extremely sensitive to the organization of the membrane and the local phospholipid content in the region of the cholesterol. This observation provides a sensitive way to detect and distinguish between different pools of cholesterol within individual cells. We have utilized this method in our studies of adipocyte ghosts where it was possible to quantitatively distinguish between the plasma membrane with a high cholesterol phospholipid ratio and the endoplasmic reticulum with a low cholesterol phospholipid ratio.

H. Hemoglobin and Oxygenation. The transport of oxygen from the lungs to the tissue is determined by the cooperative binding of oxygen to hemoglobin. The major unresolved questions about hemoglobin involve an understanding of the conformational fluctuations which permit the ligands access to the heme pocket in order to bind to the iron, as well as the dynamic pathway for the transmission of conformational changes between the hemoglobin subunits. We have initiated a program using electron spin resonance and Mössbauer spectroscopy to probe the dynamics at the iron and in the heme pocket. These studies indicate that in the region of 200°K the mean square displacement of the iron undergoes a transition associated with an abrupt increase in this displacement. These transitions take place at lower temperatures for liganded hemoglobins than unliganded hemoglobins. There is also a greater temperature dependence of the isomer shift for liganded hemoglobins which can be attributed to a greater contraction of the Fe-ligand bond distance. Above 200°K, a low spin species which appears to be a complex between iron and the distal histidine is formed with deoxyhemoglobin and methemoglobin. The formation of this complex demonstrates that even at these subzero temperatures there is adequate dynamic freedom in the ligand pocket to permit the iron to move into the heme plane and the distal histidine to be oriented in a suitable configuration to bind with the iron.

We are further trying to relate the dynamics at the heme and in the ligand pocket to the conformational changes which are involved in the cooperative binding of ligands to hemoglobin. For this purpose we have compared hemoglobin with myoglobin, a single subunit protein which is structurally similar to hemoglobin but which binds ligand without major conformational changes. In this case, no complex with the distal histidine is observed in either metmyoglobin or deoxymyoglobin, indicating that the ligand pocket is much more rigid.

I. Beta-Adrenergic Agents. Drugs of this group are extensively used to treat hypertension, cardiac arrhythmias, and angina pectoris. Numerous studies with these drugs show that the drugs' effects are due to a blockade of receptors for adrenergic hormones, which are located on the surface of cells. This year, in collaboration with Drs. Kuenzel and Augustine, the irreversible antagonist, bromoacetylalprenolol-menthane (BAAM) was used to localize the receptors responsible for therapeutic activity of beta-blockers. This compound was administered both peripherally and centrally to turkey poults, Meleagris gallopavo. Peripheral (intraperitoneal) administration of BAAM effected a significant reduction in blood pressure and heart rate. Biochemical analysis following the peripheral injections of BAAM showed a significant decrease in beta-adrenoreceptors in heart tissue, but no change in the number of beta-adrenoreceptors in brain tissue. Central (intraventricular) administration of BAAM resulted in no change in mean blood pressure or heart rate. Biochemical analysis of heart tissue following central injections of BAAM showed little or no change in the number of beta-adrenoreceptors. There was, however, a significant decrease in the number of beta-adrenoreceptors in brain tissue. These experiments clearly demonstrated that therapeutically relevant receptors are peripherally located.

The biochemical factors involved in aging and deterioration of the cardiovascular system remain puzzling. The beta-adrenergic system is clearly affected by aging but published data are scanty. On the initiative of Dr. Kusiak these problems were addressed with the following results. The cardiac beta-adrenergic coupled adenylate cyclase system was examined in young and old male Wistar rats. The number of binding sites for dihydroalprenolol, which is a probe for beta-adrenoreceptors, in membranes prepared from cardiac ventricles was 21.1 $\pm$ 2.78 (SD) fmoles/mg in 3-4 month old rats and 31.2 $\pm$ 2.20 fmoles/mg in 24 month old rats. The dissociation constant, K_D was 4.3 $\pm$ 1.8 nM and 6.7 $\pm$ 1.7 nM for young and old rats, respectively. Beta-adrenoreceptors exert their intracellular effects through activation of an enzyme, adenylate cyclase; the activation occurs via the regulatory protein. Various compounds were used to study the characteristics of activation of this adenylate cyclase in homogenates from cardiac ventricles. Basal adenylate cyclase was reduced 30% in old animals compared to young. (-)Isoproterenol alone stimulated adenylate cyclase greater than two-fold above basal in young rats and this activity was reduced by 34% in old animals. GppNHp, fluoride, and forskolin activation of adenylate cyclase above basal was reduced 38, 37, and 34%, respectively, in the old animals. The affinity of the catecholamine agonists (-)isoproterenol, (-)epinephrine, and (-)norepinephrine for stimulation of adenylate cyclase was decreased only very slightly in the old rats, but the maximum level of stimulation was reduced by a full 34%. These results suggest a reduction in the amount of functional regulatory protein in old rat ventricular tissue compared to young tissue. However, this data does not rule out the possibility of altered molecular interactions of a full complement of regulatory protein(s) with beta-adrenergic receptor and/or catalytic adenylate cyclase.

J. Lipid Soluble Vitamins. These compounds, in addition to their obvious nutritional values, have further beneficial effects. Unfortunately, to obtain a significant measure of these additional benefits, doses that already elicit toxicity are required. Thus retinoids, which are derivatives of vitamin A, have been shown to reduce the incidence of lesions in epithelium induced by carcinogens. Work done in this program focuses on lessening the toxic effects of retinoids through transforming them to a water soluble form by complexation with solubilizing agents. In previous years cyclodextrins were investigated as solubilizing agents; this year two new systems have been explored and their efficiency has been compared to that of cyclodextrins.

The first system is based on polyamino acid. Poly-L-methionine sulfoxide is a water soluble polymer containing structural elements of dimethyl sulfoxide. The preparation and radiolabeling of this polymer was performed and its bioeffects were compared with those of dimethyl sulfoxide. Poly-L-methionine sulfoxide is, similarly to dimethyl sulfoxide, a potent solubilizer of lipophilic compounds in water. The partition coefficient of poly-L-methionine sulfoxide in 1-octanol/water is only twenty times lower than that of dimethyl sulfoxide, but the former, in difference to the latter, was found not to penetrate into intracellular spaces. In contrast to dimethyl sulfoxide, poly-L-methionine sulfoxide and L-methionine sulfoxide were found to be ineffective in (a) inducing differentiation in murine erythroleukemic cells and (b) inhibiting differentiation of avian neural crest cells. This suggests that for a compound to be effective in these processes it must have the ability to penetrate into cells or into membrane proteins. Overall lack of bioactivity of poly-L-methionine sulfoxide, combined with low toxicity (2 g/kg i.v. in mouse without effect), makes this compound a suitable inert solubilizer and carrier for lipophilic drugs.

The second system studied is based on digitonin, a saponin contained in plants. Digitonin was modified by condensation with propylene oxide or with 1,4-butanediol diglycidyl ether in aqueous alkali yielding products in which some of the $-CH_2OH$ groups of digitonin were converted to $-CH_2-O-CH_2-CHOH-CH_3$ or to $-CH_2-O-CH_2-CHOH-CH_2-O-(CH_2)_4-O-CH_2-CHOH-CH_2OH$ groups, respectively. These modified digitonins were very soluble in water and chloroform and effectively solubilized lipophilic compounds into aqueous solutions; e.g., 2 mg of vitamin A or 0.6 mg of vitamin D could be dissolved per 1 ml of 5% aqueous solutions of modified digitonins. Compared to the toxicity of digitonin (LD_{50} 4 mg/kg i.v.) the toxicity of modified digitonin was greatly reduced: doses of 500 mg/kg i.v. infusion were not lethal for mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00044-10 LCMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Effect of Metals and Proteins on Nucleic Acids, Information Transfer and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Gunther L. Eichhorn, Chief, Laboratory of Cellular & Molecular Biology, NIA

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biology, NIAMDD; Department of Biophysics, Johns Hopkins University

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Section on Inorganic Biochemistry

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project focuses on the interaction of molecules concerned with genetic information transfer. A primary objective is to determine under what conditions metal ions are essential for information transfer, and under what conditions they produce errors in the information and may thus contribute to biological aging. Topics of interest are: (1) the effects of metal ions on the structure of nucleic acids, nucleoproteins and chromatin; (2) the mechanism of involvement of aluminum in Alzheimer's disease; (3) crosslinking of nucleic acid strands by metal ions; (4) the effects of metal ions on RNA polymerase; (5) metal ions and cellular aging; (6) non-invasive studies of age changes in metabolism.

PHS 6040 (Rev. 1/83)

GPO 895-100

Names, laboratory and institute affiliations, and titles of professional personnel engaged on the project:

J.J. Butzow	Commissioned Officer	LCMB NIA	
P. Clark	Research Chemist	LCMB NIA	
Y.A. Shin	Research Chemist	LCMB NIA	
E. Tarien	Chemist	LCMB NIA	
S.J. Karlik	Visiting Associate	LCMB NIA	DOD 09/30/83
R.P. Pillai	Visiting Associate	LCMB NIA	
R.P. Singhal	Special Expert	LCMB NIA	DOD 08/23/83

Project Description:

Objectives: The objectives of the Inorganic Biochemistry Section are (1) to study the effects of metals ions on the structures involved in the replication, transcription and translation of genetic information; (2) to determine how errors can be introduced into genetic information transfer by metal ions, and how these errors may affect aging; (3) to understand the interactions of proteins and nucleic acids and their mediation by metal ions; (4) to understand the role of metal ions in aging, and particularly the possible role of aluminum in aging and Alzheimer's disease; and (5) to utilize NMR techniques to monitor the effect of age on animal metabolism.

Methods Employed: (1) The interaction of metal ions, proteins, and nucleic acids are studied by circular dichroism and UV spectrophotometry to determine conformational changes, and by infrared, nuclear magnetic resonance and electron spin resonance techniques to determine interactions sites. (2) Effects of metal ions on nucleic acids and nucleoprotein are studied to determine under what conditions they serve an essential function in information transfer, and under what conditions they induce errors in information content. (3) The effect of metal ions on the enzymes responsible for genetic information transfer are studied. (4) The mechanisms by which enzymes and metal ions synthesize and degrade nucleic acids are elucidated. (5) Nuclear magnetic resonance is used as a non-invasive technique to study age changes in cellular and animal metabolism.

Major Findings:

DNA Conformation. One of the exciting recent developments in molecular biology has been the discovery that DNA can be readily interconverted between the right-handed B-conformation and the left-handed Z-conformation. Since this conversion can occur only when the DNA contains alternating GC sequences and reflects DNA sequence, it is believed that the conformational change may be important in gene regulation. We have recently discovered that the metal-complex-induced transition from B to Z DNA does not stop at this first transition, but that Z is then converted to another conformer of whose structure we were uncertain -- we called it X -- and that X is then converted into the well-established ψ-structure. We now have reasonable evidence that X-DNA is A-DNA; it has one ^{31}P NMR peak, as expected for the A conformer or the B conformer, but the latter is ruled out by the CD spectrum. We have now obtained phase diagrams that specify the domains (experimental conditions) in which the four conformers, B, Z, "A", and ψ, are to be found. We can thus predict which conformer is obtained under any conditions of DNA concentration,

metal complex inducer concentration, time and temperature. DNA modified by methylation (an important gene regulation device) or bromination is readily transformed into Z, but not into A. Both the Z and A conformers readily lead to the $\psi(+)$ structure (aggregated DNA with an intensely positive CD band).

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Significance to Biomedical Research and Program of the Institute. The participation of metal ions in every aspect of genetic information transfer and the deleterious effects on this transfer caused by undesired metal ions or essential metal ions in undesired concentrations make the study of metal ion interactions with nucleic acids of major importance. The possible relationship between aluminum accumulation and Alzheimer's disease and the discovery that the aluminum is bound to chromatin have emphasized the importance of studies on metal interaction with nucleic acids and chromatin. An understanding of the structure and function of chromatin (and therefore protein - DNA interaction), ribosomes, the nucleic acid polymerases, etc. is essential to an understanding of cellular aging. We are particularly interested in studies that show how information transfer can go wrong. Metal ions are presumably not responsible for the primary events that cause aging but we believe that they may be important factors in determining individual and geographic differences in the aging process.

Proposed Course of the Project. We intend to continue to explore the implications of the conformational flexibility of DNA that permit reaction with a single

metal complex to bring about transitions between four different DNA conformations. We shall collaborate in X-ray fiber analysis, electric dichroism and Raman studies to further characterize our X-DNA, and we shall look for the transitions in other polymers in which the alternating GC sequence that is susceptible to these transitions occurs along with other sequences that are not susceptible. We hope to learn the mechanism by which metal complexes bring about these transitions.

Having compared the template activity of B and Z DNA in RNA synthesis, we wish to study also "A" DNA and ψ DNA as templates. We want to study the mechanism for the decreased template activity of Z-DNA, with the following considerations: (1) how does the conformational change affect interaction of enzyme with template; and (2) what stages in RNA synthesis are affected: initiation, elongation or termination. We shall also try to determine whether the effect of metals on the fidelity of RNA synthesis is due to conformational change in the RNA polymerase induced by reaction with the metal ions.

We shall study aging cells and animals with NMR techniques primarily with the object of determining the effect of drugs on the metabolism of these animals. We expect that old and young animals will show rather different responses, but it is very important to determine whether this is true and to what extent.

We intend to continue our investigations into the crosslinking of Al, the relation of this crosslinking to crosslinking by other metals, and the comparison of Alzheimer and control brain autopsies by NMR techniques.

We plan to compare the uptake into cells in culture of a variety of metal ions and then study the impact of these ions on the cellular metabolism of nucleic acid molecules. These studies will be carried out with cells in different population doublings (in vitro) and with cells from donors of different age (in vivo aging).

We shall study the possible role of cytidine methylation in the aging process by measuring methylation in DNA obtained from young and old animals.

Publications:

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Eichhorn, G. L., and Marzilli, L. G. (Eds.): Advances in Inorganic Biochemistry, Vol. IV. New York, Elsevier/North-Holland, 1982.

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Hoopes, L. L. M., Brown, A., and Huang, R. C.: Methylation and rearrangement of mouse intracisternal A particle genes in development, aging and myeloma. Molecular & Cellular Biology, in press.

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Singhal, R. P.: High-performance liquid chromatography of transfer RNAs. Separation of transfer RNAs from mammalian sources. J. Chromatogr., in press.

Singhal, R. P.: Queuine: an addendum. Prog. Nucl. Acid. Res. Mol. Biol. 28: 75-80, 1983.

Singhal, R. P., and Vakharia, V. N.: The role of queuine in the aminoacylation of mammalian aspartate transfer RNAs. Nucl. Acid Res. 11: 4257-4272, 1983.

Theil, E. C., Eichhorn, G. L., and Marzilli, L. G. (Eds.): Advances in Inorganic Biochemistry, Vol. V. Iron Binding Proteins without Cofactors or Sulfur Clusters. New York, Elsevier/North-Holland, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00047-13 LCMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Structure-Function Relationships in Hemoglobin and Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Joseph M. Rifkind, Research Chemist, LCMB, NIA

COOPERATING UNITS *(if any)*

Johns Hopkins University

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Inorganic Biochemistry Section

INSTITUTE AND LOCATION

National Institute on Aging, Baltimore, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this project is to study the mechanisms involved in regulating the binding of oxygen to hemoglobin and the transport of oxygen to the tissues. The project also focuses on ways in which these functions are impaired and change with age. We have therefore studied the mechanisms involved in the oxidation of hemoglobin. Oxidation affects oxygen transport because it produces nonfunctional hemoglobin, which no longer binds oxygen. These studies have been extended to include an investigation of the stability of the entire erythrocyte and the erythrocyte membrane as well as other structure function relationships in membranes.

PHS 6040 (Rev. 1/83)

GPO 895-100

Names, laboratory and institute affiliations, and titles of professional personnel engaged on the project:

Periakaruppan Manoharan	Visiting Scientist	LCMB NIA	EOD 11/14/82
Peter Chuknyiski	Visiting Fellow	LCMB NIA	EOD 01/01/83

Project Description:

Objectives: (1) To study the binding of ligands to hemoglobin, and the role of the protein in controlling this function. (2) To study the mechanisms for maintaining hemoglobin in its functional form. (3) To study the mechanisms involved in regulating the transport of oxygen to the tissues. (4) To elucidate age-related changes in the composition and functional properties of the erythrocyte and its membrane.

Methods Employed: Various preparative procedures are used to purify erythrocyte proteins and to separate various components of the erythrocyte. Visible, UV and atomic absorption spectroscopy, as well as gel electrophoresis, are used to analyze for various erythrocyte components. The oxygenation and oxidation of hemoglobin solutions and whole erythrocytes are investigated under various conditions with and without the addition of various substances. Binding of metal ions and other small substances to hemoglobin and other erythrocyte components are studied by equilibrium dialysis. Electron spin resonance is used to observe paramagnetic spin labels and Cu(II), and to detect changes in the mobility of membrane components.

Major Findings:

A. ESR Studies on Membranes. It has been shown that the cholesterol content of biological membranes, which generally increases with age, plays an important role in regulating membrane fluidity and function. In order to relate alterations in cholesterol level with cellular function, it would be important not only to know the amount of cholesterol, but to be able to distinguish changes in the distribution and arrangement of cholesterol within cellular systems. We have found that we can address these crucial questions by supplementing chemical analysis with studies on the kinetics of the enzymatic oxidation of cholesterol. We have shown that this reaction is extremely sensitive to the organization of the membrane and the local phospholipid content in the region of the cholesterol. This observation provides a sensitive way to detect and distinguish between different pools of cholesterol within individual cells. We have utilized this method in our studies of adipocyte ghosts where it was possible to quantitatively distinguish between the plasma membrane with a high cholesterol phospholipid ratio and the endoplasmic reticulum with a low cholesterol phospholipid ratio.

B. Hemoglobin and Oxygenation. The transport of oxygen from the lungs to the tissues is determined by the cooperative binding of oxygen to hemoglobin. The major unresolved questions about hemoglobin involve an understanding of the conformational fluctuations which permit the ligands access to the heme pocket in order to bind to the iron, as well as the dynamic pathway for the transmission of conformational changes between the hemoglobin subunits. We have initiated a program using electron spin resonance and Mössbauer spectroscopy to probe the

dynamics of the iron and in the heme pocket. These studies indicate that in the region of 200°K the mean square displacement of the iron undergoes a transition associated with an abrupt increase in this displacement. These transitions take place at lower temperatures for liganded hemoglobins than unliganded hemoglobins. There is also a greater temperature dependence of the isomer shift for liganded hemoglobins which can be attributed to a greater contraction of the Fe-ligand bond distance. Above the temperature where increased fluctuations of iron occur, a low spin species which appears to be a complex between iron and the distal histidine is formed with deoxyhemoglobin and methemoglobin. The formation of this complex demonstrates that even at these subzero temperatures there is adequate dynamic freedom in the ligand pocket to permit the iron to move into the heme plane and the distal histidine to be oriented in a suitable configuration to bind with the iron.

We are further trying to relate the dynamics at the heme and in the ligand pocket to the conformational changes which are involved in the cooperative binding of ligands to hemoglobin. For this purpose we have compared hemoglobin with myoglobin, a single subunit protein which is structurally similar to hemoglobin but which binds ligand without major conformational changes. In this case, no complex with the distal histidine is observed in either metmyoglobin or deoxymyoglobin, indicating that the ligand pocket is much more rigid.

Significance to Biomedical Research and the Program of the Institute: The physiological role of hemoglobin is to transport oxygen from the lungs to the cells. The efficient uptake and release of oxygen requires cooperative oxygen binding and the proper regulation of oxygen affinity. It is also necessary to maintain the integrity of the erythrocyte and to limit the oxidation of hemoglobin in order to maintain an adequate concentration of functional hemoglobin in circulation. These studies thus help to elucidate a vital function of organisms. The aging process can involve changes in the ability of the organism to transport oxygen to certain tissues. Studies on erythrocytes are also used to compare with and to help understand changes with age in the function and structure of other more complex cellular systems.

Proposed Course of the Project: (1) We plan to extend our investigation of the dynamics at the iron and in the ligand pocket to isolated subunits, modified and abnormal hemoglobin, as well as partially liganded hemoglobin. (2) We plan to further investigate the binding sites for metal ions in hemoglobin and various membrane proteins and to attempt to elucidate the changes in functional properties which take place as a result of metal ion interaction. (3) We plan to utilize the oxidation of cholesterol as a probe for membrane organization. (4) We plan to develop models for and study effects of spin interactions between spin probes on membranes in order to develop this as a possible new probe for membrane mobility. (5) We plan to extend our studies on membrane mobility and fluidity using ESR to include fluorescent techniques. The much greater sensitivity of fluorescence over ESR will extend our capability to perform biological and aging studies. (6) We plan to study the oxygenation of whole blood to delineate the regulatory mechanisms and how they change during aging.

Publications:

Chuknyiski, P. P., Heim, J. M., and Rifkind, J. M.: Spectroscopic studies of the dynamics of deoxyhemoglobin. In Proceedings of the Third Conversation in the Discipline Biomolecular Stereodynamics, in press.

Elgavish, A., Rifkind, J., and Sacktor, B.: In vitro effects of vitamin D_3 on the phospholipids of isolated renal brush border membranes. J. Membr. Biol. 72: 85-91, 1983.

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Rifkind, J. M.: Interaction of Zinc with Erythrocytes. In Sigel, H. (Ed.): Metal Ions in Biological Systems. New York and Basel, Marcel Dekker, Inc., 1983, Vol. 15, pp. 275-317.

Rifkind, J. M., Araki, K. and Hadley, E. C.: The relationship between the osmotic fragility of human erythrocytes and cell age. Arch. Biochem. Biophys. 222: 582-589, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00088-11 LCMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Mechanisms of Cellular Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert E. Monticone, Biologist, LCMB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Studies are carried out to determine the effects of metal ions on cellular aging. The object is to determine how the permeability of the cells to ions changes with age and what effects the metal ions the penetrate the cell have on genetic information transfer within the cell.

PHS 6040 (Rev. 1/83)

GPO 895-100

Names, laboratory and institute affiliations, and titles of professional personnel engaged on the project:

Gunther L. Eichhorn Chief, LCMB LCMB,NIA

Project Description:

Objectives: Studies have been initiated on the determination of the effects of metal ions on aging in tissue culture. The objective is to find how the permeability of cells to ions changes with age and what effects the metal ions that penetrate the cell have on genetic information transfer within the cell.

Major Findings:

We have performed an extensive study of zinc accumulation in cultured human diploid fibroblasts (IMR-90). Our results show that the accumulation of zinc in these cells varies with the concentration of metal present in the culture medium in a linear manner. We have also shown that cultured fibroblasts reach maximum uptake of zinc in 2-3 hours after exposure to high levels in the medium.

Since human diploid fibroblasts have a limited in vitro lifespan, they are used as a reasonable model with which to study cellular aging. We have observed zinc uptake in IMR-90 at varying passages throughout the in vitro lifespan to determine if any change occurs in zinc permeability. Our findings show that the total zinc content of the cells approximately doubles from early to later passage. In addition, later passage cells have the capacity to accumulate higher amounts of zinc upon exposure than earlier passage cells.

The contract study "In Vitro Assessment of Human Cellular Aging" with Dr. James Smith of the W. Alton Jones Cell Science Center in Lake Placid has proceeded in a satisfactory manner. Approximately 100 fibroblast cultures have been established from skin biopsies in the past year and (CSD) colony size distribution analysis performed on them. This has increased the number of female cultures and repeat donor cultures to enable a better comparison. Progress is being made with the epidermal keratinocyte cultures and a morphological survey of explant outgrowth has been initiated on 25 individual cultures.

Proposed Course:

We shall compare the effects of other metal ions to those that have been found for zinc and attempt to determine how these metals affect the cell division process in aging human cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00046-13 LCMB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Medicinal Chemistry Applied to Problems Prominent in Senescence

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Josef Pitha Chief, Macromolecular Chemistry Section, LCMB, NIA

COOPERATING UNITS *(if any)*

Key Pharmaceuticals, Miami, FL; University of Florida, Gainesville, FL; George Washington University, Washington, D.C.; University of Maryland, College Park, MD

LAB/BRANCH

Laboratory of Cellular and Molecular Biology

SECTION

Macromolecular Chemistry Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Two groups of drugs which are heavily used by the elderly have been subjected to molecular manipulations in order to learn how their pharmacological profiles can be improved. In the group of beta-adrenergic blockers an irreversible blocker has been used to study the localization and properties of pharmacologically important drug-receptors. Furthermore, in order to form a broader basis for a rational drug design, aging changes in the beta-adrenoceptor system were studied in detail. In the field of fat-soluble vitamins and toxins new organism-compatible solubilizers have been synthesized and found to have the potential to ameliorate symptoms of hypervitaminosis or poisoning.

PHS 6040 (Rev. 1/83)

GPO 895-100

Names, laboratory and institute affiliations, and titles of professional personnel engaged on the project:

John Kusiak	Special Expert	LCMB NIA	
Grzegorz Blotny	Visiting Associate	LCMB NIA	EOD 06/15/83
Teresa Czajkowska	Visiting Associate	LCMB NIA	DOD 07/26/83
Gergely Heja	Visiting Fellow	LCMB NIA	DOD 04/17/83
Tamas Szabolcsi	Visiting Fellow	LCMB NIA	EOD 01/12/83

Project Description:

Objectives: The objective of the project is the innovative design of drugs used in age related diseases. Two classes of drugs have been studied. The first group studied are beta-adrenergic blockers, which are prominent cardiovascular agents. The objectives of this study are to prolong activity of these drugs and to diminish their side effects. The prolongation of drug activity was obtained by preparation of drug derivatives that produced an irreversible change in the tissue receptors for these drugs. The diminishment of side effects was obtained by changes in drug structure which decreased the penetration of modified drugs in the part of the body where the presence of the drug is not required. In another part of the project the water solubility of lipid soluble vitamins is increased by complexation with specially designed carriers; this process may be expected to decrease some of the toxic effects of these and other compounds.

Methods Employed: The study requires application of both chemical and pharmacological techniques. The synthetic chemical work is carried out in the section. The use of contracts for the production of starting materials for the syntheses has been requested. Pharmacological studies that use in vitro systems are also carried out in the laboratory of the section; those studies for which in vivo systems are necessary are performed outside.

Major Findings:

Beta-Adrenergic Agents. Drugs of this group are extensively used to treat hypertension, cardiac arrhythmias, and angina pectoris. Numerous studies with these drugs show that the drugs' effects are due to a blockade of receptors for adrenergic hormones, which are located on the surface of cells. This year, in collaboration with Drs. Kuenzel and Augustine, the irreversible antagonist, bromoacetylalprenolol-menthane (BAAM) was used to localize the receptors responsible for therapeutic activity of beta-blockers. This compound was administered both peripherally and centrally to turkey poults, Meleagris gallopavo. Peripheral (intraperitoneal) administration of BAAM effected a significant reduction in blood pressure and heart rate. Biochemical analysis following the peripheral injections of BAAM showed a significant decrease in beta-adrenoreceptors in heart tissue, but no change in the number of beta-adrenoreceptors in brain tissue. Central (intraventricular) administration of BAAM resulted in no change in mean blood pressure or heart rate. Biochemical analysis of heart tissue following central injections of BAAM showed little or no change in the number of beta-adrenoreceptors. There was, however, a significant decrease in the number of beta-adrenoreceptors in brain tissue. These experiments clearly demonstrated that therapeutically relevant receptors are peripherally located.

The biochemical factors involved in aging and deterioration of the cardiovascular system remain puzzling. The beta-adrenergic system is clearly affected by aging but published data are scanty. On the initiative of Dr. Kusiak these problems were addressed with the following results. The cardiac beta-adrenergic coupled adenylate cyclase system was examined in young and old male Wistar rats. The number of binding sites for dihydroalprenolol, which is a probe for beta-adrenoreceptors, in membranes prepared from cardiac ventricles was 21.1 $\pm$ 2.78 (SD) fmoles/mg in 3-4 month old rats and 31.2 $\pm$ 2.20 fmoles/mg in 24 month old rats. The dissociation constant, K_D was 4.3 $\pm$ 1.8 nM and 6.7 $\pm$ 1.7 nM for young and old rats, respectively. Beta-adrenoreceptors exert their intracellular effects through activation of an enzyme, adenylate cyclase; the activation occurs via the regulatory protein. Various compounds were used to study the characteristics of activation of this adenylate cyclase in homogenates from cardiac ventricles. Basal adenylate cyclase was reduced 30% in old animals compared to young. (-)Isoproterenol alone stimulated adenylate cyclase greater than two-fold above basal in young rats and this activity was reduced by 34% in old animals. GppNHp, fluoride, and forskolin activation of adenylate cyclase above basal was reduced 38, 37, and 34%, respectively, in the old animals. The affinity of the catecholamine agonists (-)isoproterenol, (-)epinephrine, and (-)norepinephrine for stimulation of adenylate cyclase was decreased only very slightly in the old rats, but the maximum level of stimulation was reduced by a full 34%. These results suggest a reduction in the amount of functional regulatory protein in old rat ventricular tissue compared to young tissue. However, this data does not rule out the possibility of altered molecular interactions of a full complement of regulatory protein(s) with beta-adrenergic receptor and/or catalytic adenylate cyclase.

Lipid Soluble Vitamins. These compounds, in addition to their obvious nutritional values, have further beneficial effects. Unfortunately, to obtain a significant measure of these additional benefits, doses that already elicit toxicity are required. Thus retinoids, which are derivatives of vitamin A, have been shown to reduce the incidence of lesions in epithelium induced by carcinogens. Work done in this program focuses on lessening the toxic effects of retinoids through transforming them to a water soluble form by complexation with solubilizing agents. In previous years cyclodextrins were investigated as solubilizing agents; this year two new systems have been explored and their efficiency has been compared to that of cyclodextrins.

The first system is based on polyamino acid. Poly-L-methionine sulfoxide is a water soluble polymer containing structural elements of dimethyl sulfoxide. The preparation and radiolabeling of this polymer was performed and its bioeffects were compared with those of dimethyl sulfoxide. Poly-L-methionine sulfoxide is, similarly to dimethyl sulfoxide, a potent solubilizer of lipophilic compounds in water. The partition coefficient of poly-L-methionine sulfoxide in 1-octanol/water is only twenty times lower than that of dimethyl sulfoxide, but the former, in difference to the latter, was found not to penetrate into intracellular spaces. In contrast to dimethyl sulfoxide, poly-L-methionine sulfoxide and L-methionine sulfoxide were found to be ineffective in (a) inducing differentiation in murine erythroleukemic cells and (b) inhibiting differentiation of avian neural crest cells. This suggests that for a compound to be effective in these processes it must have the ability to penetrate into cells or into membrane proteins. Overall lack of bioactivity of poly-L-methionine sulfoxide, combined with low toxicity (2 g/kg i.v. in mouse without effect), makes this compound a suitable inert solubilizer and carrier for lipophilic drugs.

The second system studied is based on digitonin, a saponin contained in plants. Digitonin was modified by condensation with propylene oxide or with 1,4-butanediol diglycidyl ether in aqueous alkali yielding products in which some of the $-CH_2OH$ groups of digitonin were converted to $-CH_2-O-CH_2-CHOH-CH_3$ or to $-CH_2-O-CH_2-CHOH-CH_2-O-(CH_2)_4-O-CH_2-CHOH-CH_2OH$ groups, respectively. These modified digitonins were very soluble in water and chloroform and effectively solubilized lipophilic compounds into aqueous solutions; e.g., 2 mg of vitamin A or 0.6 mg of vitamin D could be dissolved per 1 ml of 5% aqueous solutions of modified digitonins. Compared to the toxicity of digitonin (LD_{50} 4 mg/kg i.v.) the toxicity of modified digitonin was greatly reduced: doses of 500 mg/kg i.v. infusion were not lethal for mice.

Significance to Biomedical Research and Program of the Institute. Work in the section focuses on compounds which are used in treatments for the elderly. Treatments by antihypertensive agents, a group of drugs to which beta-adrenergic blockers belong, is required for about 31% of the elderly (W.E. Hale et al., J. Amer. Geriatr. Soc. 27: 374-377, 1979).

The work on fat soluble vitamins is related to problems of aging as well. Transformation of epithelial tissues is known to be prominent in aging and derivatives of vitamin A have the potential to bring some improvements to this area. Furthermore, there are views, often based on not completely convincing data, that anti-oxidants, i.e., vitamin E, may have a role in amelioration of aging related problems. Systematical work on the decrease of toxic effects of fat soluble vitamins may give valuable opportunities to test these compounds at much higher doses and thus, help to determine the practical value of such treatments.

Proposed Course of the Project. Continuation of the work on the above projects may hopefully lead to the knowledge necessary for the design of useful drugs.

Publications:

Pitha, J.: Physiological Activities of Synthetic Analogs of Polynucleotides. In Overberger, C.G. (Ed.): Advances in Polymer Science, Vol. 50. Berlin, Heidelberg, Springer-Verlag, 1983, pp. 1-16.

Pitha, J.: Polymer-Cell Surface Interactions and Drug Targeting. In Goldberg, E. (Ed.): Targeted Drugs, Chapter 6. New York, John Wiley & Sons, 1983, pp. 113-126.

Pitha, J., and Kusiak, J.W.: Oligomeric and polymeric β-adrenergic antagonists. Fed. Proc. 42: 279-283, 1983.

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Pitha, J., and Szente, L.: Rescue from hypervitaminosis A or potentiation of retinoid toxicity by different modes of cyclodextrin administration. Life Sci. 32: 719-723, 1983.

Pitha, J., and Szente, L.: Digitonin derivatives of low toxicity: potential solubilizers for lipophilic compounds. J. Pharm. Sci., in press.

Pitha, J., Szente, L., and Greenberg, J.: Poly-L-methionine sulfoxide: a biologically inert analogue of dimethyl sulfoxide with solubilizing potency. J. Pharm. Sci. 72: 665-668, 1983.

Pitha, J., Szente, L., and Szejtli, J.: Molecular Encapsulation of Drugs by Cyclodextrins and Congeners. In Bruck, S.D. (Ed.): Controlled Drug Delivery, Vol I. Florida, CRC Press, 1983, pp. 125-148.

The Laboratory of Molecular Aging has again had an outstanding scientific year. During FY 82-83 the Laboratory has produced 45 research papers and major reviews, published, accepted for publication or submitted, all in high quality, peer-reviewed, scientific journals. Three of these papers represent major advances in their respective fields and have appeared in the Proc. Nat. Acad. Sci. (USA). The Laboratory's research reflects a variety of individual as well as collaborative efforts both within and outside the Laboratory. They have brought credit and honor not only to the individual scientist, but also to the Gerontology Research Center, National Institute on Aging, as a locus for first-class, frontier-expanding, bio-medical research.

This report summarizes the following basic sciences projects:

(1) Mechanisms of phosphate and calcium homeostasis in senile osteoporosis and osteomalacia;
(2) Pathophysiological and hormonal regulation of membrane transport systems;
(3) Ion transport mechanisms;
(4) Regulation of intermediary metabolism.

(1) Mechanisms of phosphate and calcium homeostasis in senile osteoporosis and osteomalacia.

Senile and post-menopausal forms of osteopenia are the most common bone diseases and leading causes of morbidity and mortality in aging women. In addition to the impairment in the quality of life of the debilitated, it has been estimated that the cost of health care for broken hips, etc, in hospitals and nursing homes may run into billions of dollars. Although the development of osteoporosis probably involves many factors, including hormonal, nutritional, physical and perhaps others, the rate of bone loss has been reported to be approximately 0.5%/year after the age of 40; in post-menopausal women the rate may accelerate to 1% or more/year. Because of the urgency of the problem and the potential cost-effectiveness of the investigation, the Laboratory of Molecular Aging made a major commitment to study the mechanisms of phosphate and calcium balance as part of our effort to understand the pathophysiological bases of the osteopenia and osteomalacia. Our approach was to define the mechanisms by which phosphate and calcium are absorbed by the kidney and intestine and to determine how hormones, *e.g.* parathyroid hormone, calcitonin, glucocorticoids, and perhaps estrogen and prolactin; 1,25-dihydroxyvitamin D_3; diet; and various pathophysiological states, including aging, induce specific alterations in the activities of the membrane transporters.

(a) *Effect of 1,25-dihydroxycholecalciferol on calcium uptake in isolated intestinal cells.*

The possible clinical use of Vitamin D_3 and its metabolites in postmenopausal osteoporotic women tends to obscure the fact that little is known about the function of vitamin D and in particular about its biochemical mechanism of action. Vitamin D metabolites control calcium and phosphate homeostasis by actions primarily at three sites: bone, intestine and kidney. Last year we reported on the role of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] in enhancing phosphate reabsorption in the kidney. This year we focused on the action of 1,25-$(OH)_2D_3$ on intestinal calcium uptake. Although vitamin D was known to

increase the absorption of calcium in the intestine, the mechanism by which calcium uptake was increased is essentially unknown. The paucity of information on the mechanism stems, in part, from the lack of a model to study how the hormone affects uptake at the cell level. In our study we used isolated duodenal cells from the vitamin D-deficient and -repleted chick to examine directly the question of the action of $1,25\text{-}(OH)_2D_3$ on cell calcium uptake.

We found that calcium uptake was increased 60% after incubating duodenal cells isolated from the vitamin D-deficient chick with 1×10^{-13} M $1,25\text{-}(OH)_2D_3$ for 2 hrs. This stimulatory effect was dose dependent. The effect of $1,25\text{-}(OH)_2D_3$ was relatively specific, the potency order being $1,25\text{-}(OH)_2D_3 = 1\text{-}(OH)D_2 > 25\text{-}(OH)D_3 > 1,24,25\text{-}(OH)_3D_3 > 24,25\text{-}(OH)_2D_3 > D_3$. Kinetic studies revealed that the hormone induced a change in the V_{max} of calcium uptake. The calcium uptake induced by $1,25\text{-}(OH)_2D_3$ was blocked by protein synthesis inhibitors. This suggests a nuclear mechanism of action for $1,25\text{-}(OH)_2D_3$ although a direct liponomic effect of the hormone on the plasma membrane of the enterocyte is not ruled out.

(b) *Renal calcium transport, sodium/calcium exchange in renal tubule baso-lateral membranes, and the action of parathyroid hormone.*

An investigation of the mechanisms by which calcium is transported by the renal tubule was initiated. Thermodynamic considerations suggested that the movement of calcium from filtrate to cell across the luminal membrane was down an electrochemical gradient and, therefore, probably mediated by a facilitated diffusional system. In contrast, movement of calcium from cell to blood across the baso-lateral membrane was against its electrochemical gradient and, therefore, presumably required an active transport process.

The isolated renal cortex baso-lateral membrane vesicle, prepared as described in a previous report, possessed two mechanisms for lowering the intracellular concentration of calcium, a calcium ATPase pump and a sodium/calcium antiport carrier which pumped calcium out of the cell in exchange for sodium entering the cell down its electrochemical gradient. Our studies, to date, described some kinetic parameters for calcium and sodium in both influx and efflux modes. The transport system was membrane potential sensitive, suggesting a stoichiometry of at least 3 sodium ions for each calcium ion. Significantly, we found that the activity of the exchange in the membrane vesicle was dependent on the parathyroid status of animal from which the membrane was isolated. Calcium uptake was decreased about 40% in membrane vesicles from thyroparathyroidectomized rats and this decrement was reversed with the infusion of parathyroid hormone (1-34 peptide). This finding may represent a key to how the hormone works to enhance calcium transport.

(c) *Regulation of vitamin D_3 metabolism in the kidney.*

The interactions of Vitamin D_3 metabolites and parathyroid hormone is of crucial importance to calcium and phosphate homeostasis. Last year, this interaction was studied by examining the regulation by parathyroid hormone of phosphate uptake in renal cells from vitamin D-deficient and -sufficient chicks. This year, we studied another facet of the interrelationship between the two hormones by investigating how the synthesis of $1,25\text{-}(OH)_2D_3$ is regulated by serum levels of calcium and phosphate, parameters determined, in part, by parathyroid hormone. The synthesis of $1,25\text{-}(OH)_2D_3$ from $25\text{-}(OH)_2D_3$ is mediated by the enzyme, 25-hydroxy-vitamin D_3-1-hydroxylase. We found that the level of 1-hydroxylase activity was

highest in kidney cells isolated from chicks maintained on the low calcium, low phosphorus, vitamin D-deficient diet, partially active in cells from chicks fed the high calcium, high phosphorus, vitamin D-deficient diet and essentially inactive in cells from vitamin D-repleted chicks fed either diet. 1-Hydroxylase activity required phosphate (2.5 mM) for maximum activity, was inhibited *in vitro* by μM free-calcium and competitively inhibited by nM $1,25\text{-}(OH)_2D_3$, the product of the reaction. The apparent K_m for $25\text{-}(OH)D_3$ was 10-12 nM and the apparent V_m was 15 pmoles $1,25\text{-}(OH)_2D_3$ synthesized per 30 min per mg cell protein. Little or no 24-hydroxylase activity was observed in cells isolated from vitamin D-deficient chicks. These initial studies establish that the isolated kidney cell is a good model to study the regulation of $1,25\text{-}(OH)_2D_3$ synthesis in the kidney.

(d) *Adaptation of renal phosphate transport in response to dietary phosphorus.*

Renal adaptation to changes in phosphate intake was studied by comparing phosphate uptake by proximal tubule brush border membrane vesicles from rabbits on a relatively high or low phosphorus diet. The low phosphorus diet increased sodium gradient-dependent phosphate uptake. Uptake in the absence of sodium and in the presence of sodium, but no gradient, was not significantly affected. The phosphorus diet did not alter sodium gradient-dependent D-glucose and L-proline uptake. The low phosphorus diet increased V_{max}; affinity for phosphate was not appreciably changed. At all concentrations of extravesicular sodium, phosphate uptake was higher in membrane vesicles from animals fed the low phosphorus diet. The kinetics of the phosphate uptake system, with respect to sodium, was altered by the change in dietary phosphate. These findings suggest that adaptation involves an alteration in the rate of translocation of the sodium-phosphate carrier when energized by a sodium gradient driving force, rather than a change in the number of sodium-phosphate carrier sites. With membrane vesicles from rabbits fed a low phosphorus diet, phosphate uptake increased several-fold when the pH of the uptake medium was raised, whereas with membrane vesicles from animals fed a high phosphorus diet, the enhancement of uptake with alkalinization was relatively small. Irrespective of the diet, divalent phosphate was the probable preferred species for transport. Dietary adaptation was associated, however, with an alteration in the pH dependency of the transport system *per se*. These findings provide evidence that the adaptation of the kidney phosphate transport system to dietary phosphate load involves an intrinsic change in the sodium-phosphate carrier.

(e) *Phosphate uptake in cultured renal cells and its regulation by hormones.*

Phosphate uptake by the cultured kidney epithelial cell ($LLC\text{-}PK_1$) was studied. The uptake was sodium gradient-dependent, saturable with respect to phosphate and sodium, energy-dependent, and inhibited by arsenate and ouabain. Parathyroid hormone, dibutyryl cyclic AMP and forskolin decreased phosphate uptake. These agonists had no effect on sodium gradient-dependent α-methylglucoside uptake. Vasopressin and isoproterenol did not inhibit phosphate uptake, although these hormones activated cyclic AMP-dependent protein kinase. These findings illustrate the potential of the cultured $LLC\text{-}PK_1$ cell system as a model for further studies of phosphate transport and its regulation in the kidney.

(2) Pathophysiological and hormonal regulation of membrane transport systems

(a) *Sodium-proton exchange, metabolic acidosis, and the role of glucocorticoids.*

It has long been known that kidneys from older animals do not respond as quickly or to the same extent as younger animals when faced with an acid load. To examine more closely the molecular mechanism, we initiated a study of sodium-proton exchange in isolated renal brush border membrane vesicles and its regulation in metabolic acidosis and by glucocorticoids.

Adrenal corticosteroids are important in the regulation of acid-base metabolism and phosphate transport in the kidney. Adrenal insufficiency in humans and adrenalectomy in animals are associated with metabolic acidosis concomitant with decreases in titratable acid and ammonium excretion. States of excess adrenal steroids secretion are associated with metabolic alkalosis. Both glucocorticoids and mineralocorticoids increase when animals are faced with an acid load. However, the precise roles of glucocorticoids and of mineralocorticoids in the renal response to metabolic acidosis are not known.

We found that adrenal steroids affected transport mechanisms located in the brush border of the proximal tubule. Dexamethasone, but not aldosterone, increased the rate of sodium-hydrogen exchange, decreased the rate of sodium-dependent phosphate transport and had no affect on sodium-dependent glucose transport. In further studies of the dexamethasone effect on sodium-hydrogen exchange, we found that 60 µg/100 g·b.w. produced the maximal change in sodium-hydrogen exchange activity within 24 hours. Multiple (twice daily) injections of dexamethasone did not increase sodium-hydrogen exchange activity additionally. After a single dexamethasone injection of 60 µg/100 g·b.w. exchange activity was increased after 2 hr and became significantly greater than controls after 4 hr. In kinetic studies, we found that dexamethasone did not change the apparent affinity of Na^+ (8.1 mM) but increased the maximal velocity from 20 to 27 nmoles sodium/mg protein/5 sec.

In other studies, we evaluated the effect of metabolic acidosis on sodium-hydrogen exchange. Animals were made acidotic by the addition of 1% ammonium chloride in the drinking water. Brush border membrane isolated from these rats showed an increased sodium-hydrogen exchange activity measured as proton gradient-dependent sodium flux and sodium gradient-dependent proton flux. This change was eliminated if the acidotic animals had their adrenal glands removed. If the adrenalectomized acidotic animals were given glucocorticoid supplements, then sodium-hydrogen exchange activity increased. Changes in phosphate and ammonium excretion during acidosis were also dependent upon intact adrenals or glucocorticoid supplements.

(b) *Direct effect of glucocorticoids on the kidney demonstrated by its action in a primary cell culture system.*

A second cell culture system, the primary chick kidney cell, was developed in the laboratory. These cells were known to respond to hormones and to synthesize 1,25-dihydroxyvitamin D_3. Preliminary studies indicated that glucocorticoids had a direct affect on renal cells. We found that phosphate uptake in the cultured chick cell was strongly inhibited by glucocorticoids, in a dose dependent manner. The effect was reversed by the removal of dexamethasone.

(c) *Renal transport in the diabetic (streptozotocin-treated) rat.*

Studies were continued on the effects on diabetes and insulin on renal transport systems. Our animal model is the streptozotocin-treated rat. Streptozotocin rapidly and selectively destroys β-cells of the pancreas and the animals present

a condition resembling diabetes mellitus. At sacrifice, diabetic rats were hyperglycemic, hypoinsulinemia and ketotic; they also showed a decreased rate of weight gain compared to age-matched controls. The initial rates of sodium gradient-dependent uptake of D-glucose, phosphate, L-proline and myo-inositol were decreased in renal brush border membrane vesicles isolated from streptozotocin-diabetic rats compared to controls. Uptakes in the absence of sodium or under sodium equilibrated and short circuited conditions were not different in control and diabetic renal brush border membrane vesicles. The activity of the amiloride-sensitive sodium-hydrogen exchanger was increased in diabetes compared to controls. No difference was observed in the amiloride-insensitive sodium uptake (presumably leak pathways) between the two groups. All the effects of streptozotocin-diabetes on transport activities were reversed by insulin. These results showed that renal brush border membrane vesicles transport activities were altered in the diabetic, ketotic rat. The findings contrasted sharply with those reported for the intestinal brush border membrane vesicles from alloxan-diabetic animals.

(d) *Age-dependent changes in the molecular structure and activity of the renal membrane maltase.*

Last year, we reported on the age-dependent changes in the molecular structure and activity of the renal brush border membrane maltase; the development by hybridoma technology of clones producing monoclonal antibodies specific to the active form of the enzyme found mostly in the young adult and to the inactive form of the enzyme found with increased prevalence in the aged animal; and the separation of the two maltase species by immunoaffinity chromatography using the monoclonal antibodies as ligands. The separated proteins were cleaved with cyanogen bromide. Peptide mapping and identification of the amino-terminal residues of the peptides of the catalytically active and inactive forms of the enzyme were indistinguishable, suggesting the close homology of the "young" and the "aged" forms. The possibility that the altered enzyme may be autoimmunogenic, being the antigen inducing Heymann's glomerulonephritis is being investigated.

(e) *Phospholipid + calcium-dependent protein kinase.*

The mechanisms of action of hormones and neurotransmitters at the plasma membrane were investigated. Parathyroid hormone and α-adrenergic agonists are known to perturb membrane phospholipid metabolism and the role of a calcium + phospholipid-dependent protein kinase in these effects were studied. The enzyme was found in the rabbit kidney cortex, in both cytosolic and membrane (brush border and basolateral) fractions. The kinase was purified to homogeneity by a simple two-step procedure of DEAE-cellulose ion exchange chromatography followed by affinity chromatography. Maximal activity required the presence of: 1) a phospholipid, the most active being phosphatidic acid and phosphatidylserine; 2) a diacylglycerol; and 3) calcium. A combination of phosphatidic acid and diacylglycerol shifted the requirement for calcium to a low micromolar level. The enzyme had very low activity in the presence of calcium alone, even at millimolar levels.

(f) *Hormone-sensitive adenylate cyclase in cultured renal cells.*

Adenylate cyclase activity in cultured renal cells was studied by examining the interactions of forskolin, a diterpene activator of the cyclase complex, with parathyroid hormone and other agonists. Forskolin mimicked the action of GMP-PNP on the hormonally stimulated adenylate cyclase, increasing sensitivity and responsiveness to parathyroid hormone.

(g) *Hormonal action in the parotid cell.*

The effects of forskolin on cAMP-dependent protein kinase and amylase secretion, plus its interaction with agonists acting independently of cyclic AMP were studied in the parotid gland. Forskolin stimulated cAMP-dependent protein kinase and amylase secretion. When combined with epinephrine + propranolol (α_1), carbamylcholine, or substance P, forskolin produced a synergistic effect on amylase release.

The effects of 8-(N,N-diethylamino)octyl 3,4,5-trimethoxybenzoate (TMB-8) on various responses of parotid cell aggregates stimulated by humoral factors were studied. TMB-8 (200 μM) by itself substantially enhanced glucose oxidation (95%) and phosphatidylinositol labeling (160%), slightly increased amylase secretion (3-4% release), slightly inhibited protein synthesis (15% decrease), and substantially inhibited phosphatidylethanolamine + phosphatidylcholine labeling (30-40% decrease). TMB-8 inhibited α-adrenergically-stimulated potassium release but had little or no effect on stimulated glucose oxidation, phosphatidylinositol labeling or amylase secretion. All responses stimulated by carbamylcholine were blocked in a dose-dependent manner. None of the responses elicited by substance P were inhibited. Isoproterenol-stimulated amylase secretion was similarly unaffected. These differential effects on agonist-stimulated responses that depended on either intracellular or extracellular calcium indicated that TMB-8 did not function as a simple antagonist of intracellular calcium in the parotid.

(3) Ion Transport Mechanisms

A major effort was made to understand the relationship between calcium translocation and the calcium-dependent phosphorylation reactions which occurred in the ATPase reaction mechanism of the sarcoplasmic reticulum calcium pump. It was anticipated that information derived from these studies might be useful in developing a model to account for the decline in calcium transport activity that was found with aging in cardiac muscle sarcoplasmic reticulum.

(a) *Calcium translocation in the sarcoplasmic reticulum.*

The main catalytic pathway of the calcium pump included two consecutive acid-stable phosphorylated intermediates, the ADP-sensitive (EP) and ADP-insensitive (E*P) phosphoenzymes. The former bound calcium tightly and reacted rapidly with ADP to form ATP. The latter, which had a low affinity for calcium, did not transfer its phosphate group to ADP but could be converted to the ADP-sensitive form by raising the calcium level in the medium (calcium-jump). Although it was generally believed that calcium translocation across the membrane was coupled to the conversion of EP to E*P, this assumption had not been tested by methods with sufficient resolution to distinguish clearly the sequence of these events. Using the calcium-dependent phosphorylation reactions as an intrinsic chemical probe of the events associated with calcium translocation, we showed by means of the quench-flow technique that the calcium-depleted form of the ADP-insensitive phosphoenzyme accumulated more rapidly than calcium trapped inside of the vesicle. The calcium site on E*P which activated its conversion to EP had a low affinity for calcium ($K_{0.5}$ = 1-2 mM) and a stoichiometry greater than one (Hill coefficient= 1.7) consistent with its proposed role in calcium transport. Moreover, we found that this site could be demonstrated in the absence of ionophore, suggesting that its location was extravesicular. Since this site appeared at the extravesicular surface at a rate faster than calcium accumulated on the inside of the vesicle,

it seemed likely that during translocation calcium was detained in an intermediate compartment following its dissociation from E*P and prior to being released at the inner surface of the membrane. Despite the fact that the observed rate constants for the forward and reverse reactions of the phosphoenzyme conversion step ($EP \rightleftharpoons E^*P$) strongly favored accumulation of the ADP-insensitive phosphoenzyme, the ratio of the concentration of these intermediates stayed fairly close to one throughout the entire time course of phosphorylation. This suggested that there might be a catalytic constraint within the system which prevented these phosphoenzymes from achieving their expected steady state distribution. To explain these features of the calcium pump, a model was proposed which assumed that the functional transport unit was a dimer consisting of two catalytic subunits with an intermediate compartment for calcium located in the interface between them. The activity of the subunits was controlled by a catalytic coupling mechanism which prevented them from simultaneously existing in the same chemical or conformational state.

(b) *Calcium translocation in sarcolemmal membrane vesicles.*

The presteady state kinetics of calcium ion (Ca^{2+}) accumulation by the sodium/calcium and calcium/calcium exchange transport system in canine cardiac sarcolemmal vesicles were measured in a multimixing apparatus using EGTA to quench the reaction. When sarcolemmal vesicles pretreated with DMSO and valinomycin and loaded with 80 mM sodium chloride + 80 mM potassium chloride were mixed with and equal volume of a solution containing 160 mM potassium chloride + 50 μM calcium chloride, a burst of calcium ion accumulation was observed following a brief initial lag phase. The amplitude of the burst, obtained by extrapolation of the steady state phase to zero time, was 0.2 nmoles calcium/mg sarcolemmal protein. In the absence of valinomycin, the burst amplitude was reduced to 0.1 nmoles calcium/mg sarcolemmal protein. In the presence of an inside negative potential, created by loading valinomycin-treated sarcolemmal vesicles with 80 mM sodium chloride + 80 mM potassium chloride and mixing them with 160 mM lithium chloride + 50 μM calcium chloride, the burst amplitude was further reduced to 0.03 nmoles calcium/mg sarcolemmal protein and the lag phase extended. Addition of calcium ion to sarcolemmal vesicles suspended in a medium 100 μM calcium chloride and 160 mM potassium chloride resulted in an initial burst of accumulation which was larger (0.7 nmoles calcium/mg sarcolemmal protein) than that observed during calcium uptake coupled to sodium extrusion. In addition, the steady state rate of calcium ion/calcium exchange was 5-10 times faster (10 nmoles calcium/mg sarcolemmal protein/sec) than that of sodium/calcium exchange. These results suggested a mechanism for sodium/calcium and calcium/calcium exchange that involved a rapid translocation step followed by a slow calcium dissociation or release step.

(4) Regulation of Intermediary Metabolism

(a) *Control of mitochondrial bioenergetic reactions by calcium.*

Previously, hypothesized that there were two signals involved in increasing the activity of oxidative phosphorylation in a muscle in response to increased energy demands. One was the increased availability of ADP (the well-described form of respiratory control), and the other was the increased availability of free calcium-ions (in the concentration range 10^{-7}-10^{-6}M, pCa 7-6) to the mitochondria. The question was examined additionally by assessing whether calcium ions controlled the complete oxidation of pyruvate and fatty acids under near-

physiological conditions. The study further probed the role of the mitochondrial calcium-ion transport processes, asking whether they existed to control the pCa of the cytosol, or that of the mitochondrial matrix.

Each of the 3 enzymes catalyzing non-equilibrium (and therefore rate-limiting) reactions in the terminal oxidation of carbohydrates and fatty acids, *viz:* pyruvate dehydrogenase complex, NAD-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase, was activated by μM calcium. We showed that the proportion of pyruvate dehydrogenase in the active, dephospho-, form increased with increasing extramitochondrial calcium concentration, over the range pCa 6 to 7, when coupled, respiring heart mitochondria were incubated in the presence of calcium-EGTA buffers. Fifty percent activation was achieved at pCa 6.58, in the presence of plausibly physiological concentrations of potassium (120 mM), sodium (10 mM) and magnesium (1 mM). Omission of sodium or magnesium shifted the required pCa to markedly higher values, consistent with sodium stimulating calcium egress from the mitochondrion via an electroneutral calcium/2 sodium pathway, and with magnesium inhibiting calcium uptake via the electrogenic calcium uniporter pathway. Closely similar results were obtained when the activity of 2-oxoglutarate dehydrogenase was studied, with the addition that the continuous recording of the reduction of mitochondrial NAD (a function of 2-oxoglutarate dehydrogenase activity) allowed the effects of magnesium and sodium on dehydrogenase activation and inactivation (respectively) to be followed kinetically.

Analysis of the amount of mitochondrial calcium (*i.e.* total, ionized + non-ionized) needed for 50% dehydrogenase activation gave a value of 1 nmol/mg protein. Modification of the "null-point technique," to allow measurements 100 times smaller, showed that this corresponded to 1 μM free calcium within the mitochondrial matrix. Comparison with the results using extramitochondrial calcium:EGTA buffers (above) yielded a mitochondrial transmembrane gradient for calcium of only 2 in respiring heart mitochondria.

Studies of mitochondrial calcium transport at the small loads (nmol calcium/mg mitochondrial protein) identified as allowing modulation of dehydrogenase activity showed that the egress pathway was not-saturated (K_m = 5.4 nmol/mg or approx. 5 μM calcium). This limited the ability of heart mitochondria to buffer the pCa of the extramitochondrial fluid with any precision - a theoretical prediction which was verified experimentally. Thus, we concluded that the mitochondrial calcium transport processes existed to control the pCa of the mitochondrial matrix, not that of the cytosol as had been surmised. This allowed the matching of the activity of pyruvate oxidation and the tricarboxylate cycle to the energy needs of the muscle.

(b) *Mitochondrial calcium transport during aging.*

Last year we reported on the significant and specific decrements in heart mitochondrial calcium transport in senescence. This year our laboratory investigated the kinetics of calcium-uptake and release by kidney, liver and skeletal muscle mitochondria as a function of senescence. We found that liver mitochondria showed a large (50%) diminution in rates of calcium-release, when mitochondria from senescent (24 month) rats were compared with those of young adults (6 months). There was no change in the activity of calcium-uptake. This age-linked imbalance between the activity of calcium-uptake and release by liver mitochondria would be expected to alter the magnitude of the calcium gradient across the mitochondrial membrane and hence the sensitivity of intramitochondrial

dehydrogenases to changes in cytosolic free [calcium]. Such changes might mediate, for example, the action of α-agonists, vasopressin and angiotensin II in the liver.

(c) *Skeletal muscle mitochondrial fatty acid oxidaiton and the sparing of carbohydrate.*

Earlier studies indicated that part of the mechanism whereby cardiac fatty acid oxidation spared glucose during the stress of starvation involved inhibition in flux through pyruvate dehydrogenase. This was mediated both by a decrease in content of the active, dephospho-enzyme (PDH_A), and by an increased feedback inhibition by NADH and acetyl-CoA, the products of fatty acid oxidation. We previously showed that this mechanism was less effective in cardiac mitochondria from senescent animals, a consequence of a decreased ability to oxidize fatty acids. This correlated with decreased rates of acylcarnitine translocation and a decreased cardiac content of carnitine. This year, we asked two questions: (1) Does fatty acid oxidation predominate over pyruvate oxidation in striated muscle mitochondria, as it does with cardiac mitochondria? (2) Is this competition between substrates altered in senescence? The reason for choosing striated muscle mitochondria was that the mass of voluntary muscle was so large that any sparing of glucose by this tissue would be of great quantitative significance to the animal.

We found that the oxidation of palmitoylcarnitine and acetylcarnitine inhibited flux through pyruvate dehydrogenase (at a low and constant pyruvate concentration) but to a lesser extent than occured with cardiac mitochondria. The oxidation of acetoacetate gave approximately the same degree of inhibition as palmitoyl-carnitine, provided 2-oxoglutarate and calcium were present. Moreover, inhibition in flux reflected mainly increased feedback inhibition by acetyl-CoA and NADH, with enzyme interconversion (*i.e.* changes in PDH_A content) being less important than in the cardiac system. In particular, the interconversion system was strikingly sensitive to calcium such that at pCa values of 6.48 or lower, PDH_A content was near-maximal regardless of other effectors. These relations were investigated under different conditions of ADP-availability, corresponding to different degrees of muscular work: in general, a lesser degree of sparing of carbohydrate at the high work loads was indicated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00043-10 LMA

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Intermediary Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Richard G. Hansford Research Chemist LMA, GRC, NIA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SECTION
Intermediary Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

These studies seek to define and describe the molecular mechanisms whereby the major catabolic (*i.e.* energy-releasing) pathways of intermediary metabolism are controlled, and to investigate the ways in which these controls are altered in old-age. Such alterations are considered to underlie the decreased ability of senescent animals (including humans) to withstand environmental stresses (*e.g.* starvation, severe cold). Current investigations emphasize: (1) the role of mitochondrial Ca^{2+} transport in the control of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase in cardiac and skeletal muscles and brain; (2) the impact of aging upon the activity of mitochondrial Ca^{2+} transport, both uptake and release, in a variety of tissues, *viz*: skeletal muscle, brain, kidney and liver; (3) the effect of fatty acid and ketone oxidation on the oxidation of pyruvate, and the activity of pyruvate dehydrogenase, in skeletal muscle mitochondria; and (4) the effect of aging upon the transport of acylcarnitine species into skeletal muscle mitochondria.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel

B. Ashour	Visiting Fellow	LMA, GRC, NIA

Project Description:

Objectives: These studies seek to define and describe the biochemical mechanisms which regulate the pathways of energy-transduction in animal tissues. Alterations, sometimes quite subtle, in these mechanisms are the most plausible cause of the decreased ability of older animals to adapt to environmental stresses.

