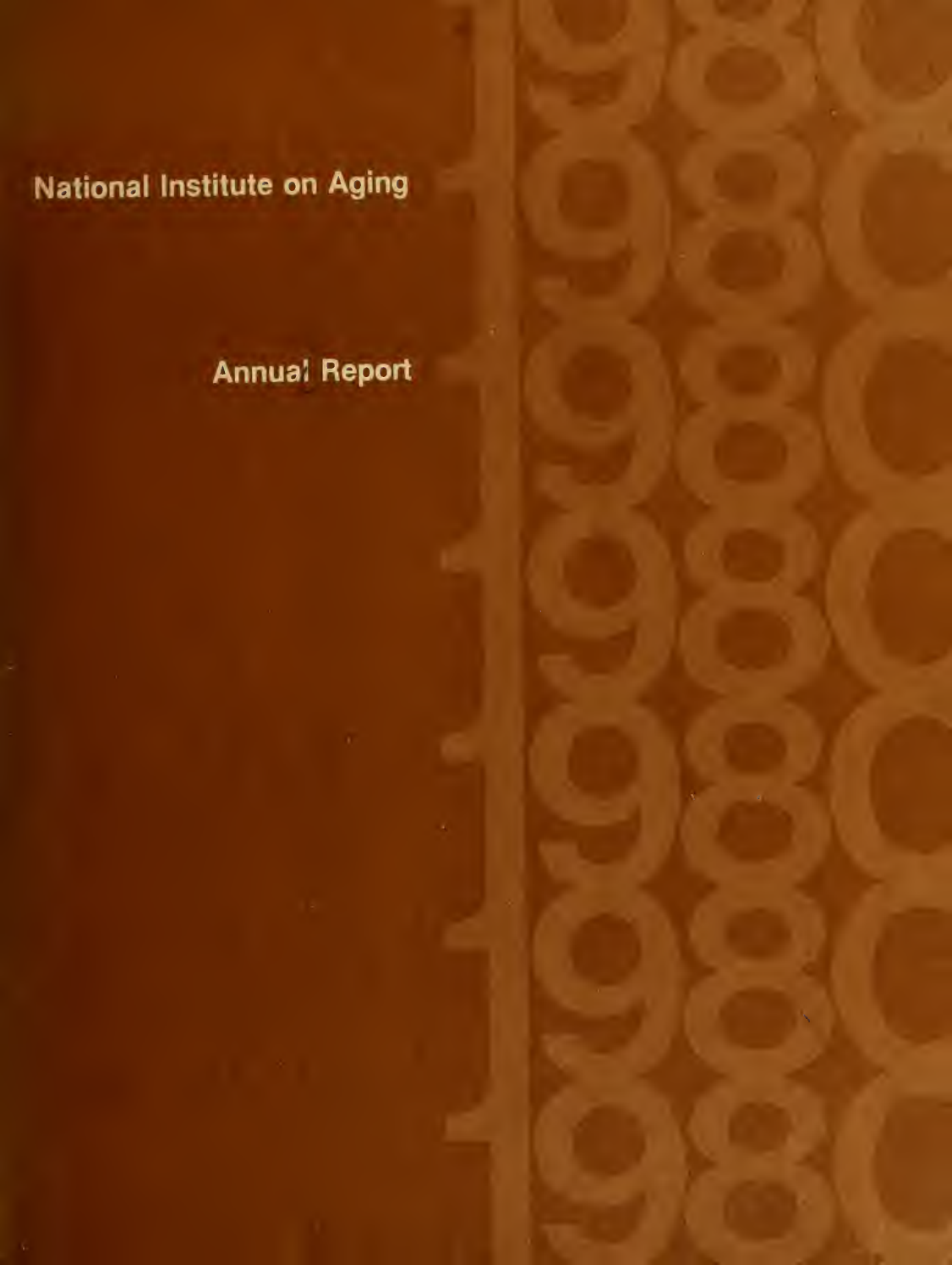
National Institute on Aging

Annual Report



National Institute on Aging

Annual Report

Oct. 1, 1979 through
Sept. 30, 1980

NIA ANNUAL REPORT

TABLE OF CONTENTS

Now in its fifth full year of operation, the NIA continues to make progress in directing and encouraging research on all facets of the aging process, including biological, social, and behavioral. The Institute is faced with an ever-increasing number of topics to be addressed, from long-term care to genetic theories of senescence. At the same time that the scope of necessary research continues to expand, the acquired knowledge on targeted areas of investigation is mounting. In the past year, changes have been made in the organization of the biomedical research and clinical medicine program, with the aim of placing more emphasis on the clinical aspects of aging and developing treatments and management strategies for age-related disorders. Some of the specific areas of growth within the last year at the NIA include the following:

Program Announcements

In FY80, four new program announcements were published in the NIH Guide to Grants and Contracts: three from the biomedical research and clinical medicine (BRCM) program and one from the social and behavioral research (SBR) program. The basic aging program of BRCM issued announcements on (a) the genetic basis of aging, using C. elegans as a model system; (b) cellular research, using differentiated human cells in culture to study age-associated alterations in differentiated functions; and (c) geriatric dermatology, focusing on the mechanisms by which skin ages.

The SBR program published an announcement encouraging research on aging as a dynamic, social process. In particular, three areas of interest include: (a) Older People in the Changing Society--research on the age composition of the population; patterns of rural and urban migration; morbidity, mortality, causes of death, and problems of dying; and age-related inequalities in income, labor force participation, voting, housing, transportation systems, and education; (b) Psychological and Social Components of the Aging Process--studies of perception and sensation, psychomotor skills, cognition and intelligence, memory and learning, and creativity and wisdom; (c) Older People and Social Institutions--research on the relationships between aging individuals and the social institutions within which they grow old, including the family; peer groups; and religious, legal, political, and leisure institutions.

In FY80, the BRCM program established the Caenorhabditis Genetics Center (CGC) at the University of Missouri. Caenorhabditis elegans, a species of nematode, is a simple, free-living organism whose stages of development and cellular and biochemical functioning mirror the life-cycle events of higher animals, including man. The CGC acquires, maintains, and distributes C. elegans, identifies mutant strains of special significance to aging research, and collects related data for distribution to interested investigators. Because of its brief life span and well-studied genetics, C. elegans is an important genetic tool for understanding the mechanisms involved in aging and longevity.

Other program announcements include the New Investigator Research Award, for newly trained investigators (not more than 5 years beyond the doctoral degree) interested in basic or clinical research projects in aging; the National Research Service Award for Short-term Training, for students in health professional schools; and the Geriatric Dentistry Awards, to stimulate the development of a curriculum in geriatric dentistry. The Institute is also continuing its work on the new NIA Academic Award. The purpose of this award is the recruitment and preparation of future academic investigators for careers in research and teaching, with special emphasis upon geriatric medicine and related clinical disciplines. The award, made to an institution, will provide a superior candidate with an opportunity for 5 years of special study and supervised experience to further his/her individual needs.

Changes in BRCM Program

During the past year, the NIA continued to consolidate its leadership with the addition of Edward Schneider, M.D., to the staff. Dr. Schneider will serve as the Medical Officer assigned to assist the NIA Director in the development of the BRCM program, the largest of NIA's three extramural programs. In addition to an expansion and reorganization of the existing components of the program, new emphasis will be placed on clinical research and training. To achieve this, a new geriatrics branch is being developed, including such sections as geriatric training, geriatric research, neurology, pharmacology, and dermatology. Other personnel additions to the BRCM staff include: Elizabeth McGuire, Ph.D., who will direct the Institute's program on nutrition; George M. Steinberg, Ph.D., who will assume primary responsibility for research on pharmacology; Mitchell Reff, Ph.D., who will develop the molecular biology component of the BRCM program; and Evan Hadley, M.D., who will assist in the new geriatric research and training area.

Long-Term Care Task Force

Because of a staggering increase in the costs of nursing home care over the past 10 years, HHS Undersecretary Nathan Stark has established a Task Force on Long-term Care which will report to the Secretary, HHS, early in FY81. Within the structure of this Task Force, the Director, NIA, will chair the Medical/Scientific Research Work Group. Among the issues under consideration is the Alzheimer's disease and related disorders initiative, a major focus of attention within the Institute over the last year. The establishment of a teaching nursing home will also be considered by the Task Force.

Experienced Pilots Act

In December 1979, NIA was assigned the major role in conducting a study of mandatory retirement for airline pilots. FAA regulations currently require pilots to retire at 60 years of age. The study will address such issues as whether the 60-year age limitation is medically warranted, and the effects of aging on the ability of pilots to perform their duties with the highest level of safety. The Experienced Pilots Act originally requested that NIH, in consultation with the Department of Transportation, undertake this study and

submit a final report to Congress by December 31, 1980. Because of the complexity and magnitude of the subject, NIA has requested an extension until September 30, 1981. A contract has been awarded to the Institute of Medicine (IOM), National Academy of Sciences, to summarize and analyze all data relevant to the issue of mandatory retirement at age 60. The IOM will present its report to NIA by March 1981, following which a consensus conference will be conducted.

Alzheimer's Disease Initiative

In the past year, major emphasis has been placed on increasing public and professional awareness of the need to gain insight into the devastating problem of Alzheimer's disease and related disorders. Research on the organic brain disorders of old age continues to be funded through a number of NIA grants. A primary goal of this research is to find the causes of Alzheimer's disease, with the hope that this will lead to guidelines on treatment or prevention.

The epidemiology, demography, and biometry program has initiated a community-based survey of victims of Alzheimer's disease and their families to provide a better understanding of patterns of occurrence. This project will characterize persons within a non-institutionalized sample who appear to have senile dementia and/or depression or to be at high risk of having these disorders. This sample will be compared with an unaffected group.

This year the Institute undertook a major role in bringing together a number of family groups around the country who are interested in encouraging family services, research, and education in the area of Alzheimer's disease. It is hoped that this national group, the Alzheimer's Disease and Related Disorders Association, will serve as the impetus for more widespread attention to the problem of Alzheimer's disease.

In early 1980, NIA staff completed a consensus development report on treatment possibilities for mental impairment in the elderly. This report, which was published in the Journal of the American Medical Association, outlines suggestions for accurate diagnosis of both reversible and irreversible mental impairment, as well as treatment possibilities for the reversible forms.

White House Conference on Aging

In preparation for the White House Conference on Aging (WHCOA), scheduled for November 30 to December 4, 1981, the NIA has appointed Daniel Cowell, M.D., to serve as the liaison with the WHCOA staff. As part of its role in the scientific activities associated with this Conference, the NIA will commission 36 scientific papers on various health topics, with popular versions for distribution to delegates at the Conference and other interested parties. The NIA will provide, or has provided, support to the Conference in the following areas: performed a demographic analysis of the population for the purpose of determining criteria for delegate selection; participated in the selection of the Advisory Council and Technical Committees; and established an NIA Council Ad Hoc Committee on the WHCOA to assist in keeping the full Advisory Council up to date on Conference progress. The Institute will

also assist the WHCOA by supplying exhibits, publications (both lay and technical), financial support, and technical advice.

WORKSHOPS AND MEETINGS

Fifth Anniversary of NIA

In May 1980, the NIA marked its fifth anniversary with a special session of the National Advisory Council on Aging. In addition to the normal review of grant applications by the council members, a special program of scientific presentations was held at the Lister Hill Center Auditorium. Among the topics discussed were the Baltimore Longitudinal Study of Aging; Genetic Heterogeneity; Aging in the Life Course in American Society; and Geriatric Medicine Needs: 1980-2030. The Director, NIH congratulated the Institute for its growth and dedication to improving the quality of life through furthering research on the aging process and on disease states associated with the elderly. Awards were presented to four individuals who have contributed to shaping the policies and legislation necessary for the birth and growth of NIA. The anniversary meeting was useful in gaining perspective on past achievements of the Institute, while increasing an awareness of the many areas of growth open for the future.

Geriatric Medicine Academic Awardees' Meeting

In 1978, the NIA introduced a new program initiative, the Geriatric Medicine Academic Award, in an attempt to meet present and future training needs of medical students and physicians in the field of geriatric medicine. This was part of the NIA's effort to assist in the development of a curriculum in geriatric medicine in those schools of medicine and osteopathy that do not have one, to strengthen and improve the curriculum in those schools that do have one, and to foster research and careers in the field of aging. The first meeting of the 15 Geriatric Medicine Academic Awardees was held on June 16-17, 1980, in Bethesda, Maryland. This meeting provided an opportunity for the grantees to meet one another and the NIA staff, to exchange information, to discuss on-going activities and future program plans, and to consider the important issue of program evaluation. Reports on accomplishments to date were given by each of the Awardees, as well as presentations by representatives of other federal agencies having a serious interest in geriatrics. Plans are under way for the second annual meeting of the NIA Geriatric Academic Awardees in 1981.

Biological Mechanisms of Aging Conference

On June 23-25, 1980, the NIA held a conference on the Biological Mechanisms of Aging. Papers were presented within seven areas of research: Mechanisms of Aging and the Human Condition; Dynamical Aspects of Senescence; Structural Pathology of DNA and the Biology of Aging; The Influence of Aging on Protein Synthesis; Posttranslational Changes in Cells and Tissues; Immunological Aspects of Aging; and Neural and Endocrine Theories of Aging. In addition, small workshops in each of these areas were held on one afternoon of the three-day conference. Each workshop chairman then presented the conclusions of the workshop participants concerning future studies and areas of investigation. Some of the

topics addressed included: the genetic basis of disease states commonly found in the elderly; the use of model systems (both theoretical and experimental) to study aging; correlations between DNA repair systems and longevity; the relationship between proteins rich in Gla--a newly discovered amino acid--and abnormal calcium deposits such as kidney stones and atheromatous plaques; the decline in immune function and the increase in autoantibodies with age; the effects of dietary restriction on longevity; and the role of dehydroepiandrosterone (DHEA) in development of breast cancer. In addition to presenting well-established results of studies in these areas, speakers and attendees aired purely theoretical ideas concerning aging and the mechanisms of disease processes. The organization of the conference was such that active participation by attendees was encouraged, and the open-ended discussions added greatly to the exchange of ideas.

Workshop on Dietary Restriction and DHEA

In July 1980, a 2-day workshop addressed the questions of dietary restriction and the effects of dehydroepiandrosterone (DHEA) on aging, blood lipids, and tumor formation in laboratory animals. A small group of researchers interested in various aspects of these two related fields of investigation met informally to present the results of their work.

Some studies on dietary restriction in laboratory rats have shown that animals given restricted diets weighed less and lived longer than rats fed ad libitum. In addition, the percent of body weight as fat mass was lower in the former group, and tumors developed later in life in restricted rats than in ad libitum rats. One investigator has observed a decrease in the incidence of some tumors (hepatomas; lung and gastrointestinal tumors) in restricted mice.

A separate but related observation by some, but not all, investigators has been the apparent antiobesity and antitumor effects of treatment with DHEA in mice. DHEA is a major adrenal steroid in man which shows a dramatic decline with age, unlike other human steroids.

Recommendations for future studies needed to confirm these preliminary findings were agreed upon by the workshop participants.

Research Frontiers in Aging and Cancer: International Symposium for the 1980's

The NIA, along with the National Cancer Institute, provided the technical and planning support for a major international symposium on aging and cancer, held in Washington, D. C., on September 21-26, 1980. The purpose of the conference was to assess the most recent scientific research in the fields of aging and cancer, and to encourage the transfer of relevant information between researchers in each field.

Approximately 45 well-known scientists, including five Nobel laureates and one who was later named Nobel laureate, presented the results of current research on such topics as organization of genetic material; regulation of gene activity; viruses in aging and cancer; and aging and cancer as genetic phenomena.

The symposium concluded with formal hearings before the House Select Committee on Aging, chaired by Congressman Claude Pepper. At these hearings, testimony was presented by the Directors of the NIA and the NCI; Lewis Thomas, M.D., president of the Memorial Sloan-Kettering Cancer Center and chairman of the symposium; and the chairmen of the eight scientific sessions.

This symposium will be the starting point for a series of future workshops to deal with other aspects of aging and cancer research. It also provided the impetus for a cooperative arrangement between the NIA and the NCI to include geriatric patients in appropriate study protocols designed to evaluate new treatment modalities.

I. ONGOING ACTIVITIES

1. Respond to inquiries by telephone, letter, and walk-ins, including those from:

   public
   media
   Congress
   other Federal agencies
   physicians
   students
   health care professionals

2. Prepare routine reports, such as:

   Special Report on Aging
   Developments in Aging
   NIH Almanac
   Scientific Directory/Bibliography
   Annual Report

3. Fill publication requests

4. Maintain NIA mailing list

5. Staff NIA exhibit

6. Coordinate NIA responses to Freedom of Information requests

7. Handle requests for speeches by/interviews with Dr. Butler

8. Provide information on NIA research and training grants

9. Screen Washington Post, Wall Street Journal, New York Times, Time, Newsweek, U.S. News and World Report, the Green Sheet, Science

10. Assist NIA staff on publications clearance, printing, and distribution procedures

II. ANNOUNCEMENTS/PRESS RELEASES

COMPLETED:

1. Research by NIA grantee Marshall on movement disorders in aged rats

2. Geriatric Medicine Academic Award

3. Conference on Endocrine Aspects of Aging

4. Aging Institute Names Ronald P. Abeles to Health Sciences Post

5. Four Honored for Contributions (NACA 5th Anniversary)

6. Dr. Jacob Brody Elected President of American Epidemiological Society

7. 5th Anniversary of the National Advisory Council on Aging

8. Three New Members Appointed to the National Advisory Council on Aging

9. NIA Announces Expanded Social and Behavioral Sciences Research Program

10. Matilda Riley Appointed NIA Associate Director

11. Dr. Leonard F. Jakubczak Joins NIA

12. Publication Announcement: A Treasure Hunt

13. Publication Announcement: Recent Developments in Clinical and Research Geriatric Medicine

14. Publication Announcement: The Older Woman: Continuities and Discontinuities

15. Publication Announcement: Special Report: 1979

16. Publication Announcement: A Guide to Medical Self-Care and Self-Help Groups for the Elderly

17. Publication Announcement: Age Page

18. Nutrition Update

IN PROCESS:

1. Dr. Edward Schneider Appointed NIA Associate Director

III. NIH RECORD STORIES

COMPLETED:

1. Dr. Riley to Head NIA's Social and Behavioral Research Program

2. Dr. Gibson Named NIA Associate Director for Planning and Extramural Affairs

3. NACA 5th Anniversary
   --announcement
   --photo story

4. NIA Celebration at Capital Children's Museum

5. Dr. Brody Elected President of American Epidemiological Society

6. NIA/NICHD Conference on Nutrition, Behavior, and the Life Cycle

7. Estrogen Conference Results

8. Sir Ferguson Anderson, Fogarty Center Scholar in Residence

9. Chinese Visit GRC (Rogers)

10. Gerontological Society Honors NIA Staff (Rogers)

11. Appointment of Drs. Wortman and Maurer

12. New NIA Advisory Council Members (Busse, Garcia-Palmieri, Martinson)

13. Senator Mathias Visits GRC (Rogers)

14. NIA Wins American Medical Writers Association Awards

15. Lifelong Dream Comes True as GRC Employee (Rogers/Ehrman)

16. Dr. Butler Wins Edward B. Allen Award

17. NIA Leading Interagency Effort to Study Important Health and Nutrition Questions: HANES Followup

IV. ANNUAL REPORTS

1. Annual Report (FY 79)

2. NIH Almanac (FY 79)

3. Scientific Directory/Bibliography 1980 (twice yearly)

4. Special Report on Aging: 1980

5. Developments in Aging: 1980

6. Program Review (FY 79)

7. Annual Report to the Congress--Public Affairs (FY 79)

8. NIA sections of NIH Information Index (every few years)

9. NIA Freedom of Information Report (FY 79)

10. NIA sections of Diabetes Special Report (1980)--editing, clearance, transmittal

11. NIA sections of Genetics Special Report (1980)--editing, clearance, transmittal

12. Opening Statement to Congress

13. NIA section of NIH Publications List

14. Annual Public Affairs Report to PHS (FY 79)

V. PROJECTS

COMPLETED:

1. Videotape on NIA for visitors and new employees

2. Refinement of NIA mailing keys

3. AoA/NIA Retreat II

4. Public relations activities for NACA 5th Anniversary
   --preparation of poster, tent cards
   --commemorative publication
   --background material on speakers
   --press relations (including contacts with media in awardees' hometowns)
   --NIH Record, NIH Alumni magazine stories
   --selection of sculpture as an award, and negotiations to commission a limited edition casting of the sculpture and a medallion with the same theme
   --public relations efforts around the sculpture on behalf of the NIA

5. Promotion of A Treasure Hunt
   --celebration at Capital Children's Museum
   --generating articles, book reviews, and media appearances
   --press release, fact sheet, biographies of authors

6. Aging: Research and Perspectives--a briefing for the press

7. Public Service Announcement to combat ageism.

8. Meeting of Alzheimer's family groups (and subsequent advice and consultation)

9. Calendar on Aging (stimulated Smithsonian to produce)

10. Summary of NIA program announcements

11. Advice to and partial support of WNET series on the brain, with considerable input to include information on aging

12. Alzheimer's Disease and Related Disorders Association
    --service on Editorial Board of ADRDA/Maryland chapter newsletter
    --liaison to ADRDA Committee on Education and Public Awareness

13. 12 tabletop exhibits and accompanying brochure holders for use when large exhibit not required or not practical to send

IN PROCESS:

1. Interagency agreement for preparation of lay publications for the White House Conference on Aging; other planning activities for WHCoA

2. Bibliographies/listings
    --general readings on aging
    --bibliographies and sources on aging
    --geriatric medicine
    --organizations concerned with aging
    --university centers in gerontology
    --journals in the field of aging
    --widowhood
    --retirement
    --older woman
    --sex and aging

3. Improving "advance" work for Dr. Butler's speaking appearances

4. Public awareness campaign on "senility"

5. Possible Sage Page series

6. New distribution channels for Age Page and other NIA publications
    --Consumer Information Center
    --Supermarket Communication Systems
    --Administration on Aging
    --State and Area Agencies on Aging

7. Research for and assistance on 10 NBC programs on aging
    --developing a quiz on basic aspects of aging
    --writing some dialogue
    --assisting in production
    --providing slides and other background material

8. Pretesting NIA publications

9. Translation of NIA information into Spanish
    --A Treasure Hunt (at the request of the Secretary, DHHS)
    --Age Pages
    --other materials as appropriate

10. TV spot(s) to promote Age Page

11. Further promotion of A Treasure Hunt
    --TV spots in English and Spanish
    --working with WDVM-TV to produce special segment for news
    --possible art exhibition plus special program at Children's Hospital or Clinical Center children's ward
    --special presentation to children at a local school

12. Developing new exhibit and revamping the existing one for display at the White House Conference on Aging

13. Updating demographic charts for lay audiences and producing a demographic fact sheet on aging

14. Updating and expanding photo and slide files to improve their usefulness to NIA staff and various media

VI. BROCHURES

COMPLETED:

1. A Treasure Hunt -- a 32-page, sturdily bound, full-color children's book designed to present older people in a positive light and combat ageism.

2. Q & A: Alzheimer's Disease -- addresses fundamental issues relating to the causes, symptoms, and treatment of Alzheimer's disease, as well as research efforts in this area.

3. Science Writer Seminars:
    --Cells and Aging
    --Does Intelligence Decline With Age?

4. Recent Developments in Clinical and Research Geriatric Medicine: the NIA Role -- a report on the efforts being made to improve the medical care of the elderly by incorporating geriatrics into the training of health care providers.

5. The First Five Years -- a compilation of abstracts of the papers presented on the occasion of the 5th anniversary of the National Advisory Council on Aging, along with biographical sketches of the speakers and a history of the development of the NIA and the NACA.

6. Research Training Opportunities at the NIA GRC -- a booklet designed to interest scientists at all levels of experience in the variety of research training opportunities available within the NIA intramural program.

7. A Guide to Medical Self-Care and Self-Help Groups for the Elderly -- a guide to self-care and self-help organizations with special interest in the elderly.

8. The Older Woman: Continuities and Discontinuities -- report of a workshop on the older woman cosponsored by the NIA and the National Institute of Mental Health.

9. Extramural Research and Training Program, Grant and Contract Summaries (assistance to Program Analysis Office)

10. Index of Current Research Grants and Contracts Administered by the NIA (assistance to Program Analysis Office)

11. Aging: Research and Perspectives--A Briefing for the Press (published by Columbia University) -- synopses of papers presented at an NIA-sponsored press briefing on the status of research on aging.

12. IoM Followup Report: A Survey of U.S. Medical School Programs in Geriatrics and Gerontology (facilitated duplication and distribution)

13. Immunological Aspects of Aging (assistance to Molecular and Biochemical Aging Program)

14. Report from the Conference on Demographic and Health Information for Aging Research (assistance to Epidemiology, Demography, and Biometry Program)

15. Second Conference on the Epidemiology of Aging -- proceedings of a conference held on March 28-29, 1977.

16. Special Report on Aging: 1980 -- a summary of NIA intramural and extramural research highlights and program accomplishments during FY 1979.

IN PROCESS:

1. Fact Sheets:
   --What Is Aging Research?
   --What Is Geriatric Medicine?

2. Skin Aging

3. New NIA Brochure

4. New A Winter Hazard for the Old: Accidental Hypothermia

5. Perspectives on Geriatric Medicine

6. Age Words: A Glossary of Gerontology

7. Biochemistry of Aging (assistance to Biomedical Research and Clinical Medicine Program)

8. Menopause and Aging

9. The Older Woman

10. Progress Reports
    --Geriatric Medicine
    --Human Performance
    --Alzheimer's Disease/Senile Dementia

11. Festschrift of NACA 5th Anniversary meeting

12. How to Get Good Geriatric Care series
    --Overview for Patients
    --Overview for Physicians
    --Arthritis

VII. AGE PAGES

COMPLETED:

1. Finding Good Medical Care for Older Americans

2. Accidents and the Elderly

3. Minorities and How They Grow Old

4. Senility: Myth or Madness?

5. High Blood Pressure

6. Aging Skin

7. Food: Staying Healthy After 65

IN PROCESS:

1. Dental Care

2. Postmenopausal Estrogen Use

3. Safe Drug Use

4. Definitions of Primary Health Professionals

5. Pain

6. Stress

VIII. ARTICLES/SPEECHES

COMPLETED:

1. Educating Professionals in Sex and Aging (Sex Education)

2. Introduction to Unlocking Home Equity for the Elderly

3. Coming of Age: The Gray Revolution and Medicine (American Pharmacy)

4. Foreword to Confusion: Prevention and Care

5. Ageism and the Corporate Executive (Conference Board magazine)

6. The Contribution of Science to the Quality of Life for the Elderly (speech to the New York State Office of Mental Health; edited for publication in Journal of Psychiatric Treatment and Evaluation)

7. Research, Gerontology, and Geriatric Medicine (American College of Physicians; edited for publication in the Annals of Internal Medicine)

8. Geriatrics and Internal Medicine (American Board of Internal Medicine; edited for publication in the Annals of Internal Medicine)

9. Aging: Agenda for the Eighties (edited for publication in the National Journal)

10. Overview on Aging: Some Social and Behavioral Perspectives (National Research Council Committee on Aging; edited for publication in proceedings)

11. Hearing and Age: Research Challenges and the NIA (Geriatrics Digest)

12. Public Interest Column: Boarding Home Fires (International Journal of Aging and Human Development)

13. Protection of Elderly Research Subjects (Clinical Research)

14. Research and Service Face the Politics of Austerity (Generations)

15. Article on NIA to accompany photo story (Washington Post)

16. Goals for Congress (Senior Citizens News)

17. The Promise of Research for Improving the Quality of Life (Grants Magazine)

18. Commentary: News from the NIA (Newsletter of the Alzheimer's Disease Association of Maryland)

19. Research: The Ultimate Service (Aging magazine)

20. Statement for Alzheimer's Disease Society (New York) Newsletter

21. Aging: Research Leads and Needs (Forum on Medicine)

22. Search for Health columns
--Heat, Cold, and the Elderly
--How We Age (Baltimore Longitudinal Study of Aging)

23. Foreword to "Old Age and Society" (special issue of the Journal of Social Issues)

24. Recent Developments in Clinical Gerontology and Geriatric Research in America (Geriatrics Review)

25. Meeting the Challenges of Health Care for the Elderly (edited for publication in the Journal of Allied Health)

26. NIA Research Interests (APHA Newsletter)

27. Letter to the Editor of Perspectives (National Council on the Aging)

28. A Glimpse Through Comroe's Retrospectroscope (by Dr. Ringler; edited for publication in proceedings of Conference on the Development and Dissemination of Biomedical Innovations)

29. Article about conference on Mental Health and the Elderly (AARP Newsletter)

30. NACA 5th Anniversary (NIH Alumni magazine)

31. Preface to The Geriatric Imperative

32. The Future of Aging Research in the United States (American Journal of Psychoanalysis)

33. Senility Reconsidered: Treatment Possibilities for Mental Impairment in the Elderly (JAMA)

34. Gerontology: A Long-Neglected Part of Medicine (Forum on Aging)

35. Section on NIA (NRTA/AARP Legislative Handbook)

36. The Federal Role in Research on Alzheimer's Disease, Senile Dementia, and Related Disorders: The NIA (presentation by Dr. Ringler at Conference on Alzheimer's Disease, Senile Dementia, and Related Disorders; edited for publication in Clinical Aspects of Senile Dementia)

37. International Horizons in Gerontology (Aging magazine)

38. Breaking Images: The Media and Aging (Columbia University)

39. Aging Now and in the 80's: Energy and Other Critical Issues (Davis Institute)

40. Helen Keller's Inner Vision and Triumphant Age: Fulfilling the Legacy (Helen Keller Centennial Congress)

41. Our Future Selves: Contributions of the NIA (NACA 5th Anniversary)

42. Aging and the Elderly (edited for Denis Prager)

43. Bringing the Old and Young Together (Capital Children's Museum)

44. The Hospital and Research in Aging (Hospitals magazine)

45. Organizing for Nutritional Research in Aging (Western Hemisphere Nutrition Congress)

IN PROCESS

1. Challenges to the American Association of Homes for the Aged

2. The Importance of Research in the Field of Aging (speech to the Indiana Governor's Conference on Aging; edited for publication)

3. Research Goals in Pharmacogeriatrics (Drug Intelligence and Clinical Pharmacy)

4. Preface to Brain Failure

5. Poverty and the Elderly

6. C. Elegans Genetics Center

7. Article for Geriatric Nursing

IX. CONFERENCE COVERAGE/PUBLICITY/ASSISTANCE

COMPLETED:

1. Biological Mechanisms in Aging

2. Estrogen Use and Postmenopausal Women

3. Hearing and Aging

4. Accidental Hypothermia "mini-consensus"

5. Endocrine Aspects of Aging

6. NACA 5th Anniversary

7. NIA/NINCDS Meeting of Alzheimer Family Groups

X. CONFERENCE SUMMARIES (PRESS/LAY)

COMPLETED:

1. To Be or Not To Be an Estrogen User?
2. Nutrition, Behavior, and the Life Cycle

IN PROCESS:

1. Biological Mechanisms in Aging
2. NACA 5th Anniversary (festschrift)

XI. TESTIMONY

COMPLETED:

1. Alzheimer's Disease (7-15-80)

XII. APPROXIMATE NUMBER OF INQUIRIES ANSWERED

1. Letters and Publication Requests: 50,000
2. Telephone Calls: 24,000

WORKSHOPS, CONFERENCES AND SEMINARS HELD, AND PROCEEDINGS PUBLISHED

DURING FY 1980

In FY 1979, the Information Office contracted with the Columbia University Graduate School of Journalism to hold a press briefing in New York City on "Aging: Research and Perspectives." The purpose of the briefing was to provide leading reporters with the background information they will need in the coming years as aging receives more and more public and media attention.

Summaries of the presentations made at the briefing were published by Columbia University during FY 1980, and the Information Office has been active in disseminating this material to interested reporters and members of the general public.

STAFF ACCOMPLISHMENTS AND ACTIVITIES IN FY 1980

A. Publications

All articles prepared or edited by the Information Office were for the signature of the Director, NIA or Deputy Director, NIA, and therefore will appear as part of their lists of staff accomplishments.

B. Presentations

1. Marian Emr spoke on "NIA Research on Alzheimer's Disease and Senile Dementia" to the Maryland Chapter of the Alzheimer's Disease and Related Disorders Association (November, 1979; Bethesda, Maryland; program-oriented).

C. Awards and Other Forms of Recognition

1. Marian Emr -- Quality Increase for performance which routinely exceeds the normal requirements for her grade, and which merits recognition on the grounds of her outstanding contributions as a Public Information Specialist and senior staff member in the NIA Information Office during a period of transition marked by numerous demands and complex staffing arrangements.

2. Judy Fernandes -- Quality Increase for contributing significantly to NIA scientific programs and public information activities, substantially beyond the normal expectations of her job.

3. Jeanne Abrahamsson -- Quality Increase for consistently producing work of the highest quality and quantity, especially for her efforts in organizing the administrative aspects of Information Office activities.

4. Maureen Mylander -- Special Achievement Award for her superb efforts in planning and organizing the NIA consensus conference on Estrogen Use and Postmenopausal Women.

5. Susan Wagner -- Quality Increase for her superior job performance, including handling the needs of a section of the Information Office located in another wing of Building 31 while performing her regular duties, and assisting Public Information Specialists with library research, responses to letters, and various administrative matters.

6. Joan Cookfair -- Special Achievement Award for her efforts in helping the Information Office cope with an increasingly large number of public inquiries during a period of staff shortage.

7. Marian Emr -- 1980 Second Award for Outstanding Medical Writing in the category of Booklets/Brochures for General Audiences, given by the American Medical Writers Association (Mid-Atlantic Chapter) for Q & A: Alzheimer's Disease.

8. Lydia Schindler (NIA contractor) -- 1980 Third Award for Outstanding Medical Writing in the category of Booklets/Brochures for General Audiences, given by the American Medical Writers Association (Mid-Atlantic Chapter) for A Guide to Medical Self-Care and Self-Help Groups for the Elderly.

9. Jane Shure, Christopher Wilson, Dagmar Wilson -- 1980 Blue Pencil Award from the National Association of Government Communicators for A Treasure Hunt.

D. Other Significant Activities

1. Promotion of A Treasure Hunt, including letters to book reviewers (resulting in several reviews); front-page mention on a Government Printing Office circular; a celebration of youth and old age at the Capital Children's Museum; radio and TV appearances for the author and illustrator of the book; a press release; and several news stories. Efforts in process include translating the book into Spanish; working with WDVM-TV to produce a special segment for the news; a possible art exhibition plus a special program at Children's Hospital and/or the Clinical Center children's ward; and a special presentation at one or more local elementary schools.

2. Launching the Age Page, a new series of fact sheets written in lay language. The purpose of the Age Page is to disseminate useful health information directly to older people, their families, and various health care providers. The aim will be to provide practical advice on prevention and self-care, and sources of additional information will be given whenever possible. Monthly publication is planned. Scheduled topics include "senility," nutrition, aging skin, drug use, high blood pressure, postmenopausal estrogen use, accident prevention, and dental care. Dissemination will be via the NIA mailing keys, the State Agencies on Aging, and supermarket information racks in over 4,500 supermarkets in the United States. Additional distribution mechanisms are being planned; suggested outlets include selected Administration on Aging mailing keys, Social Security offices, and public libraries. The Age Page has been publicized in many special-interest newsletters and a number of daily newspapers, which has generated a steady flow of requests for current and future issues.