Currently, we are studying the interaction of fatty acid and carbohydrate oxidation at the level of pyruvate dehydrogenase in various muscle tissues, having previously shown that fatty acids are less actively oxidized by heart muscle mitochondria in old-age. If true of skeletal muscles, this would be important in limiting the ability of the older animal to spare glucose during starvation.

Our studies also have the objective of delineating the role of Ca^{2+}-ions as a messenger between muscle contraction and the key energy-transducing systems of the mitochondrion. This involves the control of dehydrogenase activity by Ca^{2+}-ions, a field in the development of which this laboratory has played a large part.

Further, we are asking the question of whether changes in Ca^{2+} transport in the aging central nervous system may disrupt the pattern of activation of pyruvate dehydrogenase seen in the young adult, and thus lead to decreased availability of acetyl groups for synthesis of the transmitter substance, acetylcholine, in old age.

Methods Employed: The experimental systems used include intact mitochondria isolated from heart, skeletal muscle, brain, kidney and liver, as well as homogenates and purified enzymes. Experiments involve the use of physical techniques (*e.g.* single and dual-wavelength spectrophotometry, polarography, fluorimetry, the use of ion-specific electrode) and of radioisotopes, and also analysis involving enzymological methods.

Major Findings:

Impact of Senescence on Ca^{2+} Transport by Mitochondria from Skeletal Muscle, Brain, Kidney and Liver. We have previously reported that there are substantial and highly significant decrements in the activities of transport systems mediating both the uptake of Ca^{2+} into cardiac muscle mitochondria and the release of Ca^{2+}, when mitochondria from senescent rats (24 mos) are compared with those from young adult controls (6-9 mos). In our view, these transport processes exist to translate changes in cytosolic concentrations of Ca^{2+} into changes in Ca^{2+} concentration within the mitochondrial matrix, and thus to match the activity of 3 key Ca^{2+}-sensitive dehydrogenases (pyruvate dehydrogenase, NAD-isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase), to the energy needs of the cell (please see last year's Annual Report for more details). Changes in Ca^{2+} transport activities in senescence would be expected to alter the magnitude of the Ca^{2+}-gradient across the mitochondrial membrane (unless the decrement in the separate uptake and release pathways happened to be of similar magnitude) and thus alter the sensitivity of the central energy-yielding pathways of the cell to the work-load being supported.

This year, we have used the metallochromic indicator Arsenazo III and the dual-wavelength spectrophotometric technique to characterize the electrogenic

uniport which catalyses Ca^{2+} uptake and the electroneutral $Ca^{2+}/2Na^{+}$ antiport which catalyses release. The latter was measured in the presence of the inhibitor Ruthenium Red, to prevent re-accumulation of the released Ca^{2+}. We found that liver mitochondria from senescent rats show a marked decrease (40%, $p<0.05$) in the rate of Ca^{2+} release, but no significant change in the rate of Ca^{2+} uptake. This would be expected to increase the magnitude of the Ca^{2+} gradient across the mitochondrial membrane and alter the sensitivity of the intramitochondrial dehydrogenases to changes in cytosolic free Ca^{2+} concentration. Such changes may mediate the action of α_1-agonists, vasopressin and angiotensin II in the liver.

By contrast, there were no significant changes with aging in either transport pathway when mitochondria from kidney and hind-limb muscle were investigated. Thus the aging changes in Ca^{2+} transport are quite tissue-specific, with decrements in either both pathways (heart), one pathway (liver) or neither (skeletal muscle, kidney) being observed. It is noted that in no case was there a decline with aging in the activity of substrate oxidation or in the respiratory control ratio, with the substrate (glutamate plus malate) being the same as that used to energize Ca^{2+} transport. Thus, there is no evidence for a non-specific deterioration of mitochondrial structure and function with aging of these tissues.

The work with brain mitochondria is current and has focused on the improvement of the preparation by inclusion of a Ficoll density-gradient step and the stabilization of the mitochondria during incubation by use of a medium containing ATP and Mg^{2+} ions. Under these conditions, brain mitochondria show highly active Ca^{2+} uptake and release by a Na^{+}-dependent pathway: activities for preparations from young and old animals are currently being collected.

Dependence of Brain Mitochondrial Pyruvate Dehydrogenase Interconversion on Extramitochondrial pCa. There is good evidence that repeated electrical stimulation of cerebellar slices decreases the degree of phosphorylation of pyruvate dehydrogenase. This corresponds to an increase in the catalytically active form (PDH_A). A likely mechanism of this effect is an increased cytosolic Ca^{2+} concentration, consequent upon depolarization of the nerve cell membrane. Despite this, *in vitro* studies with synaptosomes and isolated brain mitochondria have shown very limited sensitivity of the pyruvate dehydrogenase interconversion system to Ca^{2+}, with the enzyme always being more than 70% in the PDH_A form, regardless of the presence of Ca^{2+} and other effectors.

In our recent studies, we have shown that brain mitochondria can be depleted of endogenous Ca such that the content of PDH_A is not more than 20% of that of the total enzyme. Incubation in a quasi-physiological medium containing 120 mM KCl, 10 mM NaCl and 1 mM $MgCl_2$ showed that the steady-state content of PDH_A is markedly sensitive to the pCa of the medium (as maintained with Ca^{2+}:EGTA buffers) with a 5 fold increase in PDH_A content occurring as the Ca^{2+} concentration was raised from pCa 8 to pCa 6 and with 50% activation at pCa 6.4.

Although we cannot exclude a role for the ATP/ADP and NADH/NAD^{+} ratios as effectors of pyruvate dehydrogenase interconversion on electrical stimulation of brain tissue, we consider it likely that the Ca^{2+} ion is a very important signal. We propose to repeat with brain mitochondria some of our previous experiments characterizing the response of the pyruvate dehydrogenase to Ca^{2+} in another excitable tissue, cardiac muscle. We are excited about a possible relationship

between pyruvate dehydrogenase activity and neurotransmitter synthesis (please see below).

Regulation of Pyruvate Dehydrogenase Activity in Skeletal Muscle Mitochondria, with Reference to the Oxidation of Fatty Acids and Ketones. Earlier studies indicated that part of the mechanism whereby cardiac fatty acid oxidation spared glucose during the stress of starvation involved inhibition in flux through pyruvate dehydrogenase. This was mediated both by a decrease in the content of the active, dephosphoenzyme (PDH_A), and by an increased feedback inhibition by NADH and acetyl-CoA, the products of fatty acid oxidation. We previously showed that this mechanism was less effective in cardiac mitochondria from senescent animals, a consequence of a decreased ability to oxidize fatty acids. This correlated with decreased rates of acylcarnitine translocation across the mitochondrial membrane and a decreased cardiac content of carnitine.

This year we have completed an analogous study with mitochondria from rat hindlimb muscle. This was prompted both by the physiological significance of glucose sparing in skeletal muscle, owing to the large mass of this tissue, and by the fact that reports in the literature on the effect of fatty acid oxidation on pyruvate dehydrogenase activity in skeletal muscle were quite equivocal. We found that the oxidation of palmitoylcarnitine and acetylcarnitine inhibited flux through the pyruvate dehydrogenase reaction when pyruvate was maintained at a low and physiological concentration, *i.e.* <100 μM) but to a lesser extent than occurred with cardiac muscle mitochondria. The oxidation of acetoacetate gave approximately the same degree of inhibition as palmitoylcarnitine, provided 2-oxoglutarate and Ca^{2+} were present to generate succinyl-CoA and allow activation of the acetoacetate. Moreover, inhibition in flux represented mainly increased feedback inhibition by acetyl-CoA and NADH (these coenzymes being measured in mitochondrial extracts), with enzyme interconversion - *i.e.* changes in PDH_A content - being less important than in the cardiac muscle mitochondria. In particular, the interconversion system was found to be strikingly sensitive to pCa, such that at values of 6.48 or lower, PDH_A content was near-maximal regardless of the concentration of other effectors (*i.e.* acetyl-CoA, NADH). These relations were investigated under different conditions of ADP-availability, generated by the hexokinase reaction, and corresponding to different degrees of muscular work: in general, a lesser degree of sparing of carbohydrate at the high work loads was indicated. It is felt that this study has clarified the response of skeletal muscle pyruvate dehydrogenase to the products of fatty acid oxidation and to the signals of increased work, ADP and Ca^{2+}: however, conclusions on the overall competition between fatty acid and carbohydrate oxidation need to be based also on more physiological, and complementary, studies using the perfused hind-quarter.

Effect of Aging on Acylcarnitine Translocation in Skeletal Muscle Mitochondria. This laboratory was involved in the early description of a transport process for acylcarnitine species across the mitochondrial membrane, and further established a lesser activity of this process in the heart in old-age. Currently we are measuring carnitine:^{3}H-carnitine, palmitoylcarnitine:^{3}H-carnitine and acetylcarnitine:^{3}H-carnitine exchange in skeletal muscle mitochondria, using an "inhibitor-stop" technique involving the inhibitor mersalyl, and rapid centrifugation. The $T_{1/2}$ for exchange, at 5°, is significantly ($p<0.05$) increased with carnitine and acetylcarnitine as external substrate for transport, in mitochondria

from 24 month old rats. This indicates lower carrier activity. We are currently studying the size of the intramitochondrial pool of carnitine, as this also influences overall flux through the carrier system. These results are of relevance to rates of fatty acid oxidation by skeletal muscle mitochondria (please see above) but also are of intrinsic interest in view of rather generalized changes in membrane structure and permeability that are increasingly being reported in old-age, both for the mitochondrion and other membranes.

Proposed Course of the Project. We plan to complete the study on acylcarnitine translocation in skeletal muscle mitochondria in old age.

We are very interested in the possibility that the activity of pyruvate dehydrogenase may be rate-limiting in the provision of acetyl groups for acetylcholine synthesis and are impressed by the degree of control over pyruvate dehydrogenase interconversion exerted by Ca^{2+}, in our preliminary experiments with mitochondria from brain tissue. We plan fully to characterize this response, and to examine the effects of aging: aspects of this are underway (please see above). The relevance of this is that acetylcholine synthesis has been shown to be depressed in old-age and in senile dementia of the Alzheimer's type (SDAT): further, total pyruvate dehydrogenase activity has been shown to be depressed in SDAT. Our focus will be to look for derangements of mitochondrial Ca^{2+} transport with aging and in the response of the pyruvate dehydrogenase interconversion system to Ca^{2+}.

A further interest, and possible project, involves the response of intramitochondrial Ca^{2+} in heart cells to α_1-agonists. Our evolving understanding of the pool size of mitochondrial Ca^{2+} and its function in metabolic control lead us to question the idea that α-agonists promote a primary release of mitochondrial Ca^{2+}. We anticipate instead, that the mitochondrial Ca^{2+} pool will rise, secondarily to the known increase in cytosolic Ca^{2+}. We would like to investigate this, using isolated cardiac myocytes (an experimental preparation with which we are familiar), digitonin fractionation and rapid centrifugation. These studies would include simultaneous measurement of PDH_A content, as an indirect sensor of intramitochondrial free Ca^{2+} concentration.

On the conceptual front, one of us (RGH) is developing and promoting the idea that mitochondrial Ca^{2+} transport serves the control of intramitochondrial enzymes rather than the buffering of cytosolic free Ca^{2+}. This is the theme of a review currently being written for Reviews of Physiology, Biochemistry and Pharmacology, and which will extend into the next year.

Publications:

Hansford, R. G.: Bioenergetics in Aging. *Biochim. Biophys. Acta* 726: 41-80, 1983.

Hansford, R. G. and Castro, F.: Effect of senescence on Ca^{2+}-ion transport by heart mitochondria. *Mech. Ageing Dev.* 19: 5-13, 1982.

Ashour, B. and Hansford, R. G.: Effect of fatty acids and ketones on the activity of pyruvate dehydrogenase in skeletal muscle mitochondria. *Biochem. J.*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00048-09 LMA

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Ion Transport Mechanisms.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Jeffrey P. Froehlich Medical Officer LMA, GRC, NIA

COOPERATING UNITS *(if any)*

Department of Cardiology, Johns Hopkins Univ. Medical School; Laboratory of Biochemistry, Roche Institute of Molecular Biology; Department of Physiology, Univ. Texas

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Biophysics and Intermediary Metabolism Sections

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Rapid kinetic measurements of ATP-dependent Ca^{2+} accumulation and phosphoenzyme formation in sarcoplasmic reticulum membrane vesicles were carried out in order to determine the relationship between the conformational events associated with Ca^{2+} translocation and the Ca^{2+} ATPase intermediate reactions of the Ca^{2+} pump reaction cycle. Results of these studies indicate that intravesicular Ca^{2+} accumulation is delayed with respect to the appearance of a low affinity Ca^{2+} binding site on the phosphorylated carrier. The properties of the low affinity Ca^{2+} site are consistent with its involvement in Ca^{2+} transport. The time dependence of these events suggest a translocation mechanism in which Ca^{2+} is temporarily detained in compartment located between the outer and inner membrane surfaces following dissociation from the phosphorylated carrier and prior to release on the inside of the vesicle. The presence of a dimeric functional state was inferred from the behavior of the phosphorylation reactions coupled to Ca^{2+} transport. The initial behavior of Na^{+}-dependent Ca^{2+} accumulation in cardiac sarcolemmal membrane vesicles was investigated by rapid quenching with EGTA. The time course exhibited an early burst phase which increased in the presence of inside positive charge and decreased when the inside became more negative with respect to the outside. The biphasic pattern of Ca^{2+} uptake implies that a step or steps occurring subsequent to translocation is (are) rate-limiting in the reaction cycle. Modulation of the burst amplitude by changes in the transmembrane potential occurring during the cardiac action potential afford a possible mechanism for the rapid delivery of Ca^{2+} to and removal of Ca^{2+} from the myofilaments during the excitation-contraction cycle.

Other Professional Personnel

R. W. Albers	Chief, Neurochemistry Section	LNC, NINDCDS
A. S. Hobbs	Staff Fellow	LNC, NINDCDS
T. Boulware	Cardiology Fellow	Johns Hopkins Univ.
J. P. Reeves		Roche Institute of Molecular Biology
J. L. Sutko	Assistant Professor	Dept. Physiology, Univ. Texas

Objectives: (1) To elucidate the transport mechanisms involved in the formation of ionic gradients across cellular and intracellular membranes. (2) To delineate the mechanisms responsible for the interconversion of substrate, electrical, and osmotic energy in ion transport systems.

Methods Employed: The rapid mixing quench flow technique was used to monitor the time courses of active Ca^{2+} uptake in sarcoplasmic reticulum and sarcolemmal membrane vesicles. In the Na^+/Ca^{2+} exchange experiments an outwardly directed Na^+ gradient was achieved by rapid dilution of the vesicle-containing medium with substrate solution. A new rapid quenching device which utilizes small volumes of material for determining product formation at long incubation intervals is being developed. Computer simulation of time dependent data obtained in rapid quench studies was used to test kinetic models and to determine the rate constants of reactions involved in the translocation mechanism.

Major Findings:

The mechanism of Ca^{2+} translocation in sarcoplasmic reticulum was investigated using fast kinetic methods. Results of these studies indicate that the translocation mechanism involved rapid transfer of Ca^{2+} from an external binding site to an occluded site prior to its release at the inner membrane surface.

The kinetics of the phosphoenzyme intermediate reactions of the electric organ (Na,K)-ATPase were compared in the presence and absence of K^+. Results indicate the presence of K^+-activated acceleration of the interconversion reaction favoring a mechanism involving simultaneous occupation of Na^+ and K^+ transport sites. Examination of the initial turnover characteristics of the purified kidney (Na,K)-ATPase provided additional support for a simultaneous transport mechanism.

The enzymatic partial reactions of the cardiac myofibrillar ATPase were examined with respect to Ca^{2+} sensitivity. Preliminary results indicate the presence of regulation of the steady state rate involving Ca^{2+}-activated release of reaction products.

Significance to Biomedical Research and to the Program of the Institute: Investigation of the ion transport pathways involved in muscle contractility and bioelectric phenomena will help to clarify the mechanism of active ion translocation and the process of energy transfer associated with the formation of ionic gradients. Information obtained from these investigations will serve as a baseline for future studies aimed at identifying possible age-dependent alterations in transport function.

Proposed Course of the Project: Our previous studies have demonstrated the presence of an age-dependent decline in the V_{max} for Ca^{2+} pumping in rat cardiac SR. Limitations in the amount of material available from this source have precluded a detailed investigation of the partial reactions of the Ca^{2+} pump. With the completion of a new rapid mixing device which utilizes small volumes of material these studies can now be pursued. The device will also be used to measure Na^+/Ca^{2+} exchange and Na^+-dependent glucose uptake in renal brush border membrane vesicles.

Publications:

Hobbs, A. S., Albers, R. W. and Froehlich, J. P.: Effects of oligomycin on the partial reactions of the sodium plus potassium stimulated adenosine triphosphatase. *J. Biol. Chem.* 258: 8163-8168, 1983.

Horner, R. D., Froehlich, J. P. and Moudrianakis, E. N.: Initial products of phosphorylation with AMP and [^{32}P] Pi. *J. Biol. Chem.* 258: 5618-5622, 1983.

Froehlich, J. P., Hobbs, A. S. and Albers, R. W.: Evidence for parallel pathways of phosphoenzyme formation in the mechanism of ATP hydrolysis by electrophorus (Na,K)-ATPase. *Curr. Top. Membr. Trans.*, in press.

Hobbs, A. S., Albers, R. W. and Froehlich, J. P.: ADP sensitivity of the native and oligomycin-treated (Na,K)-ATPase. *Curr. Top. Membr. Trans.*, in press.

Froehlich, J. P. and Heller, P. F.: Kinetics of accumulation of the ADP-sensitive and ADP-insensitive phosphoenzymes and their relationship to Ca^{2+} translocation in sarcoplasmic reticulum. In Fleischer, S. and Tonomura, Y. (Eds.): *Sarcoplasmic Reticulum: Structure and Function.* New York, Academic Press, in press.

Sumida, M., Okuda, H., Hamada, M., Takenaka, H., Wutras, J. M., Sarmiento, J. G. and Froehlich, J. P.: Presteady state kinetics of E-P formation and decomposition by Ca^{2+}, Mg^{2+}-ATPase in bovine aorta microsomes. In Fleischer, S. and Tonomura, Y. (Eds.): *Sarcoplasmic Reticulum: Structure and Function.* New York, Academic Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00051-03 LMA

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Phosphate and Calcium Homeostasis in Senile Osteoporosis and Osteomalacia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Bertram Sacktor Chief, Laboratory of Molecular Aging LMA, GRC, NIA

COOPERATING UNITS *(if any)*

Department of Medicine, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	4.5	2.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The mechanisms of phosphate and calcium homeostasis in senile osteoporosis and osteomalacia were studied. Findings include:

(1) 1,25-dihydroxy vitamin D_3, incubated *in vitro* with isolated intestinal cells, stimulated calcium uptake, the increase being specific for the 1,25-dihydroxy metabolite and blocked by inhibitors of protein synthesis;

(2) calcium was transported by renal tubule basolateral membrane vesicles by a sodium/calcium exchange mechanism, the system being regulated by parathyroid hormone;

(3) the establishment of the isolated renal cell as a model for studies on the regulation of the hydroxylation of 25-hydroxy vitamin D_3;

(4) the further characterization of the adaptation in renal phosphate transport in response to dietary phosphorus;

(5) the characterization of phosphate uptake in a cultured renal cell line and the demonstration that the uptake is controlled by parathyroid hormone.

Other Professional Personnel

C. T. Liang	Research Chemist	LMA, GRC, NIA
B. A. Bulos	Research Chemist	LMA, GRC, NIA
L. Cheng	Senior Staff Fellow	LMA, GRC, NIA
S. Guggino	Staff Fellow (EOD 11/82)	LMA, GRC, NIA
M. Ishida	Visiting Associate (EOD 4/83)	LMA, GRC, NIA
D. Spector	Department of Medicine	Johns Hopkins Univ.
E. Kraus	Department of Medicine	Johns Hopkins Univ.

Project Description:

Objective: Osteoporosis is a leading cause of mortality and morbidity in aging women. Studies of the mechanisms of phosphate and calcium balance are essential. This investigation was prompted by our pioneering development of technology for the isolation of specific plasma membrane vesicles, the use of these isolated membrane vesicles to define transport mechanisms and by the findings that hormones, *i.e.* parathyroid hormone, calcitonin, and perhaps estrogen, prolactin and insulin; 1,25-dihydroxy vitamin D_3; diet; and chronic uremic syndromes, induce specific alterations in the activities of the membrane transporters that are reflected in changes in the absorption of phosphorus and calcium by the kidney and intestine.

Methods Employed: Model systems representing various levels of organization were used. These range from membrane vesicles derived from the luminal (brush border) and basal-lateral segments of the plasma membranes of renal and intestinal cells; intact cells, isolated or grown in culture; to the intact animal.

Major Findings:

Senile and post-menopausal forms of osteopenia are the most common bone diseases and leading causes of morbidity and mortality in aging women. In addition to the impairment in the quality of life of the debilitated, it has been estimated that the cost of health care for broken hips, etc, in hospitals and nursing homes may run into billions of dollars. Although the development of osteoporosis probably involves many factors, including hormonal, nutritional, physical and perhaps others, the rate of bone loss has been reported to be approximately 0.5%/year after the age of 40; in post-menopausal women the rate may accelerate to 1% or more/year. Because of the urgency of the problem and the potential cost-effectiveness of the investigation, the Laboratory of Molecular Aging made a major commitment to study the mechanisms of phosphate and calcium balance as part of our effort to understand the pathophysiological bases of the osteopenia and osteomalacia. Our approach was to define the mechanisms by which phosphate and calcium are absorbed by the kidney and intestine and to determine how hormones, *e.g.* parathyroid hormone, calcitonin, glucocorticoids, and perhaps estrogen and prolactin; 1,25-dihydroxyvitamin D_3; diet; and various pathophysiological states, including aging, induce specific alterations in the activities of the membrane transporters.

(a) *Effect of 1,25-dihydroxycholecalciferol on calcium uptake in isolated intestinal cells.*

The possible clinical use of Vitamin D_3 and its metabolites in postmenopausal osteoporotic women tends to obscure the fact that little is known about the function of vitamin D and in particular about its biochemical mechanism of action. Vitamin D metabolites control calcium and phosphate homeostasis by actions primarily at three sites: bone, intestine and kidney. Last year we reported on the role of 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] in enhancing phosphate reabsorption in the kidney. This year we focused on the action of 1,25-$(OH)_2D_3$ on intestinal calcium uptake. Although vitamin D was known to increase the absorption of calcium in the intestine, the mechanism by which calcium uptake was increased is essentially unknown. The paucity of information

on the mechanism stems, in part, from the lack of a model to study how the hormone affects uptake at the cell level. In our study we used isolated duodenal cells from the vitamin D-deficient and -repleted chick to examine directly the question of the action of $1,25\text{-}(OH)_2D_3$ on cell calcium uptake.

We found that calcium uptake was increased 60% after incubating duodenal cells isolated from the vitamin D-deficient chick with 1×10^{-13} M $1,25\text{-}(OH)_2D_3$ for 2 hrs. This stimulatory effect was dose dependent. The effect of $1,25\text{-}(OH)_2D_3$ was relatively specific, the potency order being $1,25\text{-}(OH)_2D_3 = 1\text{-}(OH)D_3 > 25\text{-}(OH)D_3 > 1,24,25\text{-}(OH)_3D_3 > 24,25\text{-}(OH)_2D_3 > D_3$. Kinetic studies revealed that the hormone induced a change in the V_{max} of calcium uptake. The calcium uptake induced by $1,25\text{-}(OH)_2D_3$ was blocked by protein synthesis inhibitors. This suggests a nuclear mechanism of action for $1,25\text{-}(OH)_2D_3$ although a direct liponomic effect of the hormone on the plasma membrane of the enterocyte is not ruled out.

(b) *Renal calcium transport, sodium/calcium exchange in renal tubule baso-lateral membranes, and the action of parathyroid hormone.*

An investigation of the mechanisms by which calcium is transported by the renal tubule was initiated. Thermodynamic considerations suggested that the movement of calcium from filtrate to cell across the luminal membrane was down an electrochemical gradient and, therefore, probably mediated by a facilitated diffusional system. In contrast, movement of calcium from cell to blood across the baso-lateral membrane was against its electrochemical gradient and, therefore, presumably required an active transport process.

The isolated renal cortex baso-lateral membrane vesicle, prepared as described in a previous report, possessed two mechanisms for lowering the intracellular concentration of calcium, a calcium ATPase pump and a sodium/calcium antiport carrier which pumped calcium out of the cell in exchange for sodium entering the cell down its electrochemical gradient. Our studies, to date, described some kinetic parameters for calcium and sodium in both influx and efflux modes. The transport system was membrane potential sensitive, suggesting a stoichiometry of at least 3 sodium ions for each calcium ion. Significantly, we found that the activity of the exchange in the membrane vesicle was dependent on the parathyroid status of animal from which the membrane was isolated. Calcium uptake was decreased about 40% in membrane vesicles from thyroparathyroidectomized rats and this decrement was reversed with the infusion of parathyroid hormone (1-34 peptide). This finding may represent a key to how the hormone works to enhance calcium transport.

(c) *Regulation of vitamin D_3 metabolism in the kidney.*

The interactions of Vitamin D_3 metabolites and parathyroid hormone is of crucial importance to calcium and phosphate homeostasis. Last year, this interaction was studied by examining the regulation by parathyroid hormone of phosphate uptake in renal cells from vitamin D-deficient and -sufficient chicks. This year, we studied another facet of the interrelationship between the two hormones by investigating how the synthesis of $1,25\text{-}(OH)_2D_3$ is regulated by serum levels of calcium and phosphate, parameters determined, in part, by parathyroid hormone. The synthesis of $1,25\text{-}(OH)_2D_3$ from $25\text{-}(OH)_2D_3$ is mediated by the enzyme, 25-hydroxy-vitamin D_3-1-hydroxylase. We found that the level of 1-hydroxylase activity was

highest in kidney cells isolated from chicks maintained on the low calcium, low phosphorus, vitamin D-deficient diet, partially active in cells from chicks fed the high calcium, high phosphorus, vitamin D-deficient diet and essentially inactive in cells from vitamin D-repleted chicks fed either diet. 1-Hydroxylase activity required phosphate (2.5 mM) for maximum activity, was inhibited *in vitro* by μM free-calcium and competitively inhibited by nM $1,25\text{-}(OH)_2D_3$, the product of the reaction. The apparent K_m for $25\text{-}(OH)D_3$ was 10-12 nM and the apparent V_m was 15 pmoles $1,25(OH)_2D_3$ synthesized per 30 min per mg cell protein. Little or no 24-hydroxylase activity was observed in cells isolated from vitamin D-deficient chicks. These initial studies establish that the isolated kidney cell is a good model to study the regulation of $1,25(OH)_2D_3$ synthesis in the kidney.

(d) *Adaptation of renal phosphate transport in response to dietary phosphorus.*

Renal adaptation to changes in phosphate intake was studied by comparing phosphate uptake by proximal tubule brush border membrane vesicles from rabbits on a relatively high or low phosphorus diet. The low phosphorus diet increased sodium gradient-dependent phosphate uptake. Uptake in the absence of sodium and in the presence of sodium, but no gradient, was not significantly affected. The phosphorus diet did not alter sodium gradient-dependent D-glucose and L-proline uptake. The low phosphorus diet increased V_{max}; affinity for phosphate was not appreciably changed. At all concentrations of extravesicular sodium, phosphate uptake was higher in membrane vesicles from animals fed the low phosphorus diet. The kinetics of the phosphate uptake system, with respect to sodium, was altered by the change in dietary phosphate. These findings suggest that adaptation involves an alteration in the rate of translocation of the sodium-phosphate carrier when energized by a sodium gradient driving force, rather than a change in the number of sodium-phosphate carrier sites. With membrane vesicles from rabbits fed a low phosphorus diet, phosphate uptake increased several-fold when the pH of the uptake medium was raised, whereas with membrane vesicles from animals fed a high phosphorus diet, the enhancement of uptake with alkalinization was relatively small. Irrespective of the diet, divalent phosphate was the probable preferred species for transport. Dietary adaptation was associated, however, with an alteration in the pH dependency of the transport system *per se*. These findings provide evidence that the adaptation of the kidney phosphate transport system to dietary phosphate load involves an intrinsic change in the sodium-phosphate carrier.

(e) *Phosphate uptake in cultured renal cells and its regulation by hormones.*

Phosphate uptake by the cultured kidney epithelial cell ($LLC\text{-}PK_1$) was studied. The uptake was sodium gradient-dependent, saturable with respect to phosphate and sodium, energy-dependent, and inhibited by arsenate and ouabain. Parathyroid hormone, dibutyryl cyclic AMP and forskolin decreased phosphate uptake. These agonists had no effect on sodium gradient-dependent α-methylglucoside uptake. Vasopressin and isoproterenol did not inhibit phosphate uptake, although these hormones activated cyclic AMP-dependent protein kinase. These findings illustrate the potential of the cultured $LLC\text{-}PK_1$ cell system as a model for further studies of phosphate transport and its regulation in the kidney.

Significance to Biomedical Research and to the Program of the Institute. These studies on the mechanisms of calcium and phosphate homeostasis are requisites to the understanding of the pathophysiological bases of senile osteoporosis and osteomalacia. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: Studies will focus on the actions and interactions of vitamin D metabolites and parathyroid hormone on calcium and phosphate transports in different renal and intestinal model systems.

Publications:

Elgavish, A., Rifkind, J. and Sacktor, B.: Evidence for a direct *in vitro* effect of vitamin D_3 on the phospholipids of isolated renal brush border membranes. In Norman, A. W., Schaefer, K., Grigoleit, H. G. and Bonherrath, D. (Eds.): *Vitamin D: Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism.* Berlin, Walter D. Gruyter & Co., 1982, pp. 299-300.

Liang, C. T. and Sacktor, B.: *In vitro* effects of 1,25-$(OH)_2D_3$ on the uptake of phosphate (Pi) by chick kidney cells. In Norman, A. W., Schaefer, K., Grigoleit, H. G. and Bonherrath, D. (Eds.): *Vitamin D: Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism.* Berlin, Walter D. Gruyter & Co., 1982, pp. 437-439.

Sacktor, B., Cheng, L. and Liang, C. T.: *In vivo* and *in vitro* effects of vitamin D_3 [1,25-$(OH)_2D_3$] on the uptake of phosphate by isolated chick kidney cells. In Massry, S. G., Letteri, J. M. and Ritz, E. (Eds.): *Regulation of Phosphate and Mineral Metabolism.* New York, Plenum Press, 1982, pp. 87-95.

Elgavish, A., Rifkind, J. and Sacktor, B.: *In vitro* effects of vitamin D_3 on the phospholipids of isolated renal brush border membranes. *J. Membr. Biol.* 72: 85-91, 1983.

Cheng, L., Liang, C. T. and Sacktor, B.: Phosphate uptake by renal membrane vesicles of rabbits adapted to high and low phosphorus diets. *Am. J. Physiol.*, in press.

Cheng, L., Dersch, C., Kraus, E., Spector, D. and Sacktor, B.: Renal adaptation to a phosphate load in the acutely thyroparathyroidectomized rat: rapid alteration in brush border membrane phosphate transport. *Am. J. Physiol.*, in press.

Takenawa, T., Wada, E., Tsumita, T., Masaki, T., Filburn, C. and Sacktor, B.: Effect of parathyroid hormone, cyclic AMP and Ca^{2+} on the phosphorylation of brush border membranes in rat kidney. *Mineral Electro. Metab.*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00052-03

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Pathophysiological and Hormonal Regulation of Membrane Transport Systems.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Bertram Sacktor Chief, Laboratory of Molecular Aging LMA, GRC, NIA

COOPERATING UNITS *(if any)*

Department of Medicine, Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Laboratory of Molecular Aging

SECTION

Intermediary Metabolism Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.0	6.5	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project studies the pathophysiological and hormonal regulation of membrane transport systems and the mechanisms by which age-dependent changes perturb physiological control systems and, thus, contribute to the failure to maintain homeostasis in the aged. Findings include:

(1) sodium/proton exchange activity in rat renal brush border membrane vesicles was increased in metabolic acidosis, and this response required an intact adrenal gland or glucocorticoid supplements;

(2) glucocorticoids acted directly on renal cells in culture to inhibit phosphate uptake;

(3) renal membrane transport systems were altered in the streptozotocin-diabetic animal;

(4) peptide mapping and identification of the amino-terminal residues of the peptides of the catalytically active and inactive forms of the renal membrane enzyme maltase were indistinguishable, suggesting the close homology of the "young" and the "aged" form of the enzyme;

(5) a technique was developed for the purification to homogeneity of a phosphlipid + lipid + calcium-dependent protein kinase, and the activity of the enzyme was characterized;

(6) the hormonal responses of a primary cultured renal cell was characterized;

(7) the control of physiological responses in parotid cell aggregates was described.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel

C. Filburn	Research Chemist	LMA, GRC, NIA
L. Noronha-Blob	Senior Staff Fellow	LMA, GRC, NIA
J. Kinsella	Staff Fellow	LMA, GRC, NIA
M. Freiberg	Medical Officer (DOD 6/83)	LMA, GRC, NIA
U. Reiss	Visiting Associate (DOD 2/83)	LMA, GRC, NIA
S. El-Seifi	Visiting Fellow	LMA, GRC, NIA
T. Uchida	Visiting Fellow	LMA, GRC, NIA
D. Spector	Department of Medicine	Johns Hopkins Univ.
D. Fiertag	Department of Medicine	Johns Hopkins Univ.

Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in regulation of transport processes perturb physiological control systems and, thus, contribute to the failure to maintain homoestasis in the aged. This new information is needed as a base for the eventual development of appropriate techniques and procedures that will prevent and/or enable the aged to cope effectively with their debilities.

Methods Employed: Appropriate biochemical, physiological and endocrinological techniques for specific experimental questions are developed and/or adapted, as reported in published papers.

Major Findings:

(a) *Sodium-proton exchange, metabolic acidosis, and the role of glucocorticoids.*

It has long been known that kidneys from older animals do not respond as quickly or to the same extent as younger animals when faced with an acid load. To examine more closely the molecular mechanism, we initiated a study of sodium-proton exchange in isolated renal brush border membrane vesicles and its regulation in metabolic acidosis and by glucocorticoids.

Adrenal corticosteroids are important in the regulation of acid-base metabolism and phosphate transport in the kidney. Adrenal insufficiency in humans and adrenalectomy in animals are associated with metabolic acidosis concomitant with decreases in titratable acid and ammonium excretion. States of excess adrenal steroids secretion are associated with metabolic alkalosis. Both glucocorticoids and mineralocorticoids increase when animals are faced with an acid load. However, the precise roles of glucocorticoids and of mineralocorticoids in the renal response to metabolic acidosis are not known.

We found that adrenal steroids affected transport mechanisms located in the brush border of the proximal tubule. Dexamethasone, but not aldosterone, increased the rate of sodium-hydrogen exchange, decreased the rate of sodium-dependent phosphate transport and had no affect on sodium-dependent glucose transport. In further studies of the dexamethasone effect on sodium-hydrogen exchange, we found that 60 µg/100 g•b.w. produced the maximal change in sodium-hydrogen exchange activity within 24 hours. Multiple (twice daily) injections of dexamethasone did not increase sodium-hydrogen exchange activity additionally. After a single dexamethasone injection of 60 µg/100 g•b.w. exchange activity was increased after 2 hr and became significantly greater than controls after 4 hr. In kinetic studies, we found that dexamethasone did not change the apparent affinity of Na^+ (8.1 mM) but increased the maximal velocity from 20 to 27 nmoles sodium/mg protein/5 sec.

In other studies, we evaluated the effect of metabolic acidosis on sodium-hydrogen exchange. Animals were made acidotic by the addition of 1% ammonium chloride in the drinking water. Brush border membrane isolated from these rats showed an increased sodium-hydrogen exchange activity measured as proton gradient-dependent sodium flux and sodium gradient-dependent proton flux. This change was eliminated if the acidotic animals had their adrenal glands removed. If the adrenalectomized

acidotic animals were given glucocorticoid supplements, then sodium-hydrogen exchange activity increased. Changes in phosphate and ammonium excretion during acidosis were also dependent upon intact adrenals or glucocorticoid supplements.

(b) *Direct effect of glucocorticoids on the kidney demonstrated by its action in a primary cell culture system.*

A second cell culture system, the primary chick kidney cell, was developed in the laboratory. These cells were known to respond to hormones and to synthesize 1,25-dihydroxyvitamin D_3. Preliminary studies indicated that glucocorticoids had a direct affect on renal cells. We found that phosphate uptake in the cultured chick cell was strongly inhibited by glucocorticoids, in a dose dependent manner. The effect was reversed by the removal of dexamethasone.

(c) *Renal transport in the diabetic (streptozotocin-treated) rat.*

Studies were continued on the effects of diabetes and insulin on renal transport systems. Our animal model is the streptozotocin-treated rat. Streptozotocin rapidly and selectively destroys β-cells of the pancreas and the animals present a condition resembling diabetes mellitus. At sacrifice, diabetic rats were hyperglycemic, hypoinsulinemia and ketotic; they also showed a decreased rate of weight gain compared to age-matched controls. The initial rates of sodium gradient-dependent uptake of D-glucose, phosphate, L-proline and myo-inositol were decreased in renal brush border membrane vesicles isolated from streptozotocin-diabetic rats compared to controls. Uptakes in the absence of sodium or under sodium equilibrated and short circuited conditions were not different in control and diabetic renal brush border membrane vesicles. The activity of the amiloride-sensitive sodium-hydrogen exchanger was increased in diabetes compared to controls. No difference was observed in the amiloride-insensitive sodium uptake (presumably leak pathways) between the two groups. All the effects of streptozotocin-diabetes on transport activities were reversed by insulin. These results showed that renal brush border membrane vesicles transport activities were altered in the diabetic, ketotic rat. The findings contrasted sharply with those reported for the intestinal brush border membrane vesicles from alloxan-diabetic animals.

(d) *Age-dependent changes in the molecular structure and activity of the renal membrane maltase.*

Last year, we reported on the age-dependent changes in the molecular structure and activity of the renal brush border membrane maltase; the development by hybridoma technology of clones producing monoclonal antibodies specific to the active form of the enzyme found mostly in the young adult and to the inactive form of the enzyme found with increased prevalence in the aged animal; and the separation of the two maltase species by immunoaffinity chromatography using the monoclonal antibodies as ligands. The separated proteins were cleaved with cyanogen bromide. Peptide mapping and identification of the amino-terminal residues of the peptides of the catalytically active and inactive forms of the enzyme were indistinguishable, suggesting the close homology of the "young" and the "aged" forms. The possibility that the altered enzyme may be autoimmunogenic, being the antigen inducing Heymann's glomerulonephritis is being investigated.

(e) *Phospholipid + calcium-dependent protein kinase.*

The mechanisms of action of hormones and neurotransmitters at the plasma membrane were investigated. Parathyroid hormone and α-adrenergic agonists are known to perturb membrane phospholipid metabolism and the role of a calcium + phospholipid-dependent protein kinase in these effects were studied. The enzyme was found in the rabbit kidney cortex, in both cytosolic and membrane (brush border and basolateral) fractions. The kinase was purified to homogeneity by a simple two-step procedure of DEAE-cellulose ion exchange chromatography followed by affinity chromatography. Maximal activity required the presence of: 1) a phospholipid, the most active being phosphatidic acid and phosphatidylserine; 2) a diacylglycerol; and 3) calcium. A combination of phosphatidic acid and diacylglycerol shifted the requirement for calcium to a low micromolar level. The enzyme had very low activity in the presence of calcium alone, even at millimolar levels.

(f) *Hormone-sensitive adenylate cyclase in cultured renal cells.*

Adenylate cyclase activity in cultured renal cells was studied by examining the interactions of forskolin, a diterpene activator of the cyclase complex, with parathyroid hormone and other agonists. Forskolin mimicked the action of GMP-PNP on the hormonally stimulated adenylate cyclase, increasing sensitivity and responsiveness to parathyroid hormone.

(g) *Hormonal action in the parotid cell.*

The effects of forskolin on cAMP-dependent protein kinase and amylase secretion, plus its interaction with agonists acting independently of cyclic AMP were studied in the parotid gland. Forskolin stimulated cAMP-dependent protein kinase and amylase secretion. When combined with epinephrine + propranolol (α_1), carbamylcholine, or substance P, forskolin produced a synergistic effect on amylase release.

The effects of 8-(N,N-diethylamino)octyl 3,4,5-trimethoxybenzoate (TMB-8) on various responses of parotid cell aggregates stimulated by humoral factors were studied. TMB-8 (200 μM) by itself substantially enhanced glucose oxidation (95%) and phosphatidylinositol labeling (160%), slightly increased amylase secretion (3-4% release), slightly inhibited protein synthesis (15% decrease), and substantially inhibited phosphatidylethanolamine + phosphatidylcholine labeling (30-40% decrease). TMB-8 inhibited α-adrenergically-stimulated potassium release but had little or no effect on stimulated glucose oxidation, phosphatidylinositol labeling or amylase secretion. All responses stimulated by carbamylcholine were blocked in a dose-dependent manner. None of the responses elicited by substance P were inhibited. Isoproterenol-stimulated amylase secretion was similarly unaffected. These differential effects on agonist-stimulated responses that depended on either intracellular or extracellular calcium indicated that TMB-8 did not function as a simple antagonist of intracellular calcium in the parotid.

Significance to Biomedical Research and to the Program of the Institute: These studies define the mechanisms whereby age-dependent perturbation in physiological control systems may lead to the inability of the aged organism to maintain homeostasis. This fundamental information is needed for the eventual development of appropriate techniques and procedures which will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of Study: Studies will continue on the molecular basis of the age-dependent alteration in maltase.

Studies will continue on the mechanisms by which metabolic acidosis and glucocorticoids affect renal transport systems and the changes in the adaptation to metabolic acidosis with age.

A study of the pathogenesis of progressive glomerular sclerosis in aging will be initiated.

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Annual Report of the Laboratory of Neurosciences
National Institute on Aging

Program Overview

The Laboratory of Neurosciences (LN) at the National Institute on Aging was formed in 1978, and has embarked on a program of research on the central and peripheral nervous systems and muscle, in health, aging and disease, including dementia. In 1982, the Laboratory was divided into two sections -- a clinical section on Brain Aging and Dementia, and a basic section on Cerebral Physiology and Metabolism. The Clinical Section is located at the Clinical Center in Bethesda and is directed by Dr. Neal Cutler. Furthermore, in September 1982, a 6-bed patient care unit was established for an inpatient program to study patients with Alzheimer's and other dementias as well as normal subjects. The unit has temporary use of the 13W ward in the Clinical Center (Bethesda), and hopefully in the near future will be given a permanent ward appropriate for the program. This issue remains unresolved, however. An Outpatient Dementia Clinic also was started in 1982, as a means to screen subjects for the inpatient protocols and to establish methods for the differential diagnosis and staging of the various dementias. The basic section of the Laboratory should move to Bethesda in the fall of 1983, to provide, together with the clinical section, a concerted program in the neurosciences.

This report summarizes the following projects: (A) Brain function in aging and dementia, involving studies of normal aging, Alzheimer's disease, Down syndrome and adult autism, (B) Neuropsychology in aging and dementia, (C) Neurological function and behavior in aging and dementia, (D) Brain anatomy and chemistry in aging and dementia, and (E) Clinical pharmacokinetics and pharmacodynamics. These projects were conducted primarily by members of the clinical section on Brain Aging and Dementia.

Projects conducted mainly by the basic section on Cerebral Physiology and Metabolism include: (F) Cerebral metabolism, relation to brain function and aging, (G) Blood-brain barrier and central nervous system function, (H) Transport systems at the blood-brain barrier, (I) Drug pharmacokinetics, relation to pharmacodynamics and sensescence, (J) Pharmacology of central and peripheral catecholaminergic nervous systems, (K) Assessment of neurochemical markers in relation to age, behavior and dementia, (L) Synapse development, specificity and mechanism in culture, and (M) Function of peripheral nerve and muscle.

A. Brain Function in Aging and Dementia.

In order to examine brain function during healthy aging and in disease states, the major thrust of the clinical program has been to measure cerebral metabolism by means of a new procedure recently introduced to the Clinical Center, positron emission tomography (PET). In the last 3 years, the LN has contributed to establishing and validating the PET procedure so as to obtain accurate and consistent measures of regional cerebral metabolic rates for glucose (rCMRglc) under standardized and reproducible experimental conditions.

1. Normal aging. 18-F-2-deoxy-D-glucose (FDG) was injected intravenously in 21 healthy men aged 21 - 83 years, as a positron-emitting analogue of glucose, to examine brain metabolism. PET scanning was performed under resting conditions

when the subjects' eyes were closed and ears were plugged with cotton. It was found that rCMRglc was not correlated significantly with age in 31 pairs of bilaterally symmetrical and in 3 midline brain regions ($p > 0.05$), and that mean hemispheric glucose utilization also was not correlated significantly with age in the 21 subjects. The findings then were extended to 40 healthy subjects. The age invariance of cerebral glucose utilization indicates that cerebral functional activity does not decline with age in man in the absence of disease, at least under resting conditions. Compensatory mechanisms must exist in the senescent human brain to counteract effects of many of the morphological and neurochemical changes which have been reported.

2. Alzheimer's disease. With PET and FDG, it was demonstrated that rCMRglc was reduced in the early stages of primary progressive dementia of the Alzheimer's type. The reduction often was asymmetrical and non-uniform within the brain, and in several cases correlated with localizing neuropsychological deficits. In the later stages of Alzheimer's disease, metabolism was reduced generally throughout the brain, to less than 20% of control values, except at the occipital lobe. PET therefore can be used to examine the intensity and course of the disease process in Alzheimer's disease, and to identify affected brain regions in relation to the neuropsychological deficits of dementia.

3. Down syndrome. At least 150 developmental abnormalities and diseases may affect the human brain at an early age and cause mental retardation. Of these, Down syndrome (DS) is the most common with an established etiology. Brains of young adult DS subjects show no consistent morphological abnormalities, but brains of DS subjects have pathological and neurochemical changes that also characterize Alzheimer's disease.

We used PET and FDG to measure brain glucose utilization in 4 healthy young adult DS subjects (age 19-21 yr) and one 51 yr DS subject, as well as in aged-matched healthy controls. In the young DS subjects, the mean metabolic rate for glucose (CMRglc) equaled 6.4 mg/100 g/min, and was significantly higher than 4.6 mg/100 g/min in the aged-matched controls ($p < 0.05$). The metabolic rate in the 51 yr old DS subject was less than that of the younger DS subjects ($p < 0.05$), but not different from that in middle-age controls. The results demonstrate that young DS subjects use glucose excessively throughout the brain, and that the cognitive dysfunction in DS may be associated with a general alteration of cerebral metabolism. Unlike control subjects, aging in DS is associated with a decline in CMRglc but, in the absence of overt dementia, not to the extent noted in Alzheimer's disease.

4. Adult autism. Autism is an irreversible psychiatric disorder with onset in infancy and with a suspected neurological basis. Patients with infantile autism fail to develop emotional relationships, have delayed language development and frequently are retarded. We employed PET and FDG in adult autistics (aged 21 to 39 yr) who had been diagnosed in childhood prior to the age of 3 yr by Dr. Leo Kanner (who defined the syndrome), to examine the possible central metabolic changes associated with that syndrome. The cerebral metabolic rate for glucose (CMRglc) was about 20% higher than in age-matched controls in the frontal, parietal and temporal lobes, but there were no local defects or excesses in metabolism as compared to the controls. Values for rCMRglc were intermediate between values in controls and the higher (by as much as 40%) values in Down syndrome (see above). Like Down syndrome, there appeared to be a general excessive or perhaps inefficient rate of glucose utilization in autism. This work was performed in collaboration with Drs. J. Rapoport and J. Rumsey of the NIMH.

B. Neuropsychology in Aging and Dementia.

The Luria-Nebraska Neuropsychological Battery (LNNB) was used by Drs. J. Haxby and C. Grady, to examine cognition in 40 healthy subjects, who were PET scanned between the ages of 21 and 83 years. Eight of 11 nonredundant clinical scales and 7 of 8 localization scales were correlated significantly with age ($p < 0.05$). The most significant changes applied to measures of memory and visual processing. No significant age correlation was found for clinical scales that measured arithmetic, reading or writing. The LNNB thus measures a classical pattern of brain aging. None of the individual measures, however, was correlated with rCMRglc as determined by PET more than expected by chance, nor were results in the Wechsler Adult Intelligence Scale or the Benton Revised Visual Retention Test correlated more frequently than expected with rCMRglc. Thus, in healthy subjects at different ages, cerebral metabolism is unrelated to measurements of intelligence. On the other hand, as noted above (see Alzheimer's disease), neuropsychological deficits found in moderately advanced cases of Alzheimer's disease and suggestive of cortical dysfunction are correlated frequently with local lobular changes in rCMRglc.

C. Neurological Function and Behavior in Aging and Dementia.

Audiological analysis by Dr. C. Grady, in 30 healthy men aged 21 to 83 years, who were subjected to PET, demonstrated that pure tone thresholds were significantly and positively correlated with age at all frequencies from 250 Hz to 10000 Hz. The loss of hearing became most apparent after 50 yr of age, especially at frequencies above 1000 Hz. Of speech measures, only the low-pass filtered speech test for stimuli delivered to the left ear showed an age decline, and might be related to a right temporal lobe deficit in the older subjects. The age-decline in auditory processing indicates that, when PET is employed in subjects with unregulated auditory input, age-declines in glucose metabolism may reflect peripheral sensory defects rather than central age-changes. Auditory deprivation in young subjects can reduce CMRglc by as much as 40% in temporal lobe regions.

D. Brain Anatomy and Chemistry in Aging and Dementia.

Computer assisted tomography (CT) plus computer image processing were employed by Drs. M. Schwartz and H. Creasey to examine brain dimensions in 30 healthy men between 21 and 83 yr of age. Brain atrophy occurred in the elderly group ($p < 0.05$). Volumes of the caudate nucleus, lenticular nucleus complex and thalamus were significantly and negatively correlated with age. Ventricular volume was measured directly with CT and found to increase with age, at the expense of gray matter, of which the mass decreased with age. The mass of white matter was age invariant. Thus, despite a normal metabolic rate for glucose (CMRglc) in healthy subjects, there is a distinct pattern of regional and overall gray matter atrophy even in the absence of disease.

Brains of patients who die of Alzheimer's disease have severe defects in cholinergic neurotransmission. Dr. S. Waller examined in such brains the relation between the activity of choline acetyltransferase (CAT), the enzyme responsible for presynaptic synthesis of acetylcholine, and muscarinic receptors in post-synaptic neurons. Regions in postmortem brain samples were analyzed for specific activity of choline acetyltransferase (CAT) and muscarinic receptor binding. As compared with control brains, CAT in the Alzheimer samples was

reduced by 53-83%, but binding to the cholinergic-muscarinic receptors was elevated by 30-400%. These findings suggest that a presynaptic cholinergic defect in Alzheimer's disease is associated with up-regulation of post-synaptic receptors to acetylcholine.

E. Clinical Pharmacokinetics and Pharmacodynamics.

Dr. N. Cutler showed that, although albumin concentrations in sera of 5 young (18-33 yr) and 5 old (70 - 83 yr) healthy subjects did not differ significantly, the free fraction of salicylate in the sera of the elderly, at a therapeutic concentration of 1000 - 3000 μM, was significantly higher than in sera of the young subjects. Thus, the pharmacokinetics of salicylate will differ between elderly and young subjects.

F. Cerebral Metabolism, Relation to Brain Function and Aging.

In an animal study parallel to the clinical study of brain metabolism and aging, it was demonstrated that the regional cerebral metabolic rate for glucose (rCMRglc), as measured with 14C-2-deoxy-D-glucose (2-DG) and autoradiography, increased between 1 and 3 months of age in awake Fischer-344 rats (when the rat brain continues to grow), declined between 3 and 12 months, but then remained unchanged during maturation and senescence (between 12 and 24, and 24 and 34 months of age). As in man, resting brain metabolism did not decline during sensescence. We ascribed this to compensatory responses of the senescent rat brain to age-related neurochemical and morphological losses. In support of our hypothesis, H. Takei demonstrated absence of a senescent change in the cerebral metabolic rates for O_2 and for glucose, for the brain as a whole, as well as in overall cerebral blood flow in awake Fischer-344 rats.

The 2-DG method was employed as a pharmacodynamic tool to examine where and to what extent the brain responds to drugs with known and specific actions. The muscarinic cholinergic agonist, oxotremorine, when administered to awake rats, produced a response pattern in brain regions which could be related in part to the location of cholinergic receptors, whereas physostigmine, an inhibitor of acetylcholinesterase, produced a different response pattern. These data will eventually be applied to human studies, as losses of presynaptic cholinergic markers occur in brains of the aged and to a larger extent in brains of patients with Alzheimer's disease.

The 2-DG method also was used to evaluate central nervous system pharmacodynamics for a γ-aminobutyric acid (GABA) agonist (muscimol) and an antagonist (bicuculline). GABA is a major inhibitory neurotransmitter in the central nervous system. The patterns of the dose-dependent rCMRglc responses to these drugs were not clearly correlated with localization of markers for GABAergic synapses, which implied that factors such as neural circuitry influenced the responses. Our studies demonstrate that central nervous system actions of neurotransmitter analogues are complex and cannot be predicted on the basis of known distributions of receptors within the brain.

A quantitative method was developed by Dr. A. Kimes to examine the uptake of a fatty acid within plasma, palmitate, into individual brain regions of awake rats. Regional palmitate uptake into stable brain structures was

proportional to rCMRglc, as measured with the 2-DG technique (see above), in various brain regions. Palmitate uptake by gray matter exceeded uptake by white matter. Furthermore, palmitate uptake by brain in awake Fischer-344 rats was not correlated with age after maturity. The palmitate method should make it possible to examine the relation between brain membrane turnover and brain oxidative metabolism and function.

G. Blood-Brain Barrier and Central Nervous System Function.

Studies of the blood-brain barrier in health, disease and aging require quantitative measurements of cerebrovascular permeability and transport, as well as adequate pharmacokinetic theories to interpret drug distribution within the various brain compartments in relation to plasma concentrations and drug metabolism. Current techniques for measuring cerebrovascular permeability and transport are insensitive, semi-quantitative and can be influenced by changes in cerebral blood flow. We therefore elaborated and tested a quantitative procedure to measure blood-brain barrier permeability that can be used in conscious animals, is independent of cerebral blood flow, and is at least 1000 times more sensitive than any other currently available technique.

In 1972, we first demonstrated that the blood-brain barrier could be reversibly opened in animals by infusing a hypertonic solution of a water-soluble nonelectrolyte (e.g. urea, mannitol, arabinose) into the internal carotid artery. The effect later was shown to be caused by osmotic shrinkage of cerebrovascular endothelial cells, with consequent widening of interendothelial tight junctions. In later years, we experimentally refined the osmotic method, and quantified changes of cerebrovascular permeability in relation to infusate concentration and infusion time, and demonstrated the reversibility of the osmotic effect. We showed that, when the barrier is opened osmotically, brain metabolism is transiently stimulated, brain edema occurs and metabolism is uncoupled temporarily from cerebral blood flow. Thus, barrier integrity must be maintained continuously for normal cerebral function. In diseases which affect barrier integrity in man, changes in consciousness may be related to these central effects of barrier disruption.

On the basis of the animal studies, we initiated a Phase I clinical protocol with E.A. Neuwelt to apply the osmotic procedure in patients with metastatic brain tumors, so as to allow methotrexate and other anti-neoplastic drugs into the brain. We demonstrated with computer assisted tomography that the blood-brain barrier can be opened reversibly in humans without producing apparent neurological changes. The clinical study is being continued to see whether the osmotic procedure will be efficacious for treating brain tumors.

Because of the possible use of the osmotic method for enzyme replacement therapy in diseases such as Tay-Sachs disease, we thought it important to examine the osmotic effect in relation to tracer size. Following osmotic opening of the barrier in the rat, Z. Ziylan examined the time course of enhanced permeability to radiotracers of sucrose (mol. wt. = 340), inulin (5200) and dextran (79000), and showed that the barrier opened approximately equally to these molecules, but that it closed more slowly to the smaller sucrose tracer than to the larger tracers. These results indicate that optimized brain uptake requires understanding of the time course of barrier opening for a tracer of a given size. It also provides evidence for a pore mechanism, rather than a vesicular transport mechanism, for osmotic barrier opening.

In earlier studies, we demonstrated in the Rhesus monkey that infusion of hypertonic solutions into the carotid circulation, so as to open the blood-brain barrier, damaged the ciliary epithelium of the ipsilateral eye. We now have shown that the effect of this damage is transient, that the ciliary epithelium continues to transport ascorbate into aqueous humor following barrier opening in the monkey, mainly because of retained integrity of the nonpigmented epithelial layer, and that gross vision is unaffected by barrier opening. Similar changes have not been noted following barrier opening in man.

We also developed a theoretical model to explain the negative relation between the (cerebrospinal fluid)/(blood) protein concentration ratio and the protein molecular radius in normal human cerebrospinal fluid. The model can be used to interpret the effect of brain disease on this ratio in terms of blood-brain barrier damage. We showed that proteins enter cerebrospinal fluid through aqueous pores, either by diffusion or ultrafiltration, and possibly by vesicular transport as well.

H. Transport Systems at the Blood-Brain Barrier.

A controlled ionic composition of brain interstitial fluid is critical for normal neuronal and glial function, neurotransmission and synthetic processes. It was not known, however, to what extent ionic composition is regulated by the blood-brain barrier. Q. Smith demonstrated, clearly and for the first time, that chloride movement from plasma to brain in rats is carrier-mediated, saturable and subject to competitive interaction with other anions within the blood. The concentration dependence of chloride entry into the brain could be described by Michaelis-Menton kinetics. Thus, in addition to acting as a permeability restriction to water-soluble drugs, the blood-brain barrier has clear regulatory properties which determine brain ionic composition and thereby brain function. The extent of this regulation remains to be explored.

A new *in vivo* brain perfusion method was developed, furthermore, to quantitatively determine rates of regulated or passive transfer of various solutes at the blood-brain barrier. Unlike other current procedures, the method is free of errors caused by biotransformation in tissues other than brain, and makes it possible to accurately control the exact contents of the brain intravascular space. The method was employed to accurately determine maximum velocities of transport, and transport affinities, of a number of large neutral amino acids at the blood-brain barrier, and to further understand their role as precursors of neurotransmitters and protein synthesis in the brain.

Cerebrospinal fluid is a site for entry of drugs into the nervous system, and also constitutes a pathway for drug removal from the brain in cases of poorly penetrating drugs. Age-changes in drug responses of the central nervous system may, in some instances, be related to pharmacokinetics involving cerbrospinal fluid. Q. Smith examined spinal fluid secretion in Fischer-344 rats at 3, 12, 24 and 34 mo of age with 22Na as a tracer, and showed that secretion declined with age, but that the integrity of the brain capillary bed was unaffected in sensescent rats. His findings suggest that CSF dynamics change with age and therefore may modify pharmacokinetic parameters of centrally-acting drugs.

I. Drug Pharmacokinetics, Relation to Pharmacodynamics and Senescence.

The elderly respond differently to centrally-acting drugs than do mature adults. They are more susceptible to untoward sedation with benzodiazepines and to extrapyramidal symptoms with neuroleptics, drugs which are widely prescribed for them. Only limited information is available on the cause of the altered response.

I. Kapetanovic examined the pharmacokinetics of phenobarbital in relation to age in Fischer-344 rats. This weak acid is an antiepileptic and sedative commonly used in the elderly, and is lost from the body mainly by p-hydroxylation by the liver. The distribution volume divided by the dose of phenobarbital was higher in 34 - 36 mo old rats than in 3 - 4 mo old rats; the rate of clearance was reduced in the old rats. Furthermore, higher rate of clearance was found during continuous administration than following a bolus injection, suggesting autoinduction of metabolism in the liver. The above findings indicate that higher concentrations of phenobarbital are obtained for longer periods of time in the brains of aged rats. This may have relevance to the observation of increased toxicity of phenobarbital in elderly humans, and warrants examination in a clinical setting.

J. Pharmacology of Central and Peripheral Catecholaminergic Nervous Systems.

The Laboratory of Neurosciences previously demonstrated increased plasma concentrations of catecholamines in senescent Fischer-344 rats, but reduced responsivity of the cardiovascular and other systems to catecholamines. Increased concentrations may derive from paraganglia, extra-adrenal chromaffin tissue which are found in the para-aortic region of the rat and are abundant in the fetus and at birth but degenerate postnatally. M. Partanen demonstrated that paraganglia proliferate in senescent Fischer-344 rats, and contain large quantities of catecholamines that probably contribute to high plasma levels. Their growth may be an overall response of the sympathetic nervous system to reduced end organ sensitivity. This hypothesis, of increased activity of the sympathetic nervous system in the elderly, is consistent with the demonstration that glucose utilization, a measure of functional activity, is greater in the superior cervical ganglion of senescent than of young or mature Fischer-344 rats.