3. Planning, publicity, pre- and post-meeting publications, and extensive general assistance for the NACA 5th Anniversary meeting

4. While the NIA reorganized its extramural program and worked to compensate for the loss of several key program staff members, the Information Office filled in where gaps existed. As a result, Information Office staff replied to complex inquiries regarding research and training grants, the NIA program in neuroscience, and geriatric medicine, among other topics.

5. Implicit in our research activities on Alzheimer's disease is the need to communicate new knowledge to the general public and

to keep abreast of the public's information needs. Recognizing the role of the self-help movement in stimulating research, services, and education, the NIA and the National Institute of Neurological and Communicative Disorders and Stroke invited representatives from seven local Alzheimer family groups to come to Washington in October 1979 to discuss their common concerns and goals. This meeting resulted in the formation of the Alzheimer's Disease and Related Disorders Association (ADRDA), a national union of groups from San Francisco, Seattle, Minneapolis, Columbus, New York, Pittsburgh, and Boston. The goals of the new group are: 1) to improve public and professional education on the dementias; 2) to assist in the formation of family support groups; 3) to take an active part in research studies, including epidemiological surveys; and 4) to educate legislators to the impact of dementia and the need for government support of services and research. Both the NIA and the NINCDS maintain liaison with the ADRDA Board of Directors.

6. Spurred by the Information Office, the Smithsonian Institutions will be issuing a 1981 wall calendar featuring the works of older American artists in the National Collection of Fine Arts. Among the works that have been selected are the portrait of John Quincy Adams by Gilbert Stuart (painted when both artist and subject were 70 years old) and paintings by Benjamin West, Georgia O'Keefe, and Charles Hassam. Among its many merits, the calendar will serve to focus attention on the 1981 White House Conference on Aging and related activities across the country.

7. The Information Office spent a considerable amount of time refining the large NIA mailing key system. NIA was one of the Institutes which urged NIH to study the entire mailing key system and find ways to improve and streamline it. This commitment was translated into service on the Advisory Board to the the NIH External Mailing Key System Study and a "guinea pig" role as the test case for new techniques developed to alleviate NIH-wide problems with mailing list maintenance and usage.

8. In cooperation with the Audiovisual Branch, NIH and GRC staff, the Information Office wrote and produced a 20-minute videotape to orient new employees and visitors to the Institute as to the NIA's mission, structure, programs, and goals.

ADMINISTRATIVE CHANGES IN FY 1980

A. Personnel

1. Pamela Jones joined the office as a Public Information Specialist with major responsibility for being the editor of the Age Page and for covering selected aspects of the Biomedical Research and Clinical Medicine Program.

2. Esther Solomon joined the office as a Public Information Specialist. She will assist in the coverage of the Social and Behavioral Research Program, as well as writing articles, fact sheets, press releases, and brochures.

3. Jeanne Abrahamsson joined the office as a Clerk-Stenographer.

4. Michelle Weiss joined the office as a Clerk-Typist.

5. Clarissa Wittenberg's temporary appointment as a Public Information Specialist was renewed. She is active in press relations, planning and coordinating special projects and events, and covering the Epidemiology, Demography, and Biometry Program.

6. Maureen Mylander, a Public Information Specialist, left the office to join the Information Office of the National Eye Institute.

7. Joan Cookfair, a Secretary-Typing, left the office to join the National Endowment for the Humanities.

8. Martha Woodland, a Clerk-Typist, left to join the Division of Computer Research and Technology.

B. Space

The "exile" of a segment of the Information Office to space on the eighth floor of the A-wing ended in July after more than a year of dislocation. The Information Office now occupies space in 5C-32, 5C-34, 5C-35, and 5C-36.

C. Equipment

Modular office furniture was purchased for the three Public Information Specialists in 5C-34 to help them cope with a particularly bad allocation of space that more closely resembled a wind tunnel than an office. The Information Office also purchased videotape equipment, which includes a monitor, console, and 3/4-inch tape deck for use by the entire Institute in viewing the orientation videotape or other items of interest. Finally, the office was able to obtain a slide storage cabinet so that slide files can be organized and viewed with ease.

Report of the Social and Behavioral Research Program

MAJOR PROGRAM AREA SUMMARY STATEMENT FOR FY 1980

The SBR program area has undergone major expansion during 1980 in line with its goal of improving the understanding of the social, cultural, economic, and psychological factors that affect both the process of growing old and the place of older people in society. Research already shows the power of these factors; it shows that aging and the status of the elderly are not inevitably fixed but are subject to social modification and change. But more research is required on how these factors operate. In order to enhance the quality of life for older people and to contain the personal and social costs of health care and dependency, more knowledge is needed to strengthen the scientific basis for professional practice and public policy. Thus the expanded program is supporting studies to probe the linkages between the changing social environment and such potentially preventable or reversible decrements of old age as memory loss, chronic ill health, sensory decrements, low self-esteem, or withdrawal from active participation in social and economic roles.

PROGRAM DEVELOPMENT

In line with the mandate to the new Associate Director, the conception, substance, and scope of the SBR program area has been revised, and steps taken to set priorities and establish policies and means of implementation. A comprehensive program has been planned as follows:

1. Research Grant Programs have been outlined, as shown in Figure 1: Older People in the Changing Society (OPS), Cognitive and Biopsychological Aging (CBA), Social-psychological Aging (SA), and Older People and Social Institutions (OPI). The boundaries of these programs have been broadly designated, allowing opportunity for continuing future development and specification (See Chapter 1, Section II).

2. Special Program Initiatives have been defined, including: Psychosocial Components of Health Promotion, Behavior Genetics of Aging, Psychosocial Factors in Nutrition, Methodology for Longitudinal and Cohort Analysis. Also planned for the future are initiatives in Psychosocial Components of Geriatric Medicine, and Aging and Psychosocial Aspects of Smoking Behavior. (See Chapter 1, Section II; Chapter 3, Section II.)

3. Research Training Programs, both individual and institutional, have been continued from earlier years, with increasing emphasis on post-doctoral rather than pre-doctoral awards. Particularly unfortunate have been the fiscal constraints on training, coupled with recent increases in stipend levels, just at the time when SBR is expanding and identifying new needs for research personnel. Of special concern to SBR, as it builds bridges between psychosocial and biological aging, is the development of inter-disciplinary talent, and a special Interdisciplinary Research Award

mechanism is projected to serve this purpose (See Chapter 3, Section II).

4. Resources to be made available to grantees have been planned, including: animals and animal colonies; archives of computerized data for secondary analysis; longitudinal study populations from whom new information may be sought. (See Chapter 1, Section VII, and elsewhere).

5. Dissemination of Research Findings has been planned through such devices as: speeches and presentations at scientific meetings; preparation of an extensive SBR mailing list for direct communication with the scientific community; preparation of a volume of review essays on selected aspects of social and psychological aging (in connection with the White House Conference on Aging); plans for a series of articles and working papers issued by members of SBR staff; continuation of the Association for Advancement of Science series of volumes on Aging from Birth to Death. (See Chapter 1, Section VII).

ADMINISTRATIVE ARRANGEMENTS

The expanded staff and the administrative plans for handling this broad range of activities are outlined in Figure 2 (and see Chapter 1, Section XI). Leadership for the four emerging programs, with their associated special initiatives and resources developments, is deployed as follows:

1. Older People in Society (OPS) - Shirley Bagley
2. Cognitive and Biopsychological Aging (CBA) - Leonard Jakubczak
   Behavioral Genetics - Richard Sprott
3. Social-psychological Aging (SA) - Ronald Abeles
4. Older People and Social Institutions (OPI)
   Shirley Bagley - Ronald Abeles (acting)

Richard Suzman, a medical sociologist, will start in September 1980, as an IPA to help in planning the special initiative in Psychosocial Factors in Health Promotion and in Geriatric Medicine.

The program area is coordinated and given coherence, and new priorities and directions are set, by the Associate Director, Matilda Riley. She is assisted by Kathleen Bond in regard to Scientific Development, and by Shirley Bagley (acting) in regard to Program Development. Marilyn McMillen acts as liaison with the NIA Office of Planning and Extramural Affairs.

Support staff, not yet adequate in numbers to handle the developing program activities, consists so far of: Ellen Jaffe, Program Assistant; Dawn Sickles and Lynn Launer, secretaries to the Associate Director; Patricia King; and additional secretaries requested for 1981.

RELATION TO OTHER PROGRAMS

SBR has a complex relationship to other programs inside and outside of NIA, both as they relate to aging or as they can be understood from an aging perspective. Especially at the strategic interface between psychosocial and biological aging, SBR must be continually linked to NIA programs in

Biological Research and Clinical Medicine (BRCM), in Epidemiology, Biometry, and Demography (EDBP), at the Gerontology Research Center (GRC), and to NIH and ADAMHA programs generally. Important relationships are maintained with the National Academy of Sciences, Social Science Research Council, Institute of Medicine, and many others. It is the special task of the Associate Director to evolve coherent directions and emphases from these programs. And it is the task of all SBR professional staff to consult, cooperate, and collaborate with NIA staff and that of other Institutes, agencies, scientific and professional associations, and other institutions. The very considerable 1980 activities along these lines are detailed in Chapter 1, Sections IV, VI, VII, VIII.

SPECIAL PROGRAM EMPHASES

All these program activities are guided by four major emphases that underlie the unique character of the SBR program area:

1. The interrelatedness of old age with earlier ages, as later life is interconnected with the full life course and older people are interdependent with other people of all ages.

2. The cultural variability of age and aging, both within a single society and across societies, as this variability reveals the nature and extent of cultural influences on the aging process, the lives of older people, and the general significance of age in society.

3. The dynamic (rather than static) character of aging as a process and of social and historical change which affects the age structure of society and the ways in which individuals age.

4. The multiple facets of age and aging, as social and psychological aging processes are in continuing interplay with biological and physiological aging.

These emphases affect the program substantively, generating priorities and special initiatives; and they affect the means of implementation and the relation between SBR and other programs. Thus the dynamic (life course) emphasis (3) guides the aging programs (SA and CBA) and requires the special program initiative in METHODOLOGY that includes application and development of methods for multivariate longitudinal analysis, for comparisons of how different cohorts age, and for studies of how society as a whole is changed by the aging of successive cohorts of individuals.

Most especially, the emphasis on multiple facets of age and aging (4) conduces to cooperative arrangements with other programs within NIA and in NIH for focusing on the interplay between psychosocial and biological aging; it requires the special mechanisms being developed for stimulating and funding interdisciplinary research on aging; and it leads to the new initiatives for studying psychosocial factors in health maintenance, nutrition, and geriatric medicine.

SPECIAL NEEDS AND OPPORTUNITIES

Because social and behavioral research on aging is a new area, spanning a dozen separate disciplines, and still lacking internal integrity or scientific recognition, activity during 1980 has been directed toward two special program needs, which will become scientific opportunities for future years. (See Chapter 2, Section I).

1. Substantive integration and dissemination of the already available social science research findings, so that each new grant application can be clearly located within the relevant background, and the growing body of scientific knowledge can become cumulative. Toward this end, planning has begun for updating the 1968 volume of Aging and Society: An Inventory of Social Science Findings, conceived as a process of continual revision and publication of the most accurate and relevant information.

2. Conceptual clarification, in which aging is viewed within the changing society as a complex social, psychological, and biological process. In such a developing framework, many confused concepts can be simplified, empirical findings interpreted, and research priorities set. Toward this end, a series of NIA Social Science Workshops and a series of NIA Social Science Working Papers have been initiated (See Chapter 2, Section II).

Building on substantial earlier work in certain areas--such as cognition, minority aging, retirement, and bereavement--broad planning during 1980 has identified missing topics and mapped the broad outlines of the Social and Behavioral Research (SBR) program area as a whole. For each of the four programs which now constitute SBR, the contents, goals, and rationale were defined during FY 1980 and issued in a general Program Announcement. This Program Announcement is planned as a working document for locating current activities and for guiding development over the next several years.

In addition to these four content areas, several special initiatives have been planned during 1980. While these will engage SBR as a whole, each is administratively housed in one of the programs.

Details of this 1980 planning effort, together with an assessment of current status and needs, are outlined in the following descriptions of each of the SBR programs. Some of the findings now beginning to emerge are described under Notable Scientific Achievements.

OLDER PEOPLE IN THE CHANGING SOCIETY (OPS)

The program on Older People in the Changing Society (OPS) is concerned with research on age as a structural feature of society, both in the population and in the organization of social roles. It includes studies of the behavior, attitudes, and status of older people within the changing social and demographic structure of society as a whole. Such studies lead to understanding of the conditions influencing the health and well-being of the elderly. Relevant research is currently conducted in such disciplines as sociology, anthropology, social psychology, history, economics, political science, geography, demography, and epidemiology.

This rapidly developing program within SBR has two subprograms (Age in the Population, and Age and Societal Structures), and it takes administrative leadership in the special program initiative in Health Maintenance. This program also converges with overarching research concerns of several Institutes (such as NIH-wide initiatives in differential life expectancy and in health promotion and the prevention of disease and disability). Cooperative working relationships with other Institutes have been established.

Age in the Population

At the societal level, this program includes such research topics as:

--Age composition of the population (implications for family structure, education, income maintenance programs, etc.)

--Patterns of migration, rural-urban residence

--Morbidity, mortality, causes of death

--Distributions by sex, race and ethnicity, social class

Apart from broad descriptions of the aging population (as by Van Arsdol, R01 AG 00631) and of special ethnic populations (Serow, R01 AG 01522), increasing attention is currently centered in investigator initiated research on mortality and on migration. For example, there are projects attempting to improve the means for deriving mortality statistics (Rosenwaike, R01 AG 01550); analyses of trends in mortality (Crimmins-Gardner, R01 AG 02089) both within the United States and from an international perspective (Brenner, AG 01833); examination of underlying and multiple causes of death (Manton, R01 AG 01159); and investigation of socio-economic determinants of mortality (Rosen, R01 AG 00913). Current projects on migration deal with general patterns of migration for the elderly (Biggar, R01 AG 00891), patterns for specific racial groups (Stinner, R01 AG 01910) and migration at time of retirement (Rushton, R01 AG 01182). Planned for the future is stimulation of further research on rural-urban-suburban migration, and on sex differences in morbidity/mortality and aging and the factors underlying these puzzling differences.

Age and Societal Structures

The OPS program includes not only the older people in society, but also the age-related social structures and cultural norms in that society that constrain and influence older people's lives. This subprogram is designed to include research on such topics as:

--Laws, expectations, and social norms

--Inequalities of income

--Differential opportunity for labor force participation, education

--Differential access to political opportunities (voting, office holding)

--Environmental design (housing, communities, transportation systems)

--Age-based conflict

--Age-integration vs. age-segregation

--Life-long education vs. age-based education

--Impact of sex, race and ethnicity, and social class

While we are only beginning to develop applications in this important subprogram, a few highly promising projects focus on age-related opportunities for work and retirement. One investigator (Parsons, R01 AG 02003) is working toward an econometric model that will integrate market demand considerations for the work effort of older males into a supply model which emphasizes health constraints on behavior. Another (Pampel, R01 AG 01568) is examining the effects of societal-level changes (such as economic

development, occupational structure, economic demand, the age structure, and political organization) on aggregate retirement rates, based on cross-national, longitudinal data. A third project, (O'Rand, R01 AG 02136) is using three waves of data from the Social Security Retirement History Study to study patterns and varying conditions of retirement among older unmarried and married women. Such factors in retirement are being examined as labor force history, pension eligibility, health status, race, age, wealth, education, and household composition of the older women. Also beginning in 1980 is a cross-cultural investigation (Ulam, R01 AG 02160) of the political, economic, and social life circumstances of older people, with speical reference to work and retirement, in the Soviet Union, the People's Republic of China, and several Eastern European countries.

Social Change and Cultural Variability

In relation to all of its topics, the OPS program stresses variability from one society to another as well as changes within a given society. The status of older people is far from immutable, of course: it is drastically affected, for example, by increases in age grading, reduced economic dependency on adult children, changes in mandatory retirement ages, rapid shifts in values that can result in estranging older cohorts from the dominant popular cultures.

A few current SBR projects begin to show the variability in old people's status as members of different societies, or of different subpopulations within a society. The recent programmatic emphasis of SBR on minority aging has stimulated investigation of the variability in the status of older people across sociocultural, national, ethnic, racial, and religious groupings. At least half a dozen current projects focus on older people in varied subpopulations (Jackson, R01 AG 01294; Crawford P01 01646; Gutman, R01 AG 01343; Kuzma, R01 AG 01582; Pawson, K04 AG 00022; Van Willigen, P01 AG 01358). Thus, for example, religious doctrine often dictates dietary habits which over time influence not only health status but also longevity, as in the case of the Seventh Day Adventists currently under investigation (Kuzma).

Special Initiative in Health Promotion

Running throughout such concerns with the status of older people in society is the opportunity to understand, and perhaps to modify, those social, cultural, and psychological factors that promote health and that prevent the deficits of diseases and disability in old age. In one project, for example, the influence of culture is evident among the Samoans who show increased incidence of obesity and cardiovascular disease as they migrate successively first to Hawaii, and then to California (Pawson, K04 AG 00022). Understanding and early identification of social and behavioral risk factors associated with various disease processes is a major objective in the new SBR initiative in Psychosocial Components of Health Promotion.

Problems of Interdisciplinary Research

A serious difficulty now commanding SBR attention is that of attracting

and developing scholars competent to formulate appropriate research objectives and design research procedures for studying psychosocial components of health promotion, and disease prevention. For this purpose, it is necessary both to lay the broad groundwork in the scientific community, and to invent and test new grant mechanisms. Work is now underway on a specially constructed Interdisciplinary Research Award.

COGNITIVE AND BIOPSYCHOLOGICAL AGING (CBA)

The Cognitive and Biopsychological Aging Program (CBA) is concerned with the identification and specification of cognitive, intellectual, and perceptual changes and stabilities that occur with aging. As in previous years, a sizeable portion of the SBR program area is located here. Numerous deficits have been observed in the psychological functioning of older people. Yet, much remains to be known about which aspects of the behavior of older people are a result of their aging, and which are the results of diminished social contacts, relative lack of education, loss of employment, poor state of health, or the particular historical experience undergone by their cohort. Research in this area is potentially useful for many purposes: for specifying conditions under which average patterns of aging are variable and potentially modifiable; for seeking out universal changes with aging that persist across varied conditions; for providing baselines for the diagnosis and therapy of pathological deficits in cognition and functioning; for supplying information for the design or rewarding occupational roles for middle aged and older people, and for setting appropriate retirement policies; and so on.

Two subprograms have been defined during 1980 to address such topics as the following:

Cognitive Aging

--Perception and sensation
--Intelligence
--Psychomotor skills
--Memory, learning, attention
--Emotional arousal, motivation
--Creativity, wisdom

Biopsychological Aging

--Genetic, neurological, and endocrine bases of psychological aging
--Animal models of behavioral aging
--Biofeedback in the elderly
--Neuropsychology of aging

New Directions

Several broad intellectual currents are affecting the CBA program. One is the life-course (or life-span) approach, which pervades the SBR program, and which stresses not only the dynamic character of the aging process, but also its multidimensionality and multidirectionality. The life-course approach is being developed through the special initiative in methodology

(located in the SA program).

Second, reflected in the CBA program is the shift within experimental psychology from behaviorism and its stimulus-response paradigm to cognitive psychology and the information-processing paradigm. Over the past quarter-century, perhaps no development in the social sciences has been more radical than the revolution in our way of understanding the processes of human thinking. At mid-century, behaviorism dominated experimental psychology, which was confined to relatively simple memory and learning experiments, and to a preoccupation with laboratory rats rather than people engaged in complex thinking and problem-solving. Now, a quarter-century later, the picture has changed radically, with concomitant changes in the experimental psychology of aging. Experimental psychology has achieved a new sophistication and a new confidence both in studying with precision simple, fundamental mental processes (such as reaction times and short-term memory capacities), and in bringing into the laboratory higher-level cognitive tasks like chess playing, solving problems in mathematics or physics, understanding natural language, or making medical diagnoses. Moreover, much of the analysis and explanation of such diverse processes has been brought within a general paradigm, the information processing paradigm, without loss of operationality, and with great gains in scope, precision, and rigor.

A third intellectual strand derives from the developing bridge between information-processing psychology and neurophysiology. Since the human mind "resides" in the brain, to explain how human thinking ages requires specification of the age-related changes in neural substrates.

Special Initiative in Behavior Genetics

To give focus to the topic of aging behavior as it relates to the aging brain, a special initiative has been set in motion this year on the Behavior Genetics of Aging. Behavior genetics is important because it deals with the basic question of the degree to which genetic factors determine or predispose the individual to specific behavioral patterns in later life. Behavior genetics can also increase our knowledge of the relationship of inherited diseases to age-changes in behavior. For example, the role of genetic factors in senile dementias is a particularly important area for research.

Already underway in the CBA program, one study is examining multi-factorial influences of genes and environment in a large sample of elderly twins (McClearn, R01 AG 01591). This study complements the behavioral genetic aspects of studies of aging among the Mennonites of Kansas and Nebraska (Crawford, P01, AG 01646) and among the Scots-Irish of Northeastern Kentucky (Van Willigen, P0, AG01358), where long-term genealogical and historical information is available in depth. Such studies have high potential for understanding the relative contributions to aging of heredity and environment, affording one bridge between cognitive science and neurophysiology.

Special Initiative in Nutrition

Another major initiative which has been defined during FY 1980 concerns

the place in the life course of diet and nutrition, including behavioral processes as both antecedents and consequences. Relevant to the SBR program as a whole, the special initiative on Psychosocial Factors in Nutrition and Aging is centered administratively in the CBA program. Thus research on nutrition and longevity, obesity, feeding behavior, taste and smell, and other aspects of the nutrition/aging relationship will receive continuing emphasis in the program.

Animal Resources

Although most of the work in this program is conducted with human beings, certain of the related ideas or hypotheses can be more effectively explored or tested in animals. Considerable work in resource development has been accomplished during 1980, as described in other sections of this chapter.

THE SOCIAL-PSYCHOLOGICAL AGING PROGRAM (SA)

The Social-psychological Aging program (SA) is devoted to research on aging as a social and psychological process. In this newly defined program, research is addressed to patterns of continuity and change in social and psychological characteristics, behaviors, and environmental responses of individuals as they grow old.

Despite the vast store of information on age differences, remarkably little is yet known about aging as process. Hence research in this program is set in a dynamic framework. It aims at understanding the interplay among psychological, social, and physiological aging processes, and to specify the conditions under which functioning changes or remains stable through the middle and later years. Relevant research is currently conducted in such discipline as psychology, sociology, economics, and anthropology.

Examples of research topics to be examined in relation to social and psychological aging are:

--Subjective views of the life course

--Personality traits and processes

--Social perception and emotion

--Attitudes, self-image, life satisfaction

--Dying, attitudes toward death

--Socialization

--Sex role differences in aging

--Interpersonal relationships over the life course

--Role sequences and transitions (e.g., loss of spouse or children, retirement, multiple or successive careers, serial marriages)

--Social and cultural factors in health maintenance and functioning

--Economic implications of biological loss (e.g., memory loss).

In the SA program, in addition to a variety of special studies, two broad types of longitudinal studies are currently supported through grants:

1) Small sample, intensive studies, some of them reaching back over several decades, which can provide invaluable clues to aging processes, but are not clearly generalizable to wider populations (e.g., Eichorn, P01 AG 00365; Sears, R01 AG 01015)

2) More extensive studies, which are more nearly representative of definable populations, and which are large enough to support multivariate analysis of differentiated life course patterns and causal sequences (e.g., as used by Bossé, R01 AG 02287; or by Wan, R01 AG 01680).

Recent emphasis has been toward grants of the second type, especially where reanalysis promises richer results (as in Lieberman, R01 AG/MH 33630), or where secondary analysis of several related studies promises more generalizable findings (as in the proposed studies of retirement by Palmore, R01 AG 02023).

Relation to Other Programs

Like all programs in SBR, the SA program, though distinctive in emphasis, is related to each of the other programs. Social-psychological aging (SA) is clearly interactive with cognitive and biopsychological aging (CBA). It is also related to societal and institutional structures (OPS, OPI) in which the individual is growing older. In one sense, then, SA represents the life-course (dynamic aging) perspective on topics that can also be viewed from a structural perspective. Similarly, the SA program realtes to such special program initiatives as Nutrition or Health Promotion. Thus certain aspects of social-psychological aging may be studies as antecedents or consequences of health and health disorders, or as specific factors in the etiology of such diseases as diabetes or senile dementia.

Special Initiative in Methodology

Although varied reserach designs are required to probe the underlying mechanisms, many questions of continuity or change with aging over the life course can best be pursued by longitudinal studies which follow the same individuals over time. Therefore, the special program initiative in Methodology for Longitudinal and Cohort Analysis is centered in the SA program. A dynamic approach to aging demands application and further development of sophisticated procedures for longitudinal and causal analysis which take into account classification of differing life-course patterns and sequences of events. In addition, since differing cohorts age in differing ways, methods of cohort analysis are needed for comparing similarities and differences in life-course that stem from social and historical change.

Proposed Directory of Methodological Consultants

Obstacles to methodological advance inhere not only in the need for continuing development of new methods, but also in the all too limited utilization of existing methods and in the cost of applying them. Wider utilization of current advances in methodology could be accomplished most expeditously through use by SBR grantees of qualified consultants. Plans are being considered for preparation of a directory (by speciality and geographic location) of methodological consultants for use by SBR applicants.

Resource Development

Because longitudinal studies are costly, both in dollars and time, proposals to start new studies are viewed with great caution. However, there are a considerable number of excellent studies, ongoing under varied auspices, in which substantial past histories have already been accumulated; and there are important potentials both for secondary analysis of such studies and for cooperative arrangements for adding supplementary items to them that will focus on psychosocial aspects of the aging process. An important goal of the program is to foster the continued collection and analysis of longitudinal data, and several efforts along these lines, as planned during 1980, (and described in later sections of this report) include cooperation with other agencies for:

--Extension of ongoing longitudinal studies

--Maintenance for public use of computerized data archives

--Sophisticated analyses of existing longitudinal data.

OLDER PEOPLE AND SOCIAL INSTITUTIONS (OPI)

Research in this program refers to the relations between individuals and the several social institutions in which they are aging. Within SBR, this is the least developed of the programs, although social science research has long recognized that people's development is affected by the institutional arrangements within which the process occurs. At one extreme, for example, a child's values develop differently in a one-parent family than in a family where two parents share is socialization. At another extreme, a frail person in a nursing home is encouraged to be either dependent or independent by particular institutional arrangements and particular nurse-patient relationships. Thus the OPI program includes studies of the age structure of particular institutions, and of how institutions can shape older people's lives and can in turn be shaped by older people themselves. Systems for providing service to older people are importantly included among such institutions, although NIA emphasis (as distinct from the emphasis of some other funding agencies) is on scientific analysis of these systems generally rather than on evaluation of specific services.

This program is stimulating age-related research on such topics as:

--The family and kin networks

--Friendships, peer groups

--Economic institutions (e.g., firms, consumer organizations, labor unions)

--Religious institutions

--Educational institutions

--Political institutions (including age-based political movements)

--Health institutions

--Welfare institutions

--Leisure institutions (e.g., recreational associations, retiree organizations)

Emerging Foci of Attention

To date, the few studies funded in this program fall into three categories: the family, the polity, and health and welfare institutions. Meanwhile, efforts are being made to stimulate wider attention to aging among scholars already expert in the several institutions.

In regard to the family, we know that it provides the major support system for long-term care. We know also that increasing longevity has revolutionized the sturcture of the family: for the first time in history there are many families in which four, even five, generations are alive at the same time. What we do not yet know in full detail is how this transformed kinship system will operate, how it will affect the intergenerational relationships, influence the family's support capabilities, reinforce or change the norms of independent housing and new roles for women, and ultimately alter the ways in which people age. Projects underway here include studies of three-generation triads (grandparent - parent - offspring) in a survey of black old people (Jackson, R01 AG 01294), and in a survey of intergenerational solidarity among aged Chicanos (Markides, R01 AG/MH 01573). Relevant to the secular decline in family size is another study, which is exploring the nature of parent-adult child relationships in families of varying size (Aldous, R01 AG 02279).

In regard to the political institutions which markedly affect the lives of older people, one study is addressing the question: why do some states exert more effort for their elderly constituents than others? Under investigation are two types of relevant variables (studied at 5 year intervals from 1955 to 1975): the socioeconomic and political characteristics of the states (e.g., proportion of elderly, degree of urbanization, economic resources, life expectancy) and the nature of the "policy process" in each state(e.g., legislative and administrative arrangements, activities of special interests groups) (Klingman, R01 AG 01408). Another study addresses the possible decline in the political influence of older people as their

numbers increase. Based on analysis of budgetary trends, the argument is developed that, as public funds for the elderly claim a greater share of the human services budget, humane concerns will increasingly give way to derogatory views of "gray power" (Hudson, K04 AG 00005).

In respect to health and welfare services, there are studies underway in nursing homes and community agencies that relate attitudes of service workers toward elderly patients to the behavior of these workers toward patients (Kahana, R01 AG 000846); and studies in retirement homes that relate institutional structure to the ways in which elderly residents function (Santee, F32 AG 05129).

In one striking development, Litwak (R01 AG 00654) makes a fundamental conceptual attack on the question of how formal organizations and primary groups can work together in supporting older people with varying degrees of impairement. He reconceptualizes and simplifies informal support systems in primary groups (family, neighbors, friends) in terms comparable to the larger set of studies of formal organizations. He is now using this reconceptualization in empirical studies, for example, on the built-in strain between the primary group relationships which develop in the nursing homes (yet can lead to organizational inefficiency) and the formal requirements for institutional efficiency (which can yet lead to extreme dependency and early senility among the patients).

Proposed Initiative in Geriatric Medicine

An important extension of this program, to be planned during 1981, will be special initiative in Psychosocial Components of Geriatric Medicine. This will draw together existing work on health care institutions and on illness behavior of patients as these relate to age-changes in vulnerability to disease and disorder.

RESEARCH CONTRACTS SUMMARY FOR FY 1980

NATIONAL ACADEMY OF SCIENCES COMMITTEE ON AGING--NO1-AG-8-21111

This NIA contract (SBR's only contract during the year) was continued during Fiscal Year 1980. The contract, awarded to the Assembly of Behavioral and Social Sciences of the National Academy of Sciences, establishes the NAS Committee on Aging. The objective of the Committee is to assess the current state of knowledge, to identify promising new areas of inquiry and promising new scholars, and to stimulate aging research in three selected areas:

Stability and Change in the Family
The Elderly of the Future
The Biology and Behavior of the Elderly

In each of these areas, advisory panels were established and workshops held. The proceedings of the three workshops are undergoing final editing and review and will be published as three separate volumes.

The activities of the Committee on Aging in planning and preparing these volumes have been highly beneficial to the developing programs of Social and Behavioral Research, NIA. The Assembly of Social and Behavioral Sciences, NAS, is a neutral, unbiased organization, which can draw upon the broadest national and international sources of information and recommendations regarding research issues and needs, and which possesses the credibility necessary to provide overview and advice.

During the past two years, the Committee has organized and conducted eleven meetings of scholars from a variety of disciplines and areas of interests. In all, a total of almost 100 social, behavioral, and other scientists have been involved as participants in the workshops, in planning meetings, and as members of the Committee. These meetings and workshops were designed to illuminate a range of important scientific questions in the participants' multiple disciplines that were likely to be relevant to developing research on aging.

In addition, the Committee has maintained close contact with NIA staff, in helping to encourage a broad range of behavioral and social scientists who had not previously worked on programs relating to aging to move into this field.

It is planned to continue this contract for an additional three years. Work will be maintained in certain of the areas previously identified, such as older genetic influences on aging behavior or brain/behavior relationships among older people. In addition, attention will be turned to such new areas needing development as longitudinal methodology and aging and formal organizations.

Funds totalling $373,400 were provided by the Institute to support the work of the Committee for the period February 1, 1978 - September 30, 1980. Of these funds, $186,700 was provided in Fiscal Year 1980.

SELECTED SCIENTIFIC ACHIEVEMENTS DURING FY 1980

While many SBR projects are still in mid-stream, a wide range of interim findings can be reported. These findings vary in generality, significance, and empirical validity, but each contributes one piece to the developing mosaic of scientific understanding. Thus the selected achievements noted below are merely suggestive of the potential.

One important caveat must be kept in mind in interpreting these findings: most studies are based on contemporary cohorts of older people in our own society. Thus generalizations about the aging process or the status of older people are dangerous. Such generalizations require much further testing across other cohorts (as in historical studies) and across other societies (as in cross-cultural studies).

OLDER PEOPLE IN THE CHANGING SOCIETY (OPS)

Three projects in the subprogram in Age in the Population are contributing understanding of the factors in mortality among older people. First, a study by Rosen (R01 AG00913), which deals with socioeconomic correlates or mortality using 1973 data, corroborates earlier findings that show higher mortality (40 percent) for men with elementary school compared to those with a college education. Contray to the findings of earlier analyses, this relationship holds for men over 65 as well as for younger men. Second, preliminary findings from a carefully controlled study by Helsing (R01 AG00940) probe further into the reportedly high rates of mortality following widowhood. These tentative findings indicate that in the first year or two after widowhood, the widowed male population in most age categories does suffer a significant excess mortality even after education, smoking, frequency of church attendance, and socioeconomic status are taken into account. Specific behavioral and socioeconomic factors that appear to be related to mortality among the widowed are still being sorted out. Third, continued work by Manton (R01 AG01159), using the multiple cause mortality data, shows that chronic degenerative diseases (such as diabetes mellitus and generalized atherosclerosis) are reported as an "associated" cause of death rather than an "underlying" cause of death on the death certificates of older people. This finding suggests that analyses of mortality which rely on underlying cause data may be misleading. Mortality models using the more detailed information are being developed and tested.

One other study in this program which illustrates the effort to understand social and cultural factors in morbidity and mortality is the research conducted by Pawson (K04 AG00022). Building on earlier work showing that Samoans experience massive weight gain and increased cardiovascular risk when they move to Hawaii, preliminary findings from this new study show even greater increases in weight and in blood pressure when Samoans move to California. These associations point to cultural risk factors (such as increasing levels of industrialization from Samoa to Hawaii to California), rather than to purely genetic risk factors which should be similar for all persons of Samoan decent.

COGNITIVE AND BIOPSYCHOLOGICAL AGING (CBA)

Much of the recent advance in aging research stems from broadening use of the three approaches: (1) adoption of the information-processing paradigm, (2) the search for variability and plasticity in the aging process, and (3) the linkages to neural substrates.

In the information processing paradigm as used in the study of memory, for example, information is received and transduced by the senses and is then held briefly in one of several modality-specific sensory stores--for example, visual information is held in a visual sensory store ("iconic memory"). If the information is retrieved from sensory storage by the attention process before it is lost by decay or is overlaid by subsequent stimili, it is passed on to short-term memory--a limited capacity store in which verbal stimuli are held in terms of their auditory or articulatory features. Further rehearsal of these stimuli has the effect of transferring information to a relatively permanent long-term store. Thus, much research on aging can be interpreted as demonstrating integrity or impairment at each of these stages, the transfer of information from one stage to the next, and the registration and retrieval processes associated with each stage.