In response to stress, catecholamines are released and blood pressure is elevated. In man, cerebral metabolism and blood flow are said to be augmented by stress. These responses have been reported in rats as well. However, M. Ohata in our laboratory demonstrated that immobilization stress of awake, spontaneously hypertensive, mature Wistar-Kyoto rats did not increase cerebral blood flow, and suggested that the stress effects reported previously may not be universal. It was shown, however, that a slight increase in cerebrovascular permeability in some regions was associated with stress, and therefore might contribute to increased entry of circulating catecholamines into the central nervous system.

K. Assessment of Neurochemical Markers in Relation to Age and Behavior.

In related mouse strains, age-associated changes in several neurochemical markers such as neurotransmitter synthetic enzymes appear to be influenced by genetic variability. S. Waller examined three enzymes that are responsible for the synthesis of critical neurotransmitters, of which the function and concen-

tration might be related to animal behavior. The enzymes were choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH), responsible for the synthesis of acetylcholine, gamma-aminobutyric acid and catecholamines, respectively. The enzymes were measured in 4 brain regions of C57BL/6J and A/J mice strains, characterized by differences in activity and learning ability.

There was an approximately 30% increase in cerebellar TH activity in both strains between 4 and 24 months of age. There also were several age-associated alterations in enzyme activity that were strain-related. An example is GAD activity in the striatum between 4 and 24 months, which increased in C57BL/6J mice and decreased in A/J mice. As these enzymes have also been measured in man, and shown to be affected in certain diseases (e.g. a reduced CAT activity in Alzheimer's disease), their characterization in relation to behavior and genotype may elucidate their role in cognition and behavior in man.

L. Synapse Development, Specificity and Mechanism in Culture.

J. Thompson demonstrated that synapse formation, synapse termination, synapse specificity and neurotransmission can be studied using neurons and muscle cells in tissue culture. He detected functional synapses by inserting microelectrodes into muscle cells, and recorded miniature end plate potentials in these cells. He demonstrated that neurons from chick spinal cord form long-lived synapses with rat muscle cells in culture, but that retinal neurons form transient synapses. Each type of neuron, therefore, possesses a synapse-competent state during its development, when it can form abundant synapses with muscle cells in culture. The results suggest a mechanism of synapse specificity that is based on stabilization of correct synapses which is coupled with a synaptogenic period during development for each type of neuron. The findings may be critical for our interpretation of neuronal cell death in the aging nervous system.

In fact, J. Thompson and B. Suarez-Isla recently demonstrated that stable synapses between spinal cord neurons and muscle are due in large part to an acetylcholine receptor aggregation factor in the muscle neurons, and that this factor is absent from retina neurons. The two types of neurons also differ in their ability to modify the electrical properties of the post-synaptic membrane (slow hyperpolarizing after potential), the nature of the Ca-activated K-channel and the tetrodotoxin sensitivity of the action potential mechanism in the post-synaptic membrane. Thus, three characteristics specific to neurons which form long-lived synapses and absent from neurons which form transient connections have been clearly identified in the laboratory.

B. Suarez-Isla examined the effect of inorganic blockers of calcium permeability, such as cobalt and manganese, on the ability of retina neurons from chick embryos to extend processes critical for synapse formation, as well as the influence of calcium. He concluded that calcium fluxes through specific channels are required for competent synapse formation during development, and suggested that interference with calcium entry into cells during aging might contribute to neuronal death.

One of the major problems in studies of synapse formation in culture is the choice of the correct cell lines. J. Thompson and collaborators examined synthetic enzymes in three mouse neuroblastoma cell lines, demonstrated a

remarkable heterogeneity in these cells and showed that the presence and activity of choline acetyltransferase (CAT) is unrelated to the ability of a cell line to form cholinergic synapses.

B. Horwitz proposed a model which indicates that dendritic spines on central nervous system neurons, through their activity, provide a means to reduce competition between neighboring afferent inputs and help to organize the local integration of nerve-nerve interaction. He calculated that electrical fields generated during neuronal activity are sufficient to promote electrophoretic migration of charge metabolites within the cell or on the cell membrane, sufficient for either synaptic stabilization or enhancement and for accumulation with dendritic spines.

M. Function of Peripheral Nerve and Muscle.

The blood-nerve barrier consists of the perineurium, which surrounds the nerve, and the endoneurial capillaries which are comparable to continuous capillaries in the central nervous system. Little is known about how these barrier sites affect nerve growth and function. With electron microscopic tracer techniques, M.E. Michel and N. Shinowara characterized the perineurium of the frog sciatic nerve as a system of concentric layers of flattened cells interspersed by collagen fibers and connected by tight junctions. They showed that the cells had numerous pits and vesicles, and demonstrated with colloidal and ionic lanthanum that the vesicles were connected to the cell surfaces and never formed transcellular channels. They are examining the contribution of these vesicles to perineurial function.

P. Ask demonstrated, with the frog sciatic nerve preparation, that the nerve can act as an osmometer, in large part because the perineurium is a semipermeable membrane for osmotically induced water flow. His studies on the hydraulic properties and permeability of the perineurium, which included measurements of hydrostatic pressure within the frog nerve and the elastic properties of the nerve, provide a basis for understanding the pathological causes and consequences of peripheral nerve edema.

The capillaries of the endoneurium of the nerve form a critical part of the blood-nerve barrier, and correspond to capillaries within the brain. S. Odman and A. Weerasuriya were able, for the first time, to measure the hydraulic and permeability properties of the capillaries, and showed that nerve capillaries are leakier than central nervous system capillaries. However, M.E. Michel employed the protein tracers, horseradish peroxidase and microperoxidase, to demonstrate that the capillaries could prevent exchange of proteins with a molecular weight as low as 2000 daltons.

We examined metabolism and tension in single fibers of the frog semitendinosus muscle after excitation was uncoupled from contraction by stretch and by hypertonicity. Exposure to hypertonic solution induced an increase in both muscle tension and consumption of high-energy phosphate compounds in resting fibers, but stretch does not. During tetanic stimulation, stretch interfered with contraction but does not prevent activation, whereas hypertonicity inhibited activation as well as contraction. These results clarify the relation between metabolism and tension during the excitation-contraction process in striated muscles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00126-04 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Brain Function in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) N.R. Cutler, Section Chief, LN NIA
R. Duara, Medical Staff Fellow, LN NIA; C.L. Grady, Psychologist, LN NIA

COOPERATING UNITS *(if any)* Laboratory of Clinical Sciences, NIMH
Nuclear Medicine Department, Clinical Center, NIH
University of Lund, Department of Clinical Neurophysiology, Lund, Sweden,
Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	5.4	2.1

CHECK APPROPRIATE BOX(ES)

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

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

The regional cerebral metabolic rate for glucose (rCMRglc) was examined, as a measure of cerebral functional activity in healthy men at different ages, and in patients with primary progressive dementia of the Alzheimer's type and adult autism. rCMRglc was determined by means of positron emission tomography (PET) with 18-F-2-deoxy-D-glucose, under resting (unstimulated) conditions, when the subject's eyes were covered and his ears plugged to reduce sensory input. In 21 healthy men between the ages of 21 and 83 years, average hemispheric glucose utilization and glucose utilization in individual regions of the right and left hemispheres, did not decline significantly with age ($p > 0.05$).

In 4 young adult subjects with Down syndrome (19-27 yr), rCMRglc as measured with PET was elevated by 20-40% as compared with age-matched healthy controls, indicating that young adult Down syndrome subjects use glucose excessively throughout the brain despite their retardation. Similarly, adult patients with autism, an irreversible psychiatric disorder with onset in infancy and with a suspected neurological basis, showed elevated values of rCMRglc, to values between normal and Down syndrome subjects. Adult patients with Alzheimer's disease showed variable reductions in rCMRglc, which were distributed in the frontal, parietal and temporal lobes, and which were correlated to some extent with measured localized cognitive deficits, as determined with neuropsychological testing.

PHS 6040 cont'd

Other professional personnel.

S.I. Rapoport	Chief	LN NIA
J. Haxby	Staff Fellow	LN NIA
B. Horwitz	Senior Staff Fellow	LN NIA
M. Schwartz	Medical Staff Fellow	LN NIA
A. Kay	Medical Staff Fellow	LN NIA
H. Creasey	Visiting Associate	LN NIA
A.M. Moore	Social Worker	LN NIA
C. Vanskiver	Psychology Technician	LN NIA
M. Sundaram	Computer Aid	LN NIA
J. Rapoport	Chief	LCS NIMH
R.M. Kessler	Staff Physician	NM CC
J. Rumsey	Psychologist	LCS NIMH
D. Ingvar	Professor	University of Lund
L. Sokoloff	Chief	LCM NIMH

Project Description:

Objectives: Glucose is the major substrate for brain oxidative metabolism, and the regional cerebral metabolic rate for glucose (rCMRglc) has been examined, when employing positron emission tomography (PET) and 18F-2-deoxy-D-glucose (18FDG) as a positron-emitting isotope, as a measure of brain functional integrity and activity. The objectives of this study are to examine rCMRglc in man, as a measure of cerebral functional activity, in relation to aging, Down syndrome, autism and primary progressive dementia of the Alzheimer's type (Alzheimer's disease).

Aging - A large number of neurochemical and morphological changes occur in the human brain during aging, but their functional significance is not understood. Most previous studies of aging and cerebral metabolism have concluded, however, that metabolism declines even in the absence of disease. Our aim is to determine the relation of rCMRglc to age in healthy man, when paying particular attention to exclude from the subject population individuals with primary brain disease, or conditions (hypertension, diabetes) that might contribute to subclinical brain disease. Furthermore, measurements of rCMRglc with PET will be related to cognitive performance, as determined by a battery of neuropsychological tests.

Alzheimer Disease - We intend also to examine brain metabolism with PET in patients with Alzheimer's disease, which is the major cause of dementia in the elderly. PET will be employed for the differential diagnosis of the dementias, to examine the course of Alzheimer's disease and to relate metabolism to symptomatology and neuropsychological deficits in Alzheimer's disease.

Down Syndrome - Down syndrome is the most common cause of mental retardation with an established etiology. Although brains of young adults with Down syndrome show no consistent abnormalities, brains of subjects older than 35 yr have pathological features of Alzheimer's disease. Our aim is to examine rCMRglc with PET in Down subjects with trisomy 21, to relate the findings to cognitive defects, and to understand to what extent aging and dementia modify cerebral function in Down syndrome.

Autism is an irreversible psychiatric disorder with onset in infancy and with a suspected neurological basis, and frequently is associated with mental retardation. We will use PET to examine the possible cerebral functional defect in adult autistics.

Methods Employed:

PET scanning. A subject is placed on the bed of an ORTEC ECAT II positron emission tomograph. His eyes are covered and ears plugged with cotton to establish a "resting" state, with reduced sensory input. Both hands are heated in temperature regulated chambers. Indwelling venous catheters are inserted on the dorsa of the hands for sampling of arterialized venous blood. 18-Fluoro-2-deoxy-D-glucose (18FDG), 5 mCi, is injected i.v. as a bolus, and venous blood is taken periodically for measurement of plasma radioactivity and glucose concentration. From 45 min after injection, PET scans at one of 7 different levels above the inferior orbito-meatal line of the skull are taken with at least 750000 coincidence counts per scan. For analysis, scans are displayed on a computer screen, morphological regions of interest are outlined and, following

use of a 4-transfer constant model and equation, values for the regional cerebral metabolic rate for glucose (rCMRglc) are calculated at each region. Data are compared among individuals by statistical methods.

Down syndrome subjects were carefully selected to include those with trisomy 21 karyotype, without major cardiovascular or endocrine disorders and who were not chronically institutionalized. Psychological tests were designed to give estimates of intelligence and cognitive ability (see Annual Report No. Z01 AG 00130-01 LN).

Major Findings:

1. Healthy aging.

a. Positron emission tomography (PET) scanning with 18FDG was employed to examine hemispheric and regional rates of cerebral glucose utilization in 21 resting healthy men between the ages of 21 and 83 years. Mean hemispheric cerebral metabolic rates for glucose (CMRglc) averaged from 4.3 - 4.4 mg.100 $g^{-1}.min^{-1}$, and mean hemispheric gray matter glucose utilization, (CMRglc)gray, equaled 5.2 - 5.3 mg.100 $g^{-1}.min^{-1}$. Neither parameter was correlated with age ($p > 0.05$), nor were their right/left ratios correlated with age. The mean ratios, furthermore, did not differ significantly from 1. Regional cerebral metabolic rates for glucose, rCMRglc, at 31 pairs of bilateral and 3 midline structures, also were not correlated significantly with age. Mean rCMRglc ranged from 2.6 mg.100 $g^{-1}.min^{-1}$ at the centrum semiovale (white matter) to 6.2 mg.100 $g^{-1}.min^{-1}$ at the precentral gyrus of the frontal lobe and precuneus of the parietal lobe. The results indicate that the cerebral oxidative metabolism is not correlated with age and provide age norms for rCMRglc in healthy men (Ref. 1).

b. We now have completed PET studies on 40 healthy men with a mean age of 50 years (21 to 83 yr). We calculated ratios of rCMRglc to CMRglc (Q) for individual brain regions, and of rCMRglc in right and left homologous brain regions. In addition, we determined the relations between subtests of the Wechsler Adult Intelligence Scale (WAIS) and the Revised Benton Visual Retention Test (RBVRT) and CMRglc. The results obtained were as follows: the highest mean rCMRglc (mg.100 $g^{-1}.min^{-1}$ $\pm$ SD) was found in the right precuneus (6.52 $\pm$ 1.43, SD). The lowest mean rCMRglc in a gray matter region was found in the left inferior temporal region (3.96 $\pm$ 0.90). No significant correlations of CMRglc, rCMRglc, Q, or right/left ratios were found with age or with any of the psychological measures. These findings confirm our previous results that cerebral metabolism is not correlated with age and demonstrate that in the resting state, rCMRglc does not relate to our measures of neuropsychological performance. These findings have been published in abstract form (Duara, R., Grady, C., Renfrew, J., Schwartz, M., Robertson-Tchabo, E.A., Koziarz, B., Haxby, J., Kessler, R., Rapoport, S.I., Cutler, N.R.: Regional cerebral glucose metabolism does not change with age nor relate to psychological measures in healthy men. Neurology, 33 (Suppl. 2), 116, 1983, Rapoport, S.I., Duara, R., Horwitz, B., Kessler, R.M., Sokoloff, L., Ingvar, D.H., Grady, C., and Cutler, N.: Brain aging in 40 healthy men: rCMRglc and correlated functional activity in various brain regions in the resting state. XIth International Symposium on Cerebral Blood Flow and Metabolism, Abstract, June 20-24, 1983, Paris).

c. In addition, Q values (rCMRglc/CMRglc) for individual brain regions of 40 healthy male volunteers were entered into a 61 X 61 correlation matrix to examine regional correlations among metabolic rates. There was a notable high frequency of significant correlations among frontal and parietal regions, on the one hand, and low frequency between these regions and temporal and occipital regias. The pattern is consistent with known neuroanatomy. An abstract of this work has been published (Duara, R., Horwitz, B., Grady, C.L., Cutler, N.R., and Rapoport, S.I.: A matrix method to quantitate functional interactions among brain regions: application to state of reduced sensory input. Society Neuroscience Abstracts, 9: 1983.

2. Alzheimer's disease.

Ten patients with various stages of Alzheimer's disease were examined with PET. In individuals with the most serious cognitive defects and with the disease of longest duration, rCMRglc was reduced throughout most of the brain, by as much as 80%. In earlier cases, with minimal memory and cognitive defects, metabolism was reduced mainly in the temporal and parietal lobes. Several patients had asymmetrical reductions in metabolism, consistent with lateralizing neuropsychological deficits (see Annual Report No. Z01 AG 00130-01 LN for neuropsychology in Alzheimer's disease). Abstracts of this work have been published (Grady, C.L., Haxby, J.V., Duara, R., Rapoport, S.I., and Cutler, N.R.: Neuropsychological function and regional cerebral glucose utilization in aging and dementia. Society Nruroscience Abstracts 9: 1983; Cutler, N.R., Duara, R., Haxby, J., Creasey, H., Grady, C., Kay, A.D., and Rapoport, S.I.: Alzheimer's disease: PET and CT scanning. VII World Congress of Psychiatry Abstracts, Vienna, July 11-16, 1983).

3. Aging and Down syndrome.

We determined the cerebral metabolic rate for glucose CMRglc with PET and 18-FDG in healthy adult patients with Down syndrome (DS) with trisomy 21. PET was performed on 4 male DS subjects (aged 19, 21, 23 and 28 yr) and one female (aged 51 yr). No DS subject demonstrated obvious dementia. In the young DS subjects, the mean CMRglc was 6.3 - 6.4 $\pm$ 0.4 mg/100 g/min, significantly higher than the mean of 4.6 mg/100 g/min, in healthy age matched controls ($p < 0.05$). In addition, rCMRglc was significantly higher in most brain regions of the young DS subjects than in control subjects ($p < 0.05$). In the 51 yr old DS subject, rCMRglc was significantly less in the frontal lobe and in parts of the parietal and temporal lobes than in young DS subjects, but approximated the rate in middle-age controls. The results suggest that glucose is used excessively by the brain of young adult Down syndrome subjects, and that rCMRglc declines with age in some brain regions in Down syndrome even in the absence of clear dementia. An abstract of this work has been published (Schwartz, M., Duara, R., Grady, C., Kessler, R., White, B., Rapoport, S.I. and Cutler, N.R.: Cerebral metabolic rate is not decreased in Down's syndrome. Neurology 33 (Suppl. 2) 7, 1983.

4. Brain metabolism and autism.

Cerebral glucose utilization was measured with PET in 7 adult male autistics and 12 adult male controls. The autistics ranged in age from 21 to 39 yr (mean age 30.2 yr). Six were right handed and one was left handed, 5 had a high school education, and all were free of medical illness including seizures and were off medication for at least 10 days (5 had been medication-free for years). Four had diffuse hypotonia and 4 had some involuntary movements. The healthy controls ranged in age from 21 to 46 yr (mean age 32 yr). Eleven were right handed and one was left handed. All had a high school education and 10 had graduated from college. PET was performed in the resting state with eyes closed and ears plugged with cotton in the controls and in 5 autistics. Regional cerebral metabolism was significantly higher ($p < 0.05$), by about 20%, in the frontal, parietal and temporal lobes of the autistics than of the age matched controls, intermediate between values in Down syndrome and normal controls. This work is part of a collaborative program with J. Rapoport and J. Rumsey of NIMH. Abstracts of this work have been published (Duara, R., Rumsey, J., Schwartz, M., Kessler, R., Cutler, N.R., Rapoport, J.L., and Rapoport, S.I.: PET scan studies of adults with autism. Abstracts. National Society for Children and Adults with Autism, Annual Meeting, July 6, 1982, Omaha, Nebraska; Rapoport, J.L., Rumsey, J., Duara, R., Schwartz, M., Kessler, R., Cutler, N. and Rapoport, S.I.: Cerebral metabolic rate for glucose in adult autism, as measured with positron emission tomography (PET). Abstracts XIth International Symposium on Cerebral Blood Flow and Metabolism, June 20-24, 1983, Paris, France).

Significance to Biomedical Research and Program at the Institute: The PET studies are designed to establish baseline values of cerebral metabolism and functional activity in relation to healthy aging, and to relate these measurements to the diagnosis and understanding of brain diseases, including Alzheimer's disease, Down syndrome and autism. Very few complete clinical studies involving the noninvasive procedures of this program have been done on healthy subjects in relation to age. Our efforts will define the normal aging process in the brain, as compared to pathology in disease states. Furthermore, by combining complex new techniques which involve image processing (PET scanning, CT scanning), with psychometric, physiological and neurochemical evaluation, we shall attempt to clearly define brain processes in healthy aging, and disease states including Alzheimer's disease and Down syndrome. Our study of Alzheimer's disease, which afflicts 5-15% of the elderly population (1-3 million people in the United States), may help in the differential diagnosis of primary progressive dementias and to establish parameters against which therapeutic regimens can be evaluated.

Down syndrome, a major cause of mental retardation with a known etiology, shows the neuropathology of Alzheimer's disease after the age of 35 years. By examining the metabolic basis of cerebral dysfunction in young and old Down subjects, we may be able to understand the cerebral defect in Down syndrome that is related to retardation and perhaps understand later processes associated with dementia. Our study with autism and Down syndrome suggest that developmental disorders associated with retardation are associated with cerebral metabolic dysfunction.

Proposed Course: Data will continue to be gathered in healthy subjects at different ages, and in patients with Down syndrome, Alzheimer's disease and

autism. Data will be evaluated statistically and prepared for publication. Greater emphasis this year will be placed on Alzheimer's disease, in relation to a newly opened patient care unit and a dementia clinic.

Publications:

1. Rapoport, S.I., Duara, R., London, E.D., Margolin, R.A., Schwartz, M., Cutler, N.R., Partanen, M., and Shinowara, N.L.: Glucose metabolism of the aging nervous system. In Samuel, D., Algeri, S., Gershon, S., Grimm, V.E., and Toffano, G., (Eds.): Aging of the Brain (Aging, Vol. 22). New York, Raven Press, 1983, pp. 111-121.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00130-01 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Neuropsychological Parameters in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J.V. Haxby Staff Fellow LN NIA

C.L. Grady Psychologist LN NIA

COOPERATING UNITS *(if any)*

Rehabilitation Medicine Department, Clinical Center

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	1.5	3.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Neuropsychologically relevant mental abilities are studied in healthy men at different ages, in patients with probable Alzheimer's disease, and in adults with Down syndrome at different ages. Tests are administered to evaluate intelligence, memory, language, visuoperceptive and visuoconstructive ability, and perceptual-motor speed. Age-related differences in psychological function in our sample of healthy men, ranging in age from 20 to 83 yrs, were equivalent in magnitude to differences found in studies of non-health-screened adults. The differences were not correlated with regional cerebral metabolic rates for glucose (rCMRglc) as measured by positron emission tomography (PET), and 18-Fluorodeoxyglucose.

Neuropsychological changes in mild, early-stage cases of probable Alzheimer's disease were restricted to recent memory and also were not related to changes in rCMRglc. Neuropsychological changes in moderately advanced cases suggest local cortical dysfunction and are related to changes in rCMRglc. Older Down syndrome adults perform worse on mental abilities tests than do younger subjects; the pattern of differences is unselective.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel.

B.J. Koziarz	Psychologist	LN	NIA
J.W. Renfrew	Psychologist	LN	NIA
B. Sonies	Speech Pathologist	RM	CC
A. Cheng	Statistician	LN	NIA
M. Duffy	Psychology Technician	LN	NIA
T. McCort	Psychologist	LN	NIA

Objectives:

Mental function is known to be affected by advancing age and primary progressive dementias such as Alzheimer's disease. In normal aging the relation between psychological changes and brain changes is poorly understood. The neuropsychological deficits in Alzheimer's disease have not been related to regional neurophysiological abnormalities in vivo. Advancing age in Down syndrome is known to be associated with neuropathological changes characteristic of Alzheimer's disease, but it is not known if these changes are associated with behavioral symptoms or dementia. The aims of this project are: (1) to describe age-related differences in mental abilities in healthy men in terms of performance on neuropsychological tests known to be related to the physiological integrity of different brain regions, (2) to relate age-related differences on neuropsychological tests to regional cerebral metabolic rates for glucose (rCMRglc), (3) to assess changes in neuropsychological function associated with Alzheimer's disease and relate those changes to rCMRglc, and (4) to assess age-related differences in neuropsychological function in adults with Down syndrome and relate those differences to rCMRglc.

Methods Employed:

Neuropsychological tests are administered individually to each subject by a psychologist or psychometrist. Some of the tests are administered with the assistance of a small computer (Apple II+) with color videomonitor and response buttons.

1. For the assessment of general intelligence, the Wechsler Adult intelligence Scale (WAIS) is used.

2. As a survey of neuropsychological functions, the Luria-Nebraska Neuropsychological Battery (LNNB) is used. This test consists of 269 items that can be grouped into 14 Clinical Scales that are measures of broadly defined domains of mental ability, 30 Factor Scales that are measures of more narrowly defined abilities, and 8 Localization Scales that are reputed to predict brain lesions in eight cerebral regions.

3. Recent memory is measured with a variety of word-list learning tasks, the Benton Visual Retention Test (BVRT), the Wechsler Memory Scale, and computer assisted verbal and visual paired associate learning tasks.

4. Linguistic function is assessed with the Boston Naming Test and experimental measures of verbal repetition, immediate verbal memory, sentence comprehension, sentence formulation, and sentence anomaly detection.

5. Frontal lobe executive function is measured with the Porteus Maze Test and tests of verbal and figural fluency.

6. Visuoperceptive discrimination is measured by the Benton Facial Recognition Test.

7. Visuoconstructive ability is measured by WAIS performance subtests, the Hiskey-Nebraska Block Patterns test, and an experimental drawing test.

8. Perceptuomotor speed is measured by an experimental, computer assisted continuous performance reaction time task.

In the study of Down syndrome the following tests are employed:

1. The Peabody Picture Vocabulary Tests is used as a measure of word knowledge and general verbal intelligence.

2. Selected tests from the Hiskey-Nebraska Tests of Learning Aptitude are administered to assess immediate memory, visuoperceptive discrimination, concept formation, and three-dimensional visuospatial construction.

3. The Block Design subtest of the Wechsler Intelligence Scale for Children - Revised (WISC-R) and an experimental block design test for low-ability subjects are administered as tests of two-dimensional visuospatial construction.

4. Two verbal immediate memory span tests, one requiring a vocal response and the other requiring only a nonverbal response, and one visuospatial immediate memory test are administered. Subjects' ability to learn a sequence exceeding immediate memory span is also assessed as a measure of recent memory.

Major Findings:

1. Eight of the eleven nonredundant Clinical Scales and seven of the eight Localization Scales of the LNNB were found to be significantly correlated with age in healthy men. The Clinical Scales that demonstrated no significant age-related differences are measures of Arithmetic, Reading, and Writing. The Localization Scale that demonstrated no significant difference was the Left Parieto-Occipital Scale. The Clinical Scales that demonstrated the greatest age-related differences were measures of Memory and Visual Processes. This pattern is analogous to what has been called the "classical pattern of aging," and demonstrates that the LNNB, a test designed to assess deficits in brain-damaged subjects, is sensitive to the same age-related differences that have previously been measured with tests designed to assess differences between norml subjects.

2. Correlations have been calculated between rCMRglc in healthy men and measures of verbal intelligence (WAIS sum of verbal subtests scaled scores), performance intelligence (WAIS performance subtest scaled scores), visual memory (BVRT), and the Clinical and Localization Scales of the LNNB in healthy men. The scattered significant correlations could be accounted for by chance alone. The substantial age-related differences on these tests (the mean scores of older subjects differ from those of younger subjects by as much as 1.5 to 2 standard deviations), therefore, do not appear to be related to any alteration in rCMRglc in an unstimulated condition.

3. The age-related cross-sectional differences found on measures of performance intelligence (WAIS) and visual memory (BVRT) in our sample of unusually healthy men are of a magnitute equivalent to that found in published normative

studies with larger sample sizes. In these larger normative studies subjects are not screened for general health. These findings suggest that the increasing cumulative risk for disease and accidents associated with aging contributes little if anything to the age-related differences found in normative, non-health-screened samples.

4. Neuropsychological deficits found in mild, early-stage cases of probable Alzheimer's disease and suggestive of limbic or diencephalic dysfunction appear not to be correlated with rCMRglc. Neuropsychological deficits found in moderately advanced cases and suggestive of cortical dysfunction appear to be correlated with changes in rCMRglc. In two cases in which the only neuropsychological deficit was impaired recent memory, PET scans were within normal limits and uncorrelated with LNNB Localization Scales. In two moderately advanced cases, neuropsychological deficits were found that suggested left parietal lobe dysfunction in one subject (acalculia, impaired verbal repetition, and asyntactic comprehension) in one patient and right parietal lobe dysfunction (visuoconstructive apraxia, dressing apraxia, and left-sided inattention for visual stimuli and body image) in the other. The rCMRglc for these cortical regions corroborated these lateral asymmetries. LNNB Localization Scales also demonstrated correspondence to rCMRglc in the respective cortical areas in these two subjects. In another moderately advanced case neuropsychological deficits were equivalent for right and left hemisphere functions and rCMRglc also demonstrated no later asymmetry. LNNB Localization Scales corresponded closely to rCMRglc in this case. In a sixth severely demented case, neuropsychological function was globally and markedly impaired and the PET scan revealed diffuse, substantial reductions in rCMRglc. An abstract of this work has been published (Grady, C.L., Haxby, J.V., Duara, R., Rapoport, S.I., and Cutler, N.R.: Neuropsychological function and regional cerebral glucose utilization in aging and dementia. Society Neuroscience Abstracts, 9, 1983).

Significance to Biomedical Research and Program of the Institute: The neuropsychological studies carried out by the Section on Brain Aging and Dementia are designed to describe the changes in healthy aging and Alzheimer's disease in terms that will further the understanding of the relation between psychological changes and brain changes. Very few studies of age-related differences in mental abilities have rigorously screened subjects for the absence of neurological and non-neurological medical conditions. Our study will establish the lower limit for the magnitude of age-related differences in unusually healthy men on a wide range of neuropsychologically relevant mental abilities. Our studies of Alzheimer's disease will describe the neuropsychological heterogeneity of the clinical presentation and the course of this condition. This information may aid the differential diagnosis and management of Alzheimer's disease and may define neuropsychological areas that can be assessed in studies of the effectiveness of therapeutic regimens. Our studies of Down's syndrome will describe age-related differences in mental function and establish the similarity of these differences to neuropsychological deficits found in Alzheimer's disease.

Proposed Course: The subject sample sizes will be increased for our studies of healthy aging, probable Alzheimer's disease, and Down syndrome. Neuropsychological test performance will be related to neuroanatomical differences as measured by X-ray computerized tomography. Patients with probable Alzheimer's disease will be reassessed every six months to study the course of the disease. Initial results will be prepared for publication. Preliminary studies of more narrowly focused neuropsychological function (visuospatial functions presumably related to parietal lobe function) will be conducted on healthy men at various ages.

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00131-01 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Neurological Function in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) C. Grady Psychologist LN NIA

A.W. Kay, Medical Staff Fellow, J.Renfrew,Psychologist,A.Moore,Social Wkr,LN NIA

COOPERATING UNITS *(if any)*

Research Services Branch, NIMH
Outpatient Department, Clinical Center

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Brain Aging and Dementia

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	1.7	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Research was carried out on motor function in man in relation to aging and disease. With the use of a patient activity monitor worn on the non-dominant wrist in 14 healthy men for a period of 10 days, it was demonstrated that average wrist motor activity was lower in older individuals, primarily as a result of low activity during daytime hours. Sleep duration could be estimated from the analysis, and was not correlated with age. A quantitative neurological examination was standardized in healthy men between 20 and 80 years of age, and established curves for age related declines in coordination, speed and accuracy of movement. There was significant correlation between age and peripheral hearing sensitivity in healthy men, particularly at high frequencies, but when the effects of hearing loss due to age were taken into account, measures of speech discrimination and tympanomemetry were not related significantly with age. An outpatient Dementia Clinic was initiated at the Clinical Center in Bethesda, to screen subjects for entry into inpatient protocols and for developing instruments for the differential diagnosis and determination of progress of Alzheimer's disease.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel.

N.R. Cutler	Section Chief	LN	NIA
C.L. Grady	Psychologist	LN	NIA
S.I. Rapoport	Chief	LN	NIA
T. Colburn	Chief	RSB	NIMH
B. Smith	Engineer	RSB	NIMH
M. Schwartz	Medical Staff Fellow	LN	NIA
A.T. Pikus	Audiologist	OP	CC
A. Grimes	Audiologist	OP	CC
E.K. Vernon	Audiologist	OP	CC

Project Description: Z01 AG 00131-01 LN

Objectives:

1. Motor activity and sleep. To quantitatively measure motor activity and sleep patterns in healthy subjects in relation to age, as well as in patients with Alzheimer's disease.

2. Quantitative neurological examination. To establish and validate an easily administered battery of quantitative tests of motor, sensory and coordinative functions, so as to provide a reliable index of neurological function in relation to age and disease.

3. Hearing and aging. To measure peripheral and central auditory processing in relation to aging in healthy subjects and in patients with Alzheimer's disease and Down Syndrome. To relate audiological variables to regional brain glucose utilization (rCMRglc) as measured by positron emission tomography (PET), and to measures of psychological and cognitive function.

4. Outpatient dementia clinic. To establish an outpatient clinic to gather longitudinal data on subjects with various types of dementia and to establish criteria for the differential diagnosis of the dementias. To screen patients for inclusion in inpatient protocol studies for PET and drug protocols.

Methods Employed:

1. Patient Activity Monitor (PAM). The PAM of Colburn measures linear acceleration in digital units and provides an activity output for each hourly period over 10 days. The size of a wrist watch, it is worn on the non-dominant wrist of a subject. Data are displayed and analyzed by computer. In addition, mentally competent subjects provide information about sleep and daily living in hourly diaries during the period when they wear a monitor.

2. Quantitative neurological examination. Subjects, who are right handed and free of evidence of neurologic, medical or psychiatric disease, are administered a test battery which includes measures of stability of stance, gait, touch sensation, 2 point discrimination, rapid alternating movements of the fingers and arms, coordination, speed, accuracy and steadiness. Materials for testing include: a stopwatch, esthesiometer, 2 point discriminometer, hand held counter, keyed peg board and a MAST (motor accuracy and speed test) device.

3. Hearing and aging. A battery of audiologic tests is administered to each subject. Tests of peripheral auditory processing include pure tone thresholds (from 250 to 10,000 Hz) acoustic reflexes, speech reception threshold and speech discrimination. Tests which assess central auditory function are low-pass filtered speech and binaural fusion. For all of the above tests, the subject is seated in a sound-isolated room and the stimuli are presented over headphones via a dual-channel audiometer. Stimuli are presented monaurally except for the binaural fusion test in which the stimuli are dichotic. The staggered spondaic word test is a dichotic test, and is included because of its sensitivity to temporal cortex dysfunction. The auditory brainstem response is the average electrical response of the brain stem auditory structures to click

stimuli and is obtained from electrodes placed on the scalp. Responses are averaged and displayed using an Amplaid microcomputer.

4. Dementia Clinic. Oral presentations are given to various community groups, including the Alzheimer society, and to professional organizations, to help with recruitment of control subjects and patients. All candidates are given a physical and neurological examination, and either are (a) entered into an inpatient protocol, (b) entered on clinic roles, or (c) not seen further. Subjects on clinic roles are seen every 6 months and are administered a number of instruments to evaluate the extent of dementia.

Major Findings.

1. Motor activity and sleep. Patient activity monitors (PAM's) and self report diaries were employed to examine wrist movements over a 10 day period in 14 healthy men aged 27 to 84 yr, in their natural work and home environments. Counts/hr from the PAM were divided into high and low activity periods per day. The mean duration of low activity per day equaled 7.2 $\pm$ 0.9 (S.D.) hr in the 14 subjects compared to a mean sleep time of 8.0 $\pm$ 0.8 hr that was estimated from daily diaries. Neither the duration of the low activity period nor self-reported sleep time was correlated significantly with age ($p > 0.05$). Net counts per 24 hr day declined significantly by about 5% per decade as did counts per high activity hr ($p < 0.05$). Thus, motor activity declined with age in the 14 subjects, mainly because hourly activity during wakefulness declined. Sleep duration could be estimated by the PAM as the duration of the low activity period, but was not correlated significantly with age.

2. Quantitative neurologic examination. Studies on 30 healthy male volunteers, indicated that (1) the neurologic test in which the subject is required to stand on one leg with eyes closed demonstrated an age-decrement, (2) gross touch and 2 point discrimination did not decline with aging, and (3) age declines occurred in coordination, speed and accuracy.

3. Hearing and aging. Pure tone thresholds were significantly and positively correlated with age at all frequencies (r ranged from 0.57 to 0.82, $p < 0.05$). The increase in threshold with age was greater at the higher frequencies, with an increase of 3 dB at 250 Hz and of 13 dB at 8000 Hz. The age at onset of hearing loss at a particular frequency could not be determined due to the small number of subjects in each decade, but loss was apparent in most subjects over 50 years of age at every frequency above 1000 Hz.

Acoustic reflexes and speech measures also were correlated with age. However, these variables are highly dependent on peripheral hearing sensitivity, and separating the effect of this loss from other aging effects has been a problem in previous studies in this field. We used partial correlations to remove the effect of peripheral hearing loss from the speech and reflex measures, and found that the only measure which continued to show a relation with age was the low-pass filtered speech test when stimuli were delivered to the left ear. Since this test has been shown to be sensitive to temporal cortex dysfunction, the results indicate a deficit in the right temporal lobe in our older subjects.

However, until additional subjects are studied, we cannot rule out chance factors in this finding. An abstract of this work has been published (Grady, CL, Pikus, AT, Grimes, AM, Schwartz, M, Cutler NR and Rapoport, SI.: Alterations in auditory processing during normal aging in man. Age 5: 146, 1982).

4. An outpatient Dementia Clinic has been established at the ACRF in Bethesda. The purpose of this clinic is to screen subjects for inpatient protocols, and to standardize a battery of tests to characterize the various dementias, establish the differential diagnosis of the dementias, and to establish criteria for determining the severity of the various dementias. The clinic also is associated with family therapy and a group of members of Alzheimer families, which meets with A. Moore (Social Worker) periodically for counseling and developing support approaches for families of Alzheimer patients. It is the aim of our program to establish and identify a system of family support services that would help members to deal with the catastrophic problem of the patient, alleviate anxiety and distress in the family, enhance patient environmental therapy, and delay if possible costly and tumultuous commitment of a patient to a nursing home. This means that community services must be mobilized for training community workers and services to support a family with an Alzheimer patient. The clinic and groups are closely related to area Alzheimer organizations.

Significance to Biomedical Research and Program of the Institute.

1. Motor activity and sleep duration in man in a natural home or work environment have not been quantified, whereas such measurements have been made in laboratory settings. In order to understand the natural process of aging, and the changes that might occur in activity in Alzheimer's disease, such information is essential.

2. It would be useful to design and standardize a simple quantitative neurological examination, which could be employed in the clinic and in the medical office, to examine age-related parameters of neuromuscular and sensory function, as well as changes in these parameters in disease processes such as the dementias.

3. One of the major limitations to normal functioning in the elderly is progressive deafness, yet little has been done on understanding the physiological and environmental causes for the age decline in auditory function. Studies of various aspects of central and peripheral processes in audition, in relation to age, will establish baseline values to understand age-related decrements, and to examine the effects of environment and disease processes on audition at different ages.

4. Data collected on a well-defined clinical population can be used to establish and evaluate objective measures for the differential diagnosis of and the intensity of the various dementias.

Proposed Course:

The above studies have been recently initiated and will be continued and expanded. Data will be analyzed and prepared for presentation and publication. Methods of patient activity monitoring, quantitative neurological examination and audiology will be applied to subjects with Alzheimer's disease as well as Down syndrome.

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00132-01 LN

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* (D)
Brain Anatomy and Chemistry in Aging and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)			
A. Kay	Medical Staff Fellow	LN	NIA
H. Creasey	Visiting Associate	LN	NIA
M. Schwartz	Medical Staff Fellow	LN	NIA

COOPERATING UNITS *(if any)*
Computer Systems Laboratory, Division of Computer Research Technology
Biomedical Engineering and Instrumentation, Division of Research Services

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Brain Aging and Dementia

INSTITUTE AND LOCATION
NIA, NIH, and Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.9	.6

CHECK APPROPRIATE BOX(ES)

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

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

Cerebrospinal fluid was obtained in healthy men at different ages for evaluation of products and precursors of brain neurotransmitters and of proteins and electrolytes.

Computer assisted tomography (CT) was applied to healthy men at different ages, and analyzed by quantitative image processing techniques. Even in healthy men, brain atrophy occurs in the elderly. Gray matter volume is correlated negatively with age whereas cerebrospinal fluid volume is correlated positively with age.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel.

N. Cutler	Section Chief	LN NIA
S. Rapoport	Chief	LN NIA
James DeLeo	Computer Systems Analyst	CSL DCRT
Hal Fredrickson	Electrical Engineer	CSL DCRT
Steven Leighton	Mechanical Engineer	BEI DRS

Project Description: Z01 AG 00132-01 LN

Objectives:

1. Cerebrospinal fluid chemistry. To establish baseline cerebrospinal fluid (CSF) concentrations of biochemical markers of brain function in healthy subjects at different ages, and in patients with Alzheimer's disease and Down syndrome.

2. Brain anatomy. To use computer assisted tomography (CT) to evaluate dimensions of gray matter, white matter and CSF in brains of men at different ages and in patients with Alzheimer's and other diseases.

Methods Employed:

1. CSF. Subjects are admitted to the LN patient care unit and are placed on a low monamine diet. After 3 days, and after strict bed rest and fasting for at least 8 hours, the subject undergoes a lumbar puncture. Blood is first drawn from an indwelling intravenous line placed the day before and then a spinal tap is performed at the L3-4 interspace. Two 12 ml-aliquots of CSF are collected to establish gradients for CSF metabolites. In addition, 15 µl of CSF are collected for electrolytes, oligoclonal banding, proteins and routine diagnostic purposes.

2. Non-contrast CT scans are obtained with a GE CT/T 8800 scanner. Slices are taken parallel to and beginning at a line drawn between the lower rim of the orbit and the external auditory meatus, the inferior orbitomeatal (IOM) line). The head is scanned to the vertex. Slices are 10 mm thick, with an interslice distance of 7 mm. CT images are stored on magnetic tape and analyzed using a PDP 11/70 computer and an Evans Sutherland Picture System II analyzer.

Major Findings:

1. CSF chemistry. To date, 23 CSF specimens have been collected on 19 patients with Alzheimer's disease, 4 specimens on normal volunteers, and 4 on Down subjects. CSF was frozen at -70° and awaits analysis. The only side effect of CSF withdrawal has been a mild, postural, self-limiting headache in 5 out of 32 spinal taps, for a 15.6% incidence - similar to that reported in the literature for routine lumbar punctures.

2. Brain anatomy. Thirty healthy men between 21 and 81 yr of age were subjected to CT scanning. The scans were analyzed by computer. Generally, there was a correlation between brain atrophy and age. The volume of cerebrospinal fluid (CSF) was correlated positively with age, whereas the volume of gray matter was correlated negatively. White matter volume was unrelated to age. Thus, there is a general loss of gray matter with age even in healthy men, and an increase in cerebrospinal fluid volume. Abstracts of this work have been published. (Schwartz, M., DeLeo, J., Creasey, H., Duara, R., Renfrew, J.W., Rapoport, S.I., Cutler, N.R.: Increase in cerebrospinal fluid volume and decrease in gray matter volume in brains during aging of healthy men. Neurology, 33 No. 4 (Suppl. 2): 100, 1983; Schwartz, M., DeLeo, J., Cutler, N.R. and Rapoport, S.I.: Brain morphological alterations during normal aging. Continuing Medical Education Syllablus and Scientific Proceedings in Summary Form, American Psychiatric Association, April 1983, p. 238).

Significance to Biomedical Research and to the Program of the Institute:

1. Aging, and to a greater extent Alzheimer's disease and Down syndrome, is accompanied by a reduction in post-synaptic receptors and synthetic enzymes for a number of brain neurotransmitters, but particularly acetylcholine. By analyzing spinal fluid levels of markers for neurotransmitter synthesis or metabolism, it might be possible to infer the *in vivo* status of the systems within the brain in relation to age and disease.

2. Another way to examine brain function in relation to age and disease is by determining the dimensions of anatomic regions in the brain, and the volumes of gray matter, white matter and cerebrospinal fluid. Use of quantitative CT scanning should make this possible during life.

Proposed course: Data will be gathered, analyzed and prepared for presentation and publication.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00133-01 LN

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* (E)
Clinical Pharmacokinetics and Pharmacodynamics

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)			
N.R. Cutler	Section Chief	LN	NIA
A. Kay	Medical Staff Fellow	LN	NIA

COOPERATING UNITS *(if any)*

Pharmacy Department, Clinical Center

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Brain Aging and Dementia

INSTITUTE AND LOCATION
NIA, NIH, and Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1	.6

CHECK APPROPRIATE BOX(ES)

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

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

The binding of salicylate to serum protein from serum of elderly healthy subjects is significantly less than from serum of young healthy subjects. A higher unbound fraction of salicylate in the elderly would increase the initial volume of distribution and contribute to altered pharmacokinetics.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd.

Other professional personnel:

J. Hodes	Medical Staff Fellow	LN	NIA
D.K. Narang	Senior Staff Fellow	PHAR	CC
L.J. Lesko	Consultant to NIH, CC Pharmacy	PHAR	CC
S.I. Rapoport	Chief	LN	NIA

Project Description:

Objectives: Pharmacokinetic alterations during aging appear to conform with known physiological changes that also occur. The aims of this project are: (1) To examine the pharmacokinetics and pharmacodynamics of drugs which are administered frequently to the elderly, in relation to aging, (2) to examine drug pharmacokinetics and pharmacokinetics in disease states, and (3) to develop a recommended dosing guideline for various classes of drugs in relation to age and disease.

We initally evaluated salicylate binding to plasma protein, with diafiltration methodology, in young and elderly men.

Methods Employed: Subjects were obtained through the Outpatient Screening Clinic. The dynamics of salicylate binding to serum protein were determined with an Amicon MMC device and continuous ultrafiltration. Preliminary studies indicated that freezing and thawing of serum did not alter binding characteristics. Salicylate was analyzed with high pressure liquid chromatography. Binding parameters were obtained by fitting curves to data by non-linear least squares.

Major Findings: Five healthy young (mean 27 yr, range 18-33 yr) and five elderly (mean 73 yr, range 70-83 yr) male volunteers participated in the study. Subjects were not taking drugs at the time of the study. After an overnight fast, 20 ml of venous blood was withdrawn, allowed to sit for 1 hr at room termperature, and serum was separated by centrifugation. An aliquot of the serum was analyzed for total protein and albumin concentrations and another aliquot was frozen at $-20^{\circ}C$ for protein binding studies. The mean albumin concentrations in young sera and elderly sera were 4.13 gm/dl and 4.06 gm/dl respectively, and did not differ significantly. Similarly, there were no significant differences in total protein concentrations of the young (7.24 gm/dl) as compared to elderly sera (6.56 gm/dl).

The protein-bound fraction of salicylate within the therapeutic range of total salicylate (1000-3000 μM ranged from 0.66-0.79 for sera from elderly subjects. The free fraction in sera from older subjects was 23% at 7000 μM and 61% (at 1000 μM), significantly higher than in sera from young subjects. Computer analysis indicated few 2nd degree protein-binding sites for salicylate in sera of the older as compared to the younger subjects. A higher unbound fraction of salicylate in the elderly would increase the volume of distribution and decrease the fraction of drug within the plasma. An abstract of this work has been published (Lesko LJ, Yeager RC; Narang PK, Hodes JE, Cutler NR: Salicylate protein binding in young and elderly serum as measured by diafiltration. Clin. Pharm. Therap. 33: 257, 1983).

Significance of Biomedical Research and Program at the Institute:

A rise in the free fraction of salicylate within the therapeutic range in the elderly sera must be taken into account when interpreting total serum salicylate concentrations and adjusting drug doses, in order to prevent salicylate toxicity.

Our study indicates the need for an *in vivo* clinical study of salicylate pharmacokinetics in the elderly.

Proposed Course: To continue work on salicylates and on the pharmacokinetics of drugs in relation to age, as well as the basis of drug toxicity in the elderly.

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00125-05 LN

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* (F)
Cerebral Metabolism, Relation to Brain Function and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

T. Soncrant	Staff Fellow	LN	NIA
A.S. Kimes	NIH Research Fellow	LN	NIA

COOPERATING UNITS *(if any)*
National Institute on Drug Abuse

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION
Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.7	4.1	2.6

CHECK APPROPRIATE BOX(ES)

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

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

1) Cerebral metabolism was studied in relation to age and drug responses in unanesthetized rats. The regional cerebral metabolic rate for glucose (rCMRglc) as measured with 14C-2-deoxy-glucose, increased between 1 and 3 months when the rat brain continues to grow, but remained unchanged during senescence, suggesting that compensatory mechanisms in the brain can maintain resting function. 2) Cerebral metabolic rates for oxygen and glucose, as well as cerebral blood flow, were unrelated to age in awake Fischer-344 rats, indicating that coupling between flow and metabolism is maintained during aging of the rat brain. 3) In adult rats, central muscarinic stimulation with oxotremorine increased rCMRglc in brain areas involved in motor and cognitive function, but the pattern was more general than expected from receptor distribution. 4) Metabolic responses to agonist and antagonist drugs of γ-aminobutyric acid (GABA), a central inhibitory neurotransmitter, were not correlated with markers for GABAergic synapses, indicating that additional factors such as neural circuitry determine complex responses to drugs. GABA agonists generally decreased rCMRglc, but produced a relative increase in the dentatorubro-thalamic pathway. 5) The pharmacodynamic metabolic response to haloperidol, a dopaminergic antagonist, was related to dose and time after administration, and interpreted in terms of demonstrated pharmacokinetics of this drug. 6) A quantitative method was developed to examine incorporation of palmitate from plasma to brain as a measure of brain lipid metabolism. Regional incorporation was correlated generally with rCMRglc, and was greater in cerebral gray than white matter regions. Brain palmitate uptake decreased in Fischer-344 rats between 1 and 3 months of age, but remained unchanged after 3 months. 7) Although ketone bodies (3-hydroxybutyrate and acetoacetate) can partially replace glucose as substrates for brain oxidative metabolism, severe hyperketonemia in resting rats did not reduce calculated rCMRglc.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel.

S.I. Rapoport	Chief	LN NIA
H.W. Holloway	Biologist	LN NIA
S. Carlson	Biologist	LN NIA
W.R. Fredericks	Biologist	LN NIA
D. Jenkins	Bio Lab Techn	LN NIA
G. Pizzolatto	Visiting Fellow	LN NIA
H. Tabata	Visiting Fellow	LN NIA
P. Helen	Visiting Fellow	LN NIA
J.M. Bell	Chemist	LN NIA
E.D. London	Pharmacologist	NIDA
H. Takei	Visiting Fellow	LN NIA
M. Dam	Visiting Fellow	LN NIA

Objectives: The aims of this program are: to examine morphological, biochemical and functional changes in the brain that occur during development, maturation and aging; to study coupling between regional cerebral blood flow and metabolism; to examine cerebral metabolism in response to specific centrally-acting drugs. Specifically, projects are designed (1) to describe the time course of the regional cerebral metabolic rate for glucose (rCMRglc) during development and aging of the unanesthetized Fischer-344 rat, (2) to study global cerebral blood flow (CBF) and the cerebral metabolic rates for O_2 ($CMRO_2$) and glucose (CMRglc) in aging Fischer-344 rats, (3) to study the effects of cholinergic drugs on rCMRglc, and to relate these effects to knowledge of drug action, distribution of appropriate receptors, and peripheral and central pharmacokinetics. The drugs include oxotremorine, physostigmine, scopolamine, muscimol, THIP, bicuculline and haloperidol, (4) to examine lipid turnover in the central nervous system, by measuring palmitate incorporation into brain lipids in individual regions, and to deterime anesthetic and age effects on palmitate incorporation, and (5) to examine the relation between hyperketonemia and rCMRglc in awake rats.

Methods Employed: 1. For studies of rCMRglc, experiments are performed on partially restrained, awake rats with indwelling femoral arterial and venous catheters. 14C-2-deoxy-D-glucose (14C-2-DG), 125 μCi/kg, is injected i.v., and timed arterial blood samples are collected over the next 45-50 min until the animal is killed. Regional brain radioactivity is determined by dissection and liquid scintillation spectroscopy, or by quantitative autoradiography on 20 μ frozen sections. rCMRglc is calculated from the tissue radioactivity at kill time, the plasma histories of glucose and 14C-2-DG, and the kinetic constants for 14C-2-DG uptake and metabolism by brain (Sokoloff et al., 1977, J. Neurochem. 28: 897).

2. The cerebral metabolic rates for O_2 and glucose are obtained by placing an indwelling needle in the superior sagittal sinus of a conscious rat, and measuring the concentrations of these substances in sagittal sinus blood and femoral arterial blood. The arterio-venous differences are multiplied by CBF, as determined with 14C-iodoantipyrine.

3. Effects of central cholinergic stimulation on rCMRglc are assessed in adult rats treated with oxotremorine (0.7 mg/kg, i.p.) 2 min before i.v. 14C-2-DG, or with physostigmine (0.1 - 1.0 mg/kg, i.p.) 20 min before 14C-2-DG. Atropine methylbromide (1 mg/kg, s.c. 20 min before oxotremorine or physostigmine) is given to block peripheral muscarinic effects. Scopolamine (2.5 mg/kg, i.p.) is given 10 min before cholinergic drugs to test the specificity of drug effects on central muscarinic receptors.

4. The effects of GABAergic drugs on rCMRglc are assessed in adult rats injected i.v. with one of several doses of GABAergic agonists (muscimol, 1-7 mg/kg; 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol, THIP, 12 and 24 mg/kg), or antagonist (bicuculline, 0.3 mg/kg) prior to 14C-DG.

5. Haloperidol is injected into rats at two doses, and plasma and brain concentrations are determined by analytical techniques.

6. 14C-palmitate is injected i.v. in conscious rats, and incorporation of tracer is measured in brain regions in relation to plasma concentrations. High pressure liquid chromatography is used to measure plasma palmitate.

A model is developed and applied for direct calculation of the unidirectional incorporation of palmitate into stable brain components, as a measure of lipid turnover.

7. Blood concentrations of acetoacetate and 3-hydroxybutyrate are determined analytically in rats following 3 days of starvation or infusion of 3-hydroxybutyrate.

Major Findings

1. Age-related changes in rCMRglc in awake Fischer-344 rats. Between 1 and 3 months of age, glucose utilization tended to increase in all brain regions; statistically significant increases occurred in seven analyzed regions. Between the ages of 3 and 12 months, glucose utilization decreased significantly in 12 regions. The greatest reductions (25% or more) occurred in the striatum, inferior colliculus and pons, but the hypothalamus and thalamus, nucleus accumbens, and septum showed no statistically significant change. rCMRglc did not change in any regions examined between 12 and 24 months or between 24 and 34 months of age. The results demonstrate a rise in cerebral glucose utilization with development from 1 to 3 months, a decline between 3 and 12 months, and a constancy in the second and third years that does not reflect reported senescence-associated neurochemical and morphological cerebral changes.

On the other hand, regional cerebral blood flow (rCBF), as measured with 14C-iodoantipyrine, remained constant or even increased after 3 months of age in cerebral cortical regions of the awake Fischer-344 rat. This observation suggested that brain oxidative metabolism also remained constant with aging after 3 months, but that there might be a change in the coupling relation between glucose utilization and blood flow with age. The latter question was examined (see 2 below)(Ref. 1 & 6).

2. Cerebral blood flow (CBF) and cerebral metabolic rates for oxygen and glucose in Fischer-344 rats of different ages. The cerebral metabolic rates for 0_2 and for glucose were measured directly in conscious fasted male Fischer-344 rats at the ages of 3, 12 and 24 months, and cerebral blood flow was determined with 14C-iodoantipyrine. The metabolic rates for oxygen and glucose were obtained by multiplying blood flow by the 0_2 and glucose concentration differences, respectively, between blood in the femoral artery and in the superior sagittal sinus. Mean cerebral blood flow and the metabolic rates for oxygen and glucose did not differ significantly ($p > 0.05$) between 3 and 12 or between 12 and 24 months. Nor did the arteriovenous differences for 0_2 and for glucose change significantly with age. Because the superior sagittal sinus drains blood mainly from the cerebral cortex, the results indicate that average cerebral cortical oxidative metabolism, and the coupling ratios between the cerebral metabolic rate for oxygen and cerebral blood flow and between the cerebral metabolic rate for glucose and cerebral blood flow do not change significantly with age in the Fischer-344 rat. Therefore the "lumped constant" in the equation required to calculate rCMRglc (see section 1) may fall with age in the Fischer-344 rat and account for the calculated decline in rCMRglc between 3 and 12 months, as has been previously reported (Ref. 2).

3. Effects of a cholinergic drug on cerebral metabolism in the adult awake Fischer-344 rat. The 2-DG technique was used to examine the effects of central muscarinic stimulation on regional cerebral glucose utilization (rCMRglc) in the cerebral cortex of the unanesthetized rat. Systemic administration of the muscarinic agonist oxotremorine (OXO, 0.1 - 1.0 mg/kg ip) increased rCMRglc in the neocortex, mesocortex and paleocortex. In the neocortex, OXO was more potent in elevating rCMRglc of the auditory, frontal and sensorimotor regions compared with the visual cortex. Within these neocortical regions, OXO effects were greater in the neocortex than in the meso- or paleocortex, but no significant effect occurred in the perirhinal or pyriform cortex. OXO-induced rCMRglc increases were not influenced by methylatropine (1 mg/kg sc) but were antagonized completely by scopolamine (2.5 mg/kg ip). Scopolamine alone reduced rCMRglc in layer IV of the auditory cortex and in the retrosplenial cortex. The distribution and magnitude of the cortical rCMRglc response to OXO appeared related to the distributions of cholinergic neurochemical markers, especially high affinity muscarinic binding sites (Ref. 3).

4. Effects of γ-aminobutyric acid (GABA) agonist and antagonist drugs on local cerebral glucose utilization. The 2-DG method was used to study regional cerebral glucose utilization (rCMRglc) in rats treated with γ-aminobutyric acid (GABA) agonist (muscimol and 4,5,6,7-tetra-hydroisoxazolo 5,4-C pyridin-3-ol, THIP) and antagonist (bicuculline) drugs. It was of interest to determine if the pattern of rCMRglc responses to GABA agonists and antagonists administered systemically would reflect the known distributions of markers for central GABAergic synapses. The patterns of rCMRglc responses to muscimol and THIP generally were similar. Most brain regions showed dose-dependent decreases in rCMRglc; others showed no effects, but the red nucleus showed an increase. The GABA antagonist bicuculline produced convulsions and variable rCMRglc responses, depending on the time of administration. Bicuculline also partially antagonized the depressant effects of muscimol on rCMRglc. The magnitudes and distribution of in vivo cerebral metabolic responses to specific GABA agonists were not correlated simply with markers for GABAergic synapses. This lack of correlation indicates that additional factors, such as neural circuitry, regulate rCMRglc responses to GABAergic drugs (Ref. 4).

5. Relation between time course of haloperidol effect on local cerebral glucose utilization (rCMRglc) and haloperidol pharmacokinetics in rat brain. We demonstrated that the highest brain concentration of haloperidol (HAL) in 3 month old Fischer-344 rats is achieved after an i.p. bolus injection (Kapetanovic et al., J. Pharm. Exp. Therap. 221: 434, 1982). Because rCMRglc is coupled to brain functional activity, we examined rCMRglc in different brain regions in relation to brain HAL concentrations at 30, 60 or 90 min after HAL (0.5 mg/kg or 1 mg/kg) was injected i.p. The time course of rCMRglc following the two doses was significantly different. HAL 1 mg/kg significantly decreased rCMRglc in a few brain regions after 30 min and in most regions after 60 min. Return to control rCMRglc values was seen in most regions by 90 min. HAL 0.5 mg/kg caused greatest decreases in rCMRglc at 90 min. In addition, the peak behavioral effect (catalepsy) was reached earlier with the higher dose. Therefore, the onset of metabolic changes is related to known brain concentrations of HAL, and correlates with the time course of the behavioral effect. However, recovery of normal rCMRglc values

at 90 min after the higher HAL dose suggests that regulatory processes have occurred by then to alter the pharmacodynamic effect of the drug. An abstract of this work has been presented (Pizzolato, G., Soncrant, T.T., and Rapoport, S.I.: Relation between time course of haloperidol effect on local cerebral glucose utilization and its pharmacokinetics in rat brain. Abstracts American Society of Pharmacology and Experimental Therapeutics, August 1983, Philadephia, PA).

6. Palmitate incorporation into different brain regions in the awake rat. A quantitative method was developed to examine incorporation of the fatty acid, palmitate, from the plasma to a stable metabolic compartment of individual brain regions in awake rats. Following the i.v. injection of 14C-palmitate, brain radioactivity rose and then fell until, at 4 hr, a stable concentration was reached that was maintained for up to 24 hr. The net unidirectional uptake of plasma palmitate into this 4 hr compartment was calculated by dividing the regional brain radioactivity at 4 hr, as determined by quantitative autoradiography, by the integral of the plasma palmitate specific activity. Palmitate uptake varied from 2.0 x 10-5 μmol/g-sec at the internal capsule to 9.3 x 10-5 μmol/g-sec at the median eminence, and generally was proportional to the regional cerebral metabolic rate for glucose (rCMRglc), as measured with 2-DG. The results demonstrated that it is possible to determine net palmitate uptake into a stable metabolic compartment in individual brain regions of awake rats, that uptake by gray matter generally exceeds uptake by white matter, and that palmitate uptake is proportional to published values for regional brain oxidative metabolism. Additional studies demonstrated, furthermore, that palmitate uptake was generally reduced by barbiturate anesthesia. Abstracts of this work have been published (Kimes, A.S. and Rapoport, S.I.: Regional incorporation of 14C-palmitic acid into the brains of anesthetized rats. Society Neurosciences Abstracts, 8, 1982; Kimes, A., Sweeney, D., and Rapoport, S.I.: Relation between palmitate incorporation into brain structure and regional glucose utilization. Abstracts Eleventh International Symposium on Cerebral Blood Flow and Metabolism, Paris, June 1983).

7. Effect of development and aging of the rat on unidirectional palmitate incorporation by brain. The method to measure palmitate incorporation in the rat brain (see section 6) was employed in awake Fischer-344 rats at the ages of 1, 3, 12, 24 and 34 months. Regional palmitate uptake declined 15-30% between 1 and 3 months in all regions, but did not change significantly thereafter. Considering the fact that the maximum rate of myelin deposition in rats occurs at 20 days of age, a relatively larger uptake in 1 than 3 month old animals probably is related to the rate of myelinization. This is supported by a larger uptake by white as compared to gray matter in 1 month than in older animals. After 3 months of age, palmitate incorporation is relatively stable and demonstrates a maintenance of cerebral integrity in the absence of disease. An abstract of this paper has been published (Tabata, H., Kimes, A.S., Bell, J.M., and Rapoport, S.I.: Regional changes in the unidirectional fatty acid fluxes into the developing and aging rat brain. Society Neuroscience Abstracts, 9, 1983).

8. Relation between hyperketonemia and cerebral glucose utilization. Ketone bodies, acetoacetate (AcAc) and 3-hydroxybutyrate (3-OHB), can partially replace glucose as substrates for brain oxidative metabolism. The 2-deoxyglucose (2-DG) technique of Sokoloff to measure the cerebral metabolic rate for glucose (rCMRglc) ignores the possible influence of hyperketonemia on the calculations. Because ketone body concentrations may be elevated in many physiological conditions (e.g., starvation, stress, senescence), we evaluated the possible role of hyperketonemia on rCMRglc. Hyperketonemia was induced in awake rats by 3 days of starvation or by i.v. infusion of 3-OHB in fed rats. These treatments produced combined blood ketone body concentrations (AcAc + 3-OHB) of 1.2 to 2.4 mM. However, neither treatment significantly affected rCMRglc in 15 brain region studied. These observations demonstrate that hyperketonemia in resting awake rats does not interfere with brain uptake or phosphorylation of glucose (Ref. 5).

Significance to Biomedical Research and the Program at the Institute.

1. A knowledge of regional cerebral metabolic deficits that appear during aging could direct future research to identify particular neurotransmitter systems which may be inadequate in elderly man.

2. Our observations that resting rCMRglc does not decline with senescence of the rat, and that challenges with a cholinergic drug, oxotremorine, demonstrate functional deficits in brain regions of senescent rats, indicate that studies of cerebral glucose utilization with pharmacological probes can elucidate cerebral functional changes during aging of rats, as well as of man.

3. Measurements of brain oxygen and glucose utilization and of blood flow, are direct ways to evaluate oxidative metabolism of the cerebral cortex during aging. The age invariance of these parameters in the brain of the rat suggests that functional activity does not decline in the cerebral cortex, despite many age-related neurochemical and morphological changes.

4. Local cerebral metabolic responses to cholinomimetic drugs are relevant to the pharmacology of aging and senile dementia of the Alzheimer type. In view of reported presynaptic cholinergic losses in the human brain in aging and to a greater extent in Alzheimer's disease, treatment is being designed to enhance central cholinergic neurotransmission. Our studies suggest possible approaches to evaluation and therapy of Alzheimer's disease.

5. The relative increase in rCMRglc in components of the dentato-rubro-thalamic pathway in response to GABAergic drugs may explain the paradoxical "epileptic" action of GABA agonists. In view of the known interaction between GABAergic systems and benzodiazepines, knowledge of the distribution of the metabolic responses to GABA agonists could help explain untoward central effects of benzodiazepines in the elderly.

6. Although brain glucose utilization and oxygen consumption are frequently measured to examine brain functional activity, a method is unavailable to examine lipid turnover in individual brain regions by quantitative autoradiographic techniques. The palmitate incorporation method, developed in this laboratory, represents such an approach, and should be useful for examining the relation between brain oxidative metabolism and brain structure.

7. Haloperidol is an antipsychotic drug and dopaminergic antagonist used commonly in the elderly. Its use often results in central neurotoxicity. By examining rCMRglc in the brain as a pharmacodynamic end-point in relation to determined pharmacokinetics, it should be possible to understand the actions of the drug and to design doses more appropriate for elderly subjects.

8. Studies of brain glucose utilization in man, at different ages, have suggested that ketone bodies partially replace glucose as substrates for brain oxidative metabolism in the elderly (Dastur et al., 1963). Our observations in starved rats suggest that their contribution is minimal, however, and also that ketosis does not significantly influence measurements of rCMRglc in animal experiments.

Proposed Course. Studies of rCMRglc with dopaminergic drugs will continue and control data will be prepared for publication. Drug effects in relation to pharmacokinetics will be analyzed. The model for palmitate incorporation will be further developed and applied to anesthesia and aging.

Publications:

1. Rapoport, S.I., London, E.D., and Takei, H.: Brain metabolism and blood flow during development and aging of the Fischer-344 rat. Exp. Brain Res. Suppl. 5, 86-101, 1982.

2. Takei, H., Fredericks, W.R., London, E.D., Rapoport, S.I.: Cerebral blood flow and oxidative metabolism in conscious Fischer-344 rats of different ages. J. Neurochem. 40: 801-805, 1983.

3. Dam, M., Wamsley, J.K., Rapoport, S.I., and London, E.D.: Effects of oxotremorine on local glucose utilization in the rat cerebral cortex. J. Neurosci. 2: 1072-1078, 1982.

4. Palacios, J.M., Kuhar, M.J., Rapoport, S.I., and London, E.D.: Effects of γ-aminobutyric acid agonist and antagonist drugs on local cerebral glucose utilization. J. Neurosci. 2: 853-860, 1982.

5. Corddry, D.H., Rapoport, S.I., and London, E.D.: No effect of hyperketonemia on local cerebral glucose utilization in conscious rats. J. Neurochem. 38: 1637-1641, 1982.

6. Rapoport, S.I., and London, E.D.: Brain metabolism during aging of the rat and dog. Implications for brain function in man during aging and dementia. In Terry, R.D., Bolis, C.L., and Toffano, G. (Eds.): Neural Aging and Its Implications in Human Neurological Pathology (Aging, Vol. 18). New York, Raven Press, 1982, pp. 79-88.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00120-06 LN

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Blood-Brain Barrier and Central Nervous System Function (G)

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)			
S.I. Rapoport	Chief	LN NIA
P.J. Robinson	Visiting Fellow	LN NIA

COOPERATING UNITS *(If any)*
Experimental Morphology Section, NIA
Clinical Branch, NEI

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.7	2.7	1.0

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

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

A new method was developed in rats to examine the permeability at the blood-brain barrier to drugs and other agents. The method takes into account cerebral blood flow. Generally, permeability is related linearly to the octanol/water partition coefficient, a measure of lipid solubility. Pharmacokinetic principles for the central nervous system were established which take into account blood-brain barrier transport, intracellular drug distribution, and drug washout by cerebrospinal fluid. The principles make it possible to calculate brain concentration of drugs from measured plasma concentration curves and peripheral loss, in acute as well as steady-state drug administration regimens.

Osmotic treatment of the blood-brain barrier has been used in man for allowing anti-neoplastic agents into the brain in treatment of brain tumors. The blood-brain barrier, following osmotic treatment, closes down more rapidly to larger than smaller intravascular molecules, and this size-dependency should be taken into account in designing associated therapy of central nervous system diseases. In the Rhesus monkey, we examined one of the potential side-effects of the method, namely damage to the ciliary epithelium of the eye. The damage results in transient hypotony and loss of pigmented epithelial cells, but ascorbate transport by the ciliary epithelium into aqueous humor is unaffected.

Metabolic and respiratory acidosis increases uptake of intravenous fluorescein by the retina of the rat eye at the blood-retina barrier (retinal pigment epithelium). Uptake is not mediated by damage to the barrier, but by a pH modification of the partition coefficient of fluorescein.

A model was developed which interpreted the relations in man between plasma and cerebrospinal fluid concentrations of blood proteins of differing size, in terms of diffusion and ultrafiltration at the blood-brain barrier.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel.

Y.Z. Ziylan	Visiting Fellow	LN	NIA
N.L. Shinowara	Senior Staff Fellow	EMS	NIA
W.R. Fredericks	Biologist	LN	NIA
D.E. Gasterland	Medical Officer	CB	NEI

Objectives: The blood-brain barrier at the cerebral vasculature regulates the ionic environment of the nervous system, has specific mechanisms for transport of amino acids and glucose that support cerebral metabolism and neurotransmitter and protein synthesis, and prevents access to the brain of water-soluble drugs and other agents. The aims of this project are as follows: (1) to describe the ultrastructure of the blood-brain barrier in relation to its permeability and physiologic functions in the normal and aging brain, (2) to determine the quantitative rules that govern drug entry into the brain, (3) to develop a method to reversibly modify barrier permeability, and to apply it to understanding the central actions of systemically-administered drugs, (4) to determine how the barrier regulates cerebral metabolism and behavior, and (5) to examine drug passage across the blood-retinal barrier.

Methods Employed: A number of different methods were employed in these studies. Surgical procedures were used to open the skull and dura of anesthetized animals, and to catheterize blood vessels for infusion and plasma sampling. Neurological examinations and behavioral tests evaluated overall brain function and localized deficits. Histological methods were used to examine brain or ocular pathology, and electron microscopy was employed to evaluate transfer mechanisms at the blood-brain barrier and barrier ultrastructure. Radioisotope and fluorescent microscopic techniques including autoradiography were used to quantitate transfer of tracers from blood to brain or blood to eye. Computers were used to analyze data and to generate relevant models.

Major Findings: Blood-Brain Barrier

1. Quantitative Aspects of Drug Entry into the Central Nervous System.

Central nervous system pharmacokinetics and cerebrovascular permeability. The blood-brain barrier limits exchange of drugs between plasma and brain, and makes it difficult to predict dose-response relations for centrally-acting drugs. We therefore elaborated a pharmacokinetic model to interpret brain-blood-cerebrospinal fluid exchange, and developed quantitative experimental methodology to determine exchange in the unanesthetized rat. A radiotracer drug is injected intravenously, arterial plasma radioactivity is determined periodically thereafter, until the animal is killed and brain radioactivity is measured. The cerebrovascular permeability-area product, PA sec^{-1}, is calculated as the ratio of brain concentration (after correction for intravascular radioactivity) to the plasma concentration integral. Experiments are limited to times in which back diffusion from brain to plasma is insignificant (Ohno, K., Pettigrew, K.D., and Rapoport, S.I.: Amer. J. Physiol. 253: H299-H307, 1978). The method is 100 times more sensitive than other available methods and was used to evaluate cerebrovascular integrity in rats exposed to microwaves (see below).

2. Reversible Modification of Blood-Brain Barrier Permeability.

a. Methods and mechanism. The blood-brain barrier at cerebral capillaries is due to a continuous layer of endothelial cells that are connected by tight junctions. We demonstrated that the barrier can be opened reversibly by infusing a hypertonic solution of a water-soluble solute (e.g. arabinose or mannitol) into the carotid artery of animals or man. We also showed, with electronmicroscopy and by theoretical models, that barrier opening probably is caused by

shrinkage of cerebrovascular endothelial cells and widening of interendothelial tight junctions. The method should prove useful as a tool for studying central actions of drugs that normally do not penetrate the blood-brain barrier.

b. Quantification, time course and mechanism. To further examine the mechanism by which hypertonic solutions reversibly modify blood-brain barrier permeability, we examined barrier permeability to tracers of different size, following carotid infusion of 1.8 molal arabinose solution in rats. The tracers which were employed were 14C-sucrose (mol. wt. = 340), 3H-dextran (mol. wt. = 79000) and 3H-inulin (mol. wt. = 5200). In control brains, PA for sucrose equaled 10^{-5} sec^{-1}, whereas no measurable permeability was found for inulin and dextran radiotracers. Six min after osmotic treatment, PA for the three tracers increased dramatically. Thirty-five min afterwards, PA's also were elevated, but at this time the ratio of PA for sucrose to PA for dextran was greater than the respective ratio at 5 min. These results indicate that the blood-brain barrier, after being osmotically opened, closes down more rapidly to larger than to smaller molecules. One of the major controversies in the literature is whether blood-brain barrier opening is mediated by increased vesicular transport across the cerebrovascular endothelium, or by widening of intercellular tight junctions. The above evidence of size differentiation during reclosure supports the tight junction mechanism, as vesicles are much larger than any of the three tracers and thus would not be selectively permeant to the smaller as compared to the larger molecules (Ref. 1).

c. Effect of osmotic barrier opening by mannitol on the blood-ocular barrier. We showed previously (Rapoport, S.I.: Exp. Eye Res. 25 (Suppl): 499, 1977) that osmotic barrier opening in the Rhesus monkey is accompanied by damage to the ciliary epithelium (loss of pigmented epithelial cell layer) and a reversible fall in ocular pressure. In the present study, we examined further the morphological and physiological changes in the eye of the Rhesus monkey following damage by hypertonic solution. Twenty-five percent mannitol solution was infused into the carotid circulation on 1-4 occasions, with at least 1 week between procedures. The animals were observed clinically for 3 to 212 days, and then were killed. Within an hour after treatment, pigmented epithelial cells and protein accumulated in the aqueous humor; hypotony developed within a day. The aqueous flare and cells cleared within 2 weeks, and the hypotony was resolved within 8 to 12 weeks. More than half the monkeys had transient anisocoria. Direct and consensual pupil responses to light remained intact in untreated eyes and in treated eyes with mydriasis. About one fourth of the monkeys developed edema of the optic disk, in relation to hypotony. No monkey developed cataract, corneal opacity, vitreous or retinal change or loss of gross visual acuity. The aqueous protein concentration was slightly high a month after carotid infusion, but still considerably less than plasma concentrations. Posterior and anterior aqueous ascorbate concentrations in treated eyes were slightly below normal, but far greater than plasma concentration, indicating ascorbate is transported by the ciliary nonpigmented epithelim (Ref. 2).

3. Acidosis and tracer penetration into eye.

We reported that fluorescein permeability was increased at the retinal pigment epithelium (RPE), part of the blood-ocular barrier, in hypercapnic rats (Rapoport et al., Exp. Eye Res. 30, 129-141, 1980), but did not know the cause for this increase. We therefore examined permeability of the RPE to fluorescein,

carboxyfluorescein, Evans blue and horseradish peroxidase in rats breathing air (control) or 25% (v/v) CO_2. Arterial blood pH decreased from 7.40 in control rats to 7.24 in hypercapnic rats. Tracers were injected i.v. and allowed to circulate for 1 to 20 min before enucleation. Tissue was fixed or rapidly frozen for fluorescence microscopy. Intravascular carboxyfluorescein and horseradish peroxidase did not cross the RPE during hypercapnia, and interepithelial tight junctions remained functionally intact. However, fluorescein entered the neural retina in hypercapnic acidotic but not normocapnic rats. Fluorescein also entered the eye following NH_4Cl-induced metabolic acidosis, when $PaCO_2$ was not elevated. The results demonstrate a specific blood pH effect on ocular permeability of fluorescein but not of other tracers, and indicate that the lipid/water partition coefficient of fluorescein is increased by blood acidosis. The blood-retinal barrier remains physically intact to water-soluble agents and proteins during acidosis. An abstract of this work has been published (Shinowara, N.L., Grimes, P.A., Rapoport, S.I., Laties, A.M.: Acidosis alters fluorescein permeability across the pigment epithelium. Annual Meeting for Research in Vision and Ophthalmology Abstracts, May 2-6, 1983, Sarasota Florida).

4. Factors contributing to protein entry into cerebrospinal fluid.

The blood brain barrier restricts but does not absolutely prevent access of blood proteins to cerebrospinal fluid (CSF). Consequently, CSF protein concentrations normally are 0.5% or less than the respective plasma or serum concentrations. Furthermore CSF blood protein ratios in man, under steady-state conditions, are related inversely to protein molecular radius. These ratios were analyzed according to the pore-diffusion model of Rapoport (Microvasc. Res. 18, 105-119, 1979). It was suggested that protein enrichment along the neuraxis is related to compartmental differences in CSF flow and washout rate. The effect of a reduced flow is most evident below a complete spinal block, when protein accumulates in lumbar CSF. About 60% to 80% of blood proteins within CSF probably enter at the choroid plexus, and the remainder enter at nonchoroidal barrier sites along the neuraxis. The mechanisms that govern protein entry are selective according to hydrodynamic radius. The observed negative relation between the CSF/blood concentation ratio and radius can be accounted for if proteins enter CSF by diffusion or ultrafiltration through aqueous pores with a radius of about 117 Å, as well as by vesicular transport. Pores may represent a defect in the normally continuous tight junctions that surround and closely connect choroidal epithelial cells (Ref. 3).

Significance to Biomedical Research and to the Program of the Institute:

1. An understanding of how the blood-brain barrier regulates the brain environment and controls brain metabolism is critical for interpreting brain function in health, aging and disease. Our findings establish the ultrastructural and transport properties of the blood-brain barrier.

2. Our methods in conscious rats, together with pharmacokinetic theories that we are developing, make it possible to measure cerebrovascular permeability of drugs as a function of their physical properties, and to measure transfer constants between blood and brain compartments. It should be possible, on the basis of the findings, to predict and interpret dose-response relations of centrally-acting drugs in man and animals.

3. Reversible osmotic opening of the blood-brain barrier is the first and only method that allows normally excluded agents into the brain without producing long-term brain damage. Now that the method has been shown to be useful in man, it probably will be employed more generally for treatment of brain tumors, for enzyme replacement therapy and for augmenting drug entry into the brain in cerebral infections. The human findings set the stage for serious clinical trials with given end points for effective therapy.

4. Understanding the basis of the ocular damage which is potentially associated with the osmotic procedure is critical for decisions relating to its clinical use. The maintenence of ascorbate transport with a partially intact non-pigmented epithelium provides evidence for the localization, in the ciliary epithelium, of the transport mechanism.

5. The blood-retinal barrier influences retinal function, and often is examined in man with i.v. fluorescein. Our findings of a pH-dependent penetration of intravenous fluorescein, independent of barrier damage, are critical for evaluating clinical results with this tracer.

6. Our analysis provides a rational interpretation of CSF/plasma protein concentrations in relation to molecular size in health and disease.

Proposed Course: The pharmacokinetic rules that govern drug transfer from plasma to brain compartments will be elaborated, and models will be developed and tested to interpret dose-response relations of centrally-acting drugs in animals and man. Further application of the osmotic method will be made in human and animal studies, for its eventual use as a pharmacological tool. Additional ultrastructural studies will be performed to define the basis of increased cerebrovascular permeability following osmotic treatment.

Publications:

1. Ziylan, Y.Z., Robinson, P.J., and Rapoport, S.I.: Differential blood-brain barrier permeabilities to 14C-sucrose and 3H-inulin after osmotic opening in the rat. Exp. Neurol. 79: 845-857, 1983.

2. Gaasterland, D.E., Barranger, J.A., Rapoport, S.I., Girton, M.E. and Doppman, J.L.: Longer-term ocular effects of osmotic modification of the blood-brain barrier in monkeys. Invest. Ophthalmol. Vis. Sci. 21: 153-158, 1983.

3. Rapoport, S.I.: Passage of proteins from blood to cerebrospinal fluid. A model for transfer by pores and vesicles. In: Wood, J.H. (ed): Neurobiology of Cerebrospinal Fluid, Vol 2. Plenum Press, New York, 1982. pp. 233-246.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00129-03 LN

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Transport Systems at the Blood-Brain Barrier (H)

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Q. R. Smith Staff Fellow LN NIA
Y. Takasato Visiting Fellow LN NIA and C. -Y. Tai Staff Fellow LN NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION
Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	0

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

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

Transport mechanisms at the blood-brain barrier were studied in the rat. An in vivo brain perfusion technique was developed to examine carrier-mediated transport at the cerebral capillary endothelium. Brain perfusion with Ringer's solution or with blood did not alter cerebrovascular permeability to sucrose. Barrier permeability to nonelectrolytes was linearly related to lipid solubility. Large neutral amino acids cross the blood-brain barrier by facilitated diffusion. The cerebrovascular permeability to inorganic ions was low, comparable to a cell membrane, and followed the sequence K > Mg > Na > Cl > Ca. Chloride transport across the blood-brain barrier was by a saturable, carrier-mediated system, which was inhibited by other monovalent anions. The low permeability of the cerebrovascular endothelium to Na was maintained with age in the rat, whereas the cerebrospinal fluid transfer constant for Na fell by 18%.

PHS 6040 (Rev. 1/83) GPO 895-100

Other professional personnel.

Mark Monteferrante	Biological Aid	LN NIA

Project Description:

Objective: Neuronal function depends on the supply of essential nutrients, removal of metabolic wastes, and maintenance of a stable ionic environment. The brain capillaries, part of the blood-brain barrier, represent the major restriction to solute exchange between plasma and brain interstitial fluid, and are thought to possess facilitated and active transport systems to regulate the extracellular environment of brain cells. In addition, transport systems at the choroid plexus regulate the composition and production of cerebrospinal fluid (CSF). The aims of this project are: (1) to develop methods to study active, facilitated, and passive transport of solutes across the blood-brain barrier, (2) to determine how the transport systems regulate the composition of the brain interstitial fluid and influence brain function, (3) to examine the role of CSF in solute uptake and distribution in the central nervous system, and (4) to study functional changes in transport systems of the blood-brain barrier in relation to aging.

Methods Employed:

1. Brain perfusion technique. The right cerebral hemisphere of anesthetized rats was perfused for short periods of time by retrograde infusion of Ringer's solution or of blood into the external carotid artery, at a rate adjusted to wash out blood from the right cerebral hemisphere. Each perfusate contained a test 14C-radiotracer, and 3H-inulin to measure intravascular volume. After decapitation, samples from 6 brain regions and of infusion fluid were analyzed. Capillary fluid flow during perfusion was determined with 14C-iodoantipyrine or 14C-diazepam. The cerebrovascular permeability-area product (PA) was calculated as follows:

$$PA = -F.\ln(1 - C^*_{br} / F.C^*_{pf}.T)$$

where F = capillary fluid flow, C^*_{br} = tracer concentration in brain parenchyma, C^*_{pf} = tracer concentration in perfusion fluid, and T = perfusion time.

Carrier-mediated transport of large neutral amino acids was studied by measuring PA of an amino acid as a function of the perfusate concentration in the absence of competing amino acids. A Vmax and Km for saturable uptake, and a Kd for nonsaturable uptake, were calculated from nonlinear regression analysis of the PA data:

$$PA = Vmax/(Km + C_{pf}) + Kd$$

where C_{pf} = net amino acid concentration in perfusate.

2. Ion uptake into the central nervous system. Blood to brain transfer constants for Na, K, Ca, Mg and Cl were measured in adult rats. Radiotracers (^{22}Na, ^{42}K, ^{45}Ca, ^{28}Mg, and ^{36}Cl, 20-100 μCi/kg) were injected i.v., and timed arterial blood samples were collected until the animal was killed. Samples from 12 brain regions, from cisternal CSF, and from plasma were collected and analyzed for radioactivity. Transfer constants were calculated as,

$$k = C^*_{br \text{ or } csf} / \int C^*_{plasma} dt$$

where $C^*_{br\ or\ csf}$ = tracer concentration of brain parenchyma or CSF, and

C^*_{plasma} = tracer concentration of plasma corrected for plasma protein binding.

Plasma chloride concentration, [Cl], of pentobarbital-anesthetized rats was altered acutely from 114 to 16 mM by peritoneal dialysis with artificial interstitial fluid in which Cl was substituted by one of four replacement anions. Bilateral nephrectomy was utilized to maintain a constant plasma [Cl].

Major Findings:

A. Brain perfusion technique.

1. This technique allowed the quantitative measurement of brain uptake of solutes with PA greater than or equal to that of sucrose (PA = 5×10^{-1} sec^{-1}). Specific perfusate solute concentrations could be controlled for the study of facilitated or active transport. The method avoided errors due to radiotracer biotransformation by tissues other than the brain (e.g., liver). Perfusion for 1 min with Ringer's solution, or for 5 min with blood, did not alter the PA to sucrose. Lastly, less than 5% of perfusate in the right hemisphere was contaminated by blood. An abstract of this work has been published (Takasato, Y., Smith, Q.R. and Rapoport, S.I.: A new method to determine cerebrovascular permeability in the anesthetized rat. Society Neuroscience Abstracts, 8: 850, 1982).

2. Cerebrovascular permeability and solute lipid solubility. The cerebrovascular P to 22 nonelectrolytes was measured to determine which solute properties determine brain uptake across the blood-brain barrier. In the parietal cortex, P ranged 10^4 fold from a low of 2.2×10^{-8} cm/sec for sucrose to 6.1×10^{-4} cm/sec for iodoantipyrine. P was related linearly to the solute octanol/water partition coefficient (a measure of lipid solubility). Solute molecular weights ranged from 18 to 609 Daltons. The linear relation between permeability and solute lipid solubility is consistent with simple diffusion through an aporous lipid membrane at the blood-brain barrier.

3. Facilitated transport of large neutral amino acids. Concentration-dependent uptakes of tryptophan, leucine, isoleucine and cycloleucine were measured in anesthetized rats with the in vivo brain perfusion technique. In the parietal cortex, cerebrovascular PA for each amino acid decreased approximately 100 fold when the perfusate concentration was elevated from 0 to 20 μmol/ml. Calculated Vmax ranged from $0.93 \pm 0.07 \times 10^{-3}$ μmol/sec/g for cycloleucine to $1.05 \pm 0.08 \times 10^{-3}$ μmol/sec/g for isoleucine. In contrast to this relative constancy, Km (in μmol/ml) was 0.010 ± 0.002 for tryptophan, 0.024 ± 0.003 for leucine, 0.049 ± 0.007 for isoleucine and 0.0272 ± 0.037 for cycloleucine. A non-saturable component of uptake was not detected for isoleucine or cycloleucine. For tryptophan and leucine, Kd was significantly greater than zero and approximated PA for passive diffusion, as predicted by the octanol/water partition coefficient (see above). Significant differences in Vmax, Km or Kd were not observed among 6 brain regions. These findings show that large neutral amino acids cross the blood-brain barrier by carrier-mediated transport and

that the affinity for the amino acids (1/Km) is greater than previously reported. An abstract of this work has been published (Takasato, Y., Smith, Q.R., and Rapoport, S.I.: Kinetics of large neutral amino acid transport across the blood-brain barrier. Society Neuroscience Abstracts, 9, 1983).

B. Ion uptake into the central nervous system.

1. Regional brain transfer constants. In pentobarbital-anesthetized rats, transfer constants (k) for ^{22}Na, ^{45}Ca and ^{36}Cl ranged 3-fold, with minimal values in the frontal-parietal cortex. In contrast, there was less than 2 fold regional variation in the k for ^{42}K or ^{28}Mg. Regional k's for Na, Ca, and Cl correlated with distance from sites of CSF formation. Initial isotope uptake by cisternal CSF was 10-30 times greater than by brain tissue, so that a concentration gradient existed from spinal fluid to brain. Acetazolamide-induced inhibition of ^{36}Cl entry into spinal fluid did not alter the cerebral cortex transfer constant for ^{36}Cl, although periventricular tissue k fell by up to 50%. The transfer constant at the frontal cerebral cortex reflects ion movement primarily across the brain capillary endothelium, with minimal contribution from the CSF. The cerebrovascular permeability for influx was 50.0×10^{-8} cm/sec for K, 16.7×10^{-8} cm/sec for Mg, 8.3×10^{-8} cm/sec for Na, 5.0×10^{-8} cm/sec for Cl and 2.0×10^{-8} cm/sec for Ca. The low permeability of the cerebrovascular endothelium to ions is comparable to permeability in tight epithelia such as frog skin and toad bladder, and suggests that ion movement across brain capillaries is predominantly transcellular. An abstract of this work has been published (Smith, Q.R., Tai, C. -Y. and Rapoport, S.I.: Permeability of the blood-brain barrier to inorganic ions. Society Neuroscience Abstracts, 9, 1983).

2. Carrier-mediated transport of chloride across the blood-brain barrier. To determine the mechanism of Cl transport into the brain, the cerebrovascular transfer constant to Cl was examined as a function of plasma [Cl] when plasma Cl was replaced by isethionate in pentobarbital-anesthetized rats. In general, k to Cl decreased 2 fold as plasma [Cl] increased from 16 to 114 μmol/ml. The concentration dependence could be described by Michaelis-Menten kinetics. A Km of 43 $\pm$ 6 μmol/ml and a Vmax of $2.5 \pm 0.2 \times 10^{-3}$ μmol/sec/g were obtained for the parietal cerebral cortex. Monovalent anions competitively inhibited Cl movement across cerebral capillaries. The sequence of the permeability decrease was $CH_3SO_4 < Cl < Br < NO_3$. Although uptake of Cl by cerebrospinal fluid was 10 fold greater than by the cerebral cortex, the dependence on plasma [Cl] and the sequence of anion inhibition of cerebrospinal fluid uptake were similar to those of brain tissue. Thus, the concentration dependence of k and the reduction of k by monovalent anions are consistent with carrier-mediated transport of Cl across the blood-brain barrier. An abstract of the work has been published (Smith, Q.R., Takasato, Y., and Rapoport, S.I.: Carrier-mediated transport of chloride across the blood-brain barrier. Journal Cerebral Blood Flow and Metabolism, 3: S411-412, 1983).

3. Cerebrospinal fluid formation and capillary integrity in aging rats. The CSF transfer constant (k) for ^{22}Na reflects influx of Na mainly at the choroid plexus, and did not change significantly between 3 and 12 months of age in conscious Fischer-344 rats. Between 12 and 24 months, and between 12 and 34 months of age, the CSF k for Na fell by 6% and 18%, respectively ($p < 0.05$),

suggesting either a decline in the CSF formation rate or an increased CSF volume. The frontal cortex k for Na, 2.0×10^{-5} sec^{-1}, reflecting primarily flux of Na across the brain capillary endothelium, did not change significantly between 3 and 34 months of age. Thus, the low permeability of the cerebrovascular endothelium to Na is maintained with age. An abstract of this work was published (Smith, Q.R. and Rapoport, S.I.: Age-associated decrease in the rate of cerebrospinal fluid uptake of Na in the Fischer-344 rat. Society Neuroscience Abstracts, 8: 443, 1982).

Significance to Biomedical Research and the Program of the Institute:

1. The brain requires a balanced and continuous supply of essential amino acids to sustain protein synthesis and to provide substrates for the formation of small polypeptides and certain neurotransmitters, such as serotinin and the catecholamines. The availability of amino acids to cerebral cells is determined by the blood-brain barrier. Our studies with the brain perfusion technique are consistent with carrier-mediated transport of large neutral amino acids across the barrier, and indicate that the affinity of the transport system is 5-20 fold greater than previously thought.

2. Neuronal function is sensitive to the ionic composition of brain interstitial fluid. However, little is known about how the blood-brain barrier regulates the ionic composition of the interstitial fluid. Our observations demonstrate directly, for the first time, that the blood-brain barrier regulates brain chloride composition by carrier-mediated transport.

3. The cerebrospinal fluid can remove water-soluble drugs and metabolites from brain, and thus regulates the brain extracellular environment. Our finding that the cerebrospinal fluid transfer constant for Na falls by less than 20% during aging of the rat suggests that the spinal fluid secretion rate is maintained during aging of rats.

Proposed Course: Data will be analyzed and prepared for publication. Further work will be performed on carrier-mediated transport at the barrier.

Publications: None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG00128-03 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Drug Pharmacokinetics, Relation to Pharmacodynamics and Senescence

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)		
S.I. Rapoport	Chief	LN NIA
P. Robinson	Visiting Fellow	LN NIA

COOPERATING UNITS *(if any)*

Preclinical Pharmacology, Epilepsy Branch, NINCDS

LAB/BRANCH

Gerontology Research Center, Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	.7	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Effects of age on pharmacokinetics of a central nervous system drug were studied. Biodisposition of phenobarbital was examined in male, Fischer-344 rats of four different age groups, 3-4, 11-12, 23-24, and 32-34 months. The drug was administered intraperitoneally, either as a bolus or continuously from implanted osmotic minipumps. Plasma and brain concentrations were determined after extraction by gas chromatographic analysis with a nitrogen selective detector. Significantly higher plasma and brain concentrations were found in the older as compared to younger rats, due to a decreased apparent plasma clearance. Plasma concentrations were not directly predictive of brain concentrations, because the brain/plasma concentration ratio of the drug did not remain constant during aging. These studies are a first step to understanding of altered clinical responsivity to drugs in the elderly.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel:

R. Espinosa-Leniz	Expert	LN	NIA
D.J. Sweeney	Chemist	LN	NIA
J. Schreiber	Chemist	LN	NIA
I.M. Kapetanovic	Pharmacologist	EB	NINCDS

Project Description:

Objectives. Phenobarbital, a weak acid, is an antiepileptic and sedative which often is used by the elderly, but frequently produces side effects. It is not bound tightly to protein, but accumulates in the brain. In the body, it is metabolized by p-hydroxylation in the liver. The objective of this study is to examine the biodisposition of phenobarbital in rats of different ages, following acute or chronic administration.

More general objectives are (1) to examine age-related pharmacokinetics for centrally-acting drugs which have been reported to elicit atypical or toxic responses in elderly man. Initially, drugs without active metabolites will be examined to simplify the study of pharmacokinetic-pharmacodynamic relations, (2) to describe pharmacokinetic-pharmacodynamic relations for drugs in relation to aging, (3) to study the effects of perturbation of specific pharmacokinetic factors on drug pharmacokinetics and dynamics.

Methods Employed:

1. Experimental animals. Awake male Fischer-344 rats of different ages (3-4, 11-12, 23-24 and 32-34 months) were used. This strain, which has a mean life-span of 29 months and a maximum life-span of 35 months, is commonly used in aging research and is free of major brain pathology.

2. Drug administration. Phenobarbital was administered intraperitoneally as a bolus injection (20 mg/kg), or by continuous infusion (4.73 mg/day) with osmotic minipumps which were implanted under ether anesthesia.

3. Sample collection. Blood and brain samples were collected following decapitation. Plasma was obtained from centrifuged blood, and brain regions were dissected out immediately, on an ice-chilled metal plate, by standard techniques.

4. Determination of drug concentrations in plasma and brain samples. Tissue samples were homogenized and extracted with appropriate solvent systems. Drug concentrations were determined by gas chromatography using a nitrogen selective detector. Phenobarbital was propylated prior to analysis. For each analysis, a standard curve which bracketed the range of experimental concentrations was generated and the internal standard method was used for quantitation.

5. Data analysis. The apparent first order elimination rate constant, β, was determined from linear least-squares fitting of the terminal portions of semilogarithmic plots of drug concentration against time (t). The apparent elimination half-life $t_{\frac{1}{2}}$, was calculated as

$$t_{\frac{1}{2}} = \frac{0.693}{\beta} \qquad \text{Eq. 1}$$

The area under the curve (AUC) from time zero to infinity, was calculated using the trapezoidal rule and extrapolated to infinity by adding to it the value for the ratio between the last measured drug concentration and β.

Because intraperitoneal administration makes uncertain the fraction of the dose, F, that enters the systemic circulation, hybrid parameters Cl/F and Vd/F were determined rather than clearance, Cl, and the apparent volume of distribution, Vd, as follows,

For bolus:

$$Cl/F = \frac{dose}{AUC} \qquad \text{Eq. 2}$$

$$Vd/F = \frac{dose}{AUC \times \beta} \qquad \text{Eq. 3}$$

For continuous infusion at steady state:

$$Cl = \frac{\text{rate of drug administration}}{\text{mean steady-state plasma concentration}} \qquad \text{Eq. 4}$$

Major Findings: Significantly higher plasma and brain concentrations of phenobarbital were found in older rats following either a bolus injection of drug or during continuous intraperitoneal administration to reach a steady-state. Following a bolus injection, the apparent plasma elimination half-life $t_{\frac{1}{2}}$ (Eq. 1) was longer, and Cl/F (Eq. 2) was lower in 32-34 month than in 3-4 month old rats. Vd/F was higher in 32-34 than in 3-4 month old animals. During continuous administration, a progressive decrease in Cl/F (when normalized to body weight) was observed from 3-4 months to 23-24 months for phenobarbital. A higher Cl/F was observed in 3-4 month and 32-34 month old rats during continuous administration than following a bolus, suggesting autoinduction of metabolism. There were no age-related concentration differences in different brain regions following a bolus or during continuous administration of phenobarbital. Higher brain/plasma concentration ratios were observed in 32-34 month old rats than in some other age groups. In summary, a decrease in the apparent plasma Cl/F led to higher drug concentrations in plasma and brain, and appeared to be the predominant age-related pharmacokinetic alteration for phenobarbital. Furthermore, a higher brain/plasma concentration ratio in 32-34 month-old animals indicated that age also influenced drug distribution. Such age-related pharmacokinetic alterations may contribute to altered drug responsivity in elderly man (Ref 1.).

Significance to Biomedical Research and the Program at the Institute: Due to a lack of adequate understanding of pharmacokinetic-pharmacodynamic relations for many centrally-acting drugs, therapy in man has been largely empirical, often producing unpredicted responses. Drug toxicity in the elderly is a significant cause of iatrogenic dementia, and interferes with adequate medical treatment of the geriatric population. These studies are a first step in understanding mechanisms of toxicity, and should help to design a rational therapeutic approach for the elderly.

Proposed course: To continue to examine drug pharmacokinetics in relation to age in animal models. To develop a rational theory of pharmacokinetics and pharmacodynamics.

Publications:

1. Kapetanovic, I.M., Sweeney, D.J., and Rapoport, S.I.: Phenobarbital pharmacokinetics in rat as a function of age. Drug Metabolism and Disposition 6: 586-589, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00122-06 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Pharmacology of Central and Peripheral Catecholaminergic Nervous Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

S.I. Rapoport	Chief	LN	NIA
P. Helen	Visiting Fellow	LN	NIA

COOPERATING UNITS *(if any)*

University of Tampere, Finland
Dept of Neurosurgery, Tokyo Medical and Dental University, Tokyo, Japan

LAB/BRANCH

Gerontology Research Center, Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Immobilization stress of awake spontaneously hypertensive rats (SHR) dramatically increases circulating catecholamines, but does not significantly elevate regional cerebral blood flow or damage cerebrovascular integrity. Autoregulation of cerebral blood flow must be sufficient during stress to prevent damage to the blood-brain barrier, and prevent significant brain uptake of catecholamines.

Glucose utilization (GU), a measure of functional neuronal activity, was measured with 2-deoxy-D-glucose in sympathetic ganglia of Fischer-344 rats, and was found to increase between 12 and 24 months of age in the superior cervical ganglion. The increase may reflect a compensatory response to reduced β-receptor function in the elderly rat, and be related to increased concentrations of circulating catecholamines.

Increased circulating catecholamines in the senescent rat may be released by paraganglia, which are extra-adrenal chromaffin tissue and proliferate between 24 and 33 months of age. It was demonstrated that they contain high concentrations of catecholamines.

On the other hand, catecholamine fluorescence in single neuronal parikarya were shown to decrease with age in brain catecholamine-containing neurons (A_{12} neurons which regulate pituitary hormones), as well as in some peripheral sympathetic ganglia (hypogastric ganglion).

PHS 6040 cont'd

Other Professional personnel.

A. Hervonen	Professor of Gerontology	Univ of Tampere
W.R. Fredericks	Biologist	LN NIA
M. Partanen	Visiting Fellow	LN NIA
M. Ohata	Visiting Fellow	Tokyo Medical & Dental Univ
H. Takei	Visiting Fellow	Tokyo Medical & Dental Univ

Project Description:

Objectives:

The objectives of this project are to (1) examine the effect of immobilization stress, in unanesthetized spontaneously hypertensive rats (SHR), on regional cerebral blood flow and cerebrovascular integrity, (2) to measure glucose utilization (GU) as an indicator of functional activity, in sympathetic ganglia of rats in relation to age, (3) to examine the morphology and role of paraganglia in relation to age in rats, and (4) to measure catecholamine histofluorescence in individual neurons in peripheral sympathetic ganglia of the rat, as well as in specific brain regions in relation to senescence.

Methods Employed:

1. Regional cerebral blood flow in unrestrained awake spontaneously hypertensive rats (SHR) was examined with the use of 14C-iodoantipyrine, by the method of Ohno, Pettigrew and Rapoport (Stroke 10: 62-67, 1979). Radiotracer was infused intravenously until the animal was decapitated at 45 sec. Regional brain radioactivity and whole blood radioactivity were determined by scintillation spectroscopy.

2. The method of formaldehyde-induced fluorescence (Erlänkö, 1967) was used to demonstrate catecholamines at a cellular level. It is based on the reaction of paraformaldehyde with catecholamines, in which fluorescent isoquinoline derivatives are formed. The method involves (1) freezing of tissue samples, (2) freeze-drying, (3) paraformaldehyde treatment and (4) vacuum embedding into paraffin.

Quantitative microspectrofluorimetry. The intensity of formaldehyde-induced fluorescence is linear to catecholamine-concentration. For recordings of fluorescence, a MPV-2 Leitz microscope equipped with a photometer was used. The measuring spot was 10 µm and allowed intracytoplasmic measurements from individual neurons. Fading was prevented by an iris diaphragm on the excitation side.

For electronmicroscopy, the body was rapidly perfused with 3% glutaraldehyde in phosphate buffer (pH 7.3; 0.1 mol). Samples were embedded in EPON, sectioned and studied with an electron microscope.

Major Findings:

1. Effect of immobilization stress on regional cerebral blood flow in the conscious SHR rat. Immobilization of unanesthetized, freely breathing 10-12 month old spontaneously hypertensive rats (SHR) did not significantly alter regional cerebral blood flow (rCBF) in 13 of 14 brain regions assayed. After 5 or 15 min of immobilization, rCBF was unchanged except at the frontal lobe, where it rose significantly by 21%. Furthermore, immobilization did not increase the cerebrovascular permeability-area product for 14C-sucrose, except minimally at three brain regions. The results suggest that adequate autoregulation of rCBF is maintained when large quantities of catecholamines are released into the circulation during immobilization-induced stress. The results do not confirm the findings of a marked stress effect on cerebral blood flow, as reported by Carlsson et al. (1976) (Ref. 1).

2. Paraganglia and Age. Paraganglia (PG) are located close to sympathetic ganglia. Their cells are classified as extra-adrenal chromaffin tissue, and contain catecholamines in high concentrations. Their physiological significance is uncertain. In the rat, extra-adrenal chromaffin tissue is abundant during fetal development, but degenerates postnatally. In the young adult rat, only a few small PG are found. Because the PG are a potential source of circulating catecholamines, the finding that plasma catecholamines are elevated in unstressed aged Fischer-344 rats (Chiueh, Nespor and Rapoport, Neurobiol. Aging, 1: 157-163, 1980) led us to systematically study PG in peritoneal tissues of young and old Fischer-344 rats.

For identification of PG, samples of para-aortic tissue and of tissue containing the coeliac-mesenteric ganglion complex and the hypogastric ganglion were removed from 3- and 33-month old male Fischer-344 rats, and were processed by the formaldehyde-induced fluorescence method for visualization of catecholamines. Small PG, containing 5-30 cells per section, were found consistently in young animals. In each of 6 old animals, however, large PG containing 500-4000 brightly fluorescent cells per section were detected. Cell counting revealed a 13.5x increase in the number of PG cells between 3 and 33 months of age. Microspectrofluorimetric quantitation in the old rats showed equal amounts of catecholamines in PG cells and in adrenal medullary cells. Most PG were located in the para-aortic area (Ref. 2).

3. Glucose utilization (GU) of the sympathetic nervous system and aging. GU, as a measure of functional activity, was determined in the Fischer-344 rat in the superior cervical ganglion, the hypogastric ganglion and the coeliac-mesenteric ganglion complex at 3, 12, 24 and 32-34 months of age. GU increased significantly ($p < 0.05$) in the superior cervical ganglion between 12 and 24 months, concurrent with an increase in the circulating concentration of plasma norepinephrine in unstressed rats. Because heart rate and systolic blood pressure declined after 12 months of age, the results suggest that there is increased functional activity, related to norepinephrine release, in the superior cervical ganglion with aging, as possible compensation for decreased responsivity of peripheral receptors to circulating catecholamines (Ref. 3).

4. Catecholamine neurons in peripheral and in central nervous system and aging. Catecholamine-histofluorescence was mapped in 3, 12, 24 and 32 month-old Fischer-344 rats in peripheral sympathetic ganglia, and in the brain in epinephrine-containing neurons (A1-2), in norepinephrine neurons (A6-7) and in dopamine containing neurons (A_{8-10}). In the brain, the only significant age change was a decreased catecholamine fluorescence in A_{12} neurons, which regulate pituitary hormones. This decrease occurred between 24 and 34 months, and may be related to hormonal patterns during aging. In peripheral sympathetic ganglia, catecholamine-fluorescence decreased significantly in the hypogastric ganglion. The changes might be related to changes in the target organ, in the neurons themselves or in input to the ganglia (Ref. 2).

Significance to Biomedical Research and the Program at the Institute.

1. Increased sympathetic activity with age in the unstressed rat, as measured by increased plasma catecholamines and reappearance of the paraganglia, may be related to decreased responsivity of the cardiovascular and other systems to catecholamines. Our studies suggest a feed-back between end organ effect and sympathetic activity that is age-dependent. Because many of the changes in rats are similar to those in man (plasma catecholamines increase in man with age, and paraganglia reappear in human peritoneal tissue), the rat may be a good model for studying regulation of the cardiovascular system by the sympathetic nervous system in aged man.

2. Studies in animals and man indicated that stress modifies cerebral function, possibly by promoting entry or circulating catecholamines into the brain through a damaged blood-brain barrier. We examined spontaneously hypertensive rats in which stress induces an exceedingly high level of plasma catecholamines, and found that regional cerebral blood flow was generally unaffected, as was blood-brain barrier integrity. An intact blood-brain barrier to increased levels of circulating catecholamines during stress may help to maintain cerebral homeostasis and function.

Proposed Course: To continue to examine sympathetic ganglia and paraganglia during aging; to study catecholamine turnover by combined drug treatment and quantitative microspectrofluorimetry in sympathetic ganglia; and to study regulation of the growth of paraganglia in old animals.

Publications:

1. Ohata, M., Takei, H., Fredericks, W.R., and Rapoport, S.I.: Effects of immobilization stress on cerebral blood flow and cerebrovascular permeability in spontaneously hypertensive rats. J. Cerebral Blood Flow and Metabolism, 2: 373-379, 1982.

2. Partanen, M., Hervonen, A., and Rapoport, S.I.: Microspectrofluorometric quantitation of histochemically demonstrable catecholamines in peripheral and brain catecholamine-containing neurons in male Fischer-344 rats at different ages. In: Giacobini, E., Filogamo, G., Giacobini, G., and Vernadakis, A. (Eds.): The Aging Brain: Cellular and Molecular Mechanisms of Aging in the Nervous System, Aging, Vol. 20. New York, Raven Press, 1982, pp. 161-171.

3. Partanen, M., London, E.D., and Rapoport, S.I.: Glucose utilization in sympathetic ganglia of male Fischer-344 rats at different ages. J. Autonomic Nervous System. 5: 391-398, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00127-03 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Assessment of Neurochemical Markers in Relation to Age, Behavior, and Dementia

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Steven B. Waller Staff Fellow LN NIA

COOPERATING UNITS *(If any)*

Laboratory of Behavioral Sciences, NIA
National Institute on Drug Abuse
Dept. Pathology, University Western Ontario, Canada

LAB/BRANCH

Gerontology Research Center, Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

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

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

Age effects on neurochemical markers and the correlation between brain neurochemical markers and motor performance were tested in C57BL/6J and A/J mice. Motor performance was altered by age, and age effects varied with genetic strain. Age-associated decrements in performance were correlated with regional brain changes in cholinergic, GABAergic, and catecholaminergic neurochemical markers. Activities of choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD), and tyrosine hydroxylase (TH) were assayed in four brain regions after animals were evaluated in a battery of psychomotor tests. Significant correlations between enzyme activity and motor performance occurred most frequently in the youngest (4-mo) and oldest (24-mo) groups. Examination of age effects on the cholinergic markers, CAT activity and muscarinic binding in C57BL/6J mice revealed regional increases in CAT activity that were associated with increases in Vmax, and no changes in Km. These increases in Vmax for CAT were associated with decreases in the densities of muscarinic binding sites in the cortex and striatum and increases in the densities of muscarinic binding sites in the hippocampus. There were no age changes in the affinities of muscarinic ligands or the percent of high affinity muscarinic binding sites in any region.

A study of CAT and muscarinic binding in regions of brains of normal men and of patients who died of Alzheimer's disease revealed decreases in CAT activity that were associated with increases in total muscarinic binding. There were no differences in the percentage of total muscarinic receptor binding associated with the high affinity binding sites between control and Alzheimer's diseased tissues. These findings suggest that a presynaptic cholinergic defect in Alzheimer's disease is associated with muscarinic receptor upregulation.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel.

Milad Bitar	Staff Fellow	LN NIA
Donald K. Ingram	Staff Fellow	LBS NIA
Edythe D. London	Pharmacologist	NIDA
Melvyn J. Ball	Neuropathologist	University of Western Ontario

Project Description

Objectives: Neurochemical sequelae of aging have been described in various species. The literature presents inconsistencies suggesting that age effects are species-specific and perhaps even strain-specific. Furthermore, the functional significance of these changes has not been adequately explored. The objectives of this project are: 1) to assess brain neurochemical markers in relation to age, 2) to test correlations between neurochemical markers and behavior, and 3) to determine if age effects on neurochemical markers are related to strain.

There is substantial evidence for a cholinergic neuronal system abnormality in Alzheimer's disease. Neurochemical marker studies have consistently revealed a reduction of choline acetyltransferase (CAT), a presynaptic cholinergic element. In contrast, studies of postsynaptic cholinergic elements, i.e. muscarinic binding sites, have been inconsistent. In addition, there is increasing evidence of multiple muscarinic binding sites. The purpose of this project was to assess CAT activity, total muscarinic binding, and total muscarinic binding associated with agonist high affinity binding sites in regions of neocortex and hippocampus of normal and Alzheimer's diseased brains.

Methods Employed:

1. General methods. a. Animal studies. Mice of two genetically inbred strains (C57BL/6J and A/J) of various ages were given psychomotor tests. The mice were killed, and brains were removed and dissected. Neurochemical markers were assayed in brain regions which were frozen and stored at -70°C until the time of assay. Correlational analyses were conducted between neurochemical markers and psychomotor performance.

b. Human studies. Postmortem brain samples were taken from 17 people who died with Alzheimer's disease (AD) and 11 control subjects. The following regions were sampled: superior, middle, and inferior temporal gyrus; orbital frontal cortex; middle frontal gyrus; pre-and postcentral gyrus; parietal cortex; calcarine cortex; mammillary body; stria terminalis; hippocampal endplate cortex, H_2, and H_1-subiculum; presubiculum; entorhinal cortex; amygdala; and cingulate gyrus. The specific activity of CAT and estimates of total muscarinic binding site concentration (Bmax) and the percentage of total muscarinic binding sites associated with the agonist high affinity binding site were determined.

2. Specific methods.

a. Psychomotor tests in C57BL/6J and A/J mice. Grip strength was tested as were performances on a balance beam and rotorod, free exploration, forced exploration, and wheel activity.

b. Neurochemical markers. The activities of the neurotransmitter synthetic enzymes CAT, glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH) were assayed in various brain regions. These enzymes reflect the integrity of presynaptic cholinergic, GABAergic, and catecholaminergic neurons, respectively. Brain samples were sonicated in 20 volumes (w/v) of 0.05 M Tris-HCl, pH 7.4, containing 0.2% (v/v) Triton X-100, and were centrifuged at 4°C for 10 min at 14,000 x g. In the resulting supernatants, CAT activity was measured by the

method of Bull and Oderfeld-Nowak (J. Neurochem., 19: 935-947, 1971). GAD activity was measured by the method of Wilson et al. (J. Biol. Chem. 247: 3154-3169, 1972) and TH activity was assayed by the method of Coyle (Biochem. Pharmacol. 21: 1935-1944, 1972).

Regional muscarinic receptor binding was assessed in C57BL/6J mice as an index of the integrity of cholinoceptive neurons. Binding of ^{3}H-quinuclidinyl benzilate ([^{3}H]QNB) to muscarinic receptors was measured by the method of Ikeda et al., (Neuropharmacol. 19: 575-584, 1980). Tissues were sonicated in 800 volumes (v/w) of 20 mM Tris-HCl buffer, pH 7.4. Tissue sonicates were incubated with L-[^{3}H]QNB, 10^{-12} to 10^{-8} M, for 90 min at room temperature in a total incubation volume of 1.0 ml. Nonspecific binding, determined in the presence of 100 µM oxotremorine, was subtracted from total binding at the various concentrations of ligand. Estimates of total muscarinic receptor density (B_{max}) and affinity of [^{3}H]QNB for binding sites (K_{QNB}) were obtained by Eadie-Hofstee analysis of saturation isotherm data. Estimates of the concentration of muscarinic agonist high affinity binding sites (K_{Hi} and K_{Lo}, respectively) were obtained from assays of [^{3}H]QNB binding in the presence of 10^{-2} to 10^{-10} M carbamylcholine using tissue sonicates used to obtain [^{3}H]QNB binding saturation isotherm data. The concentration of [^{3}H]QNB used in these assays was 0.3 nM. Data were fitted to a simple mass action expression for the case of two unique binding sites for single ligand, the agonist carbamylcholine, using iterative least squares non-linear regression analysis. All neurochemical markers were reported per unit weight of protein, determined by the method of Lowry et al. (J. Biol. Chem. 193: 265-275, 1951).

Major Findings:

1. Neurochemical markers in C57BL/6J mice. CAT activity was higher in the cerebral cortex, striatum, and hippocampus of older than younger C57BL/6J mice. The age-differences were associated with changes in the V_{max} for CAT and not in the K_M of CAT for the substrates choline chloride and acetyl coenzyme A. [^{3}H]QNB binding was lower in the cerebral cortex and striatum and higher in the hippocampus of older than of younger C57BL/6J mice. Age changes in [^{3}H]QNB binding were associated with changes in the densities of muscarinic receptors (B_{max}) and not in the affinity constants for carbamylcholine (K_{Hi} and K_{Lo}) and [^{3}H]QNB (K_{QNB}) or in the percentage of muscarinic binding to high affinity sites (B_{Hi}).

2. Relation of neurochemical markers to motor performance in C57BL/6J mice. Two composite test scores were derived for each animal. The first comprised measurements of locomotor activity (ACT), and the second reflected strength and coordination abilities (SC). Test scores included under each composite correlated highly with that composite score, but not with the other composite, suggesting that the composite scores represent independent behavioral factors. Significant correlations between the composite scores and regional enzyme activities were found; however, the correlations varied with age. In most cases, the sign (+, -) of the correlation was reversed at 24 months as compared with 4 months of age. These results suggest that age differences in the correlations between performance and enzyme activities may reflect altered neurotransmitter function during senescence. In young mice, significant

positive correlations ($p < 0.05$) were observed between the ACT composite score and hippocampal CAT and GAD and cerebellar GAD, and between the SC composite score and cerebellar CAT activity. In the 18-mo group, the correlations were significantly positive between the SC composite score and hippocampal GAD activity, and significantly negative between the SC composite score and cortical GAD activity. In the aged animals (24-mo), the ACT composite score was not significantly correlated ($p > 0.05$) with any of the neurochemical measures, as was the case with the 18-mo group. However, the SC composite score was negatively correlated with cerebellar GAD and CAT and hippocampal TH activity, and positively correlated with striatal TH activity.

3. Cholinergic markers in dementia. As compared with control samples, CAT activity was significantly ($p < 0.05$) reduced in each AD brain region assayed. The reduction ranged from 53% in samples of mammillary body to 83% in samples of hippocampal endplate. Densities of muscarinic binding sites (B_{max}) were 29% to 413% greater in AD samples as compared with controls in 13 of 18 regions assayed. The greatest difference was noted in the orbital frontal cortex. The percentage of total specific [^{3}H]QNB binding that was associated with muscarinic agonist high affinity sites did not differ between AD and control brains in any of the regions assayed.

Portions of this work have been published in abstract form: (1) Waller, S. B., Ingram, D. K., Reynolds, M.A., and London, E.D.: Neurochemical changes as a function of genotype and age in mice. Society Neuroscience Abstracts, 7: 186, 1981. (2) Waller, S. B., Ingram, D.K., Reynolds, M.A., and London, E.D.: Changes in neurotransmitter synthetic enzymes as a function of genotype and age. Age 4: 143, 1982. (3) Waller, S.B., and London, E D.: Increased Vmax for choline acetyltransferase in brain regions of aged (C57B1/6J) mice. Fed. Proc. 41: 1475, 1982. (4) Waller, S.B., and London, E.D.: Kinetic studies of choline acetyltransferase and muscarinic receptors. Society Neuroscience Abstracts, 8: 143, 1982. (5) Waller, S.B., Ball, M.J., and London, E.D.: Choline acetyltransferase and muscarinic binding in Alzheimer's diseased brain. Pharmacologist, 1983. (6) Waller, S.B., Ball, M.J., and London, E.D.: Choline acetyltransferase and muscarinic binding in regions of normal and Alzheimer's diseased brains. Society Neuroscience Abstracts, 9, 1983.

Significance to Biomedical Research and the Program of the Institute:

The analysis of correlations between neurochemical and behavioral findings demonstrates the feasibility of constructing behavioral assays of neurochemical function, some of which might reflect individual differences due to environmental factors during aging. Specifically, the correlation between motor performance and neurochemical findings suggests that batteries of behavioral tests might be used to identify aged animals that are deficient in a particular enzyme, and may be used to test the efficacy of pharmacological manipulations. Because of the motor performance changes that occur in human aging, such correlations may elucidate the functional significance of postmortem neurochemical findings. Furthermore they may ultimately lead to effective pharmacological therapy for age-associated changes in motor performance.

The studies of regional cholinergic markers indicate that the presynaptic increase in CAT activity during aging of the mouse brain is related to increased levels of enzyme, and not to altered substrate affinities, and that there also exist specific age-associated changes in densities of muscarinic binding sites. These differences may reflect presynaptic compensatory mechanisms acting to maintain critical levels of cholinergic function.

A study of cholinergic markers in regions of normal and Alzheimer's diseased brains indicated that a presynaptic cholinergic defect in AD is associated with muscarinic receptor upregulation in the neocortex and hippocampus. The muscarinic receptor upregulation may reflect a postsynaptic compensatory mechanism acting to maintain critical levels of cholinergic function.

Proposed Course: Additional neurochemical analyses will be conducted to determine the relevance of correlations between neurotransmitter enzyme activity, muscarinic receptor binding and specific neurotransmitter system function. In addition, analyses will be performed to detect possible correlations between regional enzyme activities and individual psychomotor scores of A/J mice. Further studies of neurochemical markers in regional normal and Alzheimer's diseased brains are planned.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00123-05 LN

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Synapse Development, Specificity and Mechanism In Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

B.A. Suarez-Isla Visiting Fellow LN NIA
J. Cosgrove Senior Staff Fellow LN NIA

COOPERATING UNITS *(if any)*

Department of Anatomy, 567 Medical Sciences Bldg., Univ. of Ill., Urbana, IL 61801

LAB/BRANCH

Laboratory of Neurosciences

SECTION

Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

(1) Synapse formation, synapse specificity and neurotransmission were studied using neurons and muscle cells in culture. Synapses were detected and investigated by electrophysiological recording. Neurons from chick and rat retina and chick spinal cord form synapses with muscle cells. Each type of neuron possesses a synapse-competent state in its development during which it can abundantly form synapses with muscle cells in culture. In addition, developmental periods for maximal neurite extension correlate with the developmental periods for synaptogensis. These results suggest a mechanism of synapse specificity based on a synaptogenic period during development for each type of neuron. Synapse specificity can also be based on differences in synapse stabilization. Inappropriate synapses, such as those from rat or chick retina neurons on muscle cells, are transient; whereas appropriate synapses, such as those from spinal cord on muscle, are stabilized. (2) Neuroblastoma cell lines can be used as in vitro model systems for neural function. Three neuroblastoma cell lines have been characterized for their morphology, neurochemistry and ability to form synapses. (3) Neurotransmitter release can be studied in an in vitro perfusion system. Acetylcholine release increases during development and maturation of chick retina synapses. Dopamine release from rat striatal slices does not change during aging.

PHS 6040 cont'd

Other professional personnel:

D.J. Pelto	Biochem Lab Tech	LN	NIA
J.M. Thompson	Neurophysiologist	Univ of Ill.	

Project Description

Objectives: Neurons in culture form synapses with other neurons and/or muscle cells as shown by electrophysiological recording. There is a discrete developmental period for synapse formation which may be specific to the type of neuron. Older neurons appear to lose their ability to form synapses or may only form relatively few synapses, whereas neurons from younger animals have a greater capacity to form them. The objective of this study is to examine the factors that determine specific synapse formation during development and the mechanisms underlying stabilization and/or termination of synapses. In addition, the objective is to examine the effects of innervation on target cells when appropriate synapses are formed.