AGE DIFFERENCES IN SENSATION AND PERCEPTION

Even before information can affect the sensory memory, it must be transduced (converted by the sense organs from physical energy into nerve signals) and detected faithfully. Developments in signal detection theory and magnitude estimation methodology have allowed measurement of sensory events uncontaminated by nonsensory factors, and a few projects in these areas are now underway in the CBA program. Especially because of the important implications of perceptual aging for the quality of life, this area is being fostered and developed.

For example, current findings show that compared to younger subjects, elderly subjects with normal visual activity (ability to resolve small details) have a diminished ability to see large and intermediate sized objects of low contrast (Sekuler R01 AG01251). Consequently, the older person would have more difficulty than the younger person in recognizing faces, for example. These findings also suggest that simple tests of visual acuity may not adequately assess age-related defects in visual function. Similarly, the ability to discriminate food odors generally is considerably diminished in the elderly who, nevertheless, are better able than younger people to discriminate (and also more likely to prefer) fruit odors (Schiffman R01 AG00443). Finally, in rats (whose average life span is about two years), sensitivity to pain and postural reflexes seem virtually unaffected by aging, but sensitivity to visual and auditory stimuli declines rapidly beginning at about 10 months of age (Campbell R01 AG00092).

In the information-processing model, the sensory registers receive stimulation which remains relatively untransformed until it is further processed. The evidence on the effect of old age on the sensory registers, though sparse, seems to indicate that the capacities ("volumes") of these registers change little, if at all. Nevertheless, older people proces visual information less

rapidly than the young, not only through the central stages of the visual system, but also through its peripheral states (Walsh R01 AG00521).

AGE DIFFERENCES IN ATTENTION

The age-related defect in speed of processing visual information may be traced to the finding that, as compared with younger adults, older adults are slower in selectively attending to information in the initial stages of visual memory (Walsh, R01 AG00521). A similar age-related defect in attending was observed in another set of studies using a different research paradigm, viz. psychometrics. Apparently, the intellectual decline with increased age which is reflected in short-term memory loss and decreased speed is in part due to changes in the ability or the willingness to maintain close concentration attention) in effortful tasks (Horn, R01 AG 00583; Macht, F32 Ag05193). Such an age difference in attending may explain, for example, the older adult's comparative disadvantage when reading meaningful prose that contains large numbers of arguments (Hultsch, R01 AG00910), or the inability of older adults to remember specific content as well as younger adults, even though both read in similar ways (Birren, P01 AG 00137).

COMPENSATIONS AND INTERVENTIONS

Despite numerous age-related deficits, there is considerable evidence that many older people compensate, or can learn to compensate, in various ways. For example, the older person compensates to some extent for decline in speed by exercising greater persistence and carefulness (Horn, R01 AG00583). Old people are as able as young people to benefit from advance information, and are as able to maintain preparation for rapid responding (Gottsdanker, R01 AG00011). Moreover, training with a mnemonic strategy can significantly reduce the amount of energy required by the elderly in learning lists of words (Macht, F32 AG05193). And relatively short-term interventions in late adulthood and old age are successful not only increasing intellectual performance, but also in maintaining this improvement and transferring it to other related abilities (Baltes, R01 AG00403).

VARIABILITY IN THE AGING PROCESS

In addition to the enlarged scope provided by the information-processing paradigm, major advances in aging research are only beginning to derive from the focus on individual differences: that is, on variability around age group averages, rather than on these averages interpreted as"age norms." Many of the processes associated with age stem, not from any intrinsic or universal aging process, but from variable environmental causes or from potentially correctable pathologies which affect individuals quite differently. Thus high intellectual abilities, for example, are observed among some individuals at every age, and intellecutual ability is less highly correlated with age than with experiential factors such as education. A major focus requiring research attention, then, is the identification of factors that produce individual differences in cognitive aging, such as differences in health, diet, exercise, social isolation, economic disadvantage, educational level, or motivation. Particularly important are those differences by cohort, associated with the differing historical environments in which individuals grow old.

Of special relevance here are the long-term studies by Schaie (R01 AG 004380) which, though controversial, produce unexpected findings through research designs that control the sizeable differences among cohorts. Longitudinal analysis of individuals within the same cohort shows that performance on certain types of intelligence tests (of "crystallized" intelligence, presumably based on learned abilities) tend to improve over much of the life span, whereas performance on other tests (of "fluid" intelligence, presumably physiologically based) tend on the average to peak around the third decade of life. Reliable decrements until age 80 or 90 cannot be found for all abilities, nor for all individuals. A number of findings from current cross-section surveys point to possible underlying mechanisms. Thus age-related increases in "crystallized" intelligence may be traceable to age increases in the ability to retrieve material from long-term memory, and to use this material in flexible, creative ways (Horn, R01 AG00583). And in regard to problem-solving style, age is less accurate as a predictor than either education or general health status (LaRue R23 AG00745).

SOCIAL-PSYCHOLOGICAL AGING (SA)

A number of studies in the SA program are potentially of such significance as to suggest "notable scientific achievements" in-the-making.

CLUES FROM INTENSIVE LONGITUDINAL STUDIES

The long-term Berkeley Intergenerational Studies, in which the subjects are now in their 40's and 50's, have reported such findings as the following (Eichorn, P01 AG 00365):

--Small increases in IQ scores from adolescence through middle age seem to be produced by moderately improved verbal performance which counterbalance small declines in motor performance (Eichorn et. al)

--Intellectual competence in adolescence appears to predict psychological health in the middle years (Livson and Peskin)

--Children whose parents foster trust and respect in the family tend to develop into responsible adolescents and subsequently into adults able to cope successfully with critical situations (Brooks)

--Certain dimensions of human personality are found to be developmental in the sense that they tend to become increasingly salient from earlier adolescence to middle adulthood (Haan)

--The health status of adults in their middle years appears to reflect--among other factors--early adult personality (Bayer et. al)

--There is some evidence that male "investment" in work tends to peak in the fourth decade of life, then to level off (Clausen)

--Marital satisfaction, rather than declining with age, appears to increase or decrease depending on changes in life circumstances (Skolnick)

SOME FINDINGS FROM EXTENSIVE LONGITUDINAL STUDIES

A few findings are emerging from the large scale longitudinal studies in the SA program, although these findings can be interpreted only as they are integrated with the accumulating body of knowledge in related areas. For example:

- Analysis of several thousand older adults (Longitudinal Retirement History Survey) shows that high levels of life change are associated with:

  --Comparatively low health status and high use of health services

  --Tendency to change residence

  --Comparatively low participation in formal and informal social activities (Wan PO1 AG01680)

- Among men aged 50 plus, the recent retirees report no more health decline than their working peers (V.A. Normative Aging Study). However, this important association is to be examined further, with health status assessed by clinical examination (Bossé, RO1 AG02287).

SPECIAL ASPECTS OF THE AGING PROCESSES

In addition to longitudinal analyses, a number of studies of varied design probe into specific aspects of social-psychological aging. For example, some current findings are:

--Older people do not spend most of their time alone, nor do they sleep any more or less than younger persons, nor is their day devoid of obligatory activities. Even within the same environment, however, there is great individual variation in the use of time (Lawton, RO1 AG00656).

--The corrosive character of "daily hassles" appears to exacerbate the symptomatology associated with stressful life events (Lazarus RO1 AG 00799).

PSYCHOSOCIAL FACTORS IN HEALTH

Scattered studies have been launched recently in the important "bridging" area between psychosocial and biomedical aging. For example:

--In a long-term prospective study of a cohort of former medical students, men who later developed cancer were more likely than their healthy classmates to have reported a lack of closeness to parents in youth (Thomas, RO1 AG01760).

--Another longitudinal study is investigating the impact on older people's nutritional intake, health, and life satisfaction of moving to age-segregated housing (Rodin, R01 AG02455).

OLDER PEOPLE AND SOCIAL INSTITUTIONS (OPI)

This program is too new to report "achievements."

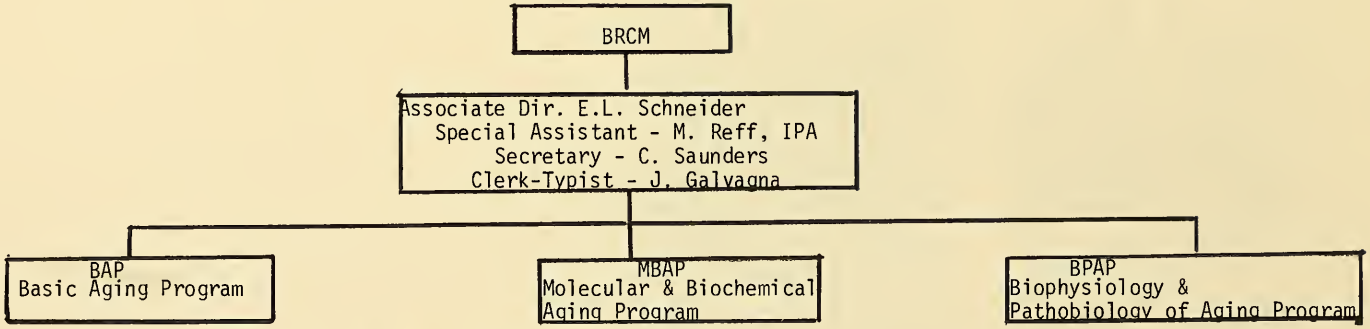
Biomedical Research and Clinical Medicine Program

BRCM Program Summary Statement for FY 1980

The Biomedical Research and Clinical Medicine Program is currently divided into three programs: a Basic Aging Program(BAP), a Molecular and Biochemical Aging Program(MBAP) and a Biophysical and Pathobiology Aging Program(BPAP). Each program is comprised of a number of subprograms as outlined in Figure 1. Studies cover an enormous breadth of research, ranging from investigations at the molecular level to the study of whole organisms. Model systems as widely diverse as E. coli and mice are utilized, and theoretical considerations as well as clinical applications are investigated. These programs have experienced considerable growth in the last few years and have expanded their activities in a number of new directions. Unfortunately this growth has not been accompanied by appropriate enlargement of staff, and the development of vital new areas such as nutrition and pharmacology have not received the attention they deserve.

One important area which has not had significant growth is clinical research. This area is of paramount importance to the goals and objectives of the Institute. While other Institutes have programs which include several age-related disorders, it is imperative that these conditions be approached with an emphasis on age-related changes that contribute to their etiology. To facilitate research in this area and also to respond to the growth of BRCM, it is proposed to reorganize this program as indicated in Figure 2. Most of BRCM subprograms will be incorporated into the Molecular and Cell Biology and Physiology of Aging Branch. A new Geriatric Branch will be established which will promote, organize and develop clinical research in geriatrics. Grants, contracts and initiatives of the present subprograms of BAP, MBAP and BPAP will be assigned to the new programs and subprograms as indicated in Table 1.

Figure 1



(GCA) GENETICS & CELLULAR AGING
(GEN) GENETICS & COMPARATIVE AGING
(CEL) GENETIC & CELLULAR RESOURCES
(DER) DERMATOLOGY
(THG) THEORETICAL GERONTOLOGY

Chief:
D.G. Murphy

Additional Personnel:

HSA - N. Das
HSA - C. Letendre
Prog. Assistant - J. Badinelli
Clerk-Typist - J. Colbert
Clerk-Typist - L. Burall
Stay-In-School - C. King

(DIA) DIABETES
(IMM) IMMUNOLOGY
(MET) INTERMEDIARY METABOLISM
(PHR) PHARMACOLOGY

Acting Chief:
B.A. Maurer

Additional Personnel:

Secretary - D. Strong

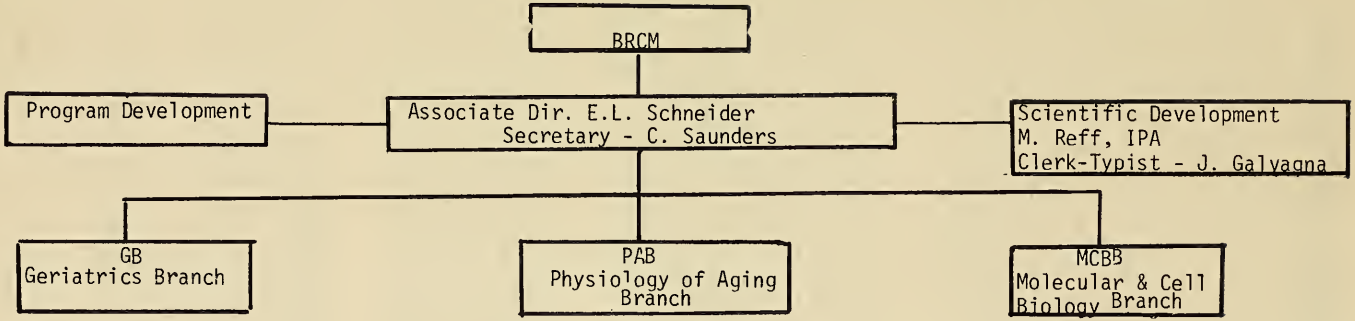
(ANM) ANIMAL MODELS & RESOURCES
(CLE) EPIDEMIOLOGY
(END) ENDOCRINOLOGY
(EXR) EXERCISE
(NUT) NUTRITION
(GER) GERIATRIC DENTISTRY
(GER) GERIATRIC MEDICINE
(SEN) NEUROSCIENCE
(PTH) PATHOBIOLOGY
(PHS) PHYSIOLOGY

Acting Chief:
B.A. Maurer

Additional Personnel:

HSA - B. Wortman (assignment)
HSA - E. McGuire (expert)
Prog. Assistant - J. Soban
Clerk-Typist - J. Kline
Clerk-Typist - A. Holland

Figure 2



(NEL) NEUROLOGY
(PHR) PHARMACOLOGY
(DER) DERMATOLOGY
(GEE) GERIATRIC EDUCATION
(GER) GERIATRIC RESEARCH

Acting Chief:
E.L. Schneider

Additional Personnel:

E. Hadley

(NUT) NUTRITION
(EXR) EXERCISE PHYSIOLOGY
(NEB) NEUROBIOLOGY
(END) ENDOCRINOLOGY
(IMM) IMMUNOLOGY
(ANM) ANIMAL MODELS

Acting Chief:
B.A. Maurer

Additional Personnel:

HSA - B. Wortman (assignment)
HSA - E. McGuire (expert)
Prog. Assistant - J. Soban
Secretary - D. Strong
Clerk-Typist - J. Kline
Clerk-Typist - A. Holland

(CEB) CELL BIOLOGY
(MOL) MOLECULAR BIOLOGY
(GEN) GENETICS
(THB) THEORETICAL BIOLOGY

Acting Chief:
D.G. Murphy

Additional Personnel:

HSA - N. Das
HSA - C. Letendre
Prog. Assistant - J. Badinelli
Clerk-Typist - J. Colbert
Clerk-Typist - L. Burall
Stay-In-School - C. King

Table 1. Grants, contracts and initiatives of BAP, MBAP and BPAP to be tranfered to new programs and subprograms.

BAP----------MCBB

CEL----------disbanded*

GEN----------GEN

GCA----------CEB

THG----------THB

----------MOL (new subprogram)

DER----------DER (in GB)

MBAP----------PAB + GB

IMM----------IMM (in PAB)

PHR----------PHR (in GB)

MET----------disbanded*

DIA----------END (in PAB)

BPAP----------GB + PAB

ANM----------ANM (in PAB)

CLE----------disbanded*

END----------END (in PAB)

EXR----------EXR (in PAB)

NUT----------NUT (in PAB)

GER----------GEE (in GB)

SEN----------NEL (in GB) and NEB (in PAB)

PTS----------disbanded*

PHS----------disbanded*

----------GER (new subprogram in GB)

*grants in these areas will be transferred to the new subprograms as appropriate.

II. BASIC AGING PROGRAM: PROGRAM REPORT

The Basic Aging program is responsible for NIA extramural research support in five areas, (see Table 1), encompassing cell biology, genetics, dermatology, theoretical biology and cellular and genetic resources. The research conducted in cell biology is primarily that involving cell culture systems. These cells, usually human in origin, are genotypically and/or phenotypically distinct from the normal, counterpart cells of the parent organism. Most research on cultured cells has been conducted on fibroblast-like cells. Staff efforts to expand cellular aging research has been directed at the use of differentiated cells in culture. This places the NIA interests at the leading edge of cell culture technology. It is difficult to obtain populations of cells of a single type, of demonstrated *in vivo* origin, maintaining key differentiated parent tissue functions *in vitro*. The NIA has encouraged both aging research on differentiated cells and the studies necessary to culture a greater range of cell types with more satisfactory expression of the *in vivo* phenotype.

Research on the genetics of aging has been pursued through comparative gerontology utilizing invertebrates, plants, and bacteria. Organisms emphasized for BAP in this area are the major laboratory organisms utilized for research in molecular genetics and developmental biology: the fruit fly, *Drosophila*; the laboratory nematode, *Caenorhabditis elegans*; protozoa, such as *Paramecium* and *Tetrahymena*; fungi, *Podospora Neurospora* and *Dictyostelium*, the colonial alga, *Volvox*; and the bacterium, *Escherichia coli*. Success in programming has largely been in research conducted on *Drosophila*. For the first time in the history of gerontology there exists, with this organism, a significant commitment to understanding the genetics of aging, senescence, and longevity.