To address these questions, neurons and their target cells are established in a culture environment which can be manipulated in order to study a) neurotransmitters, b) electrical membrane properties, c) synapse formation, d) synapse termination, and e) synapse specificity. This system allows one to study how development, aging, drugs and disease affect the above properties of neuronal connections.

Methods Employed: Striated muscle cells isolated from newborn rats grow in culture, form myotubes, develop nicotinic acetylcholine receptors and become electrically active. Neurons from chick, mouse or rat spinal cord and retina, and from neuroblastoma cell lines form synapses with these muscle cells in culture. Synapses are detected by electrophysiological recording of miniature endplate potentials with microelectrodes in target muscle cells. Electrical membrane properties such as resting membrane potential, specific membrane capacitance and resistance and parameters of the action potential are studied with conventional intracellular microelectrodes. Single channel events through acetylcholine (ACH) activated channels or Ca^{2+} dependent K^{+} channels are studied using the extracellular patch clamp technique. In addition, morphological measurements at the light and ultrastructural levels or optical fluorescent techniques are applied to cultured cells. Biochemical measurements are made of neurotransmitter levels and synthetic enzymes. Using a perfusion system, the release of neurotransmitters from retina layers or brain slices can be determined.

Major Findings:

1. Synapse development. Chick embryo retina neurons in culture extend neurites and form synapses. Seven different culture substrates were compared to determine which culture surface would produce the best neuronal survival and neurite extension. The order of survival of retina neurons during 7 days of culture is: salt-precipitated collagen > ammoniated collagen > fibronectin > air-dried collagen = polylysine > tissue culture plastic > polyornithine. Salt-precipitated collagen allowed the greatest number of cells to extend neurites. The amount of neuronal aggregation and nonneuronal cell growth also varied with the substrate. Nonneuronal cell growth promoted neuronal growth and neurite extension on several substrates (Ref. 1).

Inorganic blockers of calcium permeability such as cobalt, manganese and lanthanum added to trypsin-dissociated retina neurons from chick embryos, decrease the percent of cells that extend neurites after 24 h in culture in a concentration dependent manner. Blockers also decrease the rate of neurite growth and the

formation of synapses between retina neurons from 8 day chick embryos and rat myotubes after 24 h in culture. The concentration necessary to produce 50% inhibition of neurite extension was 95 μM for cobalt and 250 μM for manganese. Adhesion to substratum and survival of retina cells in culture were not affected at concentrations of inorganic blockers that inhibited more than 90% of neurite extension. Organic channel blockers, such as verapamil and D-600, prevented adhesion of 8 day retina neurons to substratum before affecting neurite extension. However, nitrendipine significantly inhibited neurite extension before preventing adhesion to substratum.

Growth of neurites is very sensitive to modification of extracellular calcium concentration. In medium containing 1.8 mM Ca, 0.8 mM Mg and 10% v/v fetal calf serum, about 16% of cells extend neurites after 24 h in culture. In calcium-free medium, neurite extension and cell substratum adhesion are blocked. However after addition of 1% or 10% of serum 6% and 9% of the cells extended neurites. In contrast, further increments in Ca levels, or loading of the cells with Ca after 24 h in 25 mM K^+ or 0.1 μg/ml of the calcium ionophore A23187, reduced neurite extension (Ref. 1). With normal calcium concentrations (1.8 mM), inhibition of neurite extension by a calcium channel blocker depended on the age of the donor chick embryo. 200 μM cobalt decreased neurite extension by 20% with 6 day retina cells, as compared to 90% with 14 day retina neurons.

Eight day retina neurons plated in high density on top of rat myotubes innervate 90% of the muscle cells after 24 h of coculture as determined by intracellular recording. Pretreatment of retina neurons with cobalt or manganese reduced the percent innervation in a concentration-dependent manner, but similar pretreatment of the myotubes proved ineffective. These results demonstrate the central role of calcium fluxes in neurite extension from primary dissociated neurons and suggest a mechanism for synapse termination based on impairment of calcium entry during development and aging.

It has been proposed (Ruffolo, R.R., Jr., Eisenbarth, G.S., Thompson, J.M. and Nirenberg, M., Proc. Natl. Acad. Sci. USA. 75: 2281-2285, 1978) that the sequence of neuronal birth, the relative location of the neurons within the organ, the developmental stage of neurons, and the length of their synapse competent state may restrict synapse targets to cells which have reached a developmental period in which they are receptive to forming synapses. Thus, specific neuronal connections can be formed. Synapse plasticity and/or regeneration in older animals may be restricted due to these developmental changes.

The use of primary neuronal cultures is limited by the fact that differentiated neurons do not divide in culture. Neuroblastoma cell lines, neuronal cancers which can replicate in culture, have become important in vitro model neuronal systems. In addition, they may be used as models of in vitro aging. However, few of the commonly available cell lines have been systematically compared in terms of their ultrastructure, function and neurochemistry.

Three commercially available neuroblastoma cell lines were analyzed to determine their ultrastructural, functional and neurochemical characteristics. The cell lines were Neuro-2a and NB41A3, derived from mouse neuroblastoma, and IMR-32, derived from a human neuroblastoma. Ultrastructurally, the neuroblastoma exhibited two major differences. Both neuroblastoma cell lines from the mouse

contained virus-like particles, whereas the human neuroblastoma was free of virus particles. Scanning electron microscopy revealed that the external morphology in the mouse cell lines was heterogeneous, suggesting the presence of more than one cell type, while the external morphology of the human cell line was homogenous, suggesting the presence of a single cell type. Neurochemically, choline acetyltransferase (CAT), tyrosine hydroxylase (TH) and glutamic acid dehydrogenase (GAD), synthetic enzymes for acetylcholine, catecholamines and γ-aminobutyric acid (GABA), respectively, were present in each cell line. Mean CAT activity was highest in NB41A3, but differences in CAT activity among the three lines were not statistically significant. TH activity in IMR-32 was significantly higher than in the mouse neuroblastoma cells. GAD activity was equivalent in all three cell lines (Ref. 2).

Functionally, each cell line was tested to determine its ability to form cholinergic synapses on cultured striatal muscle cells. Only the IMR-32 cells formed appreciable numbers of synapses with muscle cells as detected by electrophysiological recording.

The delineation of several characteristics of these three neuroblastoma cell lines will allow other investigators to choose the most appropriate of these cell lines for their studies. The heterogeneity of cell types of the mouse neuroblastoma may compromise biochemical measurements as each cell type may have different characteristics. Since only the IMR-32 form cholinergic synapses and have no more CAT activity than the other two cell lines, the presence of CAT is not a reliable marker for the ability to form synapses (Ref. 2).

2. Synapse stabilization. Synapses formed between spinal cord neurons and myotubes in culture are long-lived or stable, remaining for at least two weeks in culture. In contrast, synapses formed between chick embryo retina neurons, or between rat retina neurons, and myotubes are transient and are lost within one week of culture even though all muscles were innervated after 1 day of culture. Because spinal cord-muscle synapses are appropriate but retina-muscle synapses are inappropriate, the selection of appropriate synapses based on differences in synapse turnover, or stabilization, may be an important mechanism for synapse specificity.

Two aspects of synapse stabilization in this system have been studied. Spinal cord neurons possess a factor which causes aggregation of muscle acetylcholine receptors (AChR) into high density clusters. We have shown (Thompson, J.M. and Rapoport, S.I.: Absence of stable retina-muscle synapses is related to absence of acetylcholine receptor aggregation factor in retina neurons. Society Neuroscience Abstract, 8: 185, 1982) that retina neurons do not possess such a factor as studied in either retina neuron conditioned medium or cocultures of retina neurons and myotubes. The absence of an aggregating factor in retina may relate to the lack of stabilization of retina-muscle synapses, as opposed to the stabilization of spinal cord-muscle synapses.

Other studies (Suarez-Isla, B.A., Thompson, J.M. and Rapoport, S.I.: Effect of coculture and in vitro (re)innervation on the electrical properties of rat myotubes. Society Neuroscience Abstract, 8: 125, 1982) have shown that spinal cord cocultures induced a decrease in the incidence of slow hyperpolarizing after potentials in muscle cells following an overshooting action potential. Specific membrane resistance was lower in muscle cells devoid of slow hyperpolarizing

after potentials. Cultures of retina neurons and muscle do not show these changes. Thus, the process of synapse stabilization in vitro is contemporary to the induction of specific changes in the electrical membrane properties of muscle cells.

The incidence of slow hyperpolarizing after potentials (slow HAPs) in rat myotubes decreases significantly after in vitro innervation by spinal cord neurons that form stable neuromuscular synapses, but not by retinal neurons that make only transient synaptic contacts. The slow HAP is associated with a Ca-dependent K-conductance in rat muscle cells in culture (Barrett et al., Dev. Biol. 82:258, 1981) and probably maintains spontaneous contractile activity before innervation in vivo, being suppressed during maturation. To investigate whether the decrease in the slow HAP incidence after stable innervation was due to modulation of a Ca-dependent K-conductance, we compared the gating and kinetic properties of single Ca-activated K-channels ($I_{K(Ca)}$ channels) in intact membrane patches of control and cocultured myotubes, using the extracellular patch clamp technique.

The $I_{K(Ca)}$ channels were active in both control and cocultured myotubes and also in cells without HAPs. They could be observed in 80% of the patches tested, had a single channel conductance of 110 pS and in a few cases appeared in bursts followed by silent periods that ranged from seconds to minutes. Action potentials evoked by a second intracellular electrode increased transiently the probability of opening of the $I_{K(Ca)}$ channels in the patch. The fraction of time that a channel spent in the open state (f(V)) increased e-fold with 20 mV of depolarization for low levels of activity in both types of cells. The voltage dependence of the mean open or closed times was similar in both types of cells but shifts of about 30 mV along the voltage axis were observed in both cases. In contrast, the current-voltage relationship was different. $I_{K(Ca)}$ channels were blocked at lower holding potentials in cocultured cells and the I/V relationship "rolled over" at about +40 mV, showing a clear negative conductance region. In control cells, however, "roll over" became apparent only beyond +75 mV of holding potential. Application of a model of voltage dependent ion block of the channel indicated that the voltage sensitivity of the block was significantly increased in cells that have been cocultured. Further experiments with excised membrane patches are needed to test whether the neurotrophic effect has affected the Ca sensitivity of the $I_{K(Ca)}$ conductances.

3. Neurotransmitter release. The release of [^{3}H]acetylcholine (ACh) from 6-, 8-, 10-, 12-, 14- and 16-day chick embryo neural retina layers was determined using a perfusion system to collect the released neurotransmitter. After labeling the tissue with [^{3}H]choline, samples of [^{3}H]ACh released before and after stimulation by 80 mM KCl or 5 mM glutamate were collected and analyzed. The results showed a developmental increase in both basal and stimulated release. The increase was not caused by changes in [^{3}H]choline uptake or total [^{3}H]-ACh synthesized which did not increase with developmental age. The developmental increase is correlated with the time of formation and maturation of retinal cholinergic synapses in ovo, but is not correlated with the developmental decrease in cholinergic synapses formed by retina neurons with striated muscles in vitro (Ref. 3).

Significance to Biomedical Research and the Program at the Institute:

Irreversible alteration of neuronal properties during development limits the capabilities of mature and senescent neurons. Since neurons can readily form synapses only during a discrete period in their development, the number of new synapses which older neurons can form may be limited. Thus, synapse plasticity and regeneration, which may be important in learning and memory or recovery from disease or trauma, could be limited in older organisms.

Release of neurotransmitters is critical to neuronal function. The development and maturation of synapses is correlated with the development of neurotransmitter release. In one system studied during aging of rats, no deficits of dopamine release were found. Age-related deficits in motor behavior in rats are not due to deficits in release of this neurotransmitter.

Proposed Course: Neuronal and neuronal-muscle co-cultures will be used to study: (a) synapse formation and stabilization, (b) modulation of electrical membrane properties of cultured muscle cells during in vitro innervation.

Publications:

1. Thompson, J.M., and Pelto, D.J.: Attachment, survival and neurite extension of chick embryo retinal neurons on various culture substrates. Dev. Neurosci. 5: 447-457, 1982.

2. Thompson, J.M., London, E.D., and Johnson, J.E. Jr.: Ultrastructural, functional and biochemical characteristics of mouse and human neuroblastoma cell lines. Neuroscience 7: 1807-1815, 1982.

3. Thompson, J.M.: Increase in acetylcholine release from chick embryo retina during development. Dev. Brain Res. 4: 259-264, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00121-06 LN

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* (M)
Function of Peripheral Nerve and Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)		
M.E. Michel	Staff Fellow	LN NIA
C. Latker Sr Staff Fellow LN NIA, A. Weerasuriya	Visiting Associate	LN NIA

COOPERATING UNITS *(if any)* Experimental Morphology Section, NIA
Laboratory of Neurochemistry, NINCDS
Lab of Neurochem and Lab of Biophysics, Dept of Physiology, Univ of Santiago, Chile
Department of Zoology, University of Maryland, College Park, Maryland 20742

LAB/BRANCH
Laboratory of Neurosciences

SECTION
Cerebral Physiology and Metabolism

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	0

CHECK APPROPRIATE BOX(ES)

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

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

Blood-vessels of nerve are comparable to those of the central nervous system, and are lined by a continuous endothelium with intercellular tight junctions. They form part of the blood-nerve barrier, together with the perineurium. Vesicles within endothelial cells of the frog sciatic nerve endoneurial vasculature do not contribute to transcapillary exchange. Hypertension, induced by vascular perfusion of the nerve, damages the vascular endothelium by forming submembrane blisters, and increases permeability to intravascular tracers, horseradish peroxidase and microperoxidase. Cells of the vasculature and perineurium are capable of proliferation and forming an intact barrier after trauma to the nerve.

An adequate blood supply to the nerve is required to maintain nerve function and integrity. Blood flow of the nerve was measured with a laser Doppler flowmeter and radiotracer techniques, and was shown in the rat sciatic nerve to be close to that found for white matter of the brain.

Metabolism and tension were examined in single fibers of the semitendinosus muscle of the frog during fatigue produced by prolonged tetanization, and in relation to excitation-contraction uncoupling by stretch and hypertonicity. The mechanisms of uncoupling were related to energy steps in the contraction and calcium release processes.

PHS 6040 (Rev. 1/83) GPO 895-100

PHS 6040 cont'd

Other professional personnel:

P. Ask	Visiting Fellow	LN	NIA
P. Robinson	Visiting Fellow	LN	NIA
S.I. Rapoport	Chief	LN	NIA
I. Rundquist	Visiting Fellow	LN	NIA
N.L. Shinowara	Senior Staff Fellow	EMS	NIA
K.C. Wadhwani	Student Investigator	LN	NIA
J.V. Passonneau	Chief	LNC	NINCDS
V. Nassar-Gentina	Research Scientist	Univ of Santiago	
H. Levitan	Physiologist	Univ of Maryland	

Project Description:

Objectives:

1. Peripheral nerve and perineurium. Peripheral nerve function is governed in part by the local axonal milieu, which is separated from body fluids by a perineurial sheath and endoneurial capillaries (blood-nerve barrier). The objectives of this study are: (1) to study the morphology of the perineurium, (2) to examine the ability of the perineurium to act as a permeability barrier to radiotracer nonelectrolytes and to ions in normal, lead-treated and stretched nerves, (3) to measure hydrostatic pressure and volume of the endoneurial space, in relation to nerve edema mechanisms, and (4) to measure blood flow and permeability properties of the endoneurial capillaries.

2. Muscle contraction and fatigue. When a striated muscle is stimulated repetitively, its contractile force decreases and it becomes fatigued. Fatigue characterizes fast twitch muscles with glycolytic metabolism, rather than slow twitch muscles with oxidative metabolism. Muscle fatigue is thought to be caused by depletion of available energy reserves or to uncoupling of contraction from excitation. The objective of this study was to examine the excitation-contraction mechanism during muscle fatigue, induced by prolonged tetanization.

Methods Employed:

1. Peripheral nerve. For electrical and radioisotope flux studies, the perineurium of the frog sciatic nerve was isolated and mounted on cannulae within a bath of stirred Ringers solution, and perfused with Ringers solution with or without radioisotopes. Radioisotopes also could be placed in the bath. Transport through the sheath was quantified by measuring isotope exchange.

The AC impedance of the perineurium of the frog sciatic nerve was measured *in vitro* using a four electrode arrangement, where a sinusoidal current of varying frequency (1 Hz to 0.1 MHz) was imposed across the perineurium. This current was compared with the resultant transperineurial potential difference.

For morphological studies, the frog sciatic nerve was fixed with glutaraldehyde *in situ*, or *in vitro* by immersion. Rapid preservation was achieved with an osmium tetroxide-glutaraldehyde fixative. Colloidal lanthanum or lanthanum chloride was applied for 3 to 5 hours externally to small segments of nerve, or internally by injection. Horseradish peroxidase was used as an *in vitro* tracer in the bath for one hour at 5^{o} or 25^{o}C. Tissues were prepared for electron microscopic examination.

The hydraulic conductivity of the perineurium and endoneurial capillaries was measured in segments of frog sciatic nerve which were isolated and immersed in solutions with different osmolarities, or perfused *in vivo* through the aorta. Endoneurial pressure was measured with microelectrodes connected to a null-balance feedback system. Endoneurial volume was calculated from nerve diameter. Endoneurial capillary permeability was measured with an *in vivo* arrangement in which the vasculature of the hindquarters was perfused with a medium containing 14C-sucrose and a titrated blood space-space indicator.

Blood flow of the intact sciatic nerve in vivo was measured with a laser Doppler flowmeter in anesthetized rats. The flow signal was recorded and compared to flow as measured by intravenous infusion of 14C-iodoantipyrine.

2. Muscle. A single fiber was dissected from the frog semitendinosus muscle and mounted in a bath of flowing Ringers solution or of hypertonic Ringers solution at 15°C. Tension was recorded by a RCA 5734 transducer tied to one end of the fiber; the ouput was displayed on an oscilloscope face. Sarcomere length was measured by a laser diffraction technique and adjusted as necessary. The fiber was stimulated at different frequencies via external platinum electrodes, so as to produce twitches or tetanic contractions. Fibers were removed for microbiochemical measurements of glycogen, lactic acid, phosphocreatine and adenosine triphosphate (ATP).

Major Findings:

1. Morphology of blood-nerve barrier

a. Frog perineurium: vesicles and "transcellular channels." When lanthanum was applied to the outside of the fixed sciatic nerve of Rana pipiens, tracer did not enter the endoneurium, but was halted by functionally tight junctions at the inner layers of the perineurium. The perineurium consists of several concentric layers of cells interspersed with an extracellular matrix of amorphous ground substance, collagen fibrils, and fine filaments. Numerous vesicular profiles are closely associated with the surface membranes of the cells. The application of lanthanum to fixed tissue revealed that these profiles are attached to the cell surface by narrow necks, and are open to the extracellular space. Attenuated perineurial cells are filled by the vesicular structures, which often appear in electron micrographs to form continuous, patent transcellular channels. However, stereoscopic electron microscopy showed that these vesicles do not fuse with each other or with the opposite cell surface or form transcellular channels. Channel formation does not appear to contribute to the permeability of any of the perineurial layers (Ref. 1).

b. Blood vessels of the endoneurium. A 5 mm segment of frog nerve within the thigh was examined. In 1 μm thick cross sections the nerves averaged 700 μm in diameter and contained 11-18 blood vessels, with diameters of 30 to 100 μm. In thin sections, the most common vessel consisted of several attenuated endothelial cells with a basal lamina, and a pericyte and its lamina. A second type of vessel was surrounded by a dense fibrillar matrix and fibroblasts in addition to pericytes, with endothelial cells which were thicker with irregular contours and thin cytoplasmic fingers which often extended into the lumen. All endothelial cells contained Golgi apparatus, glycogen, small vesicles and dense bodies. Although the endothelial processes of some vessels could be very thin, no open or fenestrated regions were seen. In every cross-section of a blood vessel, several regions of overlapping processes appeared. The cleft between these processes was clearly open except for one or two small regions of membrane apposition suggestive of tight junctions. Intravascular horseradish peroxidase or microperoxidase did not penetrate the most lumenal of these close contacts. Endothelial cells contained numerous vesicular profiles, both attached to the cell surface and within the cytoplasm. After administration of tracer, reaction product lined the vesicles nearest the lumenal surface; only rarely did vesicles appear near the ablumenal surface and reaction product never appeared in the endoneurial space.

c. Morphology of endoneurial blood vessels of frog sciatic nerve during vascular perfusion. The ultrastructure of the blood vessels within frog sciatic nerve was examined following perfusion of the iliac artery at flow rates of 0.07-1.0 ml/min. At 0.21-0.82 ml/min, all endoneurial vessels were perfused and no vessels were collapsed or broken under light microscopy. The rounded profiles appeared prominently scattered among the axons within the endoneurial space. The total surface area of vascular endothelium equaled approximately 60% of the perineurial surface area. In all vessels examined with electron microscopy, numerous vesicles were present at both lumenal and ablumenal surfaces of the endothelial cells. At higher flow rates, no changes in vesicles or interendothelial junctions were evident; however, blebs and blisters of the lumenal membrane often appeared. In contrast to results obtained with intravascular injection of microperoxidase, when this tracer was perfused intravascularly at a flow rate 0.21 ml/min, small quantities appeared within the endoneurium. Following perfusion of the tracer at 0.82 ml/min, however, reaction product frequently flooded the endothelial cells, and was distributed within the endoneurial space. Damage to the endothelium, perhaps resulting from either hypertensive or shearing forces, appeared to cause the leakage of tracer. Neither increased vesicular transport nor opening of intracellular junctions was demonstrated. The structure of vesicles and junctions during barrier opening was further investigated by perfusing the hindlimb vasculature with hyperosmolar arabinose. Horseradish peroxidase leaked from vessels treated with the solution. No evidence of vesicular transport nor of transcellular channels was found. Intercellular junctions still consisted of the overlap or end-to-end contact of endothelial processes, and the regions of close membrane apposition were still visible. However, tracer was seen throughout the intercellular cleft of the junctional region, indicating that the tight junction had been opened.

d. Cell proliferation following sciatic nerve lesion. The permeability of the blood-nerve barrier to horseradish peroxidase increases following injury to the nerve. We examined the ultrastructure of perineurial and endothelial cells in order to see changes in vesicular or junctional morphology which contribute to this condition. Sciatic nerves of frogs were lesioned and allowed to regenerate for 2-12 wks. Tritiated thymidine (1 μCi/gm body weight) was injected intraperitoneally 48 and 4 hrs prior to killing. Radioactive thymidine was incorporated into nuclei which were actively synthesizing DNA and provided a label for dividing cells. Incorporation of (3H)-thymidine by perineurial nuclei was extensive at 2 wks but was localized to within about 1 mm of the lesion site. Only rare incorporation of label occurred in the opposite, uninjured nerve sheath. By 3 weeks, many endothelial nuclei within a similar distance of the injury were labeled. Within the lesion zone virtually all blood vessels contained at least one labeled endothelial nucleus. Most perineurial cells in both rostral and caudal stumps of the nerve were labeled. By 6 wks, the numbers of labeled cells were reduced and by 12 wks regeneration of vasculature and sheath appeared complete.

Electron microscopy of the dividing perineurial and endothelial cells at early post-lesion periods revealed abundant rough endoplasmic reticulum, numerous surface vesicles, coated pits and coated vesicles. Endothelial cells extended numerous cytoplasmic processes; the junctions between these processes consisted of single close contacts which allowed passage of horseradish peroxidase. At 12 wks and in control nerves, cells contained little rough endoplasmic reticulum,

no coated vesicles and were linked by tight junctions. Only the large number of surface vesicles was similar to that seen with the active cells of early post-lesion periods. We suggest that the increased permeability during injury is due to the increased proliferation among the cells of the blood-nerve barrier. During this time, open junctions between endothelial and perineurial cells allow passage of large tracer molecules.

Studies on the perineurium and endoneurial capillaries have been presented in abstract form: (a) Shinowara, N.L., Michel, M.E. and Rapoport, S.I.: Morphological correlates of tracer permeability through the perineurial barrier of frog peripheral nerve. Anatomical Record 202, 176A, 1982, (b) Michel, M.E., Shinowara, N.L. and Rapoport, S.I.: Vesicles within endothelial cells in frog sciatic nerve do not transport HRP in either lumenal or antilumenal directions. Neurosci. Soc. Abstr. 9, 1983, (c) Shinowara, N.L., Michel, M.E. and Rapoport, S.I.: Permeability routes of horseradish peroxidase across the frog peripheral nerve sheath, Neurosci. Soc. Abstr. 9, 1983, (d) Michel, M.E. and Rapoport, S.I.: Proliferation among cells of the blood-nerve barrier following sciatic nerve lesion. Soc. Cell Biol. Abstr., 1982. (e) Michel, M.E., Shinowara, N.L., Rapoport, S.I.: Absence of vesicle-mediated transcellular transport in the frog blood-nerve barrier. IX International Congress of Neuropathology, Abstr., 1982.

2. Endoneurial hydrostatic pressure and perineurial hydraulic conductivity. Measurements of volume and hydrostatic pressure in the frog sciatic nerve in vitro demonstrated that the intact nerve acts as an osmometer, in large part because the perineurium is a semi-permeable membrane for water flow. Endoneurial hydrostatic pressure in nerves in isotonic Ringers solution exceeded bath pressure by about 7 mm Hg. In Ringers made hypertonic by addition of sucrose, nerve volume and endoneurial pressure fell linearly in relation to increasing osmolality. The slope of the plot of pressure against volume provided a value for nerve compliance equal to 0.006 mm^2/mm Hg. Calculation indicated that the nerve has an osmotically "inactive" volume equal to 0.19 mm^3/mm, which is about 75% of the total volume of a nerve segment of unit length in normal Ringers. Perineurial hydraulic conductivity L_p equaled 7.5×10^{-13} $cm^3 sec^{-1} dyn^{-1}$, a value characteristic of nonleaky epithelia. The perineurium is an elastic tissue with a constant modulus of elasticity equal to 3×10^6 $dynes/cm^2$ when not markedly stretched, and may limit nerve swelling under pathological conditions of nerve edema (Ref. 2).

3. Electrical properties of perineurium. The DC resistance of the perineurium was 430 ohm cm^2. Impedance measurements demonstrated two dispersions in the isolated perineurium of the frog sciatic nerve, with center frequencies of 5 KHz and 20 Hz. Exposure to high conductance Ringers solution decreased the DC resistance, whereas a low conductance Ringers increased the resistance. Analysis of the data in terms of a four-element equivalent circuit (two resistors and two capacitors) suggested that small (0.1 $\mu f/cm^2$) and large (20 $\mu F/cm^2$) capacitances exist at the perineurium. The small capacitance was ascribed to six or more layers of perineurial cells. The large capacitance was ascribed to polarization of charge, perhaps at intercellular tight junctions, and was affected by experimental manipulation.

4. Permeability of endoneurial capillaries. When infusing a frog sciatic nerve with a hypertonic sucrose or NaCl solution, it was possible to measure

changes in nerve diameter and endoneurial hydrostatic pressure in relation to time. The hydraulic conductivity of endoneurial capillaries was estimated from these changes, and found to be less than that of capillaries of the central nervous system, but to approximate the conductivity of the perineurium of the nerve.

[14C]-sucrose and [3H]-inulin (blood volume indicator) were included when an isotonic vascular perfusate, and rates of uptake by the nerve of [14C]-sucrose were estimated. Results indicate that the permeability of the capillary bed to [14C]-sucrose is about 3-4 times greater than that of the perineurium. The results support the hypothesis that, under normal conditions, the endoneurial capillary is the major site of passage for substances entering and leaving the axonal environment, and that the perineurium plays a relatively passive role, defining the limits of the endoneurial space within which the endoneurial capillaries exercise their regulatory functions. An abstract of this work has been published (Rapoport, S.I. and Weerasuriya, A.: 14C-sucrose permeability of endoneurial capillaries in the frog sciatic nerve. Society Neuroscience Abstracts, 9; 1983).

5. Blood flow in the sciatic nerve of the rat. The left sciatic nerve of a pentobarbital-anesthetized rat was exposed and a laser beam from a laser Doppler flowmeter was applied. The flow signal was recorded for 15-90 min. In addition, the regional neural blood flow (rNBF) was determined by infusion of [14C]-iodoantipyrine in the femoral vein for 45 sec. Blood flow ranged from 0.09 to 0.51 ml/min/g, with a mean of 0.27 ml/min, which is comparable to flow in brain white matter of pentobarbital-anesthetized rats. rNBF was highly correlated with laser Doppler output ($r = 0.73$, $p < 0.05$). When epineurial tissue was removed from the nerve, both measures of flow fell by 50%, indicating that flow in the endoneurium is 50% of net flow. With the use of non-diffusible [57Co]-labeled microspheres (15 μ, 0.1 mCi), we calculated that about 0.22 ml/min/g is endoneurial flow and 0.32 ml/min/g is epineurial-perineurial flow, as compared to an average flow of 0.27 ml/min/g for the whole nerve. Because of correlation between laser Doppler output and rNBF, the laser method is suited for monitoring blood flow of the peripheral nerve in relation to pharmacological or physiological manipulation. An abstract of this work has been presented by Dr. Ingemar Rundquist, entitled "Measurement of blood flow in rat sciatic nerve by laser Doppler flowmetry," at the Federation of American Societies for Experimental Biology Meeting on April 4, 1983.

6. Muscle contraction. Metabolism and tension were examined in single fibers of the semitendinosus muscle of the frog (R. pipiens) at 15°C, after excitation-contraction uncoupling by stretch and hypertonicity. Interrupted tetanic stimulation at 20 Hz for 150 sec, of control fibers in isotonic Ringer at a rest sarcomere length (SL) of 2.3 μm, resulted in a steadily declining tension, stimulated glycolysis, and significantly reduced fiber phosphocreatine (PCr) and ATP concentrations. Stretching resting muscle fibers to a SL of 4.7 μm did not alter metabolite concentrations, but glucose-6-phosphate rose and PCr fell markedly when the stretched fibers were stimulated tetanically, although tension was absent. Immersion of untetanized fibers in 2.5X isotonic Ringer increased glucose-6-phosphate and elevated PCr. During the transient rise in resting tension, PCr consumption per unit tension-time integral was the same as that in

fibers stimulated tetanically in isotonic Ringer. Tetanization of fibers in hypertonic solution did not further alter metabolite concentrations or produce tension. The results indicate that exposure to hypertonicity increases both tension and consumption of high-energy phosphate bonds (~ P) in resting fibers, but stretch does not. During tetanic stimulation, therefore stretch interferes with contraction but does not prevent activation, whereas hypertonicity inhibits activation as well as contraction (Ref. 3).

Significance to Biomedical Research and the Program at the Institute.

1. Little is known about regulation of peripheral nerve function by tissues of the blood-nerve barrier, namely the perineurium and endoneurial blood vessels. Defects in these tissues may contribute to peripheral neuropathies and edema associated with diseases and aging in man. It is critical to obtain morphological and quantitative physiological information about the blood-nerve barrier in order to understand nerve function in health and disease.

2. Muscle wasting and fatigue commonly accompany senescence in man, but may be partially reversed by endurance training. Our studies on single muscle fibers demonstrate for the first time that fatigue, as caused by tetanic stimulation, has been related in single muscle cells to energy requirements for muscle contraction as well as steps of excitation-contraction coupling. It is important critical to understand the relation between function and metabolic demands in order to examine the fatigue process.

Proposed Course:

We will continue to investigate the function and morphology of the perineurium and endothelial capillaries and their regulation of the peripheral nerve environment. Furthermore, we will measure nerve blood flow under normal and pathological conditions.

Publications:

1. Shinowara, N.L., Michel, M.E. and Rapoport, S.I.: Morphological correlates of permeability in the frog perineurium: vesicles and "transcellular channels." Cell Tissue Res. 227: 11-22, 1982.

2. Ask, P., Levitan, H., Robinson, P.J. and Rapoport, S.I.: Peripheral nerve as an osmometer: role of the perineurium in frog sciatic nerve. Amer. J. Physiol. 244: C75-C81, 1983.

3. Rapoport, S.I., Nassar-Gentina, V., and Passonneau, J.V.: Effects of excitation-contraction uncoupling by stretch and hypertonicity on metabolism and tension in single frog muscle fibers. J. Gen. Physiol. 80: 73-81, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00015-25 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Baltimore Longitudinal Study of Human Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
See Attached Page.

COOPERATING UNITS *(if any)*

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

The Baltimore Longitudinal Study of Human Aging (BLSA) serves as a resource for scientists working in the field of Gerontology. It provides a well-described group of men and women between 20 and 96 years of age for studies of the mechanisms of human aging. Projects in physiology, biochemistry, psychology, nutrition, pharmacology, endocrinology, sociology, and genetics, have been carried out or are in progress.

PHS 6040 (Rev. 1/83)

GPO 895-100

Principal Investigators:

R. Andres	Chief, Clinical Physiology Branch	CPB NIA
J.D. Tobin	Chief, Human Performance Section	CPB NIA
N.W. Shock	Scientist Emeritus	NIA
S.K. Sharma	Sociologist	CPB NIA

Other:

L.J. Brant	Mathematical Statistician	CPB NIA

Other workers who are associated with the Longitudinal Study describe their involvement in their individual reports.

Project Description:

Objectives: The BLSA provides a well described group of subjects as a resource in support of a wide variety of scientific investigations in gerontology and other disciplines. While long-term planning is encouraged, important studies of shorter duration have also been undertaken. The long-term general goals of the project are to: (1) secure replicate measures of physiological, pathological, biochemical, and psychological variables on longitudinal study participants at specified intervals; (2) summarize and compare the results of testing in relation to age according to cross-sectional and longitudinal formats; (3) identify characteristics of individual participants which may be related to changes of function over time and to age at death; and, (4) determine whether the data obtained support one or another theory of the mechanisms responsible for age-related functional decrements.

Methods Employed: The Sample: Study participants are male and female volunteers recruited mostly by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every two years for an indeterminate period. Our sample continued to be highly educated, mostly married, describing themselves as financially comfortable or better, and of the group who returned for the fifth visit, 90% rated their health as good or excellent on both first and fifth visits.

Data Management: Medical records and test results are maintained in written form in the laboratory and transferred to a data retrieval and analysis system by keypunching on tabulation cards or by recording the test results directly on punched paper tape or magnetic tape. Data are maintained and used in ways which protect the privacy of participants. Sensitive material is specially encoded. Individual scientists review, evaluate and summarize the data for scientific reporting.

Major Findings: By August 1, 1983 a total of 1526 men and women (1168 men and 358 women) have participated in the testing program on one or more visits to the GRC. Since the inception of the study 299 men have died. As of July 1, 1983, 911 men have completed three or more visits for testing, 700 visited five times or more, 356 ten times or more, 212 twelve times or more, and 61 fifteen times or more. In all, these subjects account for a grand total of 7941 participant visits.

During the year, 9 women were newly admitted to the program as compared to 5 men. Of the 305 women who have joined the BLSA since January 1978, 7 have died and 46 withdrew or failed to return, leaving an active sample of 252. Two hundred twenty-eight (228) women have been tested two times; 127 have been tested three times or more.

Tables 1 and 2 show the cumulative number of Male/Female Subject Visits by Age at First Visit. Tables 3 and 4 show the cumulative number of Male/Female Active Subject Visits by Age as of July 1, 1983.

Table 1: Cumulative Number of Male Subject Visits by Age at First Visit (August 2, 1983)

Number of Visits	17-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	>100	TOTAL
1	6	158	228	242	193	154	151	32	5		1169
2	5	134	210	221	177	138	127	23	3		1038
3	4	107	187	205	159	125	105	16	3		911
4	3	90	156	194	145	114	92	11	3		808
5	2	68	137	176	136	101	78	7	3		708
6	1	41	114	164	125	96	61	6	1		609
7	1	28	104	158	109	88	56	6			550
8		18	82	146	96	80	52	5			479
9		15	73	128	88	70	45	4			423
10		12	62	108	71	63	36	4			356
11		9	39	83	58	54	27	2			272
12		8	28	64	45	45	21	1			212
13		2	16	42	37	34	16				147
14		1	8	22	28	29	10				98
15		1	3	12	19	20	6				61
16			1	6	13	17	4				41
17			1	3	7	11	2				24
18			1	2	4	9	2				18
19			1	1	2	5	2				11
20					1	2					3
21						2					2
22						1					1

Table 2: Cumulative Number of Female Subject Visits by Age at First Visit (August 2, 1983)

Number of Visits	17-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	>100	TOTAL
1	1	35	70	43	70	87	43	6	3		358
2		13	56	34	52	58	18	3	1		235
3		5	28	18	26	35	12	1	1		126
4					6	19	9	1			35
5					1	2	1				4

Table 3: Cumulative Number of Active Male Subject Visits Ages as of July 1, 1983

Number of Visits	17-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	>100	TOTAL
1		20	76	96	112	126	91	51	3		575
2		6	72	96	110	125	89	46	3		547
3		2	63	90	108	123	87	40	3		516
4		1	50	79	103	121	82	40	2		478
5			30	67	93	119	77	39	2		427
6			10	48	90	118	74	39	2		381
7			5	39	85	115	72	39	2		357
8			2	23	73	111	70	39	2		320
9				17	62	104	69	38	2		292
10				11	55	84	66	35	2		253
11				6	36	62	59	29	2		194
12				6	26	47	50	28	2		159
13				2	11	32	38	26	1		110
14				1	3	16	27	21	1		69
15				1	1	6	17	18	1		44
16					1	2	9	17	1		30
17					1	1	4	12	1		19
18					1	1	2	9	1		14
19					1		1	6	1		9
20								2	1		3
21								1	1		2
22								1			1

Table 4: Cumulative Number of Active Female Subject Visits
Ages as of July 1, 1983

Number of Visits	17-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	>100	TOTAL
1	1	24	50	46	52	67	54	8	3		305
2		5	42	42	44	51	36	6	2		228
3		3	15	25	19	31	27	3	2		125
4					2	10	18	3	1		34
5						1	3				4
6											

It is important in any long term longitudinal study to have as much information as possible on the inactive as well as the active subjects. Towards that end the Illness & Disability Study was instituted.

The objectives of the "Illness & Disability Study" are: 1) to determine the alive/dead status of those subjects who dropped out from the BLSA between 1958 and June 30, 1977 (N=299); 2) to locate and contact these subjects and gather information pertaining to their health and illness experience subsequent to their dropping out from the BLSA; 3) to ascertain the risk factors for dropping out from the BLSA and to recommend control measures directed toward attaining maximum participation; 4) to determine whether subjects who dropped out differed significantly from those subjects who remained active in the study with regard to health.

The results with regard to the first two objectives are summarized first. All but three (1%) subjects have beeen located. Seventy-eight (26%) had died subsequent to their dropping out from the BLSA. Of the remainder two hundred twenty-one (74%) subjects who were found to be alive, eighty-one (37%) returned for another visit to the BLSA, forty-four(20%) were visited in their own home by this investigator, fifty-eight (26%) responded to a mailed questionnaire, nineteen (9%) responded to a telephone conversation, six (3%) subjects refused to cooperate while one subject was dropped from the study for administrative reasons at the beginning of the study.

To ascertain the risk factors for dropping out from the BLSA, the influence of age, marital status, education, occupation, distance between a subject's residence and study center, self-health and financial assessment (all measured at first visit) and method of recruitment on attrition was examined. The 1088 subjects who joined the BLSA during thr first twenty years (1958-1977) were studied using a multiple logistic regression model. To control for death, two separate analysis were performed. Both analysis produced similar results. The analysis shows that subjects 70 years and older with less than Bachelors degree, living 500 miles or more from study center, perceiving their health as average or below and recruited by another subject who in turn has dropped out has the greatest risk of dropping out (3.5 fold). Recruiter's status shows the strongest association with the standardized risk ratio indicating a reduction in the average risk of 20% among subjects recruited by a fellow subject who remains active, while there is a two fold increase in the average risk among those whose recruiter drops out. These findings have important implications with regard to minimizing future attrition in the following ways: Identification of recruiter's status as the most important risk factor enables us to identify "population at risk" for dropping out. This can be used to suggest a number of strategies aimed at minimizing subsequent attrition.

Using the experience of the male drop-out study, a method of locating the future dropouts from the BLSA has been established. In the 1977 follow-up study, a stepwise application of five methods was performed. Though 48% of the dropouts had moved from their last known address, telephone directories were the most successful technique and accounted for 63% of the subjects found. Current/former workplace; professional associations and alumni office 2%; family physician 7%; friends and relatives who were still active in the study 7%; and other sources (medical records and correspondence file, postal service, Department of Motor Vehicle Administration, Department of Vital Statistics, funeral homes, etc.) 10% accounted for remainders. In all 99% of the dropouts were located and their survivorship status determined. Even in the absence of social security numbers, the application of this stepwise approach was successful in this study. This stepwise application is being currently applied on dropouts since June 30, 1977.

Future plans include 1) similar analysis using psychological and physical health data; 2) using Cox's Hazard Function, a survivorship analysis is in the process of exploration; 3) data on subsequent health and illness experiences of dropouts will be analyzed; 4) using the experience of male drop-out study, a method is established both operationally and conceptually for locating and contacting women drop-outs.

Statistical Consultation: The statistical activity of the Human Performance Section (HPS) provides statistical consultation to other sections involved in the BLSA as well as many other GRC investigators. One important effort was collaboration on a study of attrition among BLSA participants. Other routine consultations have been associated with research on bone and hand data, cardiovascular, body composition, and sex data pertaining to BLSA as well as other longitudinal animal data and human behavioral data of other GRC studies. Also computer algorithms and programs have been written to do specially formulated statistical analyses needed by many scientific investigators. These programs fill needs not presently available via packaged statistical programs and increase the effectiveness of the research effort.

Significance to Bio-Medical Research and the Program of the Institute: A major goal of the longitudinal program is a deeper understanding of age-related changes in the different organ systems, and their inter relationships. The relation of functional changes in an individual to age at death, age at onset of a disease, and other end points is important for understanding aging in humans and the impact of aging on society. The intensive study of multiple variables will also provide tests of risk-factor theories for specific age-related diseases.

Proposed Course: Data collection and analyses will be continued. Continued emphasis on automation of tests, data entry, and analyses should provide improved accuracy and efficiency. A summary of all aspects of this program is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00021-20 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Study of Normal Human Variability

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

C.C. Plato Sr. Research Geneticist CPB NIA

COOPERATING UNITS *(if any)*

See attached page.

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.00	0.50	0.50

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project represents an ongoing collaborative effort, involving WHO and other national and international laboratories to coordinate the collection, evaluation and interpretation of normal genetic markers. Specifically, the objectives of this project are: (A) To study the distributin of Dermatoglyphic markers in population isolates, family units, disease entities and normal control samples, and to utilize these genetic markers in understanding the etiology, development and early diagnosis of diseases or processes with late onset. (B) To determine the lateral functional dominance, grip strength, among BLSA participants, and assess their relationship to physiological processes or diseases demonstrating bilateral asymmetry. (C) Cross-sectional and longitudinal study of visual function in BLSA participants.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Investigators:

J.D. Tobin	Chief, Human Performance Section	CPB NIA
D.C. Gajdusek	Chief, Laboratory of Central Nervous System Studies	CNS NINCDS
R.M. Garruto	Sr. Staff Associate	CNS NINCDS
K.M. Fox	Biologist	CPB NIA
S. Kimura	Guest Scientist	CPB NIA
N. Gittings	Psychologist	CPB NIA

Cooperating Units:

1. W. Wertelecki
   Department of Genetics
   University of South Alabama
   Mobile, Alabama

2. J. T. Schwartz
   Division of Hospitals & Clinics
   Bureau of Medical Services
   Health Service Administration, USPHS
   West Hyattsville, Maryland

3. R. MacLennan
   International Agency for Research on Cancer
   WHO, Lyon, France

4. M. Alpers
   Institute of Medical Research
   Goroka, New Guinea

5. C. Bartsocas
   Department of Pediatrics
   University of Athens
   Athens, Greece

6. T. Kuberski, Epidemiologist
   NIMDD, Epidemiology and Field Study Branch
   Phoenix, Arizona

7. R. W. Hornabrook
   New Guinea Institute of Medical Research
   Wadestown, Wellington, New Zealand

8. Y. Ahuja
   Department of Genetics
   Osmania University
   Hyderabad, India

9. T. Steegmann
   Department of Anthropology
   State University of New York
   Buffalo, New York

10. J. Larrick
    Cetus Immune Corporation
    3400 West Bayshore
    Palo Alto, California

11. B. Schaumann
    Neurology Section
    The Veterans Administration Hospital and
    The University of Minnesota
    Minneapolis, Minnesota

12. R. G. Schamschula
    The Institute of Dental Research
    The United Dental Hospital of Sydney
    Sydney, Australia

13. M. H. Seltzer
    St. Barnabas Medical Center
    Livingston, New Jersey

14. Paul Tchen
    Groupe De Richerches De Genetique Epidemiologique
    Paris, France

Project Description:

Objectives: The objective of this project is to study normal variability in man and to relate some of these established genetic markers to physiological variability, disease entities or to processes of aging. Specifically, we are investigating: (1) Dermatoglyphic variability in normal populations, families, diseases and aging. (2) Lateral functional dominance, grip strength and muscle strength and their relationship to normal anatomical asymmetry or to diseases demonstrating bilateral asymmetry in their effects. (3) Visual acuity and color vision in BLSA participants.

Methods Employed: Digital and palmar prints collected by different groups through various methods are sent to our laboratory for evaluation and interpretation of the results. We developed new methods and computer programs for studying and analyzing the dermatoglyphic data. These methods have been accepted and are utilized by other laboratories here and abroad. In order to determine lateral functional dominance the participants were requested to perform seventeen tasks. Two of these relate to single hand involvement; two tests related to single hand fine manipulation; three tasks involved fine function of one hand with significant assistance from the subordinate hand; three tasks are single handed activities which involve whole body movement. In addition to these handedness tests, the participants were tested for foot dominance, eye dominance (Dolman Test), digital interlocking, arm folding and foot overlapping. At the completion of these tests the participant is asked whether he considers himself/herself as left handed, right handed or ambidextrous. Many participants were tested in more than one visit to determine repeatability of the tests.

Vision tests are administered with a Titmus optical vision tester which simulates vision at both 20 feet and 14 inches. Acuity is measured with both Sloan letters and Landolt Rings. Depth perception and vertical and lateral phorias are assessed. The subject is tested with glasses, if he or she wears them as well as without glasses. Color discrimination is tested with the American Optical Pseudo Isochromatic Plates.

Major Findings: Patients with breast cancer have significantly higher frequencies of whorls on their fingers than either normal controls or individuals classified as "high risk" for breast cancer because of positive family history, nulliparity or severe fibrocystic mastopathy. The frequency of digital whorls in the "high risk" group fell between that of the patients and controls.

There were no significant dermatoglyphic differences between ALS or PD patients and controls.

Dermatoglyphic variables were not associated with endenic cretinism (New Guinea).

Accurate determinations of hand dominance may be made by simply testing for hand writing or by asking the participants if they are left or right handed. Including a battery of lateral dominance tests may add very little information to the overall determination of handedness.

Frequency of left hand dominance (of all forms) is higher in male than female BLSA participants. Almost twice as high.

Younger male and female participants (under the age of 40 years) have significantly higher frequency of left hand dominance (of all forms) than participants of age groups 40-59 or 60 years and older.

Lateral functional dominance and grip strength are related to bilateral differences in bone size and mass and osteoarthritis.

Significance to Biomedical Research and the Program of the Institute: Dermatoglyphics are valuable genetic markers utilized in investigating the etiology of clinical entities. Dermatoglyphics are genetically determined, mostly through multiple genes. They are nevertheless vulnerable to intra-uterine disturbances up to the time of their final development. Once development is completed between the 3rd and 4th month of pregnancy, dermatoglyphics remained unchanged (except for size) throughout the individual's life time. Dermatoglyphics, therefore, reflect effects of genetic anomalies, diseases or intra-uterine disturbances, affecting the embryo during the first trimester of pregnancy. A final advantage in using dermatoglyphics in research is their uniqueness. That is, no two individuals (including identical twins) have identical dermatoglyphics.

Handedness and lateral dominance in general are easily identifiable manifestations of cerebral dominance. The results of the lateral dominance tests provide additional genetic polymorphic data, contribute to our efforts to understand normal anatomical asymmetry and may offer clues into the etiology of anomalies demonstrating bilateral asymmetry or unilateral occurrence.

Proposed Course: To continue the evaluation, analysis and publication of results of collected data. Expand collection of data on dermatoglyphics and breast cancer to confirm earlier results. Initiate study on possible relationship between dermatoglyphics and Alzheimer's Disease. Analyze data on bilateral differences in muscle strength and grip strength and relate them to lateral functional dominance. Explain the phenomenon of decreasing frequencies of left handed at older ages (selection or just change of habits). Study possible associations between lateral functional dominance and other normal variables or diseases.

Publications:

Seltzer, M.H., Plato, C.C., Engler, P.E. and Fletcher, H.S.: Digital and palmar dermatoglyphics and breast cancer. Breast Cancer Research and Treatment. 2:261-265, 1982.

Larrick, J.W., Plato, C.C., and Hornabrook, R.W.: Studies of endemic cretinism in Papua New Guinea: Digital and palmar dermatoglyphic patterns. Am. J. Phys. Anthrop. 61:205-210, 1983.

Wertelecki, W. and Plato, C.C.: Dermatoglyphics and Clinical Genetics. Karyogram. 9:22-26, 1983.

Plato C.C., Fox K.M. and Garruto, R.M.: Measures of Lateral Dominance. I Handedness. Human Biology. (In press).

Ahuja, J., Plato, C.C., Igbad, M.A. and Sahay, B.K.: Dermatoglyphics of Diabetes: Revisited. In Recent Advances in Human Biology. Vol. 2. Proceedings of the International Symposium on Dermatoglyphics. Today & Tommorow's Printers and Publishers. New Delhi. (in press).

Plato C.C.: The Worldwide Distribution of Dermatoglyphics. In Recent Advances in Human Biology. Volume 2. Proceedings of the International Symposium on Dermatoglyphics. Today & Tommorrows Printers and Publishers, New Delhi. (in press).

Larrick, J.W. and Plato C.C.: Digital and Palmar Dermatoglyphics patterns among natives of extreme Northwest Nepal. Human Heredity. (in press).

Plato, C.C., Garruto, R.M. and Gajdusek, D.C.: Further Studies on the Genetics of the Chamorros of Guam: Dermatoglyphics. Human Heredity. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00022-07 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Investigations of Osteoarthritis and Bone Loss

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

C.C. Plato Sr. Research Geneticist CPB NIA

COOPERATING UNITS *(if any)*

Laboratory of Central Nervous System Studies, NINCDS

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.80	0.95

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Osteoarthritis and bone loss are the two principal age related changes of the human skeleton. Even though these changes are considered inherent to aging, they may result in incapacitating ailments. The advanced cases of osteoarthritis (degenerative joint disease) produce severe restrictions of movement associated with pain. Advanced bone loss may result in osteoporosis and frequent bone fractures. Most prominent are vertebral compression fractures and fractures of the femoral neck. The following skeletal sites are involved in the present study: hand-wrist, ulna and radius and vertebral column. This project deals with the epidemiological, genetic and longitudinal aspects of osteoarthritis and bone loss among (1) the participants of the Baltimore Longitudinal Study, (2) in a sample of normal Guamanians (Chamorros), (3) among patients afflicted with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam.

Other Investigators:

J.D. Tobin	Chief, Human Performance Section	CPB NIA
D.C. Gajdusek	Chief, Laboratory of Central Nervous Systems Studies	CNS NINCDS
R.M. Garruto	Sr. Staff Associate	CNS NINCDS
W.W. Greulich	Department of Anatomy, Stanford U.	
R.T. Yanagihara	NINCDS Research Center, Guam	NINCDS
F.E. Purifoy	Staff Fellow	CPB NIA
K.M. Fox	Biologist	CPB NIA
S. Kimura	Guest Scientist	CPB NIA
K.M. Chen	NINCDS Research Center Guam	NINCDS

Project Description:

Objectives: This project deals with the epidemiological, genetic and longitudinal aspects of bone loss and osteoarthritis, the two principal age related changes of the human skeleton. Our specific objectives are: (1) To investigate, through cross-sectional and longitudinal data, the age of onset and rate of progression of osteoarthritis and bone loss at different parts of the skeletal system (hand-wrist, ulna, radius and vertebral column) of male and female BLSA participants. (2) Through familial data (sib pairs and parent offspring) to ascertain the possible genetic involvement in the etiology and progress of these diseases. (3) To study the two bone diseases in relation to normal body functions and other diseases or anomalies. (4) To compare the age of onset and rate of progression of bone loss and osteoarthritis of BLSA participants to those of other populations. (5) To ascertain the possible effects nutrition, physical activity, occupational variables and socioeconomic factors on the occurrence and severity of these diseases. (6) Bilateral asymmetry in bone weight and size of the mandible, fore limbs and hind limbs of rats. (7) Effects of physical stress on bone size and weight in rats.

Methods Employed: (A) Radiographs: Bilateral radiographs of the hand and wrist from BLSA participants, from Guamanian patients affected with Amyotrophic Lateral Sclerosis (ALS) or Parkinsonism Dementia (PD), and from non-affected adult Guamanians were evaluated for osteoarthritis and bone mineral content. All radiographs were obtained using similar film, at exposure of 1.0 second at 100 MA and 60 KVP without intensifying screens. Osteoarthritis grading of proximal, distal and metacarpophalangeal joints is carried out utilizing the internationally accepted grading system of Kellgren. Bone measurements, total width, medullary width and length, were done at the midshaft of the second metacarpal directly from the radiographs using vernier caliper. Cortical thickness was derived by subtracting medullary from the total width. (B) Single Photon Absorpiometry: Direct bone density evaluations are obtained from the distal part of the ulna and radius using Norland Bone Densitometer. (C) Dual Photon Absorpiometry: In vivo measurement by bone density and estimation of bone loss in the axial skeleton using the spine scanner developed by Lunar Radiation Corporation.

Major Findings: (A) Male and female BLSA participants loose bone with age, but bone loss in females is further accelerated after the fifth decade of life, suggesting an interaction between female climacteric and the process causing bone resorption at the endosteal surface. (B) Male and female Guamanians in general showed similar trends in bone loss as did the BLSA participants. However, the Baltimore group have earlier age of onset and higher rate of bone loss than the Guamanians. The reasons for these differences are presently being investigated. (C) Both bone measurements of the second metacarpal bone and osteoarthritis evaluations from hand-wrist X-rays demonstrated bilateral asymmetry in their expression. Regardless of population, sex, handedness or age group, right hands tend to have larger and longer second metacarpals than left hands. Differential stress excerted by the muscle on the bone, during growth years, due to hand dominance, will increase this bilateral difference among right handed and reduce it among left handed. (D) Guamanian patients with Amyotrophic Lateral Sclerosis (ALS), compared to normal Guamanian controls and patients with Parkinsonism Dementia (PD) showed striking reduction in cortical bone mass. Furthermore, significant negative correlation between percent cortical area of the second metacarpal and muscle strength and atrophy of the hands of patients suggested that the degree of bone demineralization was due primarily to disuse. (E) Bone measurement data from hand-wrist X-rays and height and weight data collected in 1947 from Guamanian children who lived through the nutritional deprivation during the Japenese occupation of Guam during the Second World War, were compared to similar data from white, black and Mexican American children of the Ten State Nutritional Survey. The results demonstrated that the Guamanian children had smaller bone and density than the three American groups, even after adjustments were made for their smaller stature and weight. These results considered together with those of evaluations of hand-wrist X-rays taken recently from Guamanian adults suggest that the hypodense bones of Guamanians are due to nutritional as well as genetic factors.

Significance to Bio-Medical Research and the Program of the Institute: Loss of bone tissue and deterioration of the joints are significant causes of disability and death in older people. Determination of the existence and degree of disease in relatively well ambulatory people provides a unique opportunity to compare bone loss and degeneration with other characteristics of these people. Nutrient intakes, activity levels and muscle strength among other variables may be compared.

Proposed Course: To continue collection of data and further evaluate and analyze collected data for both bone loss and osteoarthritis. Specifically, (1) to carry out the longitudinal study on bone loss with age from hand X-rays collected during the past twenty years. (2) To analyze bone density data collected from photon absorpiometry measurements from ulnae and radii. (3) To study age of onset and rate of bone loss in at other sites of the skeleton measured through hand-wrist radiographs and through single photon absorpiometry. (4) Investigate possible relationships between bone density and nutrient intakes, medications, life style, occupational activity, physical activity, lateral functional and physiological hand dominance and other variables. (5) Segregation analysis of family data on bone measurements. (6) Calcium metabolism assays and measurements of serum calcium.

Publications:

Plato, C.C. and Purifoy, F.E.: Age, sex and bilateral variability in bone loss and bone measurements of the second metacarpal. Growth. 46: 100-112, 1982.

Plato, C.C., Garruto, R.M., Yanagihara, R.T., Chen, K.M., Wood, J.L., Gajdusek, D.C. and Norris, A.H.: Cortical bone loss and measurements of the second metacarpal bone. I. Comparisons between adult Guamanian Chamorros and American Caucasians. Am. J. Phys. Anthrop. 59: 461-465, 1982.

Plato, C.C. and Norris, A.H.: Bone measurements of the second metacarpal and lateral dominance. In Sidhu, L. S. (Ed.): Recent Advances in Human Biology, Volume I. Human Growth. New Delhi, India, Today & Tomorrow's Printers and Publishers, 1983, pp. 159-174.

Plato, C.C.: Studies of anthropological variables at the National Institute on Aging. Research Report. Newsletter of Association for Anthropology and Gerontology. 3(4): 3, 1982.

Plato, C.C., Greulich, W.W., Garruto, R.M. and Yanagihara, R.T.: Cortical bone loss and bone measurements of the second metacarpal bone: II. Hypodense bone in post-war Guamanian children. Am. J. Phy. Anthrop. In press.

Yanagihara, R.T., Garruto, R.M., Gajdusek, D.C., Tomita, A., Konagaya, Y., Uckikawa, U., Chen, K.M., Plato, C.C., Gibbs, C.J. Jr., and Sobue, I. Calcium and Vitamin D metabolism in Guamanian Chamorros with Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia. Annals of Neurology. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00028-07 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiological & Genetic Studies of ALS/PD Complex of Guam

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
C.C. Plato Sr. Research Geneticist CPB NIA

COOPERATING UNITS *(if any)*
C & F Research Center, NINCDS

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.40	0.20	0.20

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

The purpose of this study is: 1) to investigate the genetic and epidemiological factors contributing to the very high incidence of Amyotrophic Lateral Sclerosis and Parkinsonism Dementia (ALS/PD) on Guam; 2) to evaluate the distribution of the various estalished genetic and anthropological markers among the normal Guamanian population and compare them with those of the ALS/PD patients; and 3) to acertain the effects of immobilization due to paralysis on bone density.

Other Investigators:

D.C. Gajdusek	Chief, Laboratory of Central Nervous Systems Studies	CNS NINCDS
R.M. Garruto	Sr. Staff Associate	CNS NINCDS
R.T. Yanagihara	Research Associate	CNS NINCDS
K.M. Fox	Biologist	CPB NIA

Project Description:

Objectives: The objectives of this project are: (1) To determine if ALS and PD are familial. That is, whether relatives of patients have higher risk for developing these diseases than controls. (2) To ascertain possible genetic involvement in the etiology of ALS, PD or both. (3) To study the distributions of various established genetic and non-genetic markers in the general population of Guam and compare them to those of ALS and PD patient groups. (4) To determine the degree of bone loss and osteoarthritis present in ALS and PD patients and non-affected controls (see also project Z01 AG 00022-07 CPB).

Methods Employed: Two separate approaches were followed in the efforts to study the familial and genetic aspects of ALS and PD. They are: (A) A long term prospective study, the Plato/Krooth Patient-Control Registry Panels (Registries), initiated in February 1958 and closed in February 1963. All ALS and PD cases seen on Guam during that five year period and their living 0.5 relatives (parents, sibs and offspring) and their spouses were screened neurologically and registered into the patient registry panel. Each patient was subsequently individually matched with a non-affected control of the same sex, age and village of residence. The prospective controls were further screened so that (1) they were not related (0.5 relatives or spouses) to any of the already registered patients, controls or their 0.5 relatives, (2) none of their deceased 0.5 relatives or spouses died of ALS or PD, and (3) none of their living relatives were afflicted at the time of registration with ALS or PD. Controls and their 0.5 relatives were screened neurologically. Controls and their relatives who met these requirements were entered into the Control Registry Panel. At the time of closing the Registries in 1963, the Patient Registry Panel included 126 ALS or PD patients, 1052 living 0.5 relatives and 89 living spouses. The Control Registry Panel was composed of 126 controls, 1224 living 0.5 relatives and 102 living spouses. The plan of this prospective study has been to follow-up these non-affected relatives of both patients and controls and at some future date (5-25 years) to count the number of new ALS or PD cases (recurrent cases) among the relatives of patients and controls and compare them to the respective expected number of recurrent cases in each registry panel. Expected numbers were calculated by adding the individual risks of each individual in the Registries (based

on the average annual age, sex, specific incidence of ALS and PD among all Guamanians) according to their age, sex and number of years spent in the registry. After 25 years the Registries were restudied and a report is in preparation. (B) Genetic studies through segregation analysis of 47 sibships with both parents afffectd with ALS or PD (the largest such sample available), 110 sibships with one parent affected and 126 sibships (registry control data) with neither parent affected.

Major Findings: Preliminary results the 25 year follow-up evaluation of the ALS/PD Registries showed a significantly higher risk for disease among the patients' relatives but not among the relatives of the controls. Specifically, since the establishment of the Registries, we observed 79 new ALS or PD cases among the 0.5 relatives of the patients and 16 among their spouses. The expected numbers of new cases were 27 among the 0.5 relatives and 5 among their spouses. In the control registry panel on the other hand, during the same period, we observed 29 new cases among the 0.5 relatives of controls and 7 among their spouses. The expected numbers were 33 for the 0.5 relatives and 6 among spouses which are very similar to the observed. The fact that elevated risks for the diseases were found for both the 0.5 relatives and the spouses of the patients render further support for a significant environmental contribution, in addition to genetic involvement, to the etiology of ALS and PD on Guam. Detailed presentation of the results of this prospective study and their significance to the current concept on the etiology of ALS and PD are discussed in the manuscript presently in preparation. Related genetic studies showed no significant relationship between blood groups, imunoglobin allotypes or dermatoglyphics to the etiology of ALS and/or PD.

Significance to Bio-Medical Research and the Program of the Institute: The ultimate goal of this multidisciplinary program is not only to elucidate the etiology of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam, but also to provide a model for studying the genetic and familial aspects of other neurological diseases and dementias which are for the most part diseases of old age. It will also provide information on the effect of immobilization due to paralysis on bone mineral content (bone loss).

Proposed Course: To complete manuscript and publish the results of the 25 year follow-up of the Plato/Krooth Prospective Registry Study. To complete the segregation analysis of the sibships with both, one or neither parents affected with ALS or PD, and prepare manuscripts for publication. To further investigate relationship between bone loss and ALS or PD through evaluation of longitudinal hand-wrist X-rays as well as studies in calcium and vitamin D metabolism studies.

Publications:

Garruto, R.M., Plato, C.C., Myrionthopoulos, N.C., Schanfield, S.M. and Gajdusek, D.C.: Blood groups, immunoglobin allotypes and dermatoglyphic features in patients with Amyotrophic Lateral Sclerosis and Parkinsonism Dementia of Guam. Am. J. Med. Genet. 14: 289-298, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00241-02 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Statistical Methodology for the Analysis of BLSA Data

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Larry J. Brant — Mathematical Statistician — CPB NIA

COOPERATING UNITS *(if any)*

David B. Duncan — Mathematical Statistician (IPA) — CPB NIA
Department of Biostatistics, Johns Hopkins University

Dean S. Bross — Mathematical Statistician (IPA) — CPB NIA
~~Department of Preventive Medicine, University of Maryland~~

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	0.2

CHECK APPROPRIATE BOX(ES)

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

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

The theoretical development of statistical methodology is progressing in the areas of epidemiological models, multiple comparisons and survival analysis, each of which is applicable to longitudinal studies. The research utilizes Bayesian theory and various regression methods for prospective studies. The methodology created provides original contributions to experimental testing of the simultaneous comparison of specified effects (e.g. treatments against a control or placebo), epidemiological study of disease states, survival or failure analysis of longitudinal data and other longitudinal observations representing growth and other physical changes of humans and animals. Accomplishments in the creative use of Bayesian theory in the area of multiple comparisons will fill a void in the established statistical armamentarium.

Project Description:

Objectives: The aim of this project is to create and develop statistical methodology appropriate for the analysis of BLSA data.

Methods Employed: Empirical Bayesian methods are used to solve various classes of multiple-comparisons and related simultaneous inference problems arising in many experimental situations including longitudinal studies. In addition, regression techniques for and related to stochastic process concepts such as hazard rates are used to model and compare the survival and/or failure patterns of different groups of longitudinal study subjects.

Major Findings: The need for and the wide applicability of our Bayesian approach to various classes of simultaneous inference problems has recently been demonstrated. Also being shown is how our Bayesian approach is providing solutions which give extremely flexible (adaptive) rules or procedures for the problems they are intended to solve. Experience is showing that this new approach is applicable to a very wide class of simultaneous t testing, t interval and point estimation problems that arise in a wide variety of experimental situations. In particular, new and acceptable solutions to problems such as the multigroup problem of comparing treatments or procedures, originating from one of several groups, with one another has been completed.

The method of multiple logistic regression has been theoretically modified to give a simple and practical solution for the study of attrition among BLSA participants. For example, statistics measuring attributable risk along with their corresponding standard errors have been found for this model. Other regression techniques have been used in conjunction with probit analysis to model and study the growth and nutritional status of a population of Eskimo children. The cross-sectional and longitudinal study and presentation of this data has potential usefulness in the study of other longitudinal data like that from the BLSA.

Proposed Course: Progress is continiuing in the development of a prospective model appropriate for a life-table or survival analysis of longitudinal animal data. The major objective is to model and compare the survival patterns of various groups of mice which received different anticarcinogens after receiving a carcinogen at six weeks of age. The longitudinal nature of these data and the fact that the different groups of mice have started the experiment at different times over several years give these data several features in common with the BLSA data. The BLSA is, of course, a continuously ongoing study with many more complexities in terms of the amount and types of information collected. However, the longitudinal similarities of these mice data with the BLSA and the fewer possibilities of confounding variables, etc., make this mice data set a good population to use in the development of new methods of longitudinal survival data analysis.

After the survival distribution of the mouse data has been appropriately modeled and analyzed an important multiple comparisons problem arises. This consists not only of testing the efficacy of each of the ten anticarcinogen treatments against the control, it further includes the testing of the anticarcinogen effect on each of the five classes of cancer relative to the control. Currently, investigators with a problem of this type are recommended to use Bonferroni inqualities because of the large number of simultaneous tests involved. However, the Bonferroni approach can fail to give significant differences in common situations in which many significant decisions would seem to be intuitively justified from the overall nature of the data outcome.

Also, plans are presently being made to give special attention and treatment to the many complexities and needs of longitudinal studies. For example as previously mentioned, some preliminary work has been done towards the creation and development of statistical models needed to study the problem of attrition in the BLSA. Current plans include work with survival analysis models directed towards modeling survival so as to separate direct from indirect effects or time age changes from those mediated via a searching mechanism. One possible title for this research could be "Estimation for Self Controlling Studies." The unique feature of this would be that every subject acts as his own control. This feature is a new extension of a cross-over study in which a subject in the first part of the study is compared with his experience in the second part of the study.

Publications:

Bender, T.R., Brant, L.J., and Marrell, R.W.: Streptococcal Colonization of Children in a Developing Remote Area of the United States. In Harvald, B. and Hansen, J.P.H. (Eds.): Circumpolar Health 81: Proceedings of the 5th International Symposium on Circumpolar Health. Copenhagen, Nordic Council for Arctic Medical Research, 1982, Report Series 33, pp. 415-519.

Duncan, D.B. and Brant, L.J.: Adaptive t tests for multiple comparisons. Biometrics. 39(3): (to appear September 1983).

Duncan, D.B. and Dixon, D.O.: k-Ratio t Tests, t Intervals and Point Estimates for Multiple Comparisons. In Katz, S. and Johnson, N.L. (Eds.): Encyclopedia of Statistical Sciences, 4. New York, Wiley, July 1983.

Honor and Awards:

Dr. Brant was an invited participant to the 2nd US-USSR Symposium on Longevity sponsored by the International Research and Exchange Board in November 1983.

Drs. Duncan and Brant were invited to present the seminar "Adaptive t Tests for Multiple Comparisons" at the Department of Preventive Medicine, University of Maryland, March 15, 1983.

Drs. Duncan and Brant were invited to give the President's address for the 1983 annual meeting of the Western North American Region (WNAR) of the Biometrics Society, Humboldt State University, Arcata, California, June 15-18, 1983.

Drs. Brant and Duncan gave a presentation at the Annual Meeting of the American Statistical Society on "New Adaptive Tests for Multiple Comparisons," August 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AG 00242-02 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Female Sexuality, Menopause, and Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Frances E. Purifoy Staff Fellow CPB NIA

COOPERATING UNITS *(if any)*

Jordan D. Tobin Chief, Human Performance Section CPB NIA

Clyde E. Martin Sociologist, Human Performance Section CPB NIA

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.	.	.

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The study of age and menopause-related variability in female sexual behavior was initiated in May, 1982; data is obtained by personal interview from each female participant of the Baltimore Longitudinal Study of Aging. To date, 144 (98% of those asked to participate) interviews have been done. A two-stage analysis of data from the first 130 cases involved contingency table comparisons of a pre-menopausal group aged 20-43, a middle group aged 45-55, and a post-menopausal group aged 56-79 as well as contrasts among those aged 20-39, 40-59, and 60-79 years. Focus thus far has been on 1) Age and menopause related variability in current sexual enjoyment, satisfaction, degree of arousal, adequacy of lubrication, frequency of orgasm as well as change in these measures over each women's sexual life; and 2) Birth-Cohort Contrasts in pre-marital and marital sexual behavior and current sexual attitudes - which presumably reflect variation in the socio-cultural background of the three age groups.

Project Description:

Objectives: Major objectives include 1) To obtain initial-interview information regarding past and current sexual behavior on all currently active and newly admitted female participants of the BLSA, 2) To assess the interrelationships of interview variables with each other and birth cohort, age and menopausal status, and ultimately, 3) To analyze the relationships between inter-individual and age-related variability in measures of sexuality and other physiological, psychological and sociological variables.

Methods Employed: Data is being obtained by semi-structured personal interview conducted by the principal investigator, utilizing techniques similar to those presently used on male BLSA participants by Dr. Clyde E. Martin. Questions cover the following areas: current sexual behavior, past sexual behavior, sexual attitudes, and self-assessments of sexual interest and enjoyment as well as retrospective changes in sexuality. Responses are coded in cryptic form and kept strictly confidential.

All women who are active participants of the BLSA are interviewed. There are presently no exclusion criteria, except self-exclusion for anyone declining participation. As of August 3, 1983, 144 women had been interviewed, and 5 had declined participation.

Major Findings: In the analysis which focused on age- and menopause-related variation, retrospective data showed that 71% of the oldest group, 55% of the middle, and 13% of the youngest report a decrease with age in vaginal lubrication ranging from slight to pronounced. Of those noting a pronounced decrease, most adapted to the change by using an artificial lubricant. The majority of the oldest group also noted a decrease in sexual desire, intensity of arousal, and orgasm capacity compared to a miniority of the youngest age-group. Chi-square was significant ($p<.01$) for all four variables when compared by age-group. Despite these noted changes, however, 74% of the oldest group say they have noticed no change or even an increase in sexual satisfaction with age. When asked if menopause had affected their sexuality, 82% of the oldest group, all of whom were at least 2 years past menopause, said it had either a positive or no effect; the 26% reporting a positive effect cited a decreased worry about menstruation or birth control as the reason. In a cross-sectional analysis, only 26% of the oldest group described their current degree of arousal as "intense" compared to 68% of the youngest group. However, whether this is related to the age-associated decrease is not known. Comparisons between age-groups in the percent of time orgasm is reached showed no significant differences; the majority of each age-group report that they experience orgasm 50-100% of the time. In summary BLSA women noted a decrease in vaginal lubrication, intensity of arousal, and sexual desire which did not necessarily affect degree of sexual satisfaction. Most did

not consider these changes detrimental and had no problem adapting to them. For some, however, situational or partner-related factors have interacted with aging changes, making adaptation difficult. The relationships of these and other demographic factors to the age-related changes and variation mentioned above is currently being examined.

As would be expected from women who grew up at different times of the "sexual revolution," those aged 20-39, 40-59, and 60-79 at the time of interview vary widely in reported premarital behavior and in current sexual attitues. Preliminary findings show age-related differences in age at first petting, coitus, orgasm and masturbation, with younger women reporting earlier sexual experience in each case. Only 33% of the middle and 38% of the oldest group had coitus before marriage, compared to 88% of the youngest group. The oldest age-group was also less likely to describe sexual arousal during premarital petting experience as "intense" and, for them, premarital petting was less likely to have involved genital touching. In answer to questions regarding their attitudes (positive, negative, or neutral) toward various sexual practices, the oldest age-group gave more negative responses. In summary, the data shows age-related variations in early sexual experience and current attitudes that is most likely due to the more restrictive socio-cultural atmosphere that prevailed when the older women were growing up. A more comprehensive analysis of birth-cohort differences is currently being done, with a focus on how such experiental and cultural differences might relate to the age- and menopause-related findings discussed previously.

Significance to Biomedical Research: Although myths abound, little data now exists regarding the sexuality of older females. This investigation will provide valuable information for those in medicine and aging research on a group of well-educated healthy women on whom a great deal of information about ohter physiological and psychological information is available.

Proposed Course: To continue intitial interviews of all female participants, data reduction for computer storage, and statistical analyses.

Publications:

Purifoy, F.E.: Sex histories and the study of female sexuality and aging. Abstract accepted for presentation at the Annual Meeting of the American Anthropological Association, Chicago, Illinois, 1983.

Purifoy, F.E., Martin, C.E., and Tobin, J.D.: Age-related variation in female sexual arousal and orgasmic capacity. Abstract accepted for presentation at the Annual Meeting of the Society for the Scientific Study of Sex, Chicago, Illinois, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00093-11-CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Cellular Basis of Regulation of the Humoral Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Albert A. Nordin, Research Chemist, CPB, NIA

COOPERATING UNITS *(If any)*

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.4	2.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Investigations concerned with the cellular requirements involved in the in vitro immune responses of murine lymphoid cells constitute the broad area of interest. The participation of T-cells and their products on both the antibody response, and the cell-mediated response of murine spleen cells is being investigated by using cloned, antigen-specific T-cells and selected T-cell hybridomas. The effect of lymphokines and monokines on the activation and proliferation of purified B cells constitute another area of major interest intended to establish long term cultures of functional B-cells. Information derived from these studies is applied to similar investigations relevant to understanding the effect of age on the immune response.

Jeffrey L. Curtis	Medical Staff Fellow	CPB, NIA, left 6/17/83
Jacques Proust	Visiting Associate	CPB, NIA, EOD 10/3/83

Project Description:

Objectives: The goal of this project is to establish the role of the murine lymphoid cells and their products in the in vitro immune response and to apply these findings to the understanding of immunosenescence.

Methods Employed:

(1) The in vitro culture techniques are routine methods except that a serum-free medium in which albumin, transferrin and lipids replaces the conventionally used fetal calf serum.

(2) Purified populations of antigen specific T-cells originated from either spleen or lymph node cells of immunized C57Bl/6 mice. T-cell clones were established by the limiting dilution technique and expanded and maintained in serum-free medium supplemented with T-cell growth factor(s).

(3) Purified populations of B-cells are prepared from the spleen cells of normal nu/nu mice or from nu/nu mice injected with BCL1 tumor cells by 'panning' with affinity chromatography purified antibody of the appropriate specificity.

(4) DAGG-Ficoll is prepared by modifying Ficoll by introducing carbonyl methyl amino-ethyl groups to which is added the tri-peptide glycine-glycl-alanyl with the terminal alanine substituted with a single dinitrophenol heptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings:

The effect of non-mitogenic levels of E. coli lipopolysaccharide (LPS) on the in vitro immune response to DAGG-Ficoll was investigated by limiting dilution assay. To minimize the effect of T-cells, C57Bl/6 nu/nu spleen cells were cultured in serum free medium. These studies showed that LPS induces an increase in the number of cells responsive to DAGG-Ficoll. The LPS-induced increase in the number of antigen reactive cells can also be induced by partial purified IL-1 in the absence of LPS. This strongly suggests that the enhancing effect of LPS on the immune response is mediated by the LPS-induction of IL-1 and supports the findings that IL-1 influences B-cells as well as T-cells. Definitive studies to determine if IL-1 acts directly on B-cells requires a cloned B-cell source which has proven difficult to establish. Our attempts to establish B cell lines in vitro have shown that a soluble factor is detected in supernatants of B cell cultures as early as 48 hrs after initiation of culture which markedly suppresses the proliferation of mitogen stimulated purified B-cells. Evidence suggests that this suppressive factor is generated by B-cells and is responsible in part for the repeated failures to develop B-cell lines in vitro by many laboratories including ourselves.

The quantitative determination of cytotoxic precursor cells in the spleens of old mice has shown that all mice have significantly fewer cytotoxic precursor cells than their young counterparts. This consistent reduction in cytotoxic precursor cells did not always correlate with the cytotoxic activity detected in high cell density mixed lymphocyte cultures. This discrepancy between these two methods of assessing cytotoxicity to alloantigens is most likely due to complex cellular interactions that occur at high cell density which effect the expression of cytotoxic cells. Such cellular interactions are minimized under

the conditions of limiting dilution assays since the data strongly supports that a single cell type i.e. cytotoxic precursor cells, was limiting. These studies also showed that despite optimal amounts of IL-2 cells from 24 month old mice do not respond as well as young mice. It is now well appreciated that lymphokines/monokines other than IL-2 are necessary to generate cytotoxic lymphocytes. Our experiments along these lines show that at least two soluble factors in addition to IL-2 are required for the in vitro activation and development of cytotoxic T-cells.

Significance to Biomedical Research and the Program of the Institute:

The goal of this research program is to examine the cellular populations participating in the humoral and cellular immune response. The mechanisms by which such cells function would be of significance not only to the field of immunology but would also have relevance to cell biology. It is also significant to the area of immunosenescence. The decline in immunological responsiveness with age is well established but the reasons are not at all understood. Delineation of the cellular mechanisms involved in the immune response have and will continue to explain the observed decline in the immune response of aging individuals.

Proposed Course:

Studies concerned with the enhancing effect of IL-1 on the in vitro immune response to DAGG-Ficoll will be continued in an attempt to determine the mechanism(s) involved in the recruitment of specific antigen-reactive cells. Continued attempts to propagate B-cell lines will center on regulating the suppressive factor and on developing a reliable tissue culture system that will support long term in vitro B-cell cultures.

The effect of age on the cell-mediated immune response as assessed by the quantitative estimate of the number of cytotoxic precursor cells will be continued to determine if lymphokines or monokines, other than IL-2, which control the in vitro expression of cytotoxic T-cells are regulating the response of spleen cells from aged mice. Attempts will also be made to identify these biologically active factors and to investigate their effects on the B-cell response of aged mice.

Publications:

Nordin, A. A. and Schreier, M.: T cell control of the antibody response to the T-independent antigen, DAGG-Ficoll. J. Immunol. 29, 557-562, 1982.

Nordin, A. A. and Collins, G. D.: Limiting dilution analysis of alloreactive cytotoxic precursor cells in aging mice. J. Immunol. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00095-10-CPB

PERIOD COVERED

October 1, 1982 - September 30, 1983

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

The Role of Cell Membrane Structures on Cellular Recognition

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

W. H. Adler, Medical Officer, PHS, CPB,NIA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.35	2.7	.65

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The project will define the functional capability of lymphocyte subpopulations in young and aging mice. Assays for functional ability and techniques for cellular manipulation allow for correlation of type of cell with particular function. The use of cell sorting apparatus will facilitate separation of subpopulations of cells in lymphoid tissue. The analysis of cellular function in aging animals will delineate the basis of the age associated decline in immune function.

PHS 6040 (Rev. 1/83)

GPO 895-100

		ZO1-AG-00095-10-CPB
J. E. Nagel	Medical Officer, PHS	CPB, NIA
R. K. Saxena	Visiting Associate	CPB, NIA
Q. B. Saxena	Visiting Associate	CPB, NIA
J. Curtis	Medical Staff Fellow	CPB, NIA left 7/83
M. A. Brock	Biologist	CPB, NIA

Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable subpopulations of immunologically active cells. The functional criteria and results will be compared to arrive at correlations in order to develop methods for diagnosing and describing immune deficiency and assigning certain predictive projections of immune function. Control mechanisms whereby T cell helper and suppressor subsets will be determined as part of the investigation of age-related changes in immune function. The test of immune functions will include responses to mitogens and antigens in in vitro culture conditions, responses to antigens and tumor cells in vitro and in vivo, and the development of immunologically competent cells in in vitro environments. These studies will give a better understanding of age-related immunodeficiency.

Methods Employed: The basis of most functional assays will be in vitro culture systems. These will be both short term for the investigation of mitogen, antigen and tumor cell killing responsiveness and longer time for the generation of cytotoxic lymphocytes and antibody-forming cell colonies. In vivo methods will primarily be cell transfer studies with syngeneic cells and tumor cells. The cells used will be from various mouse lymphoid organs from varying aged donors. The cells will be treated by physical separation of methods and specific antisera to eliminate certain populations, or to quantitate various populations in their overall representation in lymphoid tissue. The use of laser activated Cytofluorograf Cell Sorter with appropriate antisera will allow separation of distinct cell populations. The sorter would be used to define the cell types in lymphoid tissue, outline the changes that occur with age, and to selectively isolate specific subpopulations.

Major Findings: Using the mouse spleen natural killer cell activity as a model the effects of diet, age, and control mechanisms, it has been shown that a T cell produced factor, IL-2, can augment the spleen NK cell activity in young and old mice. This augmentation effect of IL-2 is primarily due to the development of functional NK cells from a precursor population in the spleen. There are less precursor cells in the spleens from old mice so that there is less ability to augment NK cell function in the old mice. The ability of individual NK cells whether from old or young mice, to kill the tumor target cells is exactly the same. The differences in levels of performance of spleen cells from old and young mice in NK assay systems can be explained by a) different numbers of functional NK cells, more being present in the spleens of young mice, b) different numbers of precursor NK cells which can be induced to develop into functional NK cells, less precursors being present in the spleens from old mice, and c) less inducer substance, IL-2, is able to be produced by spleen cells from old mice, which represents another piece of evidence of a T cell deficiency associated with aging.

Since various factors can change in an age associated manner in their representation in the diet, the section continues to be concerned with the effect of vitamins and proteins on immune function. Vitamin deficient mice show a loss of NK cell function, a loss of cytotoxic T cell function and a loss of T cell factor, IL-2, production. Reinstitution of these vitamins in the mouse diet results in a complete recovery to normal levels of function. The effect of protein deficiency is somewhat different than seen with a vitamin deficiency. In protein deficient mice the NK cell function remains the same on a per cell basis as compared to

normally fed mice. However, if judged on a per spleen basis the level of NK function in deficient mice is only 60% of the level found in normal mice. IL-2 production remained the same in both groups of mice. These studies will help to determine the effects of age and environmental factors on levels of immune function and whether dietary factors can increase immune function in age.

The projects concerned with cyclosporin effects on T cells have concluded. The findings were that cyclosporins not only could disrupt function of normal T cells but could kill any tumor which carried a T cell marker i.e. T cell leukemia tumors. It was ineffective on B cell or monocyte tumors. In vivo cyclosporin treatment of mice carrying T cell tumors resulted in long term survival. The mechanism of the specific effects of cyclosporin on T cells is being pursued by other laboratories.

Significance to Biomedical Research and the Program of the Institute: If the immunodeficiency of aging operates along the same lines as other physiologic conditions then a common mechanism may be reversible through rather simple measures. The T cell control data and the changes in T cells due to protein, vitamin deficiency and cyclosporin administration may mimic those changes that are seen with aging. Furthermore the control mechanisms by which the T cell populations can influence other physiologically active immunocompetent cells will be studied in future investigations.

Proposed Course: To outline the connection between morphology and function and to expand the technical ability to measure the function of immunocompetent lymphocytes and to develop methods and procedures by which the functional ability of these cells can be boosted and the interactions between different subsets of lymphocytes can be determined. Cell free active factors will be a primary focal point in these investigations.

Publications:

Saxena, R.K., Saxena, Q.B., and Adler, W.H.: Identity of effector cells participating in the reverse antibody dependent cell mediated cytotoxicity. Immunology, 46: 459-464, 1982.

Saxena, R.K.: Augmentation of mouse natural killer activity by alloantibody: A reverse ADCC reaction. In Herberman, R.B. (Ed.): Natural Cell Mediated Immunity. New York, Academic Press, 1982, pp. 449-454.

Saxena, Q.B., Saxena, R.K., and Adler, W.H.: Ethanol and natural killer activity. In Herberman, R.B. (Ed.): Natural Cell Mediated Immunity. New York, Academic Press, 1982, pp. 651-656.

Saxena, R.K., Saxena, Q.B., and Adler, W.H.: Effect of anti-HLA and anti-Beta-2 microglobulin antisera on the natural cytotoxic activity of human NK cells and monocytes. In Herberman, R.B. (Ed.): Natural Cell Mediated Immunity. New York, Academic Press, 1982, pp. 251-256.

Saxena, R.K., Saxena, Q.B., and Adler, W.H.: Decline of murine natural killer activity in response to starvation, hypophysectomy, tumor growth and beige mutation: A comparative study. In Herberman, R.B. (Ed.): Natural Cell Mediated Immunity. New York, Academic Press, 1982, pp.645-650.

Saxena, R.K., Saxena, Q.B., Collins, G.D., and Adler W.H.: Augmentation of spleen natural killer activity in mice treated with interleukin-2 preparation. Indian J. Exp. Biol. 21: 54-58, 1983.

Yanagihara, R.H., and Adler, W.H.: Direct antiproliferative effects of cyclosporin A on murine lymphoreticular tumor cells in culture. J. Biol. Response Modifiers 2: 121-132, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00096-10-CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Low Temperature Effects on Cells of Aging Individuals

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Brock, M. A., Research Biologist, CPB, NIA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of this project are to characterize age-related differences in the in vitro responses of murine lymphohemopoietic cells to mitogens, circannual rhythmicity in their functional responses to mitogens and their differential susceptibility to freezing injury. Because there are optimal cooling velocities for the cryopreservation of each cell type, T- and B-lymphocytes from young animals can be recovered with slight impairment of function using an optimal cooling rate. Changes in cellular structure and/or function with age then may be distinguishable with selective freezing injury to one or both subpopulations. Structures considered the probable sites of freezing damage are the plasma membrane and cellular membrane systems.

PHS 6040 (Rev. 1/83) GPO 895-100

W. H. Adler Medical Officer, PHS CPB, NIA

Project Description:

Objectives: To characterize the functional capacity and structure of pre- and post-mitotic cell types from aging individuals, specifically the possible age-related differences in the circannual rhythmicity of in vitro functional responses to mitogens and in susceptibility of murine and human immunocompetent cells to freezing injury. The sites of injury may be characterized with in vitro tests for structural and/or functional impairment.

Methods Employed: Cell suspensions were prepared from spleens of C57BL/6 mice. The percentage of viable lymphocytes in the unfrozen suspensions and in those that had been cryopreserved was determined using the stains, fluorescein diacetate and ethidium bromide, to identify viable and nonviable cells, respectively. Suspensions of the lymphocytes were adjusted to 15 or 30 x 10^6 cells/ml in media (RPMI-1640) containing 10% DMSO and 10% fetal calf serum. They were cooled in glass bottles at rates ranging from -0.25° to -10.0° C/min. to the low limit of -50° C using the microprocessor-controlled cooling system developed in this laboratory. After storage in the vapor phase of liquid N_2 (-196° C), the cell suspensions were rapidly thawed and the DMSO diluted before culture in vitro. The incorporation of ^{3}H-thymidine into DNA was used to assess the mitotic activity of the cells after in vitro activation of T-lymphocytes by the mitogens, phyto hemagglutinin (PHA) and Concanavalin A (Con A), and B-lymphocytes by lipopolysaccharide (LPS).

Major Findings: The viability and in vitro function of mouse splenic lymphocytes were assessed in cell suspensions from young (4-6-mo.-old), older (15-mo.-old) and senescent (23-27 mo. old) animals. Determinations were made on freshly prepared cell suspensions and on suspensions which had been cryopreserved at different rates, stored in the vapor phase of LN_2, and thawed.

Data showing rhythmicity in the functional capacity of lymphocytes from 15-mo.-old and senescent mice have been collected each month since July 1981 and compared with the previously reported activity of lymphocytes from young mice. The animals were all housed in constant environments in the absence of known entraining agents which could synchronize biological rhythms. Thus, any observed rhythms may be considered to be endogenous and similar to the circannual rhythms in other functions reported for other species. In earlier studies, in vitro functions of cells from young mice, as measured by lymphocytic responses to T- and B-cell mitogens, exhibited seasonal rhythmicity, declining to their lowest levels in December-February and then spontaneously rising to peak levels in March-April. The differences between the peak and trough values were 2-5 fold; statistical analyses highly significant correlations between paired data series. Marked differences in circannual rhythmicity of both T and B lymphocyte functions were exhibited by cells from 15 mo old and senescent mice. The amplitude of the rhythmic response of T cells to PHA was similar in young and 15-mo.-old mice but was reduced in senescent mice to 17.5% of the value for young mice (all calculations from peak levels during the first year of observations on senescent mice). The amplitude of the T cell responses to Con A was reduced in 15 mo. old mice and further depressed in senescent mice to 41% of the level for young animals. The periods (trough to trough intervals) increased with age from 12 months to 13 months in the 15-mo.-old and senescent mice. The amplitude of the rhythmic B cell responses to LPS was unchanged with age, however the periods increased from 10 months in young to 14 months in 15-mo.-old mice and to 15 months in senescent mice. These results show different effects of age on the circannual rhythmicity in the function of

B lymphocytes and of two subpopulations of T lymphocytes. Since the periods increase with age, the rhythms are not in synchrony with each other and, for the older mice, they are not in synchrony with the calendar year. Because of these changes, age-related differences in T and B lymphocyte function are not detectable during certain seasons. Furthermore, the standard errors of the means for each month are similar for all the in vitro tests for the three age groups; the "variability" reported by others is likely due to sampling during different seasons of the year.

The implications of these results include:

1. Consideration of seasonal modifications of immunotherapy if similar rhythmicity exists in humans;
2. Consideration of the seasonal differences in lymphocyte responsiveness when comparisons are made of individuals of different ages.

These data are consistent with the hypothesis that timing mechanisms are altered with age and may accompany deleterious age-associated physiological changes. The effects of cryopreservation stress on the functional recovery of lymphocytes were modified by seasons of the year and age of the donor animals. In the data analyzed to date, the rhythmic changes in recovery of cells from young mice reflect the seasonal rhythmicity exhibited by unfrozen cells. The percentage of viable, cryopreserved cells cooled at -1^{o} C/min recovered in suspensions of lymphocytes from young mice declined during the autumn months and reached its lowest point in January ($\bar{x}$ = 50%). It then rose and remained high ($\bar{x}$ = 80%) during the spring. Interestingly, the recovery of viable, cryopreserved cells from 15-mo.-old and senescent mice was uniform throughout the year at a level similar to the lowest recoveries of cells from young mice during August through January. Functional recovery of cryopreserved T and B lymphocytes from the two older age groups differed markedly from that of cells from young mice. In the latter, recovery of in vitro function of both T and B cells rose in January from October low levels and then remained high (80-100%) through the spring and summer. This pattern is similar to that for the recovery of viable, cryopreserved cells, but is phase advanced. The functional recovery of lymphocytes from both older groups of mice was reduced when compared to the young animals, and like the data on recovery of viable cells exhibited no seasonal rhythmicity. A more detailed analysis of the recoveries using cooling rates ranging from -0.5^{o} to 5.0^{o} C/min was made during the season of highest activity of unfrozen cells (March through July). Optimal recovery of viable cells was achieved with a cooling rate of -1.0^{o} C/min and declined from 78.4% using spleen cells from young mice to 59.5% for cells from senescent mice. Interestingly, the optimal recovery of functional lymphocytes preserved was with a different cooling rate, -2.5^{o} C/min. The maximum response of cryopreserved T cells to PHA was unimpaired with age, however the T cell response to Con A declined from 98% to 45% of unfrozen cells in cryopreserved lymphocytes from young and senescent mice, respectively. Activation of B cells by LPS was reduced from 98% to 66% of unfrozen cells in the young and senescent cryopreserved lymphocytes. Lymphocytes cryopreserved using several cooling rates during other seasons of the year will be assayed in the future. It appears that both the age of the animals and season of the year must be considered in assessing the efficiency of a lymphocyte cryopreservation system. Moreover, cells from older mice are modified, perhaps in ways involving the plasma membrane or cytoskeleton, so that recovery of T and B cell function differs from that of young mice.

Significance to Biomedical Research and the Program of the Institute: The reported decline in the functional capacity of lymphocytes with age may be intrinsic and/or extrinsic. These possibilities can be tested by modifying components in an in vitro system which tests functional capacity and by assessing the effects of freeze-thaw damage on lymphocytic biomembrane systems. Controlled rate cooling is a new technique that may be used to preserve lymphocytes for further study at the cellular level or for therapeutic use.

Proposed Course: Changes with age in lymphocytic resistance to the stress of cryopreservation will be continued using controlled rate cooling techniques. The characterization of age-related cooling injury to mammalian lymphoid cells will include further studies on splenic lymphocytic responses to mitogens, particularly the cytoskeleton and biomembrane systems involved in activation of the cells.

Publications:

Brock, Mary Anne: Seasonal rhythmicity in lymphocyte blastogenic response of mice persists in a constant environment. J. Immunol. 130: 2586-2588, 1983.

Brock, Mary Anne: Senescence in Campanularia flexuosa and other cnidarians. In Mitchell, D. and Johnson, T. (Eds.): Selected Invertebrate Models in Aging Research. Boca Raton, FL, CRC Press (in press).

Brock, M.A., and Adler, W.H.: Aging alters circannual rhythmicity of lymphocyte blastogenic responses of mice. The Gerontolgist (in press).

Brock, M.A., and Adler, W.H.,: Cryopreservation impairs the recovery of viable, functional splenic lymphocytes from senescent mice. Cryobiology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AG-00104-07-CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Clinical Immune Survey of the Longitudinal Project Participants

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

W. H. Adler, Medical Officer, PHS, CPB, NIA

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.05	1.9	2.15

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The immune function of participants in the Baltimore Longitudinal Study of Aging is evaluated to determine age-associated changes and the possible effects of these changes on the incidence and type of disease. Another major goal is to evaluate the ability of existing assays of immune function to provide an accurate assessment of the level of immune competence of aging individuals and to refine and develop new methods and assays to evaluate host defense mechanisms.

PHS 6040 (Rev. 1/83)

GPO 895-100

Albert A. Nordin	Research Chemist	CPB, NIA
James E. Nagel	Medical Officer, PHS	CPB, NIA
Jeffrey L. Curtis	Medical Staff Fellow	CPB, NIA, left 6/17/83
Bradley Bender	Medical Staff Fellow	CPB, NIA, EOD 6/26/83

Project Description:

Objectives: The aim of this project is to examine the morphological and functional changes of the human immune system that occur in relation to maturation and aging. Following the analysis of a large amount of previously collected data regarding the immune function of participants in the Baltimore Longitudinal Study of Aging (BLSA), it became apparent that a better understanding of normal was necessary prior to addressing questions regarding the possible presence and significance of any age-associated changes in immune function. Rather than continue to survey the immune function of large numbers of BLSA participants, it was decided to concentrate research efforts on more intensive investigations of individuals who had previously been noted to have altered immune function. Additionally, to gain perspective regarding the effects that development and illness have on immune function, a number of selected individuals, outside the BLSA, were studied. Specifically, the types of studies being performed are as follows:

1. Assessment of T helper cell function in BLSA participants previously identified to poorly synthesize Ig *in vitro*.

2. Evaluation of the production and utilization of the lymphokine IL-2 and the monokine IL-1 by individuals of various ages.

3. Examination of monocyte T-helper cell function as related to age.

4. Assessment of maturational level of T lymphocytes from aged adults by simultaneous identification of two or more T cell markers on the same cell.

5. Initiate a program to establish the HLA-A,B,C and D haplotypes of BLSA participants.

6. Examination of the effect of renal disease on natural killer (NK) cell function.

7. Evaluation of immune function in severely burned patients.

8. Assessment of the acquistion of T cell subsets by infants.

9. Evaluation of immune function is a variety of clinically defined immunodeficiency diseases.

10. Begin a prospective project to study the immune function of clinically normal individuals in low socioeconomic groups ("street people").

11. Continue and expand studies assessing age related changes in granulocyte function, in both healthy and sick individuals.

12. Analysis of BLSA immune function data specifically looking for chronobiological changes.

Methods Employed:

1. Cell Preparations
   a. All studies are being conducted in vitro. Cells are isolated by Ficoll/Hypaque or Percoll density gradient centrifugation, or electronic cell sorting, with or without the use of monoclonal antibodies.

2. Assay Systems:
   Standard techniques for the in vitro studies of immune function will be utilized. In addition:
   a. A laser activated flow cytometry system will be used in conjunction with monoclonal antibodies to study various cell subsets and to separate specific cell populations by electronic cell sorting.
   b. In vitro antibody synthesis will be quantitated using an enzyme linked immunoabsorbent assay (ELISA).
   c. IL-2 will be measured using a IL-2 dependent CTL line and IL-1 with a thymocyte assay.
   d. Tissue typing will utilize a standard cytotoxic method.

Major Findings: Until 1982, this project was conducted as a survey to determine the immunologic competency of large numbers of longitudinal study participants. Begining in FY 82, this single focus for the Sections clinical investigations was de-emphasized in favor of a more intensive and detailed examination of specific areas of immune function. Several elements of this project such as the histocompatibility typing, T helper cell and monocyte function assays continue to be limited to the study of BLSA subjects.

The findings are:

1. The total number of peripheral blood T lymphocytes identified by "pan" T cell monoclonal antibodies decrease with age. The percentage of helper cells remain constant, while suppressor cells decrease slightly.

2. A pokeweed mitogen driven assay of in vitro ability to produce immunoglobulin indicates that poor responders (mostly individuals greater than 60 years of age) have deficient T helper cell function in spite of normal representation.

3. IL-2 production of T helper cells by aged individuals is diminished. IL-1 production by human monocytes is being evaluated.

4. Preliminary studies indicate that the number of monocytes present may be critical in the evaluation of T helper cell function. Defective in vitro helper function can be boosted with increased numbers of monocytes. This may relate to an increase in IL-2 production in the presence of monocytes.

5. Dual marker studies of human T cells have been initiated. The findings to date indicate no marked age related change, although only a small number of individuals have been studied. Progress on this project is expected to significantly to accelerate with the arrival of an automated data processing system to interface with the laser flow cytometry system.

6. Tissue typing has now been performed on approximately 50 BLSA subjects.

7. Levels of natural killer cell function were variable in a group of 26 patients undergoing renal dialysis. Approximately 50% had normal numbers of Leu-7$^+$ cells and normal NK function, while the remainder had abnormalities in either or both of these parameters. Abnormality of function or cell number was not related to creatinine or urea nitrogen levels, or to length of dialysis. This group of patients is currently undergoing restudy to determine the stability of these findings and to determine if clinical condition is related to NK function.

8. Percentages of T cell subsets in the peripheral blood of infants are similar to adults. The method of cell separation is critical, since conventional cell separation yields considerable contamination by red cells. Some specimens were found to contain large numbers of cells with markers for more than one functional T cell subset. The significance of this finding is being further investigated.

9. In conjunction with clinicians at The Johns Hopkins Hospital approximately 50 individuals with clinical immunodeficiency have been investigated. Results have been thoroughly heterogeneous. Work is now underway to correlate the clinical diagnosis and laboratory findings.

10. Several other projects, including the study of burn patients and "street people" have only recently been initiated and to date no findings are yet available.

Significance to Biomedical Research and the Program of the Institute: These studies, along with other information on BLSA participants, will yield valuable information regarding age-associated diseases and immune dysfunction. With an enhanced understanding of the mechanisms underlying normal and abnormal immune function, future research can be targeted to systems that are especially vulnerable to age related changes. Specific therapy can be designed to augment the immune function.

Proposed Course: A baseline of data on BLSA participants has been established. The significance of these results will continue to be evaluated in light of new information about immune function. To establish the possible effects of factors other than age on immune function selected groups of individuals, other than BLSA subjects, will continue to be studied. The application of new technologies, such as laser cytometry, monoclonal antibodies, and electronic cell sorting to questions about age associated changes in immune function will result in better capability to examine subtle effects on immune function. We will continue to restudy BLSA participants to determine stability of immune function.

Publications:

Nagel,J.E., Chrest, F.J., and Adler,W.H.: Mitogenic activity of 12-O-tetradecanoyl phorbol-13-acetate on peripheral blood lymphocytes from young and aged adults. Clin. Exp. Immunol. 49: 217-224, 1982.

Nagel, J.E., Pyle, R.S., Chrest, F.J., and Adler, W.H.: Oxidative metabolism and bactericidal capacity of polymorphonuclear leukocytes from normal young and aged adults. J. Gerontol. 37: 529-534, 1982.

Johnson, J.P., Yolken, R.H., Goodman, D., Winkelstein, J.A., and Nagel, J.E.: Prolonged excretion of group A coxsackievirus in an infant with agammaglobulinemia. J. Infect. Dis. 146: 712, 1982.

Nagel, J.E.: Immunology. In Rothstein, M. (Ed.): Review of Biological Research in Aging. New York, Alan Liss, 1983, pp. 103.

Nagel, J.E., Yanagihara, R., and Adler, W.H.: Cells of Immune Function. In Cristofalo, V.J. (Ed.): Cell Biology. Boca Raton, FL, CRC Press, Vol. 2 (in press).

Nagel, J.E., Chrest, F.J., Pyle, R.S., and Adler, W.H.: Monoclonal antibody analysis of T-lymphocyte subsets in young and aged adults. Immunol. Comm. 12: 223-237, 1983.

Chrest, F.J., Nagel, J.E., Pyle, R.S., and Adler, W.H.: Human B cell function in reponder and nonresponder individuals. II. The role of T helper cells in promoting the PWM induced B cell production of immunoprotein. Clin. Exp. Immunol. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00010-10 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Noninvasive Assessment of the Left Ventricle in Normal Man

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. L. Fleg Staff Cardiologist CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.03	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

To determine the chest x-ray findings attributable to normal aging, we evaluated standard postero-anterior chest x-rays taken at least 10 years apart in 67 healthy men initially ages 23-76 years. The mean aortia knob diameter increased from 3.4 ± 0.6 cm to 3.8 ± 0.5 cm, and mean cardiothoracic ratio (CTR) increased from .41 ± .04 to .43 ± .04. Pulmonary abnormalities on initial chest x-ray consisted mainly of hyperinflation (27%) and increased markings (19%), both of which doubled in prevalence during follow-up. Chronic obstructive lung disease was suggested in 15% of the initial films and 21% of the final films despite the absence of clinical or spirometric abnormalities.

To define the prevalence of coronary artery disease (CAD), both overt and latent, in a free-living population, we have performed exercise thallium scintigraphy in collaboration with the Johns Hopkins University, Division of Cardiology in approximately 450 individuals from the BLSA. Preliminary analysis of these data has shown an age-related increase in the prevalence of both overt and latent CAD. We have also found that the incidence of subsequent coronary events (angina, myocardial infarction or cardiac death) in asymptomatic subjects is strikingly high in the subset whose thallium scan and exercise ECG are both abnormal.

We have also used thallium scintigraphy and exercise ECGs to define a group of men and women ostensibly free from CAD in whom left ventricular function has been measured during maximal bicycle exercise by radionuclide angiography (MUGA), also in conjunction with Johns Hopkins. In these carefully selected subjects, maximal cardiac output did not decline with age--contrary to the body of literature in less intensively screened individuals. Nevertheless, the methods of achieving maximal cardiac output were found to differ with age, the young attaining a more rapid heart rate and more complete systolic emptying, whereas the elderly depended more upon the Frank-Starling mechanism, i.e. dilatation of the heart during the filling period.

IRP/CPB-265

Other Professional Personnel:

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Project Description:

Objectives: Despite the introduction of many new cardiac imaging techniques in the last decade, the standard chest roentgenogram remains the most common diagnostic tool to evaluate cardiac anatomy. It is therefore surprising that there has been little systematic evaluation of what constitutes the normal changes of aging on this examination. The few such studies which have been performed are subject to criticism from being cross-sectional and/or failing to screen their subjects for latent cardiopulmonary disease.

Methods: Postero-anterior chest x-ray (CXR) taken at least 10 years from 67 men carefully selected for the absence of cardiovascular or pulmonary disease were analyzed in a blinded fashion by two radiologists. These men had to fulfill the following criteria throughout the study: absence of systemic disease by history, physical exam and routine laboratory tests, normal resting ECG and treadmill exercise test, normal pulmonary function tests.

Major Findings: In these healthy men over a mean follow-up period of 16.9 years, the cardiothoracic ratio(CTR) increased from .405 $\pm$ .04 to .427 $\pm$ .04 and aortic knob diameter increased from 3.4 $\pm$ 0.6 to 3.8 $\pm$ 0.5 cm; nevertheless, only 3% of men developed a CTR >.50, the commonly accepted upper limit of normal.

Significance to Biomedical Research and the Program of the Institute: Given the ubiquity of the standard CXR in clinical medicine, it is important to quantify the changes on this examination which can be attributed solely to the aging process. In this way, the practitioner may more easily separate the effects of aging versus those of disease.

Proposed Course: Although this project has been completed, it might be feasible to compare longitudinal changes in pulmonary function tests with the corresponding changes in roentgenographic appearence of the lungs.

Publications:

Ensor, R. E., Fleg, J. L., Kim, Y.C., de Leon, E. F., and Goldman, S. M.: Longitudinal chest x-ray in normal men. J. Geront. 38: 307-314, 1983.

Appendix

Contract Number: N01-AG-7-2129

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

Contractor: Johns Hopkins University, Baltimore, Maryland
Investigators: G. Gerstenblith, Asst. Prof. Medicine
M. L. Weisfeldt, Dir. Div. Cardiology
J. Weiss, Asst. Prof. Medicine
L. Becker, Assoc. Prof. Medicine
E. D. Mellits, Assoc. Prof. Biostatistics

Money Allocated: $1,152,796.00

Objectives:

A. Two-dimensional echocardiography is a relatively new technical development which allows determination of cardiac anatomy and function in much the same manner as routine echocardiography, with the important addition that an entire plane of the heart can be visualized at once rather than a simple "ice pick" view, allowing greater accuracy in the determination of heart chamber shape, size and function. The initial goal was is to examine subjects from the Baltimore Longitudinal Study over a five year period (ages 18-95) during rest and maximal semi-supine bicycle exercise to determine age-related differences in regional and global myocardial function.

B. Beginning in FY 81 multi gated acquisition MUGA scans were obtained both at rest and during exercise in subjects of all ages. This newly developed technique, which utilizes ^{99m}Technetium as a tracer provides as much information during exercise as the two-dimensional echocardiogram, but has the distinct advantage that the yield of technically satisfactory studies approaches 100% (vs 15%) overall for the ultrasound technique.

C. Thallium^{201} myocardium imaging allows non-invasive assessment of regional left ventricular blood flow combined with stress ECG and will be used to determine the incidence, severity, and prognostic implications of ischemic heart disease in Baltimore Longitudinal Study participants. The predictive values of this technique will be compared to that of stress electrocardiography and two-dimensional echocardiography.

D. Beginning in the fiscal year 81 a statistical analysis of the data influence of age on the development of ischemic heart disease was initiated. The specific goals of this analysis are: (1) to determine the prevalence, severity, and prognostic implications of ischemic heart disease in a normal aging population; (2) to determine whether age-related changes that occur in myocardial performance or structure and whether these changes are related to alterations in myocardial perfusion; (3) to determine the predictive value of electrocardiography, thallium^{201} perfusion imaging and echocardiography during exercise stress with regard to subsequent events; and (4) to relate the presence of evidence of

ischemic heart disease to the presence of specific or potential risk factors documented over a 20 year period in the study population by various investigators.

Major Findings:

A. Approximately 450 subjects have undergone two-dimensional echocardiography and this test has been phased out in FY 82. Analysis of the resting echocardiograms is in progress. Exercise tracings had been difficult to obtain, especially in elderly subjects. The echocardiographic responses to supine bicycle exercise in young subjects (mean age 31 ± 1 yr) versus old subjects (mean age 64 ± 3 yr) have been compared and the data presented at the Annual Scientific Sessions of the American Heart Association. Although echo indices of end-diastolic area (EDA) and end-systolic area (ESA) did not differ between the two groups at rest, EDA increased in the old group during exercise whereas it remained unchanged in the young. ESA, however, decreased in the young during exercise but was unchanged in the elderly. These findings indicate distinct age-related differences in the mechanisms of increasing stroke volume during exercise. The bulk of the echo data is currently being reduced for analysis.

B. To date 105 subjects have undergone bicycle stress MUGA's. Results indicate that older subjects are not able to increase their ejection fraction during exercise to the same extent as younger ones. The data also appear to confirm our two-dimensional echocardiographic findings that older subjects increase stroke volume during exercise primarily through a Frank-Starling mechanism whereas younger subjects do so by decreasing end-systolic volume. Another major finding is that, in spite of these differences during exercise those fit BLSA subject virgorously screened for coronary artery disease are able to maintain cardiac output. Likewise, at rest, no age-related decrease in cardiac output was observed. These results were presented in abstract form at the American Heart Association meetings in November 1981 and November 1982.

C. Approximately 450 subjects have received exercise thallium^{201} scintigrams. Thallium scanning has shown an increasing rate of positivity with age, even in asymptomatic subjects. We define latent coronary heart disease (CHD) only when both the exercise ECG and thallium scan are abnormal. Using these stress criteria (SC) and the standard resting criteria (RC) for CHD of angina, myocardial infarction by history or abnormal Q waves on standard ECG, we have defined the prevalence of CHD in an initial subset of the population in the table below.

Age (yr)	40's	50's	60's	70's	80's
N	41	70	73	36	10
RC	0%	13%	15%	22%	20%
RC+SC	0%	24%	37%	56%	50%

These combined figures (RC+SC) agree closely with the prevalence of CHD found in autopsy studies and suggest that stress thallium scintigraphy may provide a useful tool to enhance the detection of CHD, thus providing the epidemiological aspects BLSA with a unique feature.

D. The majority of effort in the statistical analysis portion of the contract has been spent in data definition and acquisition, and preliminary data

screening and analysis. The data has been sub-divided into mortality and 14 areas of risk. Through meetings with the appropriate investigators at the GRC and elsewhere definitions of the proper data sets have been accomplished in each of these 15 subsets. The acquisition and processing varies by area of risk. For mortality, cardiology, body composition, personality and nutrition the process is essentially complete. Glucose tolerance, physical activity and blood pressure data have been identified and are in process of being completed. A few questions remain, which are being resolved, concerning cholesterol, smoking, and family history data. Pulmonary and demographic data are still being defined. Two areas, testosterone and VO_{2max}, have been investigated and found to have insufficient data for the analyses contemplated. These areas have therefore been eliminated from further analysis. The analyses are being applied to four basis outcomes:

1. Overall Mortality
2. Cardiovascular Death
3. A "Classical" Definition of Cardiovascular Disease (using stress criteria)
4. MI (including cardiac mortality)

Each of these outcomes is being examined as a function of each of the 12 risk areas separately. In addition, the risk area variables are being considered with chronologic age as a co-variate.

Survival curves and distributions of the outcomes on both time in study and age has been completed. Cox's Hazard function analysis, age as a co-variate, has also been completed. These descriptive analyses will comprise the first part of the final presentation.

Within each risk area variables are being defined for intensity, duration and change. Then univariate, followed by multivariate, analyses are being done to define variables to be included in a final overall multivariate analysis using all areas of risk.

The first univariate analyses for the cardiac variables have been completed. These analyses have been re-examined correcting for age. Multivariate analyses of cardiac variables is presently being done.

All definitions are now complete for the personality variables and preliminary analyses have been initiated. Nutrition and glucose tolerance are the next two areas of investigation and are being "prepared" for analysis now.

Significance to Biomedical Research and the Program of the Institute: Resting two-dimensional echocardiography and MUGA scans allow detailed non-invasive analysis of cardiac structure and ventricular function and should expand our findings from M-mode echocardiography. These techniques have helped to elucidate the mechanisms for the diminution in maximal cardiac performance with age and to detect early pathological changes in cardiac muscle function.

Thallium^{201} myocardial imaging permits the non-invasive detection of coronary heart disease in an asymptomatic population. The combination of MUGA, exercise electrocardiography, and thallium scanning represents a new epidemiologic ap-

proach to the detection of this disease--the major cause of death among the elderly. The diagnosis of latent coronary artery disease should allow further insights into the natural history and the effects of various therapeutic interventions on the disease process.

Proposed Course: Currently the contract is in its fifth year. During the remainder of this fiscal year (82) and the following year, additional thallium and MUGA studies will be performed. Concomitantly, all data will be analyzed with regard to the effect of age on parameters measured. In addition, the multivariate statistical analysis of the significance of risk factors data gathered by various investigators of the Baltimore Longitudinal Study of Aging which are currently being implemented will be continued until the end product is at hand.

Staff Assessment: This contract is a direct extension of intramural research projects. Cardiovascular Section Chief, who is the Project Officer, and other members of the Cardiovascular Section have been involved in the conceptualization and design of this research. Overall the contract has been successful in meeting the objectives as set forth. It is anticipated that the targeted number of studies and data analysis will not be complete at the projected termination date of the contract, i.e. September 30, 1982. An additional year's extension is currently being sought. However, it will not be necessary to request additional funding for FY 83. For FY 84-87 a decision will be made by the Steering Committee of the BLSA as to which if any studies ought be continued and plus to extend the current or initiate additional contracts will be discussed by that body. Longitudinal assessment of cardiac blood flow and function during stress is being contemplated. Whether it will be feasible to do this testing within the scope of the intramural program or via contract mechanism is also being considered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00029-06 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Efficacy of Digitalis in Congestive Heart Failure and in Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. L. Fleg Staff Cardiologist CPB, NIA

COOPERATING UNITS *(if any)* S. H. Gottlieb, Div. Cardiology, Baltimore City Hosp., Baltimore, MD, Thomas Smith, Peter Bent Brigham Hosp., Boston, MA, Frank Marcus, Univ. Arizona, Tucson, AZ, Robert Johnson, Massachusette General Hosp., Boston, MA, Harold Strauss, Duke Univ., Durham, NC, George Steinberg, NIA, Bethesda, MD

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have previously shown in a double-blind crossover study that digitalis could be discontinued for three months without adverse clinical effect and only minor changes in cardiac size and function in 30 subjects with stable congestive heart failure (CHF) and sinus rhythm. Long-term follow-up of these patients verified this favorable early response to digitalis withdrawal. We have initiated a new study to investigate the effects of digitalis on aerobic performance and cardiac function during exercise (measured via gated blood pool scan) and its effects on cardiac rhythm in a new group of patients with chronic CHF and sinus rhythm.

Our group has helped to develop a questionnaire in conjunction with experts in cardiology at different universities. This questionnaire which will be implemented by the American Heart Association will sample representative groups of academic and practicing physicians in their current use and understanding of the effectiveness and toxicity of digitalis glycosides. The results will be important in identifying areas of consensus and areas in which major uncertainties exist; the important categories within the latter will serve as a basis for future research projects in which NIH and NIA may find a direct role.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Project Description:

Objectives:

1. Digitalis glycosides ranked fourth in a recent audit of the most frequently prescribed drugs. Given the facts that the incidence of congestive heart failure rises expontially with age, that 11% of our population is currently over age 65, and that the incidence of digitalis toxicity is highest in elderly subjects, it becomes important to demonstrate that long-term digitalis therapy for patients with chronic stable heart failure results in better cardiac function and clinical status than can be achieved without it. We are assessing the effect of chronic digoxin therapy on aerobic capacity and cardiovascular performance during exercise in outpatients with compensated CHF.

2. Although digitalis has been used to treat CHF for some two centuries, several studies in the last 20 years have shown that many patients on chronic glycoside therapy could be successfully weaned from the drug. The recent advent of vasodilator therapy for advanced CHF has further expanded the therapeutic options. It is not known, however, to what extent these recent advances have changed the physician's prescribing habits for digitalis. A questionnaire is being designed for this purpose by our group in conjunction with the American Heart Association.

Methods:

1. Twenty outpatients with compensated chronic CHF and normal sinus rhythm will be studied in a double-blind crossover protocol with digoxin and placebo. Maximal aerobic capacity (VO_2max) during treadmill exercise, left ventricular performance during maximal bicycle exercise (using gated radionuclide angiography) and arrhythmia frequency on 24 hour ambulatory ECG monitoring will be measured at baseline and after one month of each regimen. As in our previous study, clinical signs and symptoms, chest x-ray, M-mode echocardiogram and systolic time intervals will be monitored.

2. A questionnaire has been developed by ourselves and four outside experts on digitalis glycosides. This questionnaire is intended to sample physicians' current ideas and practices regarding the use of digitalis. The American Heart Association (AHA) will further refine the questionnaire and will distribute it to approximately 200 physicians in each of several categories. The AHA will then score the responses to tabulate the results and perform the appropriate statistical analyses. Our group will be responsible for interpreting these results and preparing a manuscript.

Major Findings: None.

Significance to Biomedical Research and the Program of the Institute:

1. If indeed the majority of individuals with compensated heart failure and

normal sinus rhythm can be maintained successfully without digitalis, thousands of cases of digitalis toxicity will be averted and many fatalities avoided. Since digitalis is used most commonly by elderly individuals, who because of decreased body size, diminished renal function, dosing errors, and drug interactions are at higher risk of developing toxicity than younger subjects, the avoidance of digoxin in cases of stable heart failure would have a particularly significant impact on the elderly.

2. Knowledge of physicians' current ideas and prescribing habits for digitalis should clarify the impact of recent studies demonstrating the successful weaning of many patients with CHF from digitalis. Similarly, the impact of vasodilator therapy upon these physicians' approaches to heart failure treatment will be assessed. Aspects of glycoside usage in need of further research will be identified from the questionnaire.

Proposed Course: Further studies on the efficacy of digitalis in subjects with stable congestive heart failure during exercise are planned. These will involve the effect of digitalis on maximal oxygen consumption as well as heart size and function during maximal exercise.

Publications:

Fleg, J. L., Gottlieb, S. H., and Lakatta, E. G.: Is digoxin really important in treatment of compensated heart failure? A placebo-controlled crossover study in patients with sinus rhythm. Amer. J. Med. 73: 244-250, 1982.

Fleg, J. L., Gerstenblith, G., and Lakatta, E. G.: Pathophysiology of the aging heart and circulation. In F. Messerli (Editor), Cardiovascular Disease in the Elderly. Merck, Rahway, 1983 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00032-05 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Contractile, Biochemical and Electrical Responses in Hyperthyroid Heart

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

COOPERATING UNITS *(if any)*

J. Y. Wei, Beth Israel Hospital, Boston, MA

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Changes that occur within the cardiac cell in response to β-adrenergic stimulation result in a dual effect on the mammalian cardiac contraction: a potentiating effect, manifest as an enhancement of twitch force, and a relaxant effect, evidenced by a decrease in the time to peak force and an enhanced rate of relaxation. Since these two manifestations of the relaxant effect occur concomitantly, it is not known whether they are governed by one or more than one mechanism. One approach toward an understanding of this would be to selectively block one of the relaxant effects of β-adrenergic stimulation. We have observed in the hyperthyroid heart that the effect of catecholamines to shorten time peak force does not occur, but that the enhancement of the rate of relaxation of the terminal twitch is preserved. Thus these two manifestations of the relaxant effect occur via different mechanisms. It can be hypothesized that the shortening of time to peak force results from enhanced calcium accumulation rate of sarcoplasmic reticulum, which is blunted in the hyperthyroid model, while the effect later in the twitch may be attentuable to catecholamine induced phosphorylation of troponin resulting in less calcium affinity.

Combined into Z01 AG 00226-01 CPB

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

H. A. Spurgeon	Physiologist	CPB, NIA
E. S. Beard	Chemist	CPB, NIA

Project Description:

Combined into Project Z01 AG 00226-1 CPB

Publications:

Wei, J. Y., Spurgeon, H. A., and Lakatta, E. G. Electro-mechanical responsiveness of hyperthyroid cardiac muscle to β-adrenergic stimulation. Amer. J. Physiol. 243(Endocrin. Metab. 6): E114-E122, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00033-05 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ambulatory Electrocardiography and Blood Pressure Measurement in Normal Man

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. L. Fleg Staff Cardiologist CPB, NIA

COOPERATING UNITS *(if any)*
Harold J. Kennedy, Saint Louis University School of Medicine, St. Louis, MO

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Initial ambulatory electrocardiographic work from this laboratory has characterized the normal heart rhythm patterns in healthy elderly subjects. We have extended these efforts to include younger men and women (ages 25-60). In addition, we have added a new dimension - 24 hour ambulatory blood pressure (BP) recording - simultaneous with the ambulatory ECG recording, in normal subjects as well as hypertensives and those with congestive heart failure.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Project Description:

Objectives: Blood pressure (BP) is a major risk factor for the development of coronary artery disease, heart and kidney failure and stroke. Although it is widely appreciated that BP is very labile in all individuals, changing with time of day, body position, activity level and emotional state, only recently has the technology become available to noninvasively record BP automatically over a 24-hour period. Our current efforts are directed toward characterizing the patterns of BP over a "typical" 24-hour cycle in healthy subjects over a broad age range. The technique will also be extended to hypertensives and patients with congestive heart failure.

Methods: Normal men and women across a broad age range from the Baltimore Longitudinal Study (BLS) are studied. Subjects with hypertension and congestive heart failure are also being studied and compared to age-matched mormals. In the normal subjects, heart and lung disease is excluded by history and physical exam, resting ECG, maximal treadmill exercise testing, chest x-ray and pulmonary function testing. A description of the equipment is given in last year's report. Thus far, good quality 24 hour recordings of heart rate and blood pressure have been obtained in about 80 normal BLS subjects and 5 patients with heart failure.