Cutaneous biology and dermatology are pursued through research ranging from molecular and cellular studies through clinical research. Objectives range from a knowledge of the basic biology of skin aging to the epidemiology, etiology, diagnosis, treatment and prevention of skin problems of the aging and the elderly. In addition to these objectives, gerontological and geriatric dermatology offer unique opportunities to pursue cellular aging as a model of aging for other tissues and organs, and to test concepts of the relationship between *in vitro* and *in vivo* cell biology.

Theoretical biology, mathematical and computer simulation, enables the testing of hypotheses involving more variables than can be achieved by direct experimentation. Both data-driven and abstract simulation is encouraged. Population and quantitative genetics are pursued through this field of theoretical biology.

Cell biology, genetics, dermatology and theoretical biology are the areas of primary research responsibility of BAP. In support of this research are resources, the supply and characterization of biological research materials. The three contracts supported on research resources are among the most visible of NIA research support activities. These resources are: the NIA Cell Line Repository, the Mycoplasma Contamination Testing Service, and the Caenorhabditis Genetics Service.

Progress in cellular aging has been made in understanding the control of cell proliferation, changes in metabolic activity, and the chromosomal localization and expression of specific genes which may have a role in age-associated decline of functional activity in cells.

In genetics, there has been insight gained on the difficulty of obtaining longevity mutants with Caenorhabditis, Drosophila and Podospora, and progress understanding the involvement of specific nuclear and mitochondrial genes with longevity.

In dermatology, advances have been made in understanding the ultrastructural changes and growth characteristics of actinically-damaged skin cells, in the descriptive biology of skin changes with aging, and in the photolability of skin pigments and consequent decrease in protection of skin from UV damage. Further, there is evidence that skin fibroblasts, aged in vitro, retain wound healing capacity.

Progress in theoretical biology continues to be made on a computer model simulating metabolic processes in the development and aging of Dictyostelium. This model has been shown to have the capacity to predict consequences of complex changes in metabolism or substrate, and to contribute to development of experimental design and testable hypotheses.

Long range plans to expand NIA supported research through BAP should include support of institutional training grants in all subprograms, especially in dermatology, and theoretical biology. In cell biology research training is important to the development of aging research utilizing genetic mosaic and chimeric animals. Training in genetics will include utilization of genetically well developed systems, such as Caenorhabditis. NIA goals are being reasonably met in the areas of cell biology genetics. Dermatology is emerging as a potentially strong subprogram. Theoretical biology is seriously underdeveloped, and is likely to remain so until BAP is properly staffed.

We continue to propose: 1) that the following name changes be made with BAP:

FROM	TO
Genetics and Cellular Aging	Cell Biology
Genetics and Comparative Aging	Genetics

2) that Genetics and Cellular Resources be disbanded, and that the grants and contracts of this subprogram be redistributed to remaining BAP programs. and 3), that a subprogram of Molecular Biology be added to BAP, encompassing, for example, those applications now supported through Intermediary Metabolism of MBAP.

TABLE 1: BASIC AGING PROGRAM

1) Genetics and Comparative Aging (GEN)

Comparative studies on genetic basis of aging utilizing invertebrates, plants and prokaryotes.

2) Genetics and Cellular Aging (GCA)

Studies on the mechanisms of cellular aging utilizing the technologies of cell and tissue culture, somatic-cell genetics, cell and tissue transplantation, chimeric and genetic-mosaic biology.

3) Theoretical Gerontology (THG)

Studies which emphasize computerized and mathematical models of life systems and processes of significance to gerontology, including population and quantitative genetics.

4) Dermatology (DER)

Gerontological and geriatric dermatology, basic and clinical studies on the mechanisms responsible for age-associated changes in skin.

5) Genetic and Cellular Resources (CEL)

Biologics supply, characterization, quality control in cell culture, and related services in the fields of cell culture, invertebrates, plants and prokarytes.

II. GCA: Subprogram Report

The NIA support of research on the cell biology of aging is conducted in this subprogram. The support is limited to studies in which the cells are subjected to a foreign environment or one in which the cells are genetically distinguished from cells of the same type in the host or parent organism. The research addresses the cellular level. The concepts of foreign environment and genetic distinction encompass cell, tissue and organ culture, and chimeric and genetic mosaic animals. The capacity to study human cells, especially cells with differentiated characteristics, independent of the parent organism has been emerging over the past decades through the technologies of cell culture. Research on isolated cells in culture have provided remarkable access to basic knowledge of cell structure and function. Although biochemical and morphological markers serve to identify cells as differentiated, relating them to presumed counterpart cells in vivo, such putative relationships must be interpreted with caution. Cells in culture are usually closely identified genotypically with the organism from which they are derived. However, the circumstances of the cell culture environment inevitably cause these cells to be remote phenotypically from cell in vivo. With proper appreciation of the limits of cell culture systems, they do provide models for comparative studies in cellular aging. The advantages of cultured cells as models for cellular aging research include 1) access to experimentation on human cells, 2) the ready accessibility of tissue to generate cultures from surgical procedures such as circumcision, simple skin biopsies, blood samples and autopsy, 3) the ability to study cells individually or in mass quantities, 4) the relative simplicity of, and the capacity to control, the cell culture environment as compared to the in vivo environment, and 5) the large, highly competitive community of scientists conducting basic cell biology research on cell culture systems to understand phenomena of cell structure and function other than aging.

Through Genetics and Cellular Aging, NIA encourages descriptive studies to ascertain expressions of senescence in cultured cells, and the subsequent studies to determine the mechanisms responsible for the phenomena of senescence. Somatic cell genetics, which has contributed to knowledge of chromosomal location and function of specific human genes, is a field encouraged through this subprogram to investigate the genetic control over cellular senescence.

The transplantation of cells and tissues, including serial transplantation progressively from one animal to the next, enables observation of senescence and survival features of cells that are genetically and/or morphologically distinct from those of the host. Similarly, chimeric and genetic mosaic animals offer the means to compare, within the organism, differential expressions of cell senescence and cell survival. Chimeric animals enable observation of development and senescence of cells of different genetic heritage within the normal tissue arrangement of one animal. This is made possible through emerging technologies enabling the

incorporation of genetically distinct cells into the host organism during very early embryological stages. Such chimeric animals are also obtained by combining early embryos of different genetic constitution. Genetic mosaics, on the other hand, are animals which have different phenotypic expressions of the same cell types due to alteration in genotype, or differential phenotypic expression.

Research supported in Genetics and Cellular Aging ranges from a determination of control factors involved in cell proliferation, to differential expressions of cellular senescence in genetically marked cells, in vitro and in vivo.

75 projects have been supported through this subprogram during FY 80 at an approximate cost of $ 6.96 million. This represents 56% and 62% increase in the project numbers and the project costs, respectively during a period of one year (see section II). Research supported in this subprogram includes determination of in vitro proliferative capacity of different cell types; genetic control of cellular senescence, cytogenetics and DNA repair capacity of cells and age-associated changes in the transcription and translation systems of cells. Many of these studies on cellular and molecular biology are descriptive and speculative at this stage, but with potential importance in gerontology. Some significant accomplishments have been made in these areas. These are elaborated in section IV.

Briefly, these accomplishments include:

- Establishment of primary cultures of bovine endothelial (Levine, R01-AG00839) and human endometrial (Seifter, P01-AG00374) cells. These cells exhibit limited proliferative capacity in culture and they provide an excellent opportunity to study age-associated changes in differentiated functions.

- Maintenance of young and old WI-38 cells in a chemically defined medium for a limited period of time (Phillips and Cristofalo, P01-AG00378).

- Demonstration of a linear correlation between the ratio of certain x-linked to autosomal-linked enzymes and the population doubling levels of normal diploid human fibroblast-like cells (Paz, P01-AG 00376). This ratio may be used as the cell population "age" index.

- Retention of the contractile function of non-proliferative phase III skin fibroblasts, cultured in a collagen lattice (Bell, (Stein, R01-AG 00947 and Muggleton-Harris, R01-AG 01212) and chromosomal localization of genes (Patterson, R01-AG 00029).

- Utilization of somatic cell genetics to understand regulatory mechanisms of DNA Synthesis (Shein, R01-AG-00947 and Muggleton-

Harris, R01-AG-01212) and chromosomal localization of genes (Patterson, R01-AG-00029).

- Changes in small nuclear RNA metabolism as cell division ceases and differentiation that leads to senescence ensues. (Goldstein, R01-AG-02043).

- Age-associated changes in surface components and functional complexes in human diploid fiborblast-like cells in culture (Kelley, AG 00191).

- Age-associated decrease in the LDL receptors in cultured human diploid fibroblast-like cells (Gallop, P01-AG 00376).

- Age-associated changes in receptors for hormones, growth factors and neuroeffector substances in cells in vivo and in vitro (Seifter, P01-AG 00374).

The NIA Cell-Line Repository at the Institute for Medical Research, Camden, New Jersey, provides considerable support to research activities administered through the subprogram, Genetics and Cellular Aging. This repository acquires, develops, characterizes, stores and supplies cultures, for gerontological research. The repository contains more than 350 cell-lines which include human diploid fetal lung fibroblasts (IMR-90 and IMR-91) fibroblasts from individuals with premature aging features (such as progeria, Werner s syndrome) and various epithelial cells. With a view towards providing quality control in cell culture technologies, as applied to gerontological research, NIA also maintains, through contract, the Mycoplasma contamination testing service at the Institute for Medical Research. Mycoplasma screening of the cell cultures is done free of charge for NIA grantees at this facility. Additional information on these contracts is provided in the subprogram on Genetic and Cellular Resources.

This subprogram is continually evaluated, in consultation with the scientific community, with respect to 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. A program announcement (NIH Guide 7 (2), 1978) has been issued addressing these questions. Through another program announcement (NIH Guide 9 (2), 1980), NIA is encouraging investigators to study various aspects of cell and molecular biology of aging using differentiated cell in culture. Such studies could provide valuable information on age-associated functional decline of various tissues and organs.

Through the National Research Service Awards (NRSA) this subprogram has been providing pre-doctoral (4) and post-doctoral (16) training in molecular and cellular aging (T32-AG00028-05: P.I. Dr. Melvin Stulberg; T-32-AG0069-02: P.I. Dr. George Martin) and biochemical aspects of

cellular aging (T3-AG00052-03). All trainers in these projects are outstanding scientists with international reputation in their areas of research. Although it is too early to know, it is anticipated that active participation of these young scientists in cellular aging research will considerably strengthen this area. In addition to these NSRA s, the training activities in this subprogram are supported through two individual fellowships to Drs. Sharon Pochron (F32-AG5103-02) and Dale Mosbaugh (F32-AG05195-01) who have been working at the laboratories of Dr. Renato Baserga (Temple University) and Dr. Stuart Linn (University of California at Berkeley) respectively.

Most of the applications assigned to this subprogram are reviewed by the Cell Biology, Molecular Cytology, Molecular Biology and Pathobiological Chemistry Study Sections. The field of aging research is relatively new and much of the research in this field is descriptive and speculative. The lack of understanding of such a state-of-the-art in this area sometime leads inadequate review of applications. Such deficiencies in peer review seem to have been corrected to some extent through NIA staff interactions with executive secretaries and members of the study sections and also by inclusion of scientists in the study sections with expertise in aging research. The average approval rates of the October, 1980 Council applications reviewed by these four study sections was 80% (n=42), as compared to 70% (n=25) of the October 1979 Council applications. The area of cellular aging research is growing at a rapid rate. More and more established and promising young investigators are now being involved in aging studies, as new opportunities for research in this field arise.

II. GENETICS AND COMPARATIVE AGING: SUBPROGRAM REPORT

NIA Extramural Research involving invertebrates, plants, and prokaryote organisms are supported in this subprogram. The basic mechanisms responsible for longevity and senescence remain unknown throughout the phyla. Neither the evolution of these aging phenomena, nor the relative contributions to them of either innate determinants nor the external influences of the environment are known. In contrast, the strategies of biological structure and function in development and homeostasis, at the cellular and molecular levels, are relatively well known. Cellular biochemistry and morphology are remarkably similar in all forms of life. This observation suggests that the processes responsible for senescence at the cellular level in any group of organisms may have important implications for all organisms. The great diversity of organisms, and extreme range of conditions under which populations of animals and plants have evolved and maintained themselves, offers the opportunity for examining differing bases for the expressions of longevity and senescence at the organismic level. Fundamental knowledge of the events leading to senescence in simple organisms will provide information which may guide future theory and experiments to increase an understanding of human aging. For this reason comparative aging studies are encouraged through this subprogram. This research is undertaken with the appreciation that the invertebrates, plants, and prokaryotes that are the subject of comparative research are evolutionarily remote from man and other mammals.

The laboratory organisms used for research in Genetics and Comparative Aging often have in common several features desirable to the experimentalist. These organisms are amenable to genetic studies. Further, they are relatively simple, easily cultivated under well-controlled conditions, and have short life spans. The relationships between the genome and cytoplasm, and the interactions between cells, tissues, and organs are particularly accessible to molecular dissection in these simple organisms. Populations and/or mutant lines of experimental organisms which express differential senescence and longevity, including sexual differences in longevity could enable elaboration of the fundamental mechanisms responsible. However, genetic dissection of organisms to explain senescence may be difficult to achieve as senescence probably involves interactions of multiple genes. It remains, nonetheless, one of the most promising avenues of gerontology. Research through Genetics and Comparative Aging has been especially encouraged utilizing the fruitfly Drosophila, the laboratory nematode Caenorhabditis elegans, the slime mold, Dictyostelium, the colonial flagelate Volvox, and protozoa. The research encompasses molecular genetics through the biology of aging at the cellular, organismic and population levels. Commonly, these organisms are the subject of a broad range of highly competitive research, of which gerontology is but a small part. As most such research is on developmental biology, these fields focusing on specific laboratory organisms are excellent for expansion to encompass the interests of the NIA.

The NIA has recognized the need to stimulate research in genetics since the Institute's inception in 1975. "Genetics" is the first topic presented in Our Future Selves, the Report of the Panel on Biomedical Research, from the Federal research plan on aging.

The concerted NIA staff effort to achieve a presence of genetics in gerontology has been highlighted by three recent program announcements:

The Genetic Basis of Aging: Drosophila as a Model System
(NIH Guide 7 (12). December 15, 1979).

The Genetic Basis of Aging: Protozoa as a Models
(NIH Guide 8 (8). June 5, 1979).

The Genetic Basis of Aging: C. elegans as a Model System
(NIH Guide 9 (2). January 25, 1980).

In addition to the normal NIH distribution of these announcements in the NIH Guide, the NIH developed a selective mailing to a targeted group of investigators who demonstrated potential interest and the qualifications to respond with grant applications.

The initial response of the Drosophila genetics community to the NIA solicitation has been most gratifying. NIA grant awards in the field of genetics utilizing Drosophila as a model system constitutes the first major genetics effort in the history of gerontology. Although the dollar amount in this effort is modest relative to established NIH programs, the significance for the aging field is major. The NIA grantee presence in the context of highly competitive genetics research will facilitate an increase in quality as well as the quantity of aging research.

The first of several possible genetics colonies of experimental animals recommended in Our Future Selves was established by the NIA in September, 1979. This resource, the Caenorhabditis Genetics Center (CGC) supports the biological community at large through services as mutant acquisition, storage, characterization and distribution. The CGC is further described under the Genetic and Cellular Resources subprogram.

This subprogram contributes to meet general and specific NIA extramural research goals. The successful initiation of Drosophila research should be followed by further programming efforts. This might best be done by developing the interest of highly competative scientists contacted individually by staff. The research conducted on C. elegans, although of high quality, is limited to 6 grantees. Special effort should be devoted to expanding this number of laboratories supported by the NIA.

Research highlights:

(Details of the below studies, as well as additional highlights, are provided under "scientific accomplishments", Section IV).

- Dr. Rothstein (R01-AG-00618) is accumulating evidence that age-associated changes in certain enzymes (phophoglycerate kinase and enolase) are due to post-translation conformational changes. In contrast, Dr. Lane (R01-AG-01002) shows that other enzymes (such as superoxide dismutase and beta-galactosidase) do not change with age.

- Dr. Bewley (R01-AG-1839) has shown that regulatory genes on the second Drosophila chromosome can influence th expression of the catalase gene on the third chromosome. Catalase is involved in peroxide detoxification. The accumulation of toxic metabolites has been suggested to be involved cellular aging.

- Dr. Hiraizumi (R01-AG-1934) has shown that independent genetic factors seem to be involved in determining the number of progeny a female Drosophila can produce during the fertile period, and the duration of fertility in these females.

- Dr. Reeves (R01-AG-01546) shows that mini cells (anucleate E. Coli) lose their ability to synthesize T.7 gene products with increasing age.

- Dr. Munkres' (R01-AG-00930) studies show that mutations in two genes of the fungus Neurospora, coding for catalase and mitochondrial superoxide dismutase, decrease the life-span of this organism. While these observations do not provide a direct-evidence in support of the free-radical theory of aging, they are compatible with this idea.

At present training activities in this subprogram are supported through four individual fellowships (F32-AG-05068-02, Dr. Richard Aalker; F32-AG-05083-02, Dr. Thomas McKeon; F32-AG-05121-01, Dr. Eleanor Spicer; F32-AG-05128-02, Dr. Thomas Johnson). Additional training activities in genetics aspects of aging through individual fellowhsips and National Research Service Awards have been encouraged through the program announcement mentioned above.

Most of the applications assigned to this subprogram are reviewed by the Genetics Study Section. No special problem has been encountered in reviewing aging applications by this study section. The weighted mean rate of approved of aging applications by this study section for January, May and October, 1980 Council was 92% (No. reviewed=24; 3 deferred). The NIA staff closely interact with Executive Secretary, Dr. David Remondini, of this study section. Last year Dr. Remondini gave a seminar on the activities of the Genetic Study Section at the invitation of BAP. He is planning to organize a small workshop on aging, perhaps on the use of Drosophila as a model system in aging. Dr. Remondini was very receptive

to the BAP invitation to review program announcements and take part in the Drosophila planning workshop, planning meeting and the C. elegans Genetic Center ad hoc Advisory Committee meetings. He was also receptive to the idea of sending NIA program announcements to members of the Genetic Study Section.