Major Findings: Supraventricular and ventricular ectopic beats occur in the majority of a healthy active geriatric population. High degree AV conduction disturbances, profound sinus bradycardia and ischemic repolarization patterns occur in frequently in normal elderly subjects. Twenty-four-hour ambulatory electrocardiography is significantly more sensitive than maximal treadmill exercise for detecting most types of arrhythmias in our population. Initial data on heart rhythm in younger adults and on blood pressure in all subjects are currently being collected.

Significance to Biomedical Research and the Program of the Institute: Characterization of normal BP and heart rhythm patterns over a broad age range will allow a greater understanding of blood pressure regulation mechanisms and create a standard to which subjects with various disease processes may be compared. It will also enable more rational timing of antihypertensive and anti-arrhythmic drugs and help to separate various subsets of hypertensive subjects and define their natural history and benefit from treatment.

Proposed Course: To continue collecting data until approximately 10 men and 10 women in each decade have been monitored. Because of the large number of technical failures with our current blood pressure monitoring equipment, we have ordered a newer, more reliable blood pressure recorder. This new unit should greatly facilitate data collection.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00035-04 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Fluctuations in the Intensity of Light Scattered through Diastolic Cardiac Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

COOPERATING UNITS *(if any)*

E. Marban, Cardiology Div., Depart. Med., Johns Hopkins Hospital, Baltimore, MD
W. G. Weir, Dept. of Physiol., University of Maryland, Baltimore, MD

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	2.7	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have discovered that scattered light intensity fluctuations (SLIF) are present in isolated rat ventricular muscle even under conditions formerly considered to be quiescent. Subsequent experiments indicated that SLIF are highly dependent on calcium loading of the cell and could be reversibly terminated (1) by maintaining constant calcium concentration in the myofilament space in skinned fibers or (2) in intact fibers by caffeine. These results were interpreted to indicate that cellular myoplasmic calcium concentration oscillates in diastole, producing motion of the myofilaments, which modulates the laser beam and results in SLIF. This myofilament motion which is asynchronous within a cell, and among cells, results in a small degree of diastolic force or "tone" in the muscle. Additional experiments have demonstrated SLIF in atrial, ventricular, and conduction tissues in a range of mammalian species including man and indicate the universality of this phenomenon in excitable cardiac tissues. Recently, in collaboration with the Department of Physiology at the University of Maryland, we have directly demonstrated these Ca^{2+} oscillations utilizing intracellular injects of the chemiluminescent protein, aequorin.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

A. A. Kort	Medical Staff Fellow	CPB, NIA
G. M. Bhatnagar	IPA Research Scientist	CPB, NIA
M. D. Stern	Expert	CPB, NIA

Project Description:

Objectives: (1) To determine whether SLIF which are due to Ca^{2+} oscillations within the cell are unique to rat ventricular muscle or whether they are a universal property of cardiac excitible tissues. (2) to directly determine whether SLIF are due to spontaneous cellular Ca^{2+} oscillations.

Methods: A 5 mm Helium-Neon laser is passed through cardiac tissues isolated from rat, cat, dog, guinea pig, and ferret atria and ventricles and in canine Purkinje tissues. Frog atria and ventricles are studied as well. The tissue is isometric and the bathing fluid is maintained at 29°C. Under these varying conditions, the scattered light, measured at 30° from the incident laser beam is collimated through a double pinhole system onto a photomultiplier tube. The signal is then coupled to a digital autocorrelator. The delay time of the autocorrelator function is inversely related to the frequency of intensity fluctuations of the input signal and hence provides a quantitative measure of these fluctuations. Purkinje fibers were pressure-injected with aequorin, typically, 10-20 cells in each fiber. The chamber was then shielded from external light, and light from the preparation was collected through a Lucite light guide leading to a photomultiplier tube (EMI RFI/B-293F). Light was recorded continuously as an analong signal on magnetic tape ($IV/2X10^3$ cps), and was also collected in digial form as photon counts (using discriminator control unit model 1121A and signal averager model 4203, Princeton Applied Research). Tension and membrane potential (when measured) were also recorded continuously on magnetic tape and on a chart recorder. The light records stored on magnetic tape were low-pass filtered at 15 Hz and digitized at 40 Hz on a Microlet digital oscilloscope. Data segments of 40-500 seconds duration were analyzed using a fast Fourier transform program to generate power spectra.

Major Findings: In all mammalian tissues studied, SLIF were observed on mounting of the preparation. After a period of time (10-100 minutes) tissues could be divided into three categories: (1) those in which diasolic SLIF persisted when Ca^{2+} in the bathing fluid was 1 or 2 mM; (2) those in which SLIF became absent at this $[Ca^{2+}]$, but returned when perfusate $[Ca^{2+}]$ was increased; and (3) frog tissues, which never exhibited steady state diastolic SLIF under any condition studied. As in rat ventricular muscle SLIF were not present during Ca^{2+} activation of "chemically skinned" preparations and were reversible abolished by caffeine in intact preparations. Noise analysis of the aequorin luminescence in Purkinje fibers revealed prominent peaks of power density at frequencies of 1-4 Hz; these peaks become larger and shift to higher frequencies as $[Ca^{2+}]_i$ increases. Caffeine and ryanodine abolished the $[Ca^{2+}]_i$ fluctuations, suggesting that Ca^{2+} release and uptake by the sarcoplasmic reticulum generate these events.

Significance to Biomedical Research and the Program of the Institute: The results demonstrate that during diastole isolated cardiac muscle is not quiescent, but that all mammalian cardiac tissues have the capacity to generate spontaneous cellular Ca^{2+} oscillations. This in itself is of fundamental importance in

modeling the muscle contraction and stiffness. Currently no other technique is available to monitor this oscillatory Ca^{2+} pool. The results indicate that information regarding the nature of diastolic cardiac muscle can be obtained from measurements of fluctuations in light scattered through the muscle. These fluctuations appear to be related to tissue excitability and performance.

Proposed Course: To time-gate the cardiac cycle to determine whether rapid changes occur in SLIF in the very early part of diastole whether these, like late diastolic SLIF are due to Ca^{2+} oscillations generated by the sarcoplasmic reticulum.

Publications:

Stern, M. D., Kort, A. A., Bhatnagar, G. M., and Lakatta, E. G.: Scattered light intensity fluctuations in diastolic rat cardiac muscle caused by spontaneous Ca^{++}-dependent cellular mechanical oscillations. J. Gen. Physiol. 1983 (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00036-03 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interaction of Age and Physical Conditioning on Myocardium and Vasculature

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
H. A. Spurgeon Physiologist CPB, NIA

COOPERATING UNITS *(if any)*
F. C. P. Yin, Division of Cardiology, Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Our laboratory has previously shown that aging affects myocardial function in rats, specifically that parameters measuring the duration of the isometric twitch increase with age. It has also been shown that myofibrillar ATPase activity as well as responsiveness of vascular smooth muscle (VSM) to adrenergic agents decreases as the animal ages. Short term physical conditioning reversed the changes in myocardial performance seen in the senescent heart in rats. Because of the emphasis of exercise in relationship to health, we designed a chronic exercise model in the rat to evaluate the relationship between exercise and aging. A swimming model has been developed in which daily swimming is begun in rats 5 weeks of age and continued for the duration of their lives. We have been able to obtain adequate survival to 18 months of age with isolated survivors beyond that time. Mortalities, commonly during swimming, have been analyzed as to the effect of chronic exercise on body weight and heart size. We have recently started to sacrifice the animals across a wide age range to evaluate the effect of lifelong exercise on myocardial and VSM function as well as myofibrillar ATPase activity.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
E. S. Beard	Chemist	CPB, NIA
M. F. Steinbach	Biologist	CPB, NIA
M. B. Effron	Medical Staff Fellow	CPB, NIA

Project Description:

Objectives: The present experiments are begun at 5 weeks of age and exercised throughout their lives to determine if changes in myocardial performance seen with aging can be altered or prevented by chronic forced exercise. The responsiveness of VSM will also be determined to see if the known age changes of VSM can be altered by exercise.

Methods: Rats are taken at 5 weeks of age and swum daily in warm (35°) water for 20-30 minutes per session. Details of the exercise protocol have been reported in Annual Report Z01 AG 00036-02 CPB. Weekly weight gains are recorded and mortalities are autopsied for determination of heart size. At 3, 6, 12 and 18 months of age the animals are sacrificed for studies of myocardial and VSM performance. Right ventricular papillary muscles are placed in muscle baths and isometric function is determined. A calcium dose response curve as well as a response curve for isoproterenol will be determined in each muscle. The remainder of the heart is processed to determine the myofibrillar ATPase activity after the papillary muscle is removed. The proximal descending aorta is removed at the time of sacrifice and an opened ring strip is subsequently placed in a bath. The strip is then conditioned, stimulated with a 100 mM KCl solution, and relaxation in response to varying concentrations of isoproterenol is determined. A large dose of nitroglycerin is given at the end of the experiment to determine the maximal relaxation of the tissue.

Major Findings: Weight reduction and mortality results have been previously reported in Annual Report Z01 AG 00036-02 CPB. In short, the exercise rats weigh 16% less at maturity than controls and past 16 months there is a rapid weight loss phase with an average weight of 450 gm in the exercise rats compared to controls with 63% of the swimmers lost by 18 months of age and over 90% lost in the subsequent years. The majority of the deaths occurred while the rats were swimming in the tank. Because survival past 18 months of age is poor, this age is being used as the oldest group for study of hemodynamic variables. The first group of rats have been sacrificed to determine myocardial and VSM performance, however, there is not enough data as yet to analyze.

Significance to Biomedical Research and the Program of the Institute: Because of the obvious interest in exercise, there is intrinsic value in the study as it pertains to exercise. More importantly, since there is data showing that age-associated changes in the cardiovascular system are not fixed and can be modified by exercise, determination of whether these changes can be prevented by exercise may lead to a better understanding of the process of aging.

Proposed Course: The study will be continued during the coming year. As senescence has been shown to affect the response of characteristic impedance to exercise in dogs, exercise studies will be extended to the dog model to see if con-

ditioning senescent dogs can alter the response of the characteristic response of impedance to exercise.

Publications:

Spurgeon, H. A., Steinbach, M. F., and Lakatta, E. G.: Chronic exercise prevents characteristic age-related changes in rat cardiac contraction. Amer. J. Physiol. 244: H513-H518, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00037-03 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Adaptation of Cardiac Muscle to Chronic Volume Overload is Altered by Age

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

G. D. Walford Staff Fellow DOD 07/01/83 CPB, NIA

COOPERATING UNITS *(If any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our model is the male rat subjected to chronic complete heart block at two different ages, 5 and 12 months, compared to age-matched control. We studied the response of contractile proteins to calcium by disrupting cell membranes of isolated muscle strips with the non ionic detergent Triton X-100 and then determining calcium dose-response curves for force development. Our results indicate that significantly more contractile force per unit of muscle area is developed by young, compared to older, animals with heart block or to control animals of either age. Thus an interaction of age and chronic volume overloading of the heart by complete heart block is demonstrated.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
E. S. Beard	Chemist	CPB, NIA

Project Description:

Objectives: To assess the performance of contractile proteins of isolated cardiac muscle from aniamls of different ages in whom chronic heart block has been experimentally induced.

Methods: Male, Sprague-Dawley rats of 5 mo (Y) and 12 mo (O) of age who had heart block (B) induced by closed chest electrocautary for 7 mo, had left ventricular subendocardial muscle strips excised, mounted isometrically at L_{max} and stimulated at 24 bpm while perfused with modefied Krebs solution (Ca^{2+} = 2.5 mM) and then developed force (DT) was measured. The muscles were then perfused for 30 minutes with the same buffer solution, but with Ca^{2+} = 0 and 2 mM EGTA added. Muscle membranes were then disrupted by a 30 minute perfusion with Triton X-100 (1%) dissolved in a relaxing solution containing 100 mM KCl, 7 mM $MgCl_2$, 5 mM ATP, 3 mM EGTA, 7.5 mM creatine phosphate, 0.05 mg/ml creatine phosphokinase, and 25 mM imidazole, at pH = 7.0. The muscle was subsequently washed with fresh relaxing solution without detergent, and following this, stepwise activation was produced by the addition of Ca^{2+} to achieve a given pCa. After determination of that pCa hich produced peak force, the perfusate was returned to relaxing solution without Ca^{2+} to insure that force dropped to the original baseline present prior to Ca^{2+} activation.

Major Finding: The results are analyzed for the pCa at maximum force, the maximum force itself and the slope of the force pCa curve, empressed as the Hill coefficient (n).

Group	pCa of max. force	Max. force (g/mm^2)	(N)
CY (5)	5.8	1.00 ± 0.18	3.6 ± 0.4
CO (5)	5.8	1.25 ± 0.04	4.1 ± 0.9
BY (5)	5.8	1.5 ± 0.09*	3.7 ± 0.6
BO (5)	5.8	1.25 ± 0.10	4.2 ± 1.0

(*Significant for effect of block and age by ANOVA.)

Thus, while pCa at peak force and the Hill coefficients show no effect of age or block, the peak force obtained shows an interaction of age and block, with the younger block group having significantly more force.

Significance to Biomedical Research and the Program of the Institute: The exanimation of the interaction of advancing age and diease in chronic laboratory animal models, here heart block helps our understanding of the basic properties of heart tissue, its modification by aging or time and to the challege of withstanding added stress. This understanding is the basis for a rational approach to the modification of these stresses as they might exist in mammalian species including man.

Proposed Course: This model of experimental will be used to examine the effect of a hemodynamic stres, i.e. heart block, in senescent animals.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00038-02 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Evaluation of Peripheral Blood Flow in Normal Man by Plethysmography

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. L. Fleg Staff Cardiologist CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.50	.30	.20

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Although the incidence of degenerative changes in the blood vessels is well known to increase with advancing age, quantitative data on the peripheral blood flow due to the aging process per se are lacking. Venous occlusion plethysmography has been shown to be the most accurate and reproducible method to measure the peripheral blood flow. We used this method to evaluate peripheral blood flow in the subjects of the Baltimore Longitudinal Study of Aging with ages ranging from 20 to 83 years. The study was designed to evaluate the effect of age on peripheral blood flow by venous occulsion plethysmography during rest and in response to removal of arterial occlusion and thermal stress which result in maximal flow.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel:

E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
E. S. Beard	Chemist	CPB, NIA

Project Description:

Objectives: Venous occlusion plethysmography is the most accurate, reproducible and objective non-invasive method to accurately measure blood flow in an extremity. The purpose of the study was to evaluate the effect of age on the peripheral blood flow and also to gain insight into the associated aspects of arteriolar tone and peripheral resistance. The initial goal was to determine the blood flow quantitatively in an aging population and see if there is an effect of age on the blood flow. Subsequently the study could be extended to all the participants of the BLSA on a longitudinal basis at every five to seven years and examine if there is any change in blood flow longitudinally.

Methods: The subjects lay supine on a bed after signing the informed consent. They insert the right foot and leg into the water filled plethysmography at initial temperature setting of 26°C. A large blood pressure cuff was placed in close proximity of the plethysmograph. Through a pneumatic device the pressure in the cuff could be raised or lowered instantly. The pressure in the cuff was raised sufficiently for 30 seconds at a time and the pressure at which the initial measured slope, which indexes arterial flow, is constant will be determined and will be known as venous occlusion pressure. Subsequently arterial occlusion pressure was identified by increasing the cuff pressure to 20-40 mm above the systolic blood pressure at which recorder will show no pulsatile arterial flow. Blood flow measurements were taken after getting several resting flow measurements and then following arterial occlusion of 1 to 5 minutes by rapidly lowering cuff pressure to the venous occlusion pressure. The temperature of the plethysmograph was then increased to 35°C and flow measurements as above were repeated. The volume of the limb was measured by measuring the volume of the displaced water in the plethysmograph.

Major Findings: To date 146 studies have been performed. In addition to assessing the effect of age on resting and post-ischemic blood flow, we will investigate the independent effects of smoking, blood pressure, treadmill exercise performance, medications, and coronary heart disease on peripheral blood flow by means of multivariate computer techniques currently in progress.

Significance to Biomedical Research and the Program of the Institute: Peripheral vascular disease is an important factor in the morbidity and disability in the geriatric and diabetic population. This method promises to give quantitative estimate of blood flow during normal aging process and also differentiate between normal and reduced blood flow. By determining latent vascular disease by measuring peripheral blood flow, this will enlighten us regarding the natural history of peripheral vascular disease and vascular disease due to diabetis or hypertension. Effect of different cardiovascular drugs like beta-blockers, vasodilators, and calcium antagonists on peripheral flow and resistance can be studied in normal aging man and in clinical conditions like hypertension and congestive heart failure.

Proposed Course: The current cross-sectional study has continued for the last two years. A decision on the proposed course is pending.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00039-02 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Long-term Prognosis of Asymptomatic Men with Complete Right Bundle Branch Block

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

J. L. Fleg Staff Cardiologist CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The long-term cardiac prognosis of 24 clinically healthy men with complete right bundle branch block (RBBB), identified from the 1140 men constituting the BLS population, was assessed over a follow-up period averaging 8.4 years. When compared with a control group matched for age at which RBBB appeared, men with RBBB showed no difference in the prevalence of antecedent coronary risk factors or obstructive lung disease. Their incidence of angina pectoris, myocardial infarction, valvular heart disease, cardiomegaly, congestive heart failure, advanced heart block, or cardiac death did not differ from that of the control group over the observation period. Furthermore, at latest follow-up maximal aerobic exercise tolerance and chronotropic response to maximal exercise were not impaired in men with RBBB relative to controls. However, axis deviation leftward of -30^{o} was present in 46% of RBBB men but only 15% of controls by latest follow-up. Although a PR interval prolongation greater than 40 msec developed in only 6% of control subjects over the observation period, such prolongation occurred in 29% of men with RBBB. These results support the concept that RBBB in these asymptomatic men is a manifestation of a primary abnormality of the cardiac conduction system but has no demonstrable adverse effect.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Project Description:

Objectives: Resting and exercise electrocardiographic (ECG) data have been collected on BLS participants since 1958. The long follow-up period for these carefully evaluated individuals coupled with their frequent repeat ECGs enables the detection of long-term ECG changes and assessment of the prognostic significance of various ECG abnormalities. Our initial efforts have been directed toward determining the long-term cardiac prognosis of clinically healthy men with complete right bundle branch block (RBBB).

Methods: All BLS subjects who presented with or developed RBBB in the absence of clinical heart disease were identified. Those subjects who were not available for at least one subsequent visit after the development of RBBB were excluded. In the remaining men, the initial prevalence of coronary risk factors and subsequent incidence of cardiac events were identified and compared with the corresponding values in a healthy age-match control population without bundle branch block. Aerobic exercise performance and various ECG variables were also compared between groups.

Major Findings: Asymptomatic RBBB generally occurs in older men and has no demonstrable adverse effect on long-term cardiac morbidity or mortality. RBBB in these asymptomatic subjects is commonly associated with left axis deviation and a longitudinal prolongation of the PR interval, suggestive of a more generalized abnormality of the cardiac conduction system. These results have been presented at the annual session of the American Heart Association, November, 1981 in Dallas, Texas (Circulation, 64: (4), Part II, IV-249, 1981).

Significance to Biomedical Research and the Program of the Institute: Given the increased use of the ECG as a screening tool in the general population, a substantial number of apparently healthy subjects with RBBB may be anticipated. The present data suggest that these individuals can be reassured that their likelihood of future cardiac morbidity and mortality is no greater than that of the general population.

Proposed Course: The extensive ECG files on BLS subjects allow a multitude of questions to be addressed. A longitudinal analysis of standard ECG intervals and axes in healthy subjects is planned. The prognostic significance of an abnormal Master's test and abnormal treadmill test is currently being assessed in the BLS population.

Publications:

Fleg, J. L., Das, D. N., and Lakatta, E. G.: Right bundle branch block: Long-term prognosis in apparently healthy men. J. Amer. Coll. Cardiol. 1: 887-892, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00040-02 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Thermal Sensitivity of Resting Force in Rat Cardiac Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The contractile performance of isolated myocardium is critically dependent on temperature. From 32 to 16°C, both diastolic and systolic force as well as contraction duration increase exponentially. Diastolic force approached thermal insensitivity when bathing medium calcium was removed. Diastolic force predicts by simple linear functions the entire profile of the ensuing contraction, including systolic force, rates of force development, and contraction duration. These findings are suggestive of an underlying contractile process common to both the diastolic and systolic states of the muscle.

Combined into Project Z01 AG 00035-04 CPB

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

H. A. Spurgeon	Physiologist	CPB, NIA
G. Ruano-Arroyo	Guest Scientist	CPB, NIA

Project Description:

Combined into Project Z01 AG 00035-04 CPB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00221-02 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Sodium-Calcium Dependence of Resting Force in Rat Cardiac Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

F. G. Lakatta Chief, Cardiovascular Section CPB, NIA

COOPERATING UNITS *(if any)*

G. Gerstenblith, Division of Cardiology, Department of Medicine, Johns Hopkins Medical Institutions

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In unstimulated, isolated, rat ventricular muscle, the increase in resting force (ΔRF) which occurs with an increase in external calcium concentration ($\Delta[Ca^{2+}]_e$) can be largely abolished by lowering cellular sodium content by removing sodium from the superfusate and can be potentiated by raising cellular sodium content by adding ouabain. We hypothesized that this demonstrated the activity of a membrane Na/Ca exchange (see Z01 AG 00221-01 CPB). Since this membrane exchange is well known to exhibit graded activity, we sought to extend our observations by demonstrating a graded response between $[Na^+]_i$ and ΔRF. External potassium concentration ($[K^+]_e$) was set at gradually lowered values for each experiment in the series to give graded inhibition of the Na-K pump and thus graded elevation of $[Na^+]_i$. $[Ca^{2+}]_e$ was then increased from 0 to 2 mM and the ΔRF recorded. The results show that increasing ΔRF occurs wtih decreasing $[K^+]_e$ (and thus increasing $[Na^+]_i$. Thus, not only does the ΔRF for a $\Delta[Ca^{2+}]_e$ depend on $[Na^+]_i$, but does so in a graded manner. This is further evidence for the activity of the Na/Ca exchange in controlling ΔRF in isolated rat muscle.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

G. D. Walford Staff Fellow DOD 7/01/83 CPB, NIA

Project Description:

Objectives: To demonstrate that changes in perfusate conditions which are known to cause graded changes in $[Na^+]_i$ of cardiac cells will result in directionally similar graded increases in the change in resting force that occurs when external calcium cncentration is increased.

Methods: A thin left ventricular muslce ($.3 \pm .1$ mm^2) was removed from a male, Wistar rat and mounted horizontally in a muscle bath between two lucite clamps, one of which was attached to a statham strain-guage. Superfusion at 12 cc/min with oxygenated Hepes 4.0 mM buffer solution at 29°C was maintained while the muscle was stimulated to contract by field electrodes at 24 beats per minute for one hour. The muscle was then stretched to a length at which developed force (DF) was maximum (L_{max}) and stimulated for 1 1/2 hours. The stimulator was then turned off and the superfusate was changed to increase external calcium ($\Delta[Ca^{2+}]_e$) concentration. The resting force (RF) prior to altering the superfusate, the change in RF (ΔRF) and its maximum rate of rise (Rf/dt_{max}) were measured. Prior to increasing calcium, there was a 20 minute period of calcium-free superfusion using 2 mM EGTA. This was done to establish the baseline resting force which was not dependent on external calcium. Simultaneously, $[Na^+]_i$ was altered by changing $[K^+]_e$ (to raise it); or lowering $[Na^+]_e$ (to lower it). Calcium was then added to the perfusate in the presence of this altered $[Na^+]_i$, and the ensuing change in resting force (Δ RF) was measured. In addition, the increase in calcium, whether preceeded by a calcium-free period or not, was performed when intracellular sodium content had been altered by changing external sodium or potassium.

Major Findings: The ΔRF obtained for $\Delta[Ca^{2+}]$ of 0 to 1 mM in different $[K^+]_e$ when $[Na^+]_e$ is constant at 144 mM (A) and in different $[Na^+]_e$ when $[K^+]_e$ is 0 mM (B) are given:

(A) ΔRF for different $[K^+]_e$ (mM) with $[Na^+]_e$ = 144 mM

$[K^+]_e$ (mM)	0	.25	1	3	5
RF (g/mm^2)	$2.9 \pm .6$	$2.2 \pm .4$	$1 \pm .2$	$.3 \pm .05$	$.4 \pm .1$

(B) ΔRF for $[Ca^{2+}]$ of 0 to 1 mM different $[Na^+]_e$ (mM) with $[K^+]_e$ constant at 0 mM

$[Na^+]_e$ (mM)	0	70	123	144
RF (g/mm^2)	$.3 \pm .01$	$.5 \pm .06$	$1.2 \pm .7$	$2.9 \pm .6$

Thus, for (A) lowering $[K^+]_e$, which is known to raise $[Na^+]_i$, also raises ΔRF. For (B) lowering $[Na^+]_e$, which lowers $[Na^+]_i$, lowers ΔRF. Both significant for effect of $[K^+]_e$ or $[Na^+]_e$, respectively, on ΔRF by analysis of variance.

Significance to Biomedical Research and the Program of the Institute: The modulation of resting force by the interaction of external calcium and internal sodium is most likely due to the operation of a sarcolemmal sodium-calcium exchange mechanism. This study shows the role of this mechanism in influencing the "Contractile state" of cardiac muscle as indicated by change in resting force. Thus calcium does not enter the cardiac ell merely by passive movement down the electro-chemical gradiant but enters by an active mechanism in exchange for internal sodium. The fundamental understanding of how calcium enters the cell is also of importance in a rational approach to managing pathophysiological states such as hypoxia or ischemia in which calcium entrance occurs in large quantities that are detrimental to cellular function and integrity.

Proposed Course: To examine the effect of metabolic toxins, such as cyanide, on calcium-dependent resting force.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00222-02 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Altered Mechanical Properties in Myocardium from Rats Subjected to Food Deprivation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

H. A. Spurgeon Physiologist CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

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

Numerous experiments over the last several decades demonstrate that caloric restriction increases longevity, but little evidence exists relative to direct effects on organ function. Male Wistar rats had food withheld every other day for 6 weeks to study at 24, 30 mo (EOD24, EOD30) and were compared to normal lifespan 24 mo controls (C). C are unattainable at 30 mo. Body weights (BW, gm) were (C= 504 ± 15; EOD24 = 354 ± 10; EOD30 = 349 ± 9), dry left ventricular weight, (LV, gm), relative to body size (indexed by tibia length, mm (TL) as TL/LV) were (C = .0597 ± .001; EOD24 = .037 ± .001; EOD30 = .042 ± .002) or indexed by BW as LV/BW (C =.566 ± .03; EOD24 = .456 ± .01; EOD30 = .53 ± .02) were all P<.001 versus C except EOD30 LV/BW, NS. Isolated isometric LV trabecular performance ($[Ca^{++}]$ = 2.5 mM, 29°C, L_{max}) was not statistically significantly altered by food restriction even for 30 mo. (All P< NS versus C.)

	N	Act Force	TPT	CD	$RT_{½}$	DF/DT
C	10	2.4 ± .3	156 ± 6	268 ± 11	112 ± 5	34 ± 4
EOD24	25	3.2 ± .4	147 ± 3	250 ± 7	103 ± 4	45 ± 6
EOD30	6	3.3 ± .9	146 ± 4	254 ± 9	108 ± 5	48 ± 13

Thus EOD causes marked atrophy of the heart, but does not significantly alter in vitro isometric contraction. Relative heart size per se produced by caloric restriction does not have a direct effect on myocardial performance.

Other Professional Personnel:

E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
E. S. Beard	Chemist	CPB, NIA
D. Ingram	Staff Fellow	LBS, NIA

Project Description:

Objectives: Several concurrent programs of an ongoing nature have a dimension addressed by this project. Any modification in treatment of the experimental animal such as exercise, hyperthyroidism, surgical modification, or the aging process itself produce changes in the dietary intake of the animal. It is observed that the senescent rat undergoes a precipitous loss of weight in the terminal portion of its life concomitant with the observed decreases in cardiac performance. Hypertrophy or hyperthyroidism leads to inappropriate increases in the relationship between heart size and body size (cardiac hypertrophy). It has been possible to partially dissociate the cardiac hypertrophy from functional changes in cardiovascular tissues by experimentally producing hypertrophy equivalent in magnitude to that seen in aging. The dimension associated with decreased body size has not however been addressed. The 30 to 40% reduction in body size associated with EOD feeding provides an ideal model to examine the enfluence of body size on myocardial function and further tests whether or not the decrement in cardiac performance is an obligatory function of age.

Methods: Animals from the GRC aging Wistar colony are placed on EOD feeding beginning at weaning (5 weeks) and maintained throughout life on this treatment. Animals are studied at 6, 13, 24 and 30 months of age. Age matched controls exist only for the first three groups, as normal Wistars do not live appreciably beyond 24 months. A second series, begun at 1, 7, 19 months and restricted for only 5 months provides a test of age of onset and duration of EOD feeding, when harvested at 6, 13, and 24 months. Isolated rat left ventricular trabeculae carnae from each group are studied as isometric superfused cardiac muscles and evaluated for performance as a function of bathing calcium, force-frequency relationships and responsiveness to catecholamines.

Major Findings: Comparing control to EOD animals at 24 months (the point of senescence for control animals) or comparing the same 24 month control group to EOD animals at 30 months allows the effects of age per se and of cardiac hypertrophy to be assessed. Although EOD is effective in prolonging the mean lifespan, this study revealed no significant differences in the ability of the isometric cardiac muscle to develop active force either comparing age matched controls and EOD at 24 months or comparing senescent controls (24 month) to senescent EOD (30 month). This finding is consistant with our previous observation that active force development is not compromised by age. We have, however, consistantly demonstrated prolongation in the timing parameters of the isometric twitch at senescence, but it had previously been unclear whether the modest hypertrophy which occurrs concomitantly might be responsible. In the present study, the 24 month controls and both 24 and 30 month EOD animals exhibited siginificant prolongation of twitch timing relative to young rats. Two significant points should be made here. First, twitch prolongation is not

a terminal process occurring only in the last days of an animal's lifespan, as EOD was ineffective in delaying the onset of age associated prolongation of the twitch in spite of the significant increase in lifespan of these animals. Second, since EOD animals are characterized by indexed heart size which is smaller even at senescence than that of a young adult control but EOD animals still exhibit the same degree of age-associated twitch prolongation, cardiac hypertrophy cannot, at least in this model, account for any of the functional changes noted in the isolated cardiac muscle. These findings have been presented in part at the FASEB Annual Meeting, April 1983 (Federation Proc. 42:(3), 466, 1983).

Significance to Biomedical Research and the Program of the Institute: The interrelation of nutrition and lifespan and the effects of these variables on the status of the cardiovascular system relates both to the general mission of the NIA and possibly to the status of a segment of the human population.

Proposed Course: The addition of some morphological measures from a subgroup of hearts is still in progress, as the last group of food deprived animals have just recently been sampled for morphology. Additional analysis of the functional data is expected to be suggested by the outcome of the morphological data.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00223-02 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Age on the Components of Atrioventricular Conduction in Normal Man

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. L. Fleg Staff Cardiologist CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

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

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

We have utilized a signal averaging, high resolution ECG to record His bundle potentials from the body surface of 111 normal Baltimore Longitudinal Study (BLS) volunteers ages 21 to 79. By allowing measurement of conduction time both proximal and distal to the bundle of His, this technique should enhance our understanding of the age-related changes in the cardiac conduction system. In 52 women, neither PR nor HV interval was related to age. In 59 men, the following age relationships were found:

		r	p
PR interval	= 142.4 msec + .477 age	.388	<.01
PH interval	= 105.3 msec + .427 age	.393	<.01
PR segment	= 47.6 msec + .315 age	.328	<.02
Proximal PR segment	= 10.5 msec + .267 age	.330	<.02

Thus, an age-related prolongation of PR interval is found only in men and appears to be due largely to a delay in the proximal PR segment, presumably reflecting delay within the atrioventricular junction.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Project Description:

Objectives: Although an age-related increase in the electrocardiographic PR interval has been verified by several investigators, it is not known whether this PR interval prolongation is due to slowed conduction proximal (PH) or distal (HV) to the His bundle or both. The goal of this project is to determine the site(s) responsible for this PR prolongation, using both surface recordings of His bundle activity.

Methods: Men and women from the Baltimore Longitudinal Study (BLS), found to be normal by history, physical exam, resting ECG, and maximal treadmill exercise testing, and on no interfering medications are studied. After meticulous skin preparation, electrodes are placed in the V_1, V_5, V_{6R} and ground positions and data from 512 cardiac cycles accumulated. High magnification (400X) signal averaging and filtration of random noise (Marquette MAC-1) allow identification of His bundle potentials in the majority of subject. PR, PH and HV intervals and heart rate are then measured from these recordings and compared across the age groups.

Major Findings: From an analysis of our first 111 recordings, part of which were reported at the Annual Scientific Session of the American College of Cardiology in April 1982 (Amer. J. Cardiol., 49: Part 2, 1031, 1982), the following tentative conclusions have been reached.

1. The success rate of recording surface His bundle potentials varies directly with the PR interval.

2. HV interval is not age-related in adults.

3. The prolongation of PR interval with advancing age occurs only in men and is due to conduction delay proximal to the His bundle. This delay occurs in the portion of the PR segment proximal to the His bundle depolarization, presumably reflecting delay within the AV junction.

Significance to Biomedical Research and the Program of the Institute: This noninvasive recording technique should allow a greater understanding of the age-related changes in the cardiac conduction system. The technique may be particularly useful in studying patients with disturbances of cardiac conduction and in assessing the effects of various drugs on the conduction system in normal and diseased individuals.

Proposed Course: To continue gathering data on normal BLS subjects until sufficient numbers of men and women across the entire adult age range have been examined.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00224-02 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Cardiac Myofibrillar ATPase Activity Across a Broad Age Range

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

G. M. Bhatnagar IPA Research Scientist CPB,NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

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

☐ (a1) Minors

☐ (a2) Interviews

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

As previously reported, miofibrillar ATPase activityand the duration of the isometric twitch in papillary muscles change with age and do not appear to be related. Maximum ATPase activity in detergent treated myofibrils decreased approximately twofold in rats between 2 and 6 months of age while the contraction duration of the isometric twitch increased progressively across the age span changing by approximately 30% in rats 24 months of age. It has been reported that animals treated with thyroxine increased the myosin ATPase activity and decreased the time to peak force, a component of twitch duration, and have suggested that the two parameters are related. Therefore, we treated young (2 mo) and senescent (24 mo) rats with thyroxine to produce a hyperthyroid state and were able to show that the contraction duration of the isometric twitch decreased without altering the maximum ATPase activity in either group. We conclude that age-associated prolongation of contraction duration is not fixed and can be reversed with thyroxine treatment. However, changes in myofibrillar ATPase activity are not associated with the changes in contraction duration produced by alterations of the thyroid state of the rat.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. S. Beard	Chemist	CPB, NIA
G. D. Walford	Staff Fellow DOD 07/01/83	CPB, NIA
E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
M. B. Effron	Senior Staff Fellow	CPB, NIA

Project Description:

Objectives: The purpose of the project is to determine what effect hyper and hypothyroidism will have in young (immature) and old (senescent) rats on the age associated changes in cardiac muscle. Specificaly, will the hyperthyroid state increase the myofibrillar ATPase activity and decrease the contraction duration in senescent rats and can the hypothyroid state decrease myofibrillar ATPase activity and prolong contraction duration in the immature rats? The effect of age on the rapid kinetics of myofibrillar ATPase activity will also be determined.

Methods: Rats 2, 8, and 24 months of age were treated with either intramuscular thyroxine 0.64 mg/kg for 7 days or given 2 mM propylthiouracil (PTU) solution to drink for 10 days. Purified cardiac myofibrils were prepared form the hearts using a Triton-X treatment procedure which makes the preparation free from mitochondrial and sarcolemmal membrane contamination. ATPase activity and Ca^{2+} binding were measured over the entire pCa range on these preparations. In studies related to the intact muscle preparation, a right ventricular papillary muscle from each heart was mounted in a myograph, equilibrated, optimally stretched, and isometric twitch parameters were recorded. The twitch force was expressed as tension per cross-sectional area, i.e., resting tension, developed tension, and the first derivative of developed tension. The time intervals were expressed as time to peak tension (TPT) and to half relaxation (RT 1/2); an index of contraction duration (CD) was taken as TPT + RT 1/2.

Mayor Findings: Consistent with previous reports from our laboratory, maximal myofibrillar ATPase activity was lower in the 24 month animals than in the 2 month animals (0.131 $\pm$ .005 vs 0.079 $\pm$ .004 micromolar Pi/min/mg prot. (2 mo vs 24 mo) $p<.001$) and contraction duration was increased in the 24 month animals compared to the 2 month animals (188 $\pm$ 9 vs 265 $\pm$ 9 ms (2 mo vs 24 mo) $p<.001$). Although the 7 day treatment with thyroxine elevated the plasma T4 concentration at least tenfold in both the 2 and 24 month groups, maximal myofibrillar ATPase activity was not different from control in either age group. However, CD was decreased in both groups although the 24 month rats had a larger decrease in CD than the 2 month group so that the values were not different between the two treated groups (165 $\pm$ 9 vs 170 $\pm$ 10 ms (2 mo vs 24 mo) p=NS). The CD had a tendency to increase in both the 2 and 24 month old rats but the differences from the control rats were not significant, however, PTU treatment caused a significant decrease in ATPase activity in both groups.

Significance to Biomedical Research and the Program of the Institute: This study further substantiates the findings that mechanical parameters including contraction duration and ATPase activity are not related. It also shows that the mechanical changes in cardiac muscle associated with aging are not

irreversible and must be associated with another function of the cell such as sarcoplasmic reticulum Ca^{2+} sequestration rate. These findings are imporatant in the understanding of excitation-contraction coupling in aged cardiac muscle.

Proposed Course: Measuerment of the effect of the thyroid state and age on myosin isozymes will be investigated. An attempt will also be made to measure the rapid kinetics of myofibrillar ATPase activity and its Ca^{2+} substrate, and age dependence.

Publications:

Bhatnagar, G.M., Walford, G. D., Beard, E. S., Humphries, S.H, and Lakatta, E. G.: ATPase activity and force production in myofibrils and twitch characteristics in intact muscle from neonatal, adult and senescent rat myocardium. J. Mol. Cell. Cardiol. 1983 (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00225-01 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Age on Isolated Muscle Function in Spontaneously Hypertensive Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

G. D. Walford Staff Fellow DOD 07/01/83 CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Cardiovascular Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

The spontaneously hypertensive rat (SHR) develops sustained arterial hypertension at a young age, progressive cardiomegaly, and has a high incidence of cardiovascular deaths at an age younger than its non-hypertensive control strain. It is thus an attractive model for the study of the interaction of aging and "disease." The results presented here show that: (1) intra-arterial blood pressure in the SHR is elevated at 5 months compared to controls and this is sustained until 21 months; (2) cardiomegaly is 110% at 5 months and 143% at 21 months; (3) isolated muscle at 29°C shows an interaction of age and "disease" for several parameters: (a) contraction duration; (b) developed force at short muscle lengths or in response to changes in the pattern of stimulation, and (c) recovery from hypoxia. Thus there are several important interactions of age and "disease" which are manifest in the performance of isolated cardiac muscle.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

Project Description:

Objectives: To examine the interaction of advancing age on blood pressure, heart size and isolated muscle performance of cardiac muscle from spontaneously hypertensive rats (SHR) compared to non-hypertensive age-matched controls.

Methods: Intra-arterial blood pressure was measured in anesthetized animals of two age groups (5 mo and 21 mo in each) which were later sacrificed, had their hearts weighed and an isolated left ventricular subendocardial muscle studied in modified Krebs solution (Ca^{2+} 2.5 mM) at 24 bpm, L_{max} and 29°C for resting force (RF) developed force (DF), and contraction duration (CD). These same parameters were measured at short muscle lenghs (.87 L_{max}, .92 L_{max}, 95 L_{max} and .97 L_{max}), under the influence of a paired stimulus and during and after hypoxia (20 mm/dy).

Major Findings: Blood pressure was greater in SHR than Wistar-Kyoto rats (WKY) at 5 mo (160 ± 15 vs 118 ± 10 mm Hg) and did not change significantly with age (178 ± 20 vs 122 ± 7 mm Hg). The ratio of SHR to WKY heart size was 110% at 5 mo and increased to 143% at 21 mo, showing marked increase in cardiomegaly with advancing age (or time per se). The CD was markedly prolonged in 21 mo SHR (see below) pre-hypoxia or pre-H, while RF and DF were similar. The response of CD to hypoxia (H) was the same in all groups, measured as percent shortening, but the overshoot of CD on reoxygenation (R) showed both an age and age SHR effect (see below):

	Pre-H (msec)	H (% Control)	R (% Control)
SHR (5 mo)	205 ± 4.8	93.9 ± 1.8	115.3 ± 4.7
SHR (21 mo)	250 ± 7.3*	93.3 ± 1.0	133.4 ± 6.7*
WKY (5 mo)	212 ± 6.7	91.9 ± 3.8	112.3 ± 3.3
WKY (21 mo)	219 ± 6.7	92.3 ± .75	123.9 ± 7.1°+

(n = 8 in each, $p<.05$ for effect of age vs 5 mo SHR* or WKY° by paired t-test; $p<.05$ for effect of the interaction between age and SHR on CD prolongation with R).

The response of RF, DF and CD to short muscle lengths did not vary among groups. For paired stimulation, the amount of potentiation of DF did not vary but the stimulus interval at which maximum potentiation occurred was longer in SHR than WKY regardless of age.

Significance to Biomedical Research and the Program of the Institute: The interaction of advancing age and a physiological stress, here the hypertension and cardiomegaly found in the SHR, and in our understanding of the basic properties of heart muscle, their modification by age or time and also to the challenge of added stress. This understanding is the basis for a rational approach to the modification of these stresses as they might exist in mammalian species including man.

Proposed Course: To study this model for response to hypoxemic stress while observing changes in metabolic parameters.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00226-01 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Excitation-Contraction in Isolated Cardiac Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

M. C. Capogrossi Staff Fellow CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Cardiovascular Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.8	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have dissociated myocardial muscle cells from newborn rats and from adult rats and rabbits. These preparations are being used to study the contractile, electrophysiological, and biochemical characteristics in a variety of different conditions. In particular we have been able to identify in these isolated cells a longitudinally propagating wave which occurs spontaneoulsy when the cell is not being electrically stimulated and is considered at rest and has a normal resting membrane potential. These "waves" are likely to represent the phenomenon of calcium induced calcium release and are the cause of the scattered light intensity fluctuatibns (SLIF) which our laboratory has studied in the past in multicellular preparations. These conclusions are drawn by the fact that SLIF and the "waves" respond in the same way to changes in the extracellular calcium or sodium and to the addition of caffeine to the medium.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel:

E. G. Lakatta — Chief, Cardiovascular Section — CPB, NIA

Project Description:

Objectives: (1) To study the phenomenon of calcium induced-release of calcium (CICR) in isolated myocardial cells; (2) to determine how different perturbations such as changes in temperature, $[Ca^{2+}]_o$, age of the animal, caffeine, verapamil, and hypoxia affect CICR, and (3) how CICR affects the mechanical and electrophysiologic properties of the cardiac muscle during rest and stimulated contractions.

Methods: Isolated myocardial muscle cells are obtained from rats of different ages and rabbits through a Langendorff perfusion with a low free $[Ca^{2+}]$, collagenase containing medium. The dissociated single cells are then resuspended in a physiologic medium containing millimolar concentrations of Ca^{2+} and studied on the stage of a diavert Leitz microscope. The cells are induced to contract through field stimulation via platimum electrodes placed in the bathing medium. The cell image is projected on a video monitor and the edge movement quantified with a video dimension analyzer. Cells are impaled with a microelectrode for simultaneous measurements of E_m in some studies.

Major Findings: (1) We have previously studied spontaneous mechanical oscillations in multicellular preparations with the use of scattered light intensity fluctuations (SLIF) (Lakatta and Lappe, J. Physiol. (Lond.) 315: 369-394, 1981; Stern et al., J. Gen. Physiol. 82: 119-153, 1983). In isolated myocardial muscles cells we have observed spontaneous longitudinally propagating waves which show the same species and Ca^{2+}-dependence of SLIF and respond in the same way to decrease in $[Na^+]_o$ and high doses of caffeine. (2) Low temperature (23°C) has a marked effect on decreasing the number and velocity of the waves. (3) Senescent rats exhibit lower frequency of waves than adult rats as might be expected from studies of the SR of old animals (Froehlich et al., J. Mol. Cell. Cardiol. 10: 427-438, 1978). (4) The length of the interval separating two waves is directly proportional to the velocity of the second wave. (5) These waves are seen in cells with a RMP of -80 to 85 mV and their occurrence is accompanied by a small 1-3 mV depolarization. (6) A subthreshold electric stimulus is often capable to trigger a wave.

Significance to Biomedical Research and the Program of the Institute: Single cells represent an ideal preparation in which to study CICR. The opportunity to observe this phenomenon, directly measure how frequently it occurs and how rapidly it propagates across the cell greatly enhances our ability to understand it and to determine its significance in cardiac physiology and physiopathology.

Proposed Course: To expand our work in CICR to determine how it is affected by physiologic variables and how it affects the mechanical and electrophysiologic behaviour of the heart muscle. We will also try to determine if a connection exists between the phenomon of "graded response" and CICR.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00227-01 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Excitation-Contraction in Rat Myocardium: Alterations with Adult Aging

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

COOPERATING UNITS *(if any)*

Jeanne Y. Wei, Beth Israel Hospital, Boston, Massachusetts

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

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NIA, NIH, Baltimore, Maryland 21224

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0.8		

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☐ (a1) Minors

☐ (a2) Interviews

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

Simultaneous measurements of the transmembrane action potential (TAP) and isometric contraction were made in right ventricular papillary muscles isolated from senescent and young adult rat hearts. In muscles contracting at the peak of the length-tension curve, contractile tension developed in response to excitation (DT), and the maximum rate of tension development (dT/dt), were not age-related; contraction duration (CD) was 17% greater in senescent than in young adult ($p < .001$); resting membrane potential (RMP) was not age-related. TAP time above zero (T_{pos}) and integrated area above zero (A_{pos}), and times to 75% (T_{75}) and 90% (T_{90}) repolarization were approximately twofold greater in senescent than in young adult ($p < .001$). An increase in $[Ca^{2+}]_e$ to 2.5 mM: increased DT, dT/dt, CD, T_{pos}, and A_{pos} in both age groups; decreased T_{75} in both groups; but increased T_{90} in sensecent only. The transient change in A_{pos} with each beat following the step increase in $[Ca^{2+}]_e$ was highly correlated with the changes in DT and dT/dt. The changes in steady state T_{75} and T_{90} due to the change in $[Ca^{2+}]_e$ was significantly correlated with those in CD, DT and dT/dt in senescent but not in young adult. We conclude that TAP and contractile parameters in rat myocardium demonstrate interrelationships similar to those observed in other species; both TAP and CD are prolonged in senescent versus young adult; a prolonged and greater extent of depolarization appears to be related not only to the prolonged CD in senescent but may also have a role in determining the peak force developed in response to excitation.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Professional Personnel:

E. S. Beard	Chemist	CPB, NIA
M. S. Steinbach	Biologist	CPB, NIA

Project Description:

Objectives: The purpose of the preent study was to simultaneously measure TAP and contraction in isometrically loaded cardiac muscles isolated from adult and senescent rats specifically to determine: (1) whether the prolonged contraction in senescent muscles is associated with changes in TAP and (2) whether a relationship could e demonstrated between TAP and contractile force or duration parameters.

Methods: Papillary muscles were mounted horizontally in a chamer between two clips, one of which was attached to a strain gauge (Statham UC2) for force measurement, and the other to a micrometer for precise control of changes in the muscle length. Contractile parameters and TAP were measure as described in detail previously (Wei, Spurgeon & Lakatta: Amer. J. Physiol. 243: E113-E122, 1982). Measurements were made in low (0.375 mM) and high (2.5 mM) perfusate $[Ca^{2+}]$. Briefly, a compliant Ag-AgCl wire served to couple the microelectrode filled with 3M KCl to a high input impedance preamplifier. Each action potential signal with concomitant isometric twitch was monitored on an oscilloscope and recorded on-line in digital form at 0.5 msec intervals on a Raytheon RDS 500 computer and stored on magnetic tape which was subsequently edited to discard beats in which a mechanical artifact was present in the tap recording, or in which the resting membrane potential (RMP) was less negative than-65 mV. The following parameters subsequently were measured by a computer program: RMP, action potential amplitude (Amp), the difference between RMP and peak positive potential; the maximum rate of depolarization (dV/dt); the extent of depolarization above zero (OS); the time of depolarization above -40 mV (T_{40}); integrated area above -40 mV (A_{40}); time to 75% (T_{75}) and 90% (T_{90}) repolarization. Resting force, developed force in response to excitation, the difference between total and resting force, and the maximum rate of force development were normalized for muscle cross-sectional area (CSA) and expressed as tension: RT = resting tension; DT = developed tension, dT/dt = maximum rate of tension development. The time from the stimulus to peak tension (TPT) and RT 1/2, the time for peak developed tension to fall 50% and their sum (CD) were also computed. Action potentials were measured in approximately 10 beat in each of 3-4 cells in each muscle for each condition. The average values of TAP parameters from these impalements and the average parameters of the concomitant contractions were taken as the values to represent a given muscle.

After electrical and mechanical parameters were measured in the actively contracting muscle in $[Ca^{2+}]_e$ of 0.375 mM, $[Ca^{2+}]_e$ was increased to 2.5 mM. These levels of $[Ca^{2+}]_e$ were specifically chosen because under present experimental conditions, maximum twitch force is achieved approximately at $[Ca^{2+}]_e$ of 2.5 mM over the range of $[Ca^{2+}]_e$ 0.375 to 2.5 mM relative twitch force strength varies substantially, i.e. from maximum to approximately 30% of maximum. On a rare occasion, the microelectrode remained satisfactorily impaled within a given cell and permited the recording of the TAP during the transient state following the increase in $[Ca^{2+}]_e$ to 2.5 mM. Following an

equilibration period of approximately 20 minutes in $[Ca^{2+}]_e$ of 2.5 mM, after which the contractile activity attained a new steady state, the measurements of TAP from several cells and isometric twitch parameters were repeated. Occasionally, when microelectrode impalement remained stable in a given cell following these measurements, $[Ca^{2+}]_e$ was lowered back to 0.375 mM in order to record the resultant transient in TAP.

Following the experiment, each muscle was removed from the clamps, blotted,weighed, and CSA was calculated, assuming a cylindrical shape and a density of 1.0.

All results are expressed as the mean $\pm$ standard error of the mean for N muscles in each group. Statistical analysis of the effect of ae, $[Ca^{2+}]_e$, and the age-$[Ca^{2+}]_e$ interaction was accomplished by analysis of variance with appropriate consideration of unbalanced data. Relationships between $[Ca^{2+}]_e$ dependent changes in twitch and TAP parameters was examined by least square linear regression. Student's t-test was employed where appropriate, and $P<.05$ was taken as the level of significance.

Major Findings: The major findings of the present study are (1) that the cardiac contraction in RV papillary muscles from the senescent rat heart is prolonged compared to that from younger adult hearts; (2) that the simultaneously measured TAP exhibits a greater extent of depolarization and is prolonged in senescent versus adult muscles; (3) that steady state TAP and twitch duration indices exhibit modest correlations in senescent but not in younger adult muscles when the reponses to a change in $[Ca^{2+}]_e$ in both are compared; (4) that in response to an increase in $[Ca^{2+}]_e$, changes in steady state T_{75} and T_{90} are highly correlated with changes in DT and dT/dt in senescent but not younger adult muscles; (5) prolongation of neither TAP nor contraction can be directly attributed to ventricular hypertrophy.

Signigicance to Biomedical Research and the Program of the Institute: The duration of Ca^{2+} activation of the myofilaments is an important determinant of twitch duration, especially the first two-thirds of the twitch, or approximately to the time of CD in the present study. This is determined in large part by the magnitude of the transient increase in $[Ca^{2+}]_i$ and its rate of removal which begins very early with respect to the twitch time course and approaches the resting level at about the time between TPT and RT 1/2 in rat myocardium. Thus, even though TAP depolarization is 90% complete well before the TPT in rat cardiac muscle, a voltage dependence in either the extent to which myoplasmic Ca^{2+} increases with excitation, or of the time of onset or rate of removal of Ca^{2+} might be expected to affect TPT, RT 1/2 and CD. Prolongation of the action potential, the, may be, at least in part, causally related to prolonged contractile activation in the senescent heart.

Proposed Course: (1) to measure contractile and parameters and TAP in isolated single cardiac cells; (2) to determine what specific age-related alterations occur in the ionic currents that underly prolonged TAP in senescent cells.

Publications: None.

ENDOCRINOLOGY SECTION

Annual Report Summary for October 1, 1982 to September 30, 1983

The Endocrinology Section is engaged in a variety of projects that are directed to the goal of better understanding the biochemical and physiologic bases of age-related alterations of hormone and neurotransmitter secretion and action. The studies involve intact human subjects, animal models, tissue and cell culture techniques, and cell-free systems.

Age-related alterations of β-adrenergic mediated lipolysis in the rat have been under study by our group for a number of years. We have shown a clear decrease of β-adrenergic hormone responsiveness with age in rat fat cells. However, the difficulties of using this system as a model of aging are several. The results using our GRC rats proved to be very different from those of our colleagues at the University of Texas who used the Fisher 344 strain. Our animals showed less of an age decrement (±50% vs. 90%) for the Texas group and did so only at very advanced age, while the Fisher rats showed a progressive change from early maturity through mid-life. This qualitative and quantitative difference demanded elucidation. We considered two possibilities: 1) a strain (i.e., genetic) difference; and 2) an environmental factor. Since the rats in the two studies were fed different diets, we considered based on the results of other studies in the literature that diet was a significant factor. A study of the effect of diet on lipolytic responsiveness has been continued in this past year. Because of earlier financial constraints we have only recently been able to implement a study using the Fisher rat.

In exploring the issue to diet the following facts have emerged. First, both the amount of fat in the diet and the type of fat (degree of saturation) are important variables for the hormone-sensitive lipolytic response. A relatively high fat diet, but one which is comparable to that used by others with the Fisher 344, is strikingly inhibitory to lipolytic responsiveness. Second, the composition of dietary fat has a profound effect on the lipid composition of cell membranes and the latter in turn controls the activity of numerous cell membrane enzymes, including the hormone-sensitive adenylate cyclase system. However, within the same animal the effect of altered dietary fat is not the same in every tissue. Thus, while little or no effect of a diet high in unsaturated fat in fat cell membranes, the membranes of liver cells are grossly affected and exhibit increased hormone-responsiveness to both catecholamines (epinephrine) and the polypeptide hormone, glucagon. We have further shown that while receptor numbers can change during dietary manipulation, they do not correlate with alterations of hormone-sensitive adenylate cyclase. Coupling of receptors to the remainder of the adenylate cyclase complex may be affected.

It is obvious from these complex results that age is only one of many variables in the control of lipolysis and that a great deal of work remains to be done in this area if we are to begin to understand the interrelationships and mechanism(s) of the age-effects. On the other hand, the fat cell system is still one of the best defined, single-cell systems available for a biochemical dissection of age-related alteration of hormone action.

Because we believe that the fat cell offers unique advantages for aging research, we have established a facility for in vitro culture of fat cells. Preadipocytes are being obtained from animals of varying ages. The immediate questions to be answered are whether age of animal will program the developing fat cells to exhibit the same type of biochemical change (decreased lipolytic responsiveness) that one sees in vivo. If so, a valuable new approach to in vitro cellular aging will be available. Preliminary results are encouraging.

Over five years ago, we reported that in the liver of old rats a doubling of epinephrine-sensitive adenylate cylase activity was apparent. Some of our evidence at that time suggested that the mechanism seemed to involve some alteration of the liver cell membrane, but we wished as a first step to determine if β-adrenergic receptors were involved. Did these increase in number as cyclase activity increased? If not, our notion that an intramembrane event was involved seemed even more reasonable. However, despite a huge effort we could not quantitate β-receptors in adult/senescent livers. The reasons were largely technical. However, we persisted and finally discovered that a new β-antagonist (^{125}I-pindolol) is a suitable compound for this purpose. Remarkably, our data indicate that β receptors increase three-fold, i.e., more than the two-fold increase of epinephrine-sensitive adenylate cyclase. The β-receptor affinity is unchanged. No similar increase of β-receptor number has ever been seen under any other physiologic or pathophysiologic condition, including aging. Studies of animals at 6, 12, 18, and 24 months show that there is no simple relationship between the increase of β-receptors and the increase of cyclase. Further studies of the causes of these changes should be highly rewarding and are outlined below.

During the past year we had the opportunity to examine the mechanism of action of forskolin, a plant-derived diterpene activator of adenylate cylcase. This compound is used as a probe of cyclase alterations, hence the need for precise information on its mechanism of action. Contrary to earlier notions, the compound appears to facilitate the interaction of N_s and the catalytic unit rather than act directly on the catalytic unit.

We have also completed a clinical prospective study of the effect of severe illness on thyrotropin (TSH) secretion. Severely ill patients, usually elderly, exhibit the phenomenon of markedly decreased levels of thyroid hormones, the cause of which has been unexplained. We have now shown, for the first time, that thyrotropin (TSH) secretion in man is severely inhibited during severe stress. The study has significant therapeutic implications.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 AG 00011-11 CPB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hormones and aging. I. Adenylate cyclase and hormone action.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
R.I. Gregerman, Chief, Endocrinology Section, Clinical Physiology Branch, NIA

COOPERATING UNITS *(if any)*

Department of Surgery, Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

The project includes studies on the biochemistry of hormone-sensitive adenylate cyclase in a variety of tissues. Their purpose is to explore the mechanisms by which age produces alterations of hormone-responsiveness in biological membranes, with special emphasis on the relationship between adenylate cyclase and hormone receptors. Dietary effects on the components of the adenylate cyclase system are also under study. The mechanism of the age-related decrease of catecholamine-sensitive lipolysis is being studied in comparative measurements in two strains of rat (GRC-Wistar and Fisher 344) which exhibit different age-related changes. Aging in fat cells is being studied in tissue culture of pre-adipocytes from these rats. The phenomenon of stress-related inhibition of thyrotropin (TSH) secretion and development of low thyroxine (T_4) in severely ill humans ("euthyroid sick syndrome") is under study.

PHS 6040 (Rev. 1/83)

GPO 895-100

Project Description:

Other:	E. M. Dax	Expert	CPB	NIA
	L. Zhongding	Visiting Fellow (EOD 10/82)	CPB	NIA
	J. Kirkland	Guest Worker (EOD 4/82)	CPB	NIA
	E. Pavlov	Visiting Associate (EOD 7/83)	CPB	NIA

Objectives: A portion of this project explores age-related effects on the hormone-sensitive adenylate cyclase system. The action of many hormones is at the cell surface where each hormone combines with its specific receptor protein and thereby initiates a series of intracellular reactions. The hormones which act in this manner include peptides (e.g., glucagon) and proteins (e.g., thyrotropin) and amines (e.g., epinephrine). The result of the hormone-receptor interaction is activation of the membrane-bound enzyme, adenylate cyclase (AC) which catalyzes the conversion of adenosine triphosphate (ATP) to the cyclic nucleotide, adenosine-3'-5'-monophosphate (cAMP; cyclic AMP). This key compound in turn phosphorylates and thereby activates a number of enzymes (protein kinases), an event which initiates or accelerates many cellular processes (e.g., the breakdown of fat (lipolysis); the permeability of membranes to water; relaxation of smooth muscle in bronchi, etc.). The intracellular concentration of cAMP is regulated by phosphodiesterases, enzymes which degrade cAMP. Recently, it has become apparent that some hormones can initiate inhibitory effects through the AC system.

The biochemistry of adenylate cyclase (AC complex) has been extensively studied in recent years. The characteristics of the hormones receptors (glycoproteins) have been elucidated. Methods have been devised for quantitation of these receptors. A key protein which interacts with both the receptor and the catalytic portion of the cyclase is called the GTP-binding regulatory protein (formerly termed G/F, now termed N_s). This coupling protein has now been purified to homogeneity. Recently, an inhibitory coupling, protein N_i, has also been isolated. Several other less well designed proteins stimulate AC activity. The AC complex interacts with and its activity is determined by the composition of the lipid matrix of the cell membrane.

A number of age-related alterations of hormone action are known in which changes of the AC system may play a role. It has been an objective of our laboratory over the years to identify the AC alterations that are age related, and to elucidate the biochemical mechanisms by which these age-related changes occur. In this regard, we have developed methods for quantitation of receptors and of adenylate cyclase. Activator proteins have been identified. Since hormone receptors and AC are part of the cell membrane, our work is a probe of age effects on cell membranes.

Methods employed: Tissues used are from experimental animals (mainly the rat) and from man (surgical specimens). Tissue homogenates, isolated cells, and cell membranes are employed, cells being isolated by enzymatic (collagenase) digestion and membranes by density gradient centrifugation, etc. Adenylate cyclase is quantitated by a labeled substrate assay in which α-^{32}P ATP is converted to a α^{32}-cAMP and the latter quantitated after isolation on columns. Use of two marker isotopes (^{3}H-cAMP and ^{14}C-cAMP) enables precise correction of losses due to destruction during incubation and chromatography. Receptor quantitation utilizes labeled hormone antagonists. Protein factors are isolated by standard techniques of protein fractionation (gel and ion-exchange chromatography, etc.).