II. CEL: Subprogram Report

Research in this subprogram supported by grants includes studies on cell lineages and population characteristics of human diploid cells in culture, the establishment, isolation, and characterization of longevity-mutant nematodes, and the research training of post-doctoral scientists in the technologies of tissue-specific cell line development. Resources supported by contracts are: 1) the NIA Cell Line Repository, which supported acquisition, characterization, and distribution of cell lines of special utility to NIA grantees, prospective grantees, and other gerontologists; 2) the Mycoplasma Contamination Testing Service, contributing to quality control in cell culture laboratories for the same group of investigators, 3) the Caenorhabditis Genetics Center (CGC) for acquisition, characterization and distribution of C. elegans wild type and mutant strains. Descriptions of the cell line and mycoplasma resources have been published:

Das, N.K. and D.G. Murphy, 1978 National Institute on Aging Cell Line Repository Experimental Aging Research 4: 321-331.

Das, N.K. and D.G. Murphy, 1978 National Institute on Aging Mycoplasma Testing Service. Experimental Aging Research 4: 333-341.

All contracts, supported by this subprogram, have been providing excellent services in gerontological research. The demand for the IMR-90/91 cell-lines (human fetal lung fibroblasts), developed by the Contractor for an eventual replacement of WI-38 cells, has been heavy. These cell-lines accounted for more than one half of all cell-lines shipped during the past year. Cultures of human skin fibroblasts from the Baltimore Longitudinal Study have been included in the NIA Cell-Line Repository and they are now available for gerontological research. The Mycoplasma Contamination Testing Service provides a valuable function in quality control in cell culture technologies. Approximately one half of NIA grantees working in cellular aging have been taking advantage of this service. Fifty-two out of 822 cell cultures screened so far (in two years) have been found to be contaminated with mycoplasmas. Elimination of these cultures from grantee laboratories not only improved the standardization of experimental studies, but was significantly cost-effective. During the past 8 months the CGC has made significant progress. It has already acquired, verified and frozen 140 strains of C. elegans. Some of these strains have been supplied to investigators.

We recommend terminating this subprogram, redistributing the contracts and grants currently managed here into the appropriate subprograms of Molecular and Cellular Aging Program. Specifically, the Mycoplasma and Cell-Line contracts would go to the Cell Biology subprogram, the CGC to the Genetics subprogram.

I. DER: Subprogram Report

While the age-associated changes of skin may initially cause adults only modest physical discomfort, they create very marked concern associated with self-image and appearance-based age stereotyping. As a person becomes older, serious skin-related discomforts develop the appearance of skin become more than matched by serious discomforts such as chronic dryness and itching. Millions, if not billions of dollars are spent to ameliorate these problems. However, few of these remedies have a sound basis in research derived knowledge. Others may be of questionable value, and may even aggravate an existing skin problem. The mechanisms responsible for age-associated changes in skin are unknown. Even the basic descriptive biology of the changes associated with skin aging remain inadequately studied. A concerted research program on skin aging is needed to provide the knowledge base for interceding with environmentally induced skin aging, and perhaps even modifying the rate of intrinsic processes. Skin, while complex in nature, is one of the most accessible organs. The molecular and cell biology of aging skin may therefore serve as a model system for studying aging processes.

Research on skin is being pursued at both the basic biological level to understand the mechanisms of skin aging, and at the clinical level to better define the nature and extent of the skin problems with which the elderly are confronted. Unfortunately, there is no clear interface currently between the biomedical, molecular and cellular research and the clinical research. At the basic end of the spectrum, in vitro skin cell biology will be pursued in the subprogram of Genetics and Cellular Aging. The cell culture model most frequently examined is the skin derived fibroblast-like cells. Another system with increasing interest is the cultured keratinocyte. An understanding of the relationship between data derived from cell-culture models and that derived from intact skin may provide a rational interpretation for cell-culture findings. Specifically, the dermatology subprogram will seek to support studies which determine the range and adequacy of both clinical and home remedies for skin problems of the aged. The diagnosis, treatment, and prevention of skin aging problems will be the focus of clinical studies, information on such questions as biochemistry of the dermis and epidermis, changes in microvasculature, elastin, collagen, mucopolysaccharides, the basal lamina, pigmentation, keratinization, hair follicles, sebaceous secretion, apocrine glands, sweat glands, hair and nails. Basic questions of particular interest to the elderly will be addressed where possible. These include such concerns as the change in capacity of skin to adapt to modifications in surface area, e.g. following weight loss, the reasons for skin sagging and appearance of wrinkels, and the changes in aging skin which reduce its effectiveness as a barrier to environmental insult, e.g. chemicals, temperature extremes.

Skin, the most accessible of organs is probably the organ most likely to yield the earliest of fundamental knowledge of the aging process in man. It is anticipated that clinical advances may also be forthcoming in the near future. Although the dermatology subprogram is just beginning to be developed, several of the grantees have already made noteworthy contributions. Noninvasive techniques for assessing various skin parameters are being developed by Dr. Albert Kligman and his associates in Philadelphia. They have succesfully demonstrated that a number of skin changes occur during the course of normal aging which are pertinent to some of the clinical problems which are particularly discomforting to the elderly (e.g., decreased sebum production, decreased number of sweat glands, increased wound healing time, decreased visible reaction to chemical agents). Dr. Eugene Bell and his collaborators have successfully grown skin fibroblasts in culture and shown that certain differentiated functions such as contractile ability are retained. Successful use of homologous fibroblasts grown on collagen matrices as skin grafts has been reported. This may well be applied successfully to treatment of decubitus ulcers. A biosynthetic and photosynthetic study of the skin pigment, phaeomelanin, is well underway in the laboratory of Dr. Miles Chedekel at Johns Hopkins University. They hope to demonstrate that this pigment, found in skin and hair of redheads, is photolabile and that its destruction explains increased photosensitivity and increased skin cancers seen in people of Celtic origin. Over 92 strains of skin fibroblasts including samples from people of different ages, psoriatic patients, and those with other diseases with skin involvement have been cultured in Dr. Martin Carter s laboratory at Yale University. Growth characteristics, DNA repair capability, ultrastructural features, and keratin changes are being simultaneously determined to give a comprehensive picture of changes in skin fibroblasts with aging.

A program announcement, "Gerontological and Geriatric Dermatology," which appeared on September 26, 1979, has received a moderate response. A new initiative in decubutus ulcers is being planned for FY 1981 through a planning meeting followed by a workshop. It is anticipated that a program announcement on decubutus ulcers would follow the conference.

The first meeting of the ad hoc Interagency Working Group on Dermatology met in May to identify plans and areas of interest of eight Federal agencies. The minutes of this meeting were prepared in the form of a report.

The need for well trained investigators capable of studying the spectrum of problems in cutaneous biology is evidenced by the lack of good grant applications. Training grants would seem to be an excellent mechanism to bring investigators into the field. One highly meritorious training grant has been pending for over a year.

An excellent resource for NIA grantees in dermatology is the NIA Cell-line Repository from which many skin-derived cell lines are available. As need arises, we will recommend additional lines being added.

The dermatology program remains small and the response to programming activities has been limited. Until May of 1980 no full time staff person was available to handle the program. Development of interest in geriatric and gerontological dermatology has also been inhibited by travel restrictions on program staff. The new initiative in decubutus ulcers is being planned to consider topics ranging from the biochemistry of wound healing to clinical studies of the effectiveness of various treatments.

It is hoped that the visibility of NIA s dermatology program will be improved.

The Executive Secretary of General Medicine A Study Section, Dr. Harold Davidson, has been helpful and supportive. The Study Section now has sufficient expertise such that grant applications in dermatology are adequately reviewed.

II. THG: Subprogram Report

NIA extramural research involving simulation and modeling, and research on population genetics are supported in this BAP subprogram of Theoretical Gerontology. Simulation studies are encouraged both as data-driven and as theoretical models. Data-driven studies are those in which the modeling and computer simulation are dependent upon experimental data for development. Experiments, for example, can be designed to quantify all interdependent metabolites and metabolic processes within the scope of the simulation model. The model integrates these data, and analyzes relationships based on unknown or hypothetical pathways and interdependences.

The simulation models become valuable when sufficiently sophisticated to predict functions describable as experimentally testable hypotheses. Data-driven simulation research is encouraged at the molecular/biochemical, physiological, and cellular (cell-lineage) levels. The basis for support of such studies is the probable need for integration of many variables to achieve knowledge of mechanisms of senescence or longevity. This concept recognizes the extreme complexity of an organism, and the possibility that single-variable, cause and effect experimentation may perturb the rate of aging in an organism, but not necessarily explain underlying aging mechanisms.

The abstract modeling enables elaboration of mathematical concepts of heuristic value to gerontology. This type of simulation can provide a guide to gerontological theory development, however its usefulness ultimately lies in an ability of the models to predict biological functions testable in the laboratory. Although hypotheses testable on laboratory animals are the goal of NIA-supported simulation research, the abstract simulation may contribute most directly to the development of concepts and computer models for data-driven simulation.

Knowledge of the fate of individual cells and their progeny is of paramount importance to the understanding of senescence in dividing cell populations. In the organism this information is sought from the zygote through embryogenesis, development, and eventually through cell attrition and tissue involution that appears related to senescence. Similarly, in clones of protozoa, and populations of cells in culture, a knowledge of cell lineages is essential to the design of experiments addressing senescent changes at the cellular level. Simple eutelic metazoans, such as nematode Caenorhabditis, offer promise of complete analysis of all cell lineages. This will enable determination of any age-associated cell loss, and enable prediction of the role and mechanism of such loss. In large organisms, and those in which development is less deterministic, a more generalized lineage analysis is achievable, and may have similar value in predicting the role of senescent changes in populations of cells.

Theoretical Gerontology remains undeveloped. Professional staff time has not been available to implement plans for the sub-program nor is there any significant unsolicited grant application activity in this area.

Two studies are supported through this sub-program. One is a computer model of renal homeostasis under a grant which has been active less than a year. The second is a very successful study of the computer analysis of aging in the slime mold, Dictyostelium, a research activity in its 11th year of NIA grant support. Progress on this study is further elaborated in section V, below.

Training of investigators in theoretical biology addressing aging is highly desirable and may be essential to effective development of the sub-program. A coordination of existing resources, most notably computer programs and facilities, will probably be key to efficient implementation of theoretical gerontology objectives. For example, Dr. Wright at the Boston Biomedical Research Institute has expressed interest in making available to investigators conducting developmental and aging studies on Dictyostelium the computer program she has developed. Collaboration of this type, facilitated by the NIA, would be mutually beneficial to the originator of the computer program and the experimentalist wishing to integrate data and utilize the simulation capability of the program in experimental design and "pre-testing" of hypotheses.

The community of investigators in theoretical biology is generally of the opinion that NIH has a limited, perhaps inadequate, capability for the peer review of theoretical biology applications. Thus, any serious programming effort in theoretical gerontology should be accompanied by discussions with DRG and with leaders in the theoretical biology field to assess the accuracy of this view, identify specific issues, and otherwise assure a peer review capability.

II. Molecular and Biochemical Aging Program (MBAP): Program Report

The Molecular and Biochemical Aging Program consists of four subprograms: Immunology, Pharmacology, Intermediary Metabolism and Diabetes. This program is primarily focused on physiological aspects of the aging process and thus includes those programs associated with the normal physiological processes of aging in the intact organism.

The most striking effect on aging has been observed subsequent to a change in diet. It has been established that dietary restriction profoundly influences the immune system and can extende the maximum lifespan of mice. Immunological studies on the effect of diet have been supported by the Immunology subprogram and have been conducted by Dr. Goidl under a program project grant. Dr. Goidl's data indicated that dietary restriction sustained T-cell function and extended lifespan two to three fold in tumor prone strains of mice. Dietary restriction also delayed the development of both autoantibodies and circulating immune complexes, both of which are characteristics of an aging immune system. His research has indicated that dietary restriction delayed the pathological changes in the glomeruli, coronary arteries, renal interstitium and renal tubules in two different strains of mice. In the MLR strain of mice, dietary restriction delayed the onset of lymphoproliferative disease. C3H/Bi mice on restricted diets did not form mammary tumors, produced less antibody to the murine mammary tumor virus, had lower levels of serum immune complexes and produced less mammary tumor virus.

Of major importance to the understanding of the aging process, is the regulation of those immunological modifications that occur with time. The involution of the thymus is a major landmark in the development of the immunological system. One study on the role of the thymus is being conducted in a program project grant at the Cornell Medical School and the Sloan-Kettering Memorial Institute. Studies supported by this program project grant have indicated that the adoptive transfer of young thymocytes or the administration of thymic hormones to old mice reversed age-associated immune deficiency. Also, under a contract with the University of Alabama, the long-term effect of the administration of four thymic hormone preparations to three strains of mice is being evaluated. Although this contract is in the early stages, it is anticipated that significant data will be forthcoming on the role that thymic hormone plays in the aging immunological process and possibly on the life span of mice. The involution of the thymus has also been associated with a reduction in the level of chromosomal protease. These studies, supported by the Intermediary Metabolism subprogram, have produced evidence which suggests that this protease may be involved in the involution of the thymus through its effect on chromatin structure. The effect of aging on another

endocrine system was studied by Dr. Christopher Widnell with support from the Intermediary Metabolism subprogram. He identified an age-related effect of corticosteroid and tri-iodothyronine on the activity of glucose 6-phosphatase in old and young rats as an indicator of endoplasmic reticulum activity. Not only did this enzyme activity decrease with age, but his data suggested that this enzyme, and the endoplasmic reticulum, is probably influenced by a complex endocrine interaction. The Diabetes subprogram has been supporting work by Dr. Eve P. Reaven on the effect of aging on beta-cell structure and function. Her data indicated that as rats aged from 2 to 18 months, the number of beta cells increased and the islet insulin content doubled. In contrast, however, glucose-stimulated insulin release decreased progressively with age. Thus, insulin secretion per beta cell was decreased with age in spite of a concomitant increase in the storage of insulin. Thus, aging appears to produce a profound reduction in glucose-stimulated insulin release from beta cells.

Recent pharmacologic research has clearly indicated that the elderly respond significantly differently to various drugs than do the young. Dr. Kramer°s studies, supported by the Pharmacology subprogram have indicated that the elderly display more heterogeneity in their response to theophylin than the young, and that the metabolites of theophylin are eliminated more slowly in the elderly. The research of Dr. Marcus M. Reidenberg has produced evidence which may require the modification of the intact nephron hypothesis to allow for changes in the tubular secretion of some compounds that may occur with age. His studies on the slowing of renal tubular secretion of both procainamide and acetylprocainamide with aging suggest that drug dosages for the elderly must take renal function into consideration. The research of Dr. Robert C. Smith has indicated that long-term treatment with neuroleptic drugs may be responsible for a super-hypersensitivity to dopamine and norepinephrine. This research may have implications for the syndrome of tardive dyskinesia in man.

In support of its research objectives, MBAP funded 31 applications in FY 80 at a total direct cost of $2,541,963. A detailed description of these applications as to type and cost is included in the following section. No contracts were awarded during FY 80. However, one active contract is ongoing in the Immunology subprogram to study the effects of long-term administration of thymic hormones in mice.

Program activity in FY 80 included the publication of a program announcement on a "Special Emphasis Research Career Award (SERCA): Diabetes Mellitus in the Elderly". This SERCA announcement is designed to foster an interdisciplinary approach to the metabolic and endocrinologic

aspects of diabetes in the elderly by encouraging qualified individuals to acquire indepth experience and skills in the basic and clinical scientific disciplines associated with diabetes. Program activity in the Immunology program included a meeting of an immunology task force. The responsibilities of this task force were: 1) to develop a definition of immunology and aging consistent with current research in the field in general; 2) to develop appropriate terminology and to describe immunological research related to aging; and 3) to define and make recommendations for animal and cellular resource needs. This report will assist the program director for the Immunology program in the updating of the referral guidelines and for program planning. A publication entitled "Immunological Aspects of Aging" is being completed during this fiscal year and is expected to be in print by the end of FY 80. A second paper entitled "Age Associated Changes of Acetyl Salicylic Acid" is currently is press. The authors are: S.I. Baskin, L. Smith, L.A. Hoely, Paul I. Levy and Allan H. Goldfarb. Although no scientific presentations were made by MBAP program staff, several presentations were made to identify the research programs at NIA and the subprogram of MBAP in particular. These are identified in greater detail in Section 7 of this report and include presentations by Dr. Lester Smith at the University of South Florida and Dr. Bruce A. Maurer at the Missouri Academy of Sciences.

II. DIA: Subprogram Report

The diabetes subprogram of the molecular and biochemical aging program supports research aimed at understanding the mechanisms responsible for the development of diabetes in the elderly as well as the etiology and pathogenesis of this disease. At the present time this program contains only a modest number of grants covering a variety of scientific topics within diabetes research.