Major Findings: Biochemistry of the Adenylate Cyclase System.

1) Human fat cell cyclase. We have previously studied the human AC system and described a number of differences from that of the rat. As part of the further characterization of this system in anticipation of age-related measurements with human material, we have further examined the guanine nucleotide requirement of the catecholamine (hormone) stimulated enzyme. These studies further emphasize the difference of human AC from that of all other species studied to date.

In previous work we showed that GTP was essential for hormone stimulation of the human fat cell AC system, as it is for other species, although its effects in the human system were unique. In order to study this and related problems further, we examined the action of the GTP antagonist, GDP-β-S, to see whether GTP could be displaced from crude membranes and whether an earlier described salt effect could be antagonized. To our surprise, GDP-β-S, an antagonist or partial agonist in every other system studied, proved to be a potent agonist for human AC, although it was also a GTP antagonist. Furthermore, the action of GDP-β-S was stimulated by salts. Accordingly, we studied the action of GDP in the human AC system. In previously studied AC systems, GDP cannot substitute for GTP. A few reports of GDP substituion for GTP have been recently rejected as artefacts of membrane transphosphorylation which can be inhibited by UDP. However, in the human system, GDP behaves much like GDP-β-S and its effect is not blocked by UDP. Stimulation by hormone is not supported by GDP. Our findings with human adipocyte AC show, for the first time in any system, potent stimulation of hormone-independent adenylate cyclase activity by GDP. Theories of the role of guanine nucleotides in enzyme activation which postulate that hydrolysis of GTP to GDP always inactivates the system are inadequate in their present forms to explain our results. On the other hand, the data are consistent with models in which GTP (or its analogues) promotes receptor-mediated enzyme activation more effectively than GDP, and in which hormone facilitates GDP release from its regulatory unit binding site. Na^+ appears to act on N_s or the N_s-catalytic unit complex rather than on the catalytic unit alone. These results have been submitted for publication.

2) Mechanism of AC activation by forskolin. The remarkable, naturally occuring diterpene drug, forskolin, has been shown to activate adenylate cyclase both in vivo and in vitro and to promote hormone action. The drug should be a useful probe of age-related changed of AC, provided that its mechanism of action is precisely known. A number of observations suggest that the activator stimulates AC by acting on the catalytic component of the AC complex. However, we attempted to define the mechanism of drug effect in a more definitive fashion. For this purpose we studied the effect of forskolin on the AC of bull sperm which is not associated with regulatory protein (N_s) or receptors. This AC can be fused with red cell membranes which contains N_s. Forskolin was then shown to have no effect on AC alone but did enhance AC fused to N_s. This result suggests that the action of the drug is on the N_s-AC interaction rather than on AC catalytic unit alone. This work has recently been published.

3) Age-related alterations of β-adrenergic mediated lipolysis. Our published studies using GRC rats gave results very different from those reported from the University of Texas where the Fisher 344 strain has been utilized. GRC animals show less of a decrement than the Texas group (± 50% vs. 90%) and did so only at very advances age, while the Fisher rats show a progressive change from early maturity through mid-life, by which time most of the change has occured. Two possible explanations are under consideration. The first is a strain difference

(genetic) and the second, an environmental factor. The rats of the two studies were fed quite different diets. Because the Fisher 344 rat was not available to us until recently, we first explored the effect of diet. The GRC rats received ordinary rat chow (5% fat, variable degrees of unsaturation) while the Fisher rats received a synthetic diet (10% fat as corn oil !unsaturated1). There is information in the literature that suggests that fat content and type may affect lipolytic responsiveness, but the issue has not been well studied. In order to determine whether diet affects hormone responsive lipolysis we have now fed GRC rats synthetic diets containing about 10% fat while varying the degree of saturation of the fat. It is now clear that the amount of fat in the diet is an important variable for the hormone-sensitive lipolytic response. When our GRC rats are fed a relatively high fat diet (10% fat), which is comparable to what had been used by the Texas group for the Fisher 344, there was striking inhibition of lipolytic responsiveness as compared to results with lower fat in the diet. This inhibition of lipolysis in the GRC rat points up a strain difference in response to dietary manipulation and at the same time makes it unlikely that diet alone is responsible for the different results in the two published studies. The effect of dietary fat does not appear to be mediated by the adenylate cyclase complex, since the hormone-sensitive activities appear little affected by dietary fat. We suspect that adenosine, a lipolysis inhibitor produced by fat cells, may be the key substance, but this aspect needs much further work.

The genetic aspect is now being further explored. Fisher 344 rats have been obtained which have been fed ordinary rat chow. After adaptation to the GRC laboratories, the lipolytic responsiveness is being studied using isolated adipocytes, as in our earlier published report. If the difference between the two strains is apparent in our hands and with the present diet, the genetic aspects of the lipolytic responsiveness will be clearly identified.

4) Dietary fat as a determinant of hormone-sensitive adenylate cyclase in liver. The availability of tissues from the animals used in the experiments on lipolysis (above) led us to examine the effect of varying the degree of dietary fat on hormone-sensitive adenylate cyclase in liver. Unlike fat, in which adenylate cyclase was not affected, the liver membranes were greatly affected by dietary manipulation. The greater the degree of unsaturation of dietary fat, the greater the hormone-sensitive adenylate cyclase response. Catecholamine response, like that for glucagon, was doubled by the highly unsaturated diet. The mechanism of this change does not appear to be related to altered receptors as determined by labeled antagonist (^{125}I-pindolol) or agonist (^{125}I-glucagon) binding. Receptors for glucagon are highest in those tissues showing the lowest adenylate cyclase activities (diet high in saturated fat). In the case of β-receptors, the increase of epinephrine-sensitive cyclase (diet high in unsaturated fat) is not accompanied by increased β-receptors. Presumably, coupling of the cyclase complex is affected. This work is being extended and prepared for publication. The results appear to be of general importance in defining dietary fat as a major determinant of hormone responsiveness that is mediated by adenylate cyclase. The results no doubt reflect diet-related alterations of membrane lipids.

5) Adipocytes as model cells for the study of cellular aging in vitro. Studies to date of cellular aging in tissue culture have not utilized cells with a specialized and complex but yet reasonably understood hormone-sensitive response such as that of lipolysis. We believe that the cultured fat cell offers a model in which the role of genetic factors can be defined by comparative studies of tissues from GRC vs. Fisher 344 animals. Furthermore, the mechanism of age-related alteration of hormone response can be studied in a fashion not possible in vivo.

A number of laboratories have shown that fat when digested by collagenase yields not only intact adipocytes but a pre-adipocyte fraction that can be cultured. These pre-adipocytes can be grown to confluence and repeatedly subcultured. With suitable manipulations, the preadipocytes (fibroblast-like appearance) differentiate as shown by production of fat globules and development of certain enzymes.

We have now established a suitable culture laboratory and have succeeded in growing cells from rats of varying ages. The very preliminary results indicate that preadipocytes contain adenylate cyclase but no β-adrenergic receptors. Differentiation can be forced by several means. The preadipocyte cells from young animals appear to grow more rapidly than those from older animals. We expect to study the appearance of receptors during maturation and the lipolytic responsiveness of the cells derived from both GRC and Fisher 344 rats of varying ages. If the cells derived from old animals show an effect of age on lipolytic responsiveness, the results will have an important implication for the mechanisms of aging.

6) β-adrenergic receptors of liver and their relationship to age-related changes of adenylate cyclase. Several years ago we reported a 2-fold increase of adenylate cyclase activity in the liver of old rats. A number of mechanisms were considered to explain these results: a) altered β-adrenergic receptor number, b) alteration of the interaction of components of the membrane bound system, perhaps due to changes of membrane components (lipids), c) altered number of cyclase catalytic molecules within the membrane. At that time the third possibility was considered least likely. We presented some evidence for the second, i.e., alteration of the membrane, but we could not - for methodologic reasons - examine the first possibility of altered receptor number. Now, after several years of methodologic development in our laboratory, we have perfected a technique for quantitation of β-receptors in livers. Recent results show a rather remarkable 3-fold increase of β-adrenergic receptors in the livers of old animals. Although we might be tempted to relate this 3-fold increase of receptors to the 2-fold increase of adenylate cyclase in a simple causal fashion, other results suggest a more complex set of changes. Thus, receptors increase only modestly (50%) in the livers of 18 month rats (old but not senescent) while their cyclase has already increased 2-fold. By 24 months, receptors increase more than cyclase (3-fold vs. 2-fold).Finally, a few of the oldest animals, very large increases of receptors are associated with unusually low cyclase activities. Glucagon-sensitive cyclase does not increase in old animals. Receptors for glucagon can now be measured, but we have not yet performed these important parallel experiments. These results indicate that there is no simple, progressive relationship between β-receptor number and catecholamine-sensitive cyclase activity during aging.

This system demands further investigation as the understanding of these changes may elucidate the impact of aging on membranes and hormone action. Questions which can be addressed are: a) Are receptors increased or merely unmasked? b) What are the roles of the guanine nucleotide regulatory proteins (N_s and N_i) in these phenomena? c) Can in vitro biochemical alterations of membrane lipids - with resultant effects on coupling - produce similar effects to those produced by in vivo manipulation? d) How do membrane lipids change during aging and why do they change?

Significance to Biological Research and the Program of the Institute. The current studies are producing the basic information with which we can hope to understand the mechanisms of age-related changes of hormone responsiveness as they exist in the adenylate cyclase system and in more "distal" biochemical events. The interrelationships between receptors and adenylate cyclase activity and aspects of the biochemistry of adenylate cyclase continue to be explored.

Proposed Course of the Project: The extensions of the work described have been presented under the appropriate reports above.

Publications:

Pourmotabbed, G., Chou, H. J., and Gregerman, R. I.: A pepstatin-dextran conjugate as an inhibitor of proteinase-free human renin. Enzyme, 28:343-347 (]982).

Dax, E. M. and Partilla, J. S.: Adrenergic ligand liposolubility in membranes: Direct assessment in a β-adrenergic binding system. Mol. Pharmacol., 22:5-7 (]982).

Gregerman, R. I.: Aging and the Neurophysiology of Thyrotropin. In Meites, J. (Ed.): Neurobiology of Aging. New York, Plenum Press, in press.

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Gregerman, R. I.: Thyroid diseases, Ch.55. In Andres, Bierman, and Hazzard (Eds.): Principals of Geriatric Medicine. McGraw-Hill, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00012 - 10 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

G. Roth, Research Chemist, Endocrinology Section, CPB, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project is mainly involved in relating age, the various components mediating hormone actions (including receptors) and the biological responsiveness of hormone sensitive tissues.

PHS 6040 (Rev. 1/83)

GPO 895-100

Project Description:

Other:	M. Haji	Visiting Fellow (Departed 6/83)	CPB	NIA
	R. Chuknyiska	Visiting Fellow (EOD 1/83)	CPB	NIA
	B. Baum	Dental Officer	PCB	NIDR
	J. Rifkind	Research Chemist	LCMB	NIA
	M. Blackman	Guest Scientist	CPB	NIA
	D. Ingram	Staff Fellow	LBS	NIA

Objectives: This project attempts to elucidate the mechanisms by which the actions of hormones are altered during aging.

Methods Employed: Whole animals, isolated tissues, and defined cell populations in short term culture are used. Hormone receptors, located either on the cell surface or intracellularly, are studied qualitatively and quantitatively by measuring specific binding of labeled steroids, catecholamines and other hormones to tissues, cells and subcellular fractions. Affinity chromatography is used to isolate hormone receptors for measurement of synthetic and degradative rates as well as purification for preparation of antisera. Radioimmuno- and conventional assays are used for determination of hormone levels. Standard enzymatic, immunochemical and physiochemical techniques are employed. Hormonal control of various cellular metabolic processes such as nutrient transport and utilization are measured by standardized radiochemical techniques. Macromolecular biosynthetic processes are also assessed.

Major Findings: 1) Estrogen action in rat uterus and pituitary during aging.

Attempts have been made to explain the differential age changes in estrogen stimulation of LH and PRL release from rat pituitary. Only slight stimulation of total RNA synthesis by estrogen can be observed at either age. Thus is will be essential to use cDNA probes specific for the LH and PRL mRNA's to quantitate the synthesis of these respective messages as a function of age. However, since basal levels of LH and PRL release are altered concommitantly with age changes in estrogenic stimulation, reduced LH and increased PRL release in response to estradiol may simply reflect changes in the proportions of lactotroph and gonadotroph cells with age.

In contrast, estrogenic effects in rat uterus seem to be uniformly reduced during aging and are at least partially due to loss of cytoplasmic receptors. In conjunction with receptor loss, delayed and decreased stimulation of uterine nuclear RNA polymerase II is observed following estrogen administration in vivo. In order to further elucidate the mechanisms responsible for these changes, we have established a broken cell system of isolated nuclei and cytoplasmic receptors which exhibits time and concentration dependent estrogenic stimulation of nuclear RNA polymerase II. By examining various combinations of receptors and nuclei from young and old rat uteri, it was determined that both of these components from senescent animals are less effective than those from young counterparts in elevating polymerase II activity. In order to better understand these defects, a system for measurement of cytoplasmic estrogen receptor translocation into nuclei and attachment to chromatin acceptor sites has been employed. We have shown for the first time that such a system is sensitive to estradiol injection in vivo, in that nuclei obtained from treated animals exhibit less available nuclear acceptor sites than those from saline treated control rats. Preliminary experiments reveal no differences in translocation following estrogen administration when equal concentrations of receptors from young and old animals are employed. Thus, changes in polymerase II stimulation may be due to active alterations in receptor activation of transcription.

2) Alpha adrenergic action in rat parotid cells.

Efforts have continued to elucidate the mechanisms by which alpha adrenergic action is altered during aging in rat parotid cells, and to distinguish between differential patterns of changes in various adrenergic responses. Since epinephrine stimulated potassium release is maximal within one minute, attempts are being made to measure binding of epinephrine to alpha adrenergic receptors after a one minute exposure. Even though concentrations of these receptors are not reduced during aging, loss of affinity between 3 and 12 months of age may conceivably be reflected in reduced binding and might possibly account for reduced stimulation of potassium release over this period.

In contrast, epinephrine stimulation of glucose oxidation is most markedly reduced between 12 and 24 months of age. However, like the potassium release response, we have recently found that age differences in glucose oxidation can be obliterated if sufficient calcium can be made to enter aged cells. This can be achieved by increasing calcium concentrations in the presence of the ionophore, A23187. Thus, despite different termporal patterns of loss, both alpha adrenergic processes are calcium dependent and impairments in response seem to be due to altered calcium mobilization. Impaired calcium mobilization during aging now appears to be a widespread phenomenon, and may explain many dysfunctions in calcium dependent processes.

3) Dopaminergic action in rat corpus striatum.

We have recently ameliorated the well characterized loss of striatal dopaminergic receptors and biochemical and behavioral responsiveness during aging by 6-hydroxydopamine and prolactin administration as well as by dietary restriction. Attempts to understand the mechanisms involved in these manipulations have yielded the following information.

First, administration of low concentrations of highly purified rat prolactin by osmotic mini pump do not elevate circulating prolactin levels. Despite greater prolactin stimulation of striatal dopamine receptor concentrations in senescent animals, no differences between age groups in serum prolactin levels are observed following treatment. It is therefore possible that prolactin may stimulate release of other factors which mediate the increase in dopamine receptors, since old rats normally have higher prolactin levels than young, despite lower dopamine receptor levels.

Second, dietary restriction retards the age associated loss of dopamine receptors, both the D_3 subtype as measured by ADTN binding and the D_1 and D_2 subtypes as measured by spiperone binding. We have recently expaned our initial study which only examined 24 month old restricted rats to include the entire lifespan. Young restricted rats have receptor concentrations comparable to ad libitum fed counterparts but levels remain nearly constant until after 12 months of age while those of the ad libitum group exhibit a marked progressive decrease until death. Marked reduction in receptor concentrations is seen only between 24 and 30 months in restricted animals. In addition, 2 week restriction does not alter receptor levels in ad libitum fed 24 month old rats.

Proposed Course of the Project:

1) Prior to the use of cDNA probes to quantitate estrogen dependent LH and PRL mRNA production during aging, reliable methods to isolate and quantitate gonadotroph and lactotroph cell populations must be devised. At best, density sedimentation methods only yield 30% purity for the lactotroph population. Thus, improvement or other methods need to be utilized to quantitate cell populations during aging.

Measurement of uterine estrogen receptor translocation and binding to nuclear acceptor sites will be completed. If the lack of age differences persists, isolated nuclei and chromatin must be examined for selective estrogen dependent transcriptional capability. cDNA probes for particular estrogen dependent messages will also be useful for the uterine system.

2) Short term binding of epinephrine to parotid cell alpha adrenergic receptors will be completed to determine whether any age changes occur prior to the calcium mobilization events. In any case, calcium mobilization mechanisms will be further examined with radioactive calcium as well as with selective inhibitors and potentitors of the biochemical events mediating this process. Finally, 3,4-diaminopyridine will be employed in the first in vivo attempt to overcome deficits in calcium dependent parotid functions. This compound has been shown by others to be a non-toxic short term stimulator of calcium release.

3) Further elucidation of the mechanisms responsible for loss of dopamine receptors and responsiveness will be attempted. Modulation of membrane fluidity by cholesterol and lecithin will be employed to determine whether some receptors become "hidden" in the membrane with increased age. In addition, in vivo, receptor biosynthetic roles will be measured following injection of irreversible dopamine antagonists into old and young rats. Results of the above experiments will be applied toward an understanding of the mechanisms by which 6-hydroxydopamine, prolactin and dietary restriction modulate normal age-related impairments in the dopaminergic system.

Publications:

Roth, G.S.: Age-related changes in hormone action: The role of hormone receptors. In Adelman, R.C. and Roth, G.S. (Eds.) Endocrine and Neuroendorcrine Regulatory Mechanisms During Aging. Boca Raton, FL, CRC Press, p. 51, 1982.

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Ito, H., Baum, B.J., Uchida T., Hoopes, M.T., Bodner, L., and Roth, G.S.: Modulation of rat parotid alpha adrenergic responsiveness at a step subsequent to receptor activation. J. Biol. Chem. 257: 9532, 1982.

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Roth, G.S.: Altered mechanisms of hormone/neurotransmitter action during aging: receptor and post-receptor events. In Adelman, R.C., Cristofalo, V.J., Roberts, J. and Baker, G.T. (Eds.): The Fourth Philadelphia Symposium on Aging, New York, Alan R. Liss (in press).

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Joseph, J.A., Roth, G.S. and Whitaker, J.R.: Importance of striatal dopamine receptor changes in senescence. In Cristofalo, V.J., Roberts, J. and Baker, G. (Eds.): Intervention in the Aging Process, New York, Alan R. Liss (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00013-09 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Hormones, hormone receptors, and aging. III. Aging and human endocrine regulation.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

S. M. Harman, Senior Investigator, Clinical Physiology Branch, NIA

COOPERATING UNITS *(if any)*

Department of Medicine, Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Endocrinology and Human Performance Sections

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

These studies gather data on function of pituitary gonadal and pituitary thyroid endocrine systems in normal aging men in the Baltimore Longitudinal Study on Aging (BLSA), and data on daily reproductive hormone secretion patterns of normal cycling women comparing young and middle aged premenstrual females. Measurements of pituitary response to hypothalamic releasing factors as well as circulating levels of sex steroid hormones and their relationship to sexual behavior in aging men are being undertaken. Measurements of women's daily excretion of reproductive hormones is being used to follow events of the menstrual cycle.

?HS 6040 (Rev. 1/83) GPO 895-100

Project Description: Z01 AG 00013-09 CPB

Other:	R. Cropper	Clinical Staff Fellow (resigned 7/1/83)	CPB	NIA
	M. Blackman	Guest Scientist	CPB	NIA

Objectives:

A. Background - Steroid hormones secreted by the gonad play an important role in regulating body economy throughout the lifespan. In the male, testosterone, the major testicular steroid hormone, not only maintains secondary sex characteristics and sexual function, but is responsible for positive nitrogen balance and maintenance of increased muscle mass, for skeletal integrity, and possibly for such diverse functions as rate of healing and general level of aggressiveness. Steroid secretion by the testis is maintained by the pituitary hormone, LH, which is itself regulated by hypothalamic secretion of another hormone LHRH. This intricate control mechanism is in turn inhibited by rising plasma levels of testosterone, forming a complete system with its own feedback device to assure constancy of function. A second pituitary hormone, FSH, also under hypothalamic regulation by LHRH, controls testicular production of germ cells. LH, FSH, (and TSH) are glycoprotein hormones consisting of two subunits, a common α subunit and a β subunit which confers hormonal specificity on the molecule. The pituitary gland also produces prolactin, the hormone responsible for induction of lactation in women. The secretion of prolactin is normally repressed by a hypothalamic inhibitory factor and can be stimulated by hypothalamic TRH, the same substance which is responsible for pituitary release of TSH. This latter hormone, the thyrotropic hormone, stimulates thyroid incorporation of iodine into thyroxine, secretion of thyroid hormones into the blood and growth of thyroid tissue. Prolactin is known to inhibit gonadal function in the male both by decreasing pituitary release of gonadotropins and by some direct interference with testicular production of androgens. There also is an increased incidence of impotence in men with elevated prolactin.

In women the hypothalamic-pituitary-ovarian system is responsible for maintenance of normal ovulatory menstrual cycles. In each cycle FSH stimulates maturation of a cohort of ovarian follicles, only one of which normally reaches full maturity. LH causes follicular cells to produce estrogens (and androgens). Estrogen acts to promote a midcycle peak LH secretory episode which induces ovulation by the mature follicle. This follicle then becomes a corpus luteum which secretes progesterone, the second major uterotrophic steroid hormone required for successful reproduction. In addition to important stimulatory effects on the uterus and maintenance of female secondary sex organs (breasts, vagina) in the developed state, estrogens help protect women from osteoporosis (loss of bone calcium) and probably from atherosclerotic coronary disease as well.

B. Current knowledge - Previous investigators have described a decrease in the circulating level of testosterone after age 60, an increase in plasma protein binding of testosterone, which further lowers the level of "free" (and thus, presumably, bioavailable) testosterone, and an increase in circulating levels of female hormones (estrone and estradiol). At the same time the plasma FSH and LH levels have been found to increase, suggesting a primary failure of the testis, which releases the pituitary from feedback inhibition control. Some evidence also exists for a reduction in pituitary function with age in that response of the pituitary to exogenous LHRH appears to be somewhat reduced. Various investigators have found elevations of free α subunit in older humans but whether this represents

unbalanced synthesis of α and β chains (i.e., loss of efficiency) is not clear. Since study populations have not been well-defined in terms of variables which may affect hormone secretion such as nutrition and obesity, alcohol and tobacco consumption, and health and general level of activity, it is difficult to interpret the significance of this data to aging, per se. Furthermore, none of the available studies has a longitudinal design, which makes their interpretation subject to all of the difficulties which characterize cross-sectional aging studies. We have published results showing that in BLS men there are no changes in sex steroid hormones from age 25 to 89. Nonetheless, there does seem to be a rise in LH and FSH. Furthermore in a follow-up study in 180 men we found a correlation of sexual frequency and testosterone in men over 60 which was not mediated by body type, smoking, alcohol consumption, or the incidence of coronary artery disease.

A number of studies have suggested that thyroid failure with low thyroxine and elevated TSH may be more common in elderly people. Pituitary release of TSH in response to TRH also seem impaired with age according to some sources. A preliminary report has described an increase in prolactin in aging men.

A number of studies of perimenopausal menstrual cycles in women have suggested that altered patterns of hormone secretion, particularly increased FSH, decreased estrogen, and altered lengths of the preovulatory (follicular) and/or post-ovulatory (luteal) phases of the cycle may precede menopause. Whether these changes are primarily due to altered ovarian vs. hypothalamic-pituitary physiology and to what extent a particular pattern is characteristic of an individual woman is not known.

C. Present studies - Using a well-characterized group of men from the Baltimore Longitudinal Study on Aging, the effects of age on testicular function and pituitary-gonadal regulation independent of illness, excess alcohol consumption, obesity, etc. are being defined. In addition, we have studied effects of age on pituitary-thyroid dynamics. The effects of illness on sex hormones are being studied as a compliment to the aging studies referred to above. It is hypothesized that some previous work showing sex steroid and gonadotropin alterations in aging men done by other investigators may have been influenced by the inclusion of subjects with significant chronic illness.

We are also measuring excreted hormone levels in a number of young and middle-aged cycling women for 90 sequential days (3 cycles) in order to investigate alterations of cyclic ovarian function with age.

Methods employed: (1) Plasma gonadotrophins prolactin and TSH are assayed using double antibody radioimmunoassays. The TSH assay used a special concentrating technique which enabled us to distinguish variation within the normal range by increasing sensitivity 10-fold. (2) For the study of the menstrual cycles, first morning voided urines (whose hormone levels correlate strongly with 24 hr urinary values) are being collected from female volunteers in their 20's and early 30's and mid to late 40's with regular menstrual cycles for 90 sequential days. Glycoprotein hormones are extracted from these urines by concanavalin A-sepharose mini-columns which enables us to purify and concentrate LH and FSH 3-10 fold. Fractions containing estrogens and progesterone are prepared for radioimmunoassay using high performance liquid chromatography. (3) Plasma testosterone and dihydrotestosterone are measured with radioimmunoassay using Florisil separation. (4) Plasma progesterone, estrone, and estradiol are assayed using standard radioimmunoassay methods. (5) Plasma thyroxine, thyroid binding globulin, triiodothyronine, and reverse triiodothyronine were assayed using routine radioimmunoassay methods. (6) The fraction of free testosterone in plasma is estimated by an ion-exchange column method developed in our laboratory. (7) Blood samples were obtained from

a healthy, non-obese subgroup of the BLS population before and after constant intravenous infusion of 96 μg of TRH over 4 hours to test for pituitary TSH secretory reserve. (8) Data is analyzed using sophisticated computerized biostatistics.

Major Findings:

A. Aging and the pituitary thyroid axis. Findings suggest that healthy ambulatory older men may have a modest degree of reduction in T_4 secretion and a slight increase in T_4 binding which together produce a small but significant decrement in indices of free thyroid hormone. In addition the secretion of T_3 and or peripheral conversion of T_4 to T_3 appears mildly impaired and the decrease in serum T_3 and free T_3 indices is more pronounced than for T_4. Physiologically significant reductions in thyroid hormone effect should lead to compensatory elevation of plasma TSH, but in our study only a nonsignificant trend in that direction was noted, nor was there an augmentation of TSH response to TRH as is seen in the hypothyroid state. Alternative explanations for these latter findings are: a) the thyroid hormone reduction is physiologically insignificant, or b) a coexisting intrinsic aging defect occurs in the thyrotropic cells of the pituitary which prevents the compensatory response. The importance of these observations is that they already separate the effects of illness from those of aging in man. Unlike the results of several other studies, T_3 levels do change at advanced age but TRH responses are unaffected. A manuscript describing these findings has been prepared and submitted.

B. Longitudinal follow-up of male reproductive system. Samples are being obtained, but have not yet been analyzed, for a 5-6 year longitudinal follow-up of our previous study on aging effects on sex steroids and gonadotropins in BLS men. In that study, we demonstrated that no "male menopause" occurred in healthy, active, middle class men from age 25-89. To confirm and extend this conclusion, which was obtained from a cross-sectional analysis of 76 men, we are collecting repeat serum samples on most of the same individuals in order to do longitudinal analysis. To date, 42 men have been "followed up". The major sex steroids, steroid binding to globulin, and gonadotropin concentrations will be analyzed in these samples, and compared with the previous values.

C. Sex hormones and illness studies. Preliminary results show that both benign and malignant illness alter sex hormones and sex hormone binding to reduce free testosterone compared with young controls. Further studies of age-matched healthy controls are required to complete this study.

D. Menstrual cycle study. At this point 80% of samples have been collected. LH assays are nearly complete on those samples, FSH assays are underway, and the steroid methodology is being worked out. Preliminary results show a striking loss of LH midcycle ovulatory "spikes" in the older women despite continued regular cycles. This finding is unprecedented and, if confirmed, would suggest that an aging defect may occur in the hypothalamic-pituitary axis of women.

Significance to Biomedical Research and the Program of the Institute. The studies described and proposed not only add to the fund of normative data on aging but also illustrate the necessity of a sophisticated design and statistical approach to experiments on aging humans. Furthermore, they go beyond description in attempting to elucidate changes seen with age in terms of responsible physiologic mechanisms (e.g., protein hormone synthesis and assembly of subunits). Therefore they should help point the way toward a deeper scientific understanding of the nature of aging changes in human metabolism.

Proposed Course of the Project:

A. Comparison of parenteral vs. oral cyclic estrogen-progestin replacement therapy on estrogen responsive tissues in young hypogonadal vs. post-menopausal women. Hormone replacement has been shown to be beneficial in menopausal women both in relieving acute symptoms of estrogen deficiency and, most importantly, in protecting against osteoporotic fractures. However, oral sex hormones have been associated with a number of serious risks including increased incidence of endometrial carcinoma, heart disease, thrombophlebitis, gall bladder disease, and possibly, breast cancer. Good evidence suggests that the cancer risk may be reduced or eliminated by supplying estrogen cyclically with at least 10 days of progestin at the end of the cycle. Furthermore, it appears that the other risks may be mediated by absorption of estrogen into the portal circulation so that it reaches the liver in pharmacologic rather than physiologic concentrations. We plan to study the physiologic responses of young and older women to 3 graded doses of transcutaneously deliver estradiol with oral progestin and compare these cycles with 2 cycles of standard (optimal) oral therapy. Variables studied will include indices of vaginal cytology, bone resorption, plasma hormone levels, serum levels of hepatic-synthesized proteins, endometrial histology, serum lipoproteins, and plasma changes associated with intravascular clotting. The study should provide evidence whether parenteral therapy has less effect on those variables thought to be associated with risks of hormone replacement and also on what the optimal dose of estrogen should be for young vs. older women.

B. Diurnal variations of testosterone and gonadotropins in aging males. An early morning increase in T with return to baseline levels by about noon has been described in normal young men. A preliminary report at the Endocrine Society (1981) meeting suggests that this diurnal rhythm may be absent in older men. We plan to use constant withdrawal pumps to get integrated half hourly blood samples from ambulatory volunteers over a 24 hour period in order to investigate this issue in BLSA men.

Publications:

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Harman, S.M.: Relation of the neuroendocrine system in reproductive decline in men. In Meites, J. (Ed.): Neuroendocrinology of Aging, New York, Plenum Publishing Corp., in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AG 00023-08 CPB

PERIOD COVERED

October 1, 1982 to September 30, 1983

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

Hormones and aging. Testis, pituitary, and hypothalamic function.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

S. M. Harman, Senior Investigator, Clinical Physiology Branch, NIA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Secretory cells from rats pituitaries are studied in vitro to compare their physiology in old and young animals. Production of TSH in response to TRH and production of LH and FSH and their subunits in response to LRH have been measured in order to investigate altered function of pituitary secretory cells. Deficient function of aged pituitary cells in vitro has been found. Castration increases LRH responsiveness of gonadotrophs of aged and young rats to the same extent. Experiments to define the biochemical differences in glycoprotein hormone synthesis in aged pituitary cells are being undertaken as well as experiments to investigate effects of LHRH in vivo pre-treatment on in vitro function of pituitary cells.

Project Description: Z01 AG 00023-08 CPB

Other: M. Blackman Guest Scientist CPB NIA

Objectives: The Leydig cells of the testis secrete testosterone, a hormone essential to male reproductive development and function, which also has important actions at sites as diverse as bone, muscle, skin, and central nervous system. A number of studies have shown reduced function of Leydig cells with age both in animals and in man. Leydig cell function is normally under the control of the pituitary hormone, LH, which is secreted under the influence of the hypothalamic releasing factor, LRH. LH interacts with Leydig cell receptors to activate the enzyme adenylate cyclase. The resultant increase in cAMP leads to catalytic phosphorylation and activation of a system of protein kinases, which in turn alter the cell's internal metabolism and lead to hormone production and secretion. One of the goals of our studies has been define and investigate the nature of the defect appearing in an animal Leydig cell system with advancing age. Studies in our own and other laboratories have suggested that the Leydig cell deficiency in older rats is at least in part due to reduction in LH activity and thus may be a result of altered hypothalamic-pituitary function. For this reason we are now changing the emphasis of our research to focus on the pituitary gonadotropic cells.

Methods Employed: Matched pairs of young (4-9 months) and old (22-26 months) rats from the GRC Wistar colony are killed by decapitation and the testis removed and partially digested with collagenase. Tubular and interstitial elements are separated by filtration. The number of viable Leydig cells in each preparation is estimated using a histochemical (3-β-hydroxy-dehydrogenase) stain and the trypan blue exclusion technique. Short term incubations are then carried out with varying doses of human chorionic gonadotrophin (hCG, and LH-like hormone) or Dibutyryl cyclic AMP to determine cell production of testosterone. Cell membrane binding capacity and affinity for radioactivity labelled hCG is determined to estimate receptor number and quality. Testosterone is analyzed by radioimmunoassy using Florisil for separation of bound and free hormone. In secretion experiments, pituitaries from animals, intact or castrated, and treated or untreated with LHRH, TRH, etc., are removed, pooled, digested with collagenase, and after 24 hours of preincubation cell function is assessed by adding various doses of stimulating substances (LRH, TRH, dibutyryl cAMP, etc.) to the media and measuring secretory products (LH, FSH, TSH, and α, LHβ, FSHβ, and TSHβ subunits) released. In biosynthetic experiments, quartered pituitaries from young and old rats are incubated with radiolabeled amino acids and/or sugars along with varying concentrations of LHRH. Immunoreactive glycoprotein hormones and their subunits are obtained from cell lysates by immunoprecipitation and uptake of labelled compounds into various protein fractions and/or glycosidic side chains is estimated after gel chromatography of the glycoprotein.

Major Findings: A. Leydig cells. Continued experiments have confirmed our preliminary results that the "aging" defect is reversible with hCG treatment in vivo, and that serum FSH and LH are reduced in aged rats. hCG treatment improves performance of old cells despite 85 to 90% reduction in available membrane receptors for hCG in both young and old cells. Similar levels of cAMP are found in young and old cells. These findings suggest that the "aging defect" in old rats may be mainly an effect of gonadotropin deficiency rather than an age related alteration of intrinsic Leydig cell metabolism.

Repeated experiments have also agreed that one or more of the enzymes in the steroid synthetic pathway proximal to the final step may be deficient in activity and that either the final enzyme in the pathway (17α keto reductase) is deficient or lacks some important cofactor(s).

B. Measurements of serum gonadotropin levels and gonadotropin subunit secretory activity of pituitary cell suspensions from young and old intact and castrate male rats have been made. Reductions in secretion of intact LH and FSH by pituitary cells in vivo and in vitro before and after castration and after in vitro stimulation with LHRH have been documented as well as unbalanced excess secretion of α subunit by cells of old rats. Stimulation of cells in vitro with LHRH reverses the relationship of molar α/(LH + FHS) ratios in young vs. old rats, an effect similar to that seen in young and old men after in vivo LHRH stimulation. A pilot project has been undertaken comparing uptake of ^{35}S labeled methionine (new protein) and ^{3}H labeled glucosamine (glycosylation) into LH, FSH, and α subunits in in vitro pituitary preparations from young and old rats with and without LHRH in the culture media. This project requires mastery of technology not previously employed by our laboratory, including immunoprecipitation, SDS slab gel electrophoresis, and autoradiographic scanning. No data are as yet available.

Significance to Biomedical Research and the Program of the Institute: If the nature of aging is to be understood, the precise biochemical defects in the function of differentiated cells of aging animals as well as the defects hindering the replacement of such cells from populations of less differentiated cells must be investigated. Since the characteristic response pattern and details of many of the intermediate steps in the metabolism of Leydig cells is known, and since secretory and synthetic mechanisms of pituitary and hypothalamic neurosecretory cells are being worked out, these models seem to be well suited for the study of aging processes, both on a cellular basis and from the point of view of an interacting multicomponent homeostatic system.

Proposed Course: A. Effects of aging on Leydig cell response to gonadotropin. No further investigations are planned.

B. Effect of age on pituitary glycoprotein hormone and hormone subunit synthesis. We plan to expand investigations of the details of biochemical events leading to assembly and secretion of "finished" glycoprotein hormone. They will include "pulse chase" experiments to clarify the timing of various stages of hormone production, effects of exogenous stimulation in vivo with LHRH, to clarify whether the age-induced alterations are intrinsic to the pituitary cell, or represent a hypothalamic deficiency, and the development of novel methodology to label and sort pituitary cells by cell type. These methods may include labelling of cells with fluorescent microbeads coupled to an LHRH analogue and sorting of these cells following by electron microscopic confirmation of specificity and/or a new technique of micro-plaque hormone assay which enables investigation of quantitative hormone production by individual cells in vitro. These techniques will give us improved estimates of per-cell secretory rates and enable us to work with the biochemistry of pure cell types.

If additional resources were available we would propose establishing a laboratory to investigate age-related changes in protein synthetic mechanisms at the genetic level, particularly as they affect hormone synthesis. This would involve application of the latest methodologies including use of cDNA probes to identify

specific genes, (in the case of our lab genes specifying protein hromone sequences) and specific endonucleases to determine degree of methylation (inactivation) of individual gene sites. Such research might shed light on whether aging is associated with the irreversible "shutting down" of gene sites expected to be active and/or activation of gene sites expected to be inactive in various tissues. This type of research is labor intensive and would require expansion of personnel, ideally one Ph.D. level person with training in molecular biology and one post-docotral training position for a Ph.D. or M.D. level person with similar training and interests, and also two additional support staff persons, one chemist and one microbiologist.

Publications:

Tsitouras, P.D., Kowatch, M.A., Blackman, M.R., and Harman, S.M.: In vivo hCG administration reverses the testosterone secretory defect of Leydig cells from old rats despite apparent receptor down-regulation. J. Gerontol., in press.

Report of the Planning and Extramural Affairs Program

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OFFICE OF PLANNING AND EXTRAMURAL AFFAIRS

Introduction

During the past year, the results of three major projects of importance to the Institute's planning process were published: A National Plan for Research on Aging: Toward an Independent Old Age; the Report of the National Advisory Council on Aging for a National Plan of Research on Aging; and An Inventory of Federally Supported Research on Aging. In addition, policy guidelines for extramural activities were formulated, the scientific review process was formalized, and a Management Information System to characterize the scientific content of NIA research projects was developed. Based on this framework for its planning and policy functions, OPEA is now implementing a major shift in the way its activities are conducted. Organizational linkages are being established among related activities in the OPEA offices. This coordination will permit more efficient utilization of personnel and resources and will result in more effective support for the NIA's planning, evaluation and legislative functions, extramural review, data analysis and technical information development, and grants and contracts management.

The Planning and Evaluation Office (PEO) is formalizing its activities to meet the requirements of internal review of research programs by the Director, NIA, for his consideration of research opportunities and options in current and subsequent fiscal years. Planning and evaluation activities increasingly focus on assessment of program development and analysis of biomedical research trends in order to form the basis for decisions concerning the Institute's research directions. In addition, these activities will disclose scientific activities that may indicate the need for new program emphases relevant to the Institute's priorities.

The Scientific Review Office (SRO) represents one of the more critical functions at NIA, i.e., review of grant applications for scientific merit. This office is now structured to provide scientific review for a variety of applications from small grants (R03s) to manpower development and research training grants to large multi-project program projects. This office also provides technical review for all types of research contracts from cellular aging and animal resources to multi-focused population studies.

The Program Analysis and Technical Information Office (PATIO) has completed the scientific component of the Management Information System which provides the elementary bases for more detailed analyses of the Institute's extramural program activities. This office has also completed a study on word processor and communications systems suitable for use by NIA. As a first step, the basic word processing components of this system will be introduced in all OPEA offices early this fall to provide a word processor and information transfer capability within OPEA offices. If proven feasible, this system can be expanded to all NIA offices to replace the present individual word processors.

The transfer of the Grants and Contracts Management Office (GCMO) is a recent change in the organizational structure of OPEA. The activities of this office are closely aligned with review of grants and contracts and the preparation for Council review of applications. This change will facilitate the efficient conduct of the interrelated functions of the GCMO and the other OPEA offices.

Office of the Associate Director (AD/PEA)

The Office of the Associate Director conducts an array of activities central to the functional responsibilities of the NIA. A major responsibility which falls directly under AD/PEA is the formulation and implementation of internal policies and procedures and compliance with NIH and departmental directives.

- o During FY 1983 guidelines were developed, in collaboration with PATIO, for the consideration of the percentile ranking of applications in the determination of HPR/LPR recommendations. It was decided that, rather than adopt another funding system, this information can be used to identify applications which may warrant special action by Council.

- o Procedures for Advisory Council review of applications proposed for HPR/LPR action were developed to define the Council's function and to facilitate discussions by primary and secondary reviewers. This statement summarizes the criteria upon which Council members may base such recommendations and clarifies NIH policy for the scientific appraisal performed by the initial review group.

- o Draft revised guidelines were prepared for the assignment of applications by the Division of Research Grants. These guidelines establish the Institute's research interests and priorities and describe the factors to be considered in research areas which overlap the programs of other Institutes. In some cases, agreements on application assignment in these overlapping areas have been formally negotiated with the organizations involved.

The AD/PEA represents the Institute at the weekly meetings of the NIH Extramural Policy and Management Committee (EPMC), the major forum for information dissemination and coordination of policies and procedures among NIH components.

- o Issues raised at these meetings are presented to NIA staff at the NIA Committee for Policy and Program Management meetings, along with other issues which require discussion and/or resolution within the Institute. In FY 1983 the function of this committee was strengthened so that these meetings, attended by representatives of each NIA program and office, have become an invaluable means of exchanging information and addressing problems concerning various administrative activities which cross-cut the Institute, such as contract status, budget projections, changes in policies and regulations, activity scheduling, and funding strategies.

AD/PEA also handles all Council review activities including preparation of agendas and management of Council-related meetings, acquisition of recommendations for special actions from NIA staff and Council members and arrangements for Council reviewers, format of summary statement books and special action folders, and development of the formal funding action document.

- o Due to continuing concerns with the Advisory Council review of conference grant applications (R13s), the procedures for these activities were modified in FY 1983. Rather than sending the R13s directly to Council for

review and scoring, the applications now receive a primary review by the Aging Review Committee (AGE). The applications are then presented to Council, with an accompanying staff position statement.

- The management of special actions was also modified. All such actions proposed by NIA staff are now presented to the Director at the pre-Council meeting for his review and recommendation. At the Council meeting each special action is presented individually, with comments by primary and secondary reviewers, as described in the HPR/LPR discussion above. In addition, each Council member is polled three days before the meeting to obtain any items proposed for special action, and these applications are included in the agenda with the staff proposals. These changes have provided all participants a better opportunity to evaluate the relative merit of each action.

- The Institute-wide post-Council session was formalized. All staff members are requested to attend and to be prepared to discuss issues and action items which have arisen during the Council meetings. AD/PEA then prepares an implementation memorandum, indicating specific individuals who are assigned responsibility for each item.

- The Orientation Handbook for Advisory Council members was revised and updated, with the assistance of PATIO, to provide more concise information on the Institute's organization and current programs, the structure and function of its review groups, and the application review process through which research proposals are selected for funding.

AD/PEA, in collaboration with SRO, develops and implements policies and procedures for all Institute activities related to the submission and review of applications for research grants, contracts and agreements, and for coordination and review of proposed program announcements and requests for application.

- In FY 1983 special directives were prepared for the submission of program project applications to be published in the NIH Guide to Grants and Contracts. This statement defines the intent of this mechanism and the features which distinguish it from other support mechanisms, research priority areas for which this grant would be appropriate, Institute policies concerning specific eligibility factors, the preapplication process and procedures for submission, and the subsequent review process.

In addition to review activities, AD/PEA supports the Institute's Council in its advisory role.

- This year it provided direction to and coordination of activities associated with the development of the Council's response to the National Plan for Research on Aging, which has now been transmitted to the Secretary, DHHS.

AD/PEA conducts NIA oversight activities, including resolution of fraud and abuse issues, quality control of all products generated by PEA, and interpretation and application of related NIA and NIH policies and procedures.

- A draft of a formal NIA Policy and Procedure statement was prepared to "clarify the process whereby all instances of real or apparent misconduct involving research, research training, and related activities conducted or funded by the NIA are investigated." This document summarizes general NIH policy on this issue and presents specific NIA policy, outlines the precise procedure to be followed when an incident occurs, and describes the areas of responsibility for each Institute official involved. After a trial period in Bethesda, these guidelines will be issued formally for all of NIA.

- Although research misconduct covers a whole range of fraudulent practices, during the past year the NIA had only one problem related to utilization of resources and another regarding reimbursement of research subjects. In general, NIA has been able to intervene in the early stages of questionable activities and prevent problems from occurring.

- AD/PEA continued its editorial review of summary statements prepared by the Scientific Review Office. These statements are assessed in terms of clarity, accuracy, format, and adherence to NIH policy. This review has resulted in a substantial decrease in the number of comments received from applicants concerning their critiques.

Following the NIA-sponsored workshop on "Senile Dementia of the Alzheimer's Type: Ethical Issues in Clinical Research," a reference document was prepared. This document presents principles to be considered in research activities involving mentally impaired subjects for whom "informed consent" may be difficult or impossible to obtain. It is now being prepared for publication.

Planning and Evaluation Office

The planning, evaluation, and legislative components of this office establish the framework and processes for the assessment of NIA goals, objectives, and research strategies; the establishment of research priorities; the consideration of research support allocations; the development of new programs; the modification of existing programs and their evaluation; and the coordination of legislative activities.

PEO is responsible for the formulation and conduct of the annual review process which encompasses instructions for preparation of the annual NIA Planning Sourcebooks, Program Review sessions between the Director, NIA, and the program officials, and follow-up activities to implement decisions reached at these sessions.

- The Planning Sourcebook was further revised, in consultation with program and administrative staff, to compile information for a number of purposes, including the Director's program reviews, preparation of the Annual Report and the Report to Council, development of research and operating budgets and trend analyses, and procurement planning. In addition, the Gerontology Research Center has adapted a modified version of this format to certain aspects of its reporting needs so that this year the Institute's entire intramural program is represented in this document as well.

- The Fifth Report to Council on Program was prepared for the National Advisory Council. It describes program objectives and current areas of support, outlines research advances for the past year, and discusses anticipated program development and expansion. This resource document and verbal presentations by each program director provided the Advisory Council with a comprehensive account of the status of the Institute's research activities.

PEO coordinates selected activities important to the planning process.

- The Contracts Advisory Committee was convened twice during FY 1983, first in December 1982 to conclude all actions required for FY 1983 funding, and again in June 1983 to consider all research contracts and agreements proposed for FY 1984 funding. Summaries of the discussions at these meetings were forwarded to the NIA Director to assist in his decisions on contract funding for the Institute.

- The Conference Review Committee met to evaluate proposed conferences and workshops to be sponsored in whole or in part by the NIA during FY 1984. Procedures have been modified based on staff comments and reporting requirements so that this Committee now serves in a consultative rather than an approval capacity. A summary of this meeting will also be forwarded to the NIA Director for his consideration.

- A tracking system was designed and implemented to monitor the status of all research contracts and agreements, both active and pending. An integral part of this system is the Research Contract and Agreement Status Report which lists each document with related administrative and funding information and actions that have occurred since the previous report. The report is circulated once a month for comments and corrections, which are then included in the next month's report. This system results in up-to-date information which can then be incorporated into the Research and Development Contract Procurement Plan which the PEO prepares each quarter for inclusion in the NIH procurement report. It also provides NIA program staff with information upon which to base decisions for future contracting activities.

- A computer-based system was implemented to produce the Institute Funding List. This type of approach reduces the incidence of data entry errors and the possibility of grant applications being overlooked. It also introduces a high degree of flexibility into the funding list process and facilitates the speedy disposition of any last-minute modifications. The system has been expanded to provide preliminary lists which are used by the programs to assist in funding decisions.

- Plans are underway to update the Inventory of Federal Research on Aging. Several issues concerning the structure and scope of this second inventory are being considered, based on the response to the first document from both contributors and users. Among these issues are methods to elicit consistent reporting from contributing agencies as well as a determination of those agencies to be included; characterization of potential users and the content and format that will most effectively meet their needs; and specification of automation goals, such as links to other data bases. When decisions on these issues have been reached, procedures for data collection and assembly will be developed for implementation in FY 1984.

- A method for classifying NIA research projects into basic, applied, and developmental (BAD) categories was developed by PEO and approved by NIH. This method is based on an algorithm which is applied to all competing records. The algorithm defines conference grants (R13s) and large scale clinical trials as 100% developmental. Projects that include human subjects are 100% applied. All other projects are considered 100% basic.

- The formation of the Institute's Information and Data Steering Committee was approved, and the first of its regularly scheduled meetings will be held in the fall of FY 1983. The Committee will be chaired by the Deputy Associate Director, OPEA, and will include representatives from each NIA program and office.

PEO participates in the NIH and departmental planning process.

- During the past year the PEO Chief and selected staff represented the NIA at the NIH Planning and Evaluation meetings where NIH-wide planning and evaluation issues are discussed and policies proposed.

- Background material was prepared for the NIA Director's planning/appropriations briefings with the Director, NIH. These materials included an agenda for the planning session, high priority areas to be funded under the FY 1984 President's Budget, high priority areas which could not be adequately emphasized under the FY 1984 budget, trans-NIH research areas, and program issues likely to be raised at the appropriations hearings.

- The NIA portion of the NIH Research Plan for FY 1985, which covers high priority areas of scientific opportunity to be funded in FY 1985, as well as program changes, was submitted to the Office of the Director, NIH.

This office coordinates and develops NIA program evaluations and evaluation plans including those required by DHHS and those developed by the Institute to fulfill its legislative mandate.

- Based on reviews of the evaluation panel reports for the NIA Animal Models Development Program and the NIA Genetic and Cellular Resources Program (both completed in FY 1982), implementation plans were developed which addressed each of the panels' recommendations. The implementation plan and current status of the implementation were presented to the National Advisory Council.

- The data collection phase of the Geriatric Medicine Academic Award (GMAA) evaluation was completed, and the information from this survey was presented at the May 1983 annual meeting of GMAA awardees.

- The development of the Institute's scientific data base (also an evaluation project) is nearing completion, pending only final programming for the NIA Management Information System. Details of this activity are presented in the Program Analysis and Technical Information Office section of this report.

- An evaluability assessment for the implementation of the Report of the National Advisory Council on Aging on the research plan was approved for set-aside funding in FY 1983. This project will establish baseline data to be used in the measurement of the status of aging research in terms of the recommendations detailed in the report. Recently developed bibliometric analysis techniques will be utilized to develop quantitative measures for tracking research change and affirming research opportunities and needs identified in the report.

- Another new evaluation project has been initiated to determine the economic impact of senile dementia on the family and society.

- Three proposals for FY 1984 evaluation projects were submitted to NIH. The first is the replication of the bibliometric techniques which will provide the computerized data needed to track research change. The second is a full-scale evaluation for the GMAA. The third is the development of methodology and base-line data for the NIA Small Grant Award program.

PEO also monitored and analyzed legislative developments relevant to NIA; responded to legislative questions from the agency and the public regarding legislative issues; and represented the Institute at NIH meetings of legislative officers, briefings for NIH legislative officers, and other required functions for NIH legislative staffs.

Scientific Review Office

The Scientific Review Office is responsible for providing initial merit review on a variety of grants, contracts and special awards. The staff handles a diverse group of applications in the basic and clinical aspects of biological, behavioral and social sciences.

As the budget, size and scope of the programmatic activities of the NIA continue to increase, so does the volume of applications to be reviewed. In FY 1983, a record number of program projects (P01) applications, including 18 Teaching Nursing Home Applications, were site-visited and reviewed. The Small Grant (R03) mechanism has been in place for a full year and has demonstrated an ability to attract an impressive number of high quality proposals. FY 1983 was also a record year for the review of contracts, with a total of 11 reviewed by this office.

In FY 1983, the Scientific Review Office completed a total of 3 chartered committee and 32 ad hoc review meetings. Four ad hoc reviews (one T32 Training Grant and three K08 Academic Awards) were conducted by conference telephone calls, at a substantial savings to the Government.

In addition to the reviews, this office also provided a summary of outside opinions obtained by mail for all conference grants (R13s) received by the Institute. For FY 1983, nine R13s were reviewed, each one in effect representing an ad hoc review meeting.

During the year, the SRO also completed a revision of the Institute Referral Guidelines and provided the AD/PEA with advice on the implementation of these guidelines with respect to acceptance by DRG and other BIDs.

Other major accomplishments during the year include the production of revised review documents for ad hoc and chartered IRG members; the production of new P01 guidelines to be issued by the NIA; and the reduction of the number of K07 review meetings to one per year. The SRO, in recognition of the need for increased review capabilities once again submitted a charter for a second review committee, the Geriatrics Review Committee, to review clinically oriented applications.

Program Analysis and Technical Information Office

This office is responsible for planning, developing, and managing an NIA scientific and administrative Management Information System. This system includes data retrieval and reporting components, with associated analytical and interpretive emphasis to provide a basis for decisions concerning NIA policies and programs, as well as information in response to inquiries from both within and outside the Institute.

During FY 1983 the Systems Design and Implementation group expanded the automated Fiscal Ledger System to provide current information on the NIA budget status, existing commitments and the balance of funds as yet uncommitted; implemented the Animal Models Inventory Data Base System, which keeps a census for each animal breeding center and permits principal investigator and transaction file information to be entered interactively; developed a small grants data base including both NIA administrative and IRG data to be used for review purposes; and began developing an expense data base for grant review, site visits and related IRG expenditures including meetings, consultant fees, travel, etc.

In the first six months of FY 1983 the Data Management and Reporting group responded with over 3000 standard and ad hoc computer reports for NIA staff and other individuals and groups.

The Scientific Classification Group was responsible for the completion and maintenance of the NIA MIS Evaluation Project. Herner & Company completed work on this contract during the period October 1980 to March 1983, using NIH set-aside funds in the amount of $339,000. The workscope included (1) abstracting all NIA projects active from FY 1978 through FY 1981 and (2) coding projects active from FY 1978 through January 1983 using the NIA Multi-Axis Coding System (MACS), which consists of scientific terminology describing the thrust and methodology of each project. Information sources included the original application from the principal investigator, the summary statement describing the project, and progress reports. The 1034 abstracts were all proofread, keyboarded and corrected by Herner & Company personnel with interim and final edits by PATIO staff, as well as review by the principal investigator. Descriptive codes were assigned, keyboarded and matched to the abstracts. The abstracts are now being formatted and supplemented with administrative data in preparation for publication, and programming is being developed for retrieval of the information according to the MACS codes.

During FY 1983 the Technical Information Development and Analysis group carried out ongoing activities, including:

- Update and revision of the NIA Council Orientation Handbook for distribution to the National Advisory Council on Aging

- Technical editing of summary statements generated by the NIA Aging Review Committee and ad hoc review groups

- Submission of material for annual research requests from other Institutes including population research, senile dementia, cardiovascular and blood research, diabetes, Down's syndrome, and maternal and child health

- Provision of program data for BRCM and BSR annual program reviews

- Preparation of statistical packages for presentation to the Institute's Advisory Council, including specialized tables of approval rates and priority score distribution for applications to be reviewed by the Council

Special projects completed by this group during FY 1983 include:

- Analysis of NIA Small Grant Awards for presentation to Council

- Preparation of an inventory of NIA prevention research to update the NIH/DHHS publication on this topic for FY 1982

- Compilation of background material on the Institute's training and manpower development program for presentation by the Acting Director, NIA, to the May 1983 Council meeting

- Assembly of comparative data for NIA staff consideration concerning the relationship between raw scores and percentile ranking for FY 1981 and FY 1982 NIA grant applications

- Analysis of the percent increase in funds between the last type 5 award and the next type 2 award to see whether NIA's support followed the same trend as NIH as a whole

- Development of a projection model based on past funding trends to estimate paylines in future Council rounds. The system is now being monitored to evaluate its applicability and to make any necessary modifications before implementation.

Grants and Contracts Management Office (GCMO)

The fundamental role of the Grants and Contracts Management Office is to provide program officials and the grantee community with technical assistance and expertise in the business and other nonprogrammatic areas of grants administration.

The staff is responsible for all business management and fiscal aspects associated with the review, negotiation, award, and administration of grants. This office also acts as liaison for contract management between the NIH Division of Contracts and Grants and the NIA project officers for all NIA contract activities and provides advice to ensure the required execution of all documents for contracts and agreements.

During the past year the Grants and Contracts Management Office participated with top level staff in the development and evaluation of program plans and the overall management of the grant and contract programs. It also provided daily assistance to the scientific staff in the conduct of the grants and contracts management operations of the Institute.

- GCMO staff reviewed, made recommendations on, and prepared over 1000 communications to the grantee community for a variety of requests, inquiries, and notices. It also participated in conference calls with program staff and grantees to provide quick assessment of special problems and efficient handling of difficult situations.

- It responded to requests for guidance on policy interpretation from NIA scientific staff.

- Several conferences were held with principal investigators, administrative counterparts at grantee institutions and executive secretaries of DRG in order to assure compliance with NIH policies and procedures in the funding and management of grants.

- Reports of Expenditures were reviewed and analyzed; Statement of Appointment of Trainee forms were verified and processed; Termination Notices were verified; and Pay Back obligations were tracked during this reporting period.

- GCMO specialists and the Grants Management Officer participated in 16 site visits from May 1982 through April 1983. Staff provided in-depth analysis of program project and grant applications to alert and advise reviewers of potential administrative problems and provided on-site guidance for policies and procedures, documented budget and administrative recommendations, and translated comments into recommended levels of support. Financial summaries and budget charts were then incorporated into the official summary statement for the Aging Review Committee, ad hoc review committees, and the National Advisory Council.

- Staff specialists also attended and provided on-the-spot assistance at review sessions by translating budgetary recommendations into total dollars for final voting by the committees and presentation to Council. Administrative grant or contract policy issues were addressed by GCMO staff at each meeting.

The GCMO, acting as a liaison between the NIH Division of Contracts and Grants and the NIA project officers for NIA contract activities, provided advice to ensure the required execution of all documents for contracts and interagency agreements.

- Thirty-six incremental funding actions, new and renewal awards, and fourteen modifications to active contracts were handled by this office, with related review and interpretation of associated correspondence and preparation of documentation.

- Nine interagency agreements were processed requiring discussions with the project officers, review of the agreement, initiation and processing of the "Agreement Clearance and Extract Record" and review of the "Project Objective and Progress Report."

- Seventy vouchers were processed after review to ensure that adequate funds were available in each contract.

- The GCMO is also responsible for ensuring that all project officer training requirements are met. As of this writing, all staff members assigned as project officers on a contract or interagency agreement have successfully completed the Basic and/or the Advanced Course.

Activity on the Chairman's grant produced over 300 travel vouchers to be audited and approved. In addition, monthly reconciliation of fiscal data was carried out to verify debits and credits to the Chairman's grant account. Deposits were made routinely to ensure adequate balances for timely reimbursement, and fiscal data were entered into the NIH computer system for use by the Division of Financial Management in preparing Internal Revenue Service reports concerning consultant services.

During the past year the GCMO carried out its responsibility for precouncil operations which included receipt and distribution of applications and summary statements to appropriate NIA program staff; establishment of new official grant records; distribution of worksheets and resumes of applications assigned to Initial Review Groups; daily verification of accuracy of the summary statements; issuance of Action/Change Notifications to update NIH computer systems; and monitoring of applications to verify changes in assignments, IRG reviews, principal investigators, inactivations, etc.

Council books of summary statements and special action folders were prepared for three regular Council meetings and one special Council meeting during FY 1983. Fellowship review books were also prepared for NIA Executive Staff secondary review.

Post-Council activities included transmission of verification and notification for all Council actions on applications into the DRG computer system. Also, letters of notification of Council actions on individual applications were provided to the grantee community. While these letters are principally form letters, approximately 50% of these letters must document individual recommendations for budgetary adjustments. Finally, memoranda to other NIH BID's were prepared to document actions on dually assigned applications.

Responsibilities for the administrative management of the Institute's research and training grant programs include a variety of support mechanisms such as research projects, program projects, research careers, Institutional training, and fellowships. Accomplishments in this area during FY 1983 included the

processing of 600 competing and noncompeting awards and the preparation of approximately 130 revised awards to implement changes in budgets precipitated by grantee requests and other administrative actions.

The NIH 7000 computer terminal and a CT45 hard copy printer have enabled the awards processing staff to produce original award statements on a daily basis using the "Interactive Awards System." Additionally, this system is used to account for all extramural funds.

The Fiscal Ledger System, a data base by which the GCMO monitors status of the commitments and obligations of grant and contract funds for the current fiscal year, has been updated by GCMO staff on a daily basis. In addition, a Grants Status Report on pending applications has been issued on a monthly basis following each Council. This report allows all of the NIA scientific staff to be constantly aware of the status of pending awards in order to communicate the most up-to-date information to the grantee community.

Grant close-out activities included the review of terminated grants to ensure that all reporting requirements have been completed by the grantee. Final technical reports and final expenditure reports were requested and reviewed. To date there are 200 such terminated grants completed and awaiting shipment to the Federal Records Center. Further information is presently required on 48 other grants.

In addition, all unfunded, disapproved and withdrawn applications must be removed from the official files on a yearly basis and sent to the Federal Records Center. At present 269 files are ready for shipment.

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