An area of particular interest to the NIA is understanding the mechanism responsible for the development of glucose intolerence in the elderly. What remains to be elucidated is whether the alterations in glucose metabolism reflected by glucose intolerance, is a normal physiological effect of aging or whether in fact they represent the emergence, with aging, of a disease process. Current studies are focused on areas such as body compos tion, obesity, and serum lipids, which will be important in attempting to make this distinction. In addition, the effects of changes in glucose metabolism are being correlated with biological, medical and physiological variables which may change with age and which may be affected by changes in glucose metabolism. Efforts are also underway to elucidate the mechanism responsible for the decline in glucose tolerance with age. This research is centered on the physiology of the glucose-insulin relationship and includes studies on beta cell responses to hyperglycemia as well as the sensitivity of peripheral tissue to insulin. In all of these studies the interrelation of aging, diabetes and obesity is being considered. An important aspect of this work is to determine the effect of aging on the ability of beta cells to respond to challenge and to determine whether age has any influence on the sensitivity of target tissues to insulin. Studies conducted with human subjects have thus far revealed that loss of tissue sensitivity to insulin may be an important factor in the development of glucose intolerance with aging. In another NIA supported study the effect of age on beta cell function is being examined in isolated islets of Langerhans obtained from young and old rats. The results of this study indicate that as rats age from two to eighteen months, the number of beta cells per islet increased, but the number of insulin granules released decreased progressively with age. The detailed examination of these processes in rats and other animal models may provide important insight which can be useful in analyzing the same processes in humans.

The major administrative problem facing the diabetes program is its association with comparable programs in other institutes, especially NIAMDD. Although relations with NIAMDD have been very good among the program people, the substantial overlap between the NIA and NIAMDD programs has presented several problems with regard to the assignment of research applications. Some improvement in this problem might be gained by attempting to narrow the focus of the

NIA diabetes program to topics of particular importance for aging, and to work out some agreement with NIAMDD and NHLBI on the assignment of research applications. The major training problem in this area continues to be the recuitment of quality clinical investigators.

II. IMM: Subprogram Report

The objectives of the immunology program are to understand: 1) The fundamental molecular and cellular changes responsible for the decline of immune competence with age 2) the relationship between immune dysfunction and age-related pathologies at the molecular, cellular and organismic levels using human and animals models 3) the development of replacement therapies and other treatment modalities to augument immune function and 4) the application of new knowledge to clinically relevant situations involving elderly subjects. Toward this end, the immunology program currently supports the following grants: 4 Postdoctoral Individual National Research Service Awards; 3 Program Project Grants; 29 Traditional Research Project Grants; 2 Exploratory/Developmental Grants; 5 New Investigator Research Awards; and 3 Institutional National Research Service Awards.

A comparison of a variety of immunolgical parameters of the aged with those of young individuals demonstrated : 1) an increase in the amount of immune complexes; 2) an increase in the number of suppessor T cells; 3) a decrease in thymic hormones and; 4) an increase in the amount of auto-antibodies. These characteristics of the immunological status of the aged have been implicated in their association with: 1) the reduced ability to resist infection 2) the inability to eliminate neoplastic cells and 3) the deterioration of biological function through an auto-immune mechanism. These immunological findings are probably the results of a continuum of change occuring in the immune system with time which reflect a regulatory process.

Research supported by the immunology program is investigating various parameters of these modifications.

Research is in progress concerning the role of thymic hormones in a longitudinal and cross-sectional study in three strains of mice. These are being conducted by Dr. Hiramato through a contract with the University of Alabama. It is hoped that this long term study will identify those immune functions which can be sustained by long term administration of various thymic hormone preparations. The studies to be conducted on this contract were stimulated by a variety of research, including the Program Program Project Grant headed by Drs. Weskler and Good. Studies supported by this Program Project Grant indicated that the adoptive transfer of of young thymocytes or the administration of thymic hormone to old mice, reversed age-associated immune deficiences.

With regard to the regulation of the immune system, Dr. Henry Clayman at the University of Colorado Medical Center, has studied the immuno-regulation of B cells. He has identified an apparent age-associated change in the FC receptor in aging B cells which may provide important insights in the

understanding of development of auto-reactive B cells in the aged. It is known that the elderly have a reduced capacity to resist infection. In this regard Dr. Masakazu Matsumoto, School of Veteraninary Medicine Medicine, Oregon State University, have been conducting studies to determine the influence of aging on the production of secretory antibody in the respiratory secretions of mice. His studies indicated that secretory antibody to tetanus toxid in the upper respiratory secretions of CDI mice appeared to decrease with age. An understanding of the modified ability of the aged to generate secretory antibody in respiratory secretions may lead to ways of improving the immunologic response of the aged to respiratory infections or vaccines.

With regard to human aging research, Dr. Roy Walford of the University of California, Los Angeles, has been studying Down's syndrome as a model of accelerated aging in man. He has identified several immunologically related paramaters in patients with Down's syndrome that appeared to be similar to those that occur in a normal aged population. He found that the number circulating of B lymphocytes were decreased, that the lymphocytes of Down's patients have decreased abilities to respond proliferatively to phytohemagglutihin. This probably reflects a defect in both T-lymphocyte and monocyte populations. There was additionally an increase in the amount of immune complexes, anti-immunoglobulin and anti-thyroid antibodies in Down s syndrome. Thus, Down's syndrome may be a good model for accelerated aging in man.

One of the most significant findings which relates to the immunology of the aging has been the effect of diet. Dr. Goidl, working with Dr. Weksler on a Program Project Grant, has been studying the effect of diet on life span, disease and immune function in mice. He has found that dietary restriction of the short lived, autoimmune prone, strains of mice mice was found to sustain T cell function and to extend life span two to three fold. Dietary restriction also delayed the development of auto-antibodies and circulating of immune complexes in both strains of mice and delayed the pathological changes in the glomeruli and coronary arteries of one strain of mice and in the renal interstitium and renal tubules in a second strain of mice. Even when the dietary restriction was delayed until the mice were three months of age, when autoimmunity had already developed, life span could be prolonged. Dietary restriction not only delayed the autoimmune disease of MLR mice but also delayed the onset of lymphoproliferative disease seen in this strain. The effect of dietary restriction on tumor formation was studied in the C3H/Bi strain of mice. Mice feed restricted diets did not form mammary tumors, produced less antibody to the murine-mammary tumor virus, had lower levels of serum immune complexes and produced less mammary tumor virus.

In support of these basic research programs, the immunology program has additionally funded two training grants. One, located at the University of Alabama in Birmingham under Dr. Raymond Hiramato is supporting 3 trainees during FY80. Also, Dr. Robert Good is the Director of a training grant that the Sloan-Kettering Institute for Cancer Research in New York City. His training grant is supporting 5 trainees during this fiscal year.

The immunology program during FY80 has progresssed satisfactorily. There have been several new grants that have received good priority scores and this reflects the attraction of new, good immunologists to the field and also reflects an improvement in the general status of the discipline of immunology of aging.

II. MET: Subprogram Report

The objective of the intermediary metabolism subprogram is the understanding of the decline of catabolic and anabolic events that occur during the aging process. Of special interest to this subprogram are those aged-related modifications which have their manifestations in various disease entities. The molecular and biochemical events involved in intermediary metabolic activities are multi-factorial and complex. The role of the adrenal cortex the hypothalmus, and adenohypophysis are involved in complex interactions resulting in the control of the metabolism of carbohydrates, lipids and proteins. Experiments are being conducted on the metabolism of macromolecules such as carbohydrates, lipids, proteins DNA and RNA, lipo-proteins, collagen and connective tissues as well as other molecules within various organs and tissues. Experiments on regulation and control of protein systhesis governed by genetics and possibly environmental fictors, are under investigation in man and animal models. The role of membranes in control of transport and energy production is also being explored. In its attempt to achieve these objectives the intermediary metabolism subprogram in FY 80 is funding 1 Postdoctral Individual National Research Service Award, 34 Traditional Research Project Grants, 2 Program Projects Grants, 4 Exploratory/Developmental Grants, 1 New Investigator Research Award, and 2 Institutional National Research Service Awards.

The elucidation of the role of the endocrine system in metabolic events is the subject of the research of Dr. Christopher Widnell. His research has indicated that the combined administration of corticosteroid and triiodothyronine has an effect on old rats that is significantly different from the effect of administration of either hormone separately. These data underscore the complex inter-relationships that exist in the endocrine system. In a similar type of study Dr. Chae has investigated a chromosomal protease in the thymus. This research has significance for metabolic events in the aging process since the involution of the thymus has a significant impact on the development of the immunological system. He studied the chromosomal protease using a model for thymus involution which involved the treatment of rats with hydrocortisone and X-ray. Dr. Chae's data indicated a close correlation between the loss of thymus weight, the number of viable T-lymphocytes, the level of nucleolytic degradation and chrosomal protease in the thymuses of these rats.

The decline in the activity of the thyroid and para-thyroid organs during aging may have a significant effect on bone resorption and calcium mobilization and conservation. Dr. Leroy Klien has been studying these relationships in dogs. His data indicated that parathyroid hormone and vitamin 1,25-D3 both have a significant effect on bone resorption and calcium conservation. These in turn, are necessary for an understanding of bone remodeling and have significance

for the treatment of osteoporosis in the aged.

In an attempt to understand those events which lead to conformational changes in protein structure, Dr. George Nemethy has constructed a computer model to identify various bio-physical parameters which have an effect upon the conformation of macromolecules. Using this computer model Dr. Nemethy has been able to judge the effect of peptide hydration, modes of stable packing of triple helices, and beta-bend formation on molecular conformation. Using this model Dr. Nemethy has been able to gain insight into the conformation and folding of collagen-like peptides. Information gained from this work will probably contribute to the understanding of the molecular basis for the mechanical, physical and chemical behavior of collagen, which undergoes significant modification during the aging process.

The Intermediary Metabolism Program is currently supporting two training grants. One, at Boston University with Dr. Marott Sinex as the Program Director, supports eight trainees and the second, at the Philadelphia Geriatric Center with Dr. Richard Adelman as Program Director, currently supports two trainees.

There has been very little programmatic activity in this sub-program. This is due to lack of a Program Director identified specifically with this sub-program. Due to the expected reorganization of BRCM, it is anticipated that the grants that are currently funded by this program will be incorporated into the various sub-programs of BRCM, particularly the Endocrinology subprogram of PAP and the Molecular Biology subprogram of MCBP.

II. PHR: Subprogram Report

The research objectives of the pharmacology program include: 1) examining changes in the distribution, metabolism and pharmacokinetics of therapeutic drugs as a function of age; 2) investigating the nature of drug interaction in aged patients receiving multiple drugs, 3) examining the paradoxical reaction to drugs in the elderly and; 4) searching for nutritional factors which may affect drug efficacy or toxicity in the aged. In FY 80, the pharmacology program supported: 2 Postdoctoral Individual National Research Service Awards; 9 Traditional Research Project Grants; 3 Exploratory/Developmental Grants; 4 New Investigator Research Awards and; 1 Institutional National Research Service Award.

An important area of pharmacologic research is drug absorption. Dr. William Hayton of Washington State University was awarded a New Investigator Research Award to study the effects of aging on the capacity of the gastrointestinal tract to absorb orally administered drugs. Four drugs were studied using the rat model: tetracycline, bretylium tosylate, riboflavin and sulfisoxazole acetyl. The aged rat appeared to have a reduced ability to absorb tetracycline, riboflavin and sulfisoxzole acetyl. Bretylium tosylate on the other hand, appeared to have an increased rate of absorption. Not only do the aged animals respond more irregularly to drugs than younger animals but they may have less subtle responses. Some drugs, although useful in younger people, have toxic effects in the elderly. Dr. Robert Smith of the Texas Research Institute of Mental Sciences, a New Research Investigator Awardee, has demonstrated an increased uptake of dopamine and norepinephrine after termination of chronic neuroleptic drugs.

This indicates that chronic treatment with neuroleptic drugs might have an important effect on pre-synapatic neurons as well as post-synaptic receptors and may be responsible for the super-sensitivity to these drugs. Additionally, this super-sensitivity might be related to the syndrome of tardive dyskinesia in man.

Dr. Jay Roberts, the Principal Investigator of a Program Project grant, has studied the effect of age on atrial and ventricular pacemaker activity and the response of these pacemakers to drugs. Unlike the depressant effects of quinidine and lidocaine, which are altered in the elderly, phenytoin effects were not influenced by age. This finding indicated that inhibition of automaticity by drugs is not necessarily influenced by age and suggests that the membrane ionic channels and the drugs affecting these channels have different factors involved in their action, and these respond differently during the aging process. Thus it is likely that certain drugs may prove to be more effective in treating cardiac rhythm disturbances in the elderly.

A second study of this Program Project grant was a determination of the effect of age on the response of the cardiovascular system to drugs which affect the autonomic nervous system. Studies of the effect of cholinergic drugs on the vagus nerve suggested that there was a decrease in the activity of the cholinergic nervous system and in the cholinergic receptor responsiveness during aging. It was clearly shown that the decrease in heart rate associated with aging is due, not to increased cholinergic activity, but rather that the slowing of the heart rate with age is due to changes developing in the sinus pacemaker itself.

The effect of drugs on metabolism is the subject of the research of Dr. Sidney Strohs of the University of Nebraska. He has determined the effect of age on tissue glutathione levels, activities of mixed function mono-oxygenases and hepatic cytochrome P-450 content in mice. These studies have demonstrated that decreases in glutathione levels and microsomal mixed function mono-oxygenase activities occur with advanced age. These factors may contribute to a decreased rate of metabolism, and an increased susceptibility to drugs, foreign chemicals and disease processes which occur with advanced age.

The pharmacology program is currently funding a training grant with the Massachusetts General Hospital, Dr. David Greenblatt, Program Director. This training grant is supporting one trainee in the area of geriatric pharmacology.

The pharmacology program as identified in this report has not been very effective and falls short of meeting the general and specific goals of the program. The principal cause for this failure has been the inability of NIA to identify a suitable pharmacologist to direct this program.

During this fiscal year, an interagency initiative on drugs and the elderly with NIA as the lead agency, has been identified by HHS. NIA is currently in the process of identifying meaningful objectives of this initiative and the paths to achieve these objectives. A Grants Associate has been identified and will spend three weeks at NIA to assist in this process. It is anticipated that a pharmacologist, at least on an interim basis, will be identified to direct this initiative for the next several months.

II. BPAP: Program Report

The Biophysiology and Pathobiology of Aging Program (BPAP) consists of nine subprograms: Neuroscience, Nutrition, Endocrinology, Geriatric Medicine, Pathobiology, Physiology, Exercise, Epidemology and Animal Models and Resources. This program is primarily focused on pathologies that are associated with the aging process. A second major goal of BPAP is to support qualified applications to improve the quality of curricula in geriatric medicine and dentistry. The Animal Models program is distinct from the other programs in BPAP in that it is focused on providing animal resources to NIA grantees. Thus, the Animal Models program is supported by contracts.

Of all the pathological manifestations of the aging process, senile dementia of the Alzheimer's type (SDAT) is the most debilitating. It is characterized by neuropathologic alterations which consist of neurofibrillary tangles in the neurons of the frontal and temporal cortex and of the hippocampus. The Neuroscience program of BPAP is currently supporting several research projects which are studying SDAT. One of these has identified that the nuclear region of hippocampal neurons from elderly SDAT patients has a high concentration of intranuclear aluminum. Aluminum was not detected in the neurons from the hippocampus of elderly non-demented subjects. The underlying mechanisms for this positive correlation between the presence of aluminum and SDAT remains to be elucidated. A physiological manifestation of SDAT is the deficit of enzymes related to the synthesis and degradation of acetylcholine in the cerebral cortical tissue. Research performed during this fiscal year has demonstrated a positive correlation between reduced choline acetyltransferase and psychological evaluation in individuals with demonstrable pathological features of SDAT. Thus, it appears that there is a major deficiency of an essential neurotransmitter in SDAT. This information may prove to be valuable in the generation of effective therapy for SDAT. The loss of memory is an age-related pheonemon that has wide-spread effect upon the day-to-day function of the elderly. Dr. Schwartz of the University of Pennsylvania has hypothesized that lexical loss frequently seen in dementia is qualitatively different from amnesic aphasia which results from a focal brain insult. His preliminary data suggested that within the general population of demented patients there probably exists a subset of patients which show pronounced and early disruption of word-finding loss, the mechanism of which is different from that which is responsible for similar symptoms in aphasias. This research, may produce a refinement of the diagnosis of SDAT and distinguish it from similar age-related dysfunctions.

One apparent consequence of the aging process is sensory modification. The subprograms of BPAP are supporting research in this area. One such study on taste has indicated that the function of taste may not be necessarily lost with age. The apparent loss in taste intensity may not be organic in origin, but an age-related behavorial modification which results in the alteration of perceived taste.

The Nutrition subprogram is supporting studies on the wide-spread effects of nutrition on aging. Modifications in diet may have an effect upon the metabolism of aging tissue. In one study, the cell free protein synthetic activity of the testicular tissue of 30-month-old rats was reduced by 50% when compared to younger animals. In another study, the principal investigator is attempting to evaluate not just the effect of what is eaten but the time at which it is eaten. He will try to identify whether groups of mice on different circadian rhythms and fed either ad libidum or a restricted diet will live to the same age. In a more clinically oriented study, Dr. Griminger of Rutgers University is conducting studies which are designed to determine whether mice subjected to specific traumas such as surgery may have specific nutrients requirements. Dr. Goodwin at the University of New Mexico is taking a much broader look at the role of nutrition in the elderly. He will determine the nutritional status of 250 healthy elderly through the use of a clinical examination of their diet and biochemical assays. He will study these subjects over a period of five years and will determine the consequences of subclinical milnutrition, as well as the relationship between nutritional status and depressed immune function in the elderly.

The male and female sex hormonal systems exhibit marked changes with aging. MBAP, through its Endocrinology program, is currently supporting a number of projects which are attempting to delineate these changes and their consequences. One such study has indicated that adipose tissue may be a major site for the formation of both estrone and estrogen, and that the availability of plasma androstenedione may determine how much of these hormones are formed in extraglandular tissues. Another study has indicated that the accumulation of dihydrotestosterone in the aging prostate and the stimulatory effect of estradiol may be involved in the pathogenesis of prostatic hyperplasia in aging males.

In supporting its research objectives, BPAP funded 46 grants in FY 80 for a total of $4,258,472. A detailed description of these applications as to type and cost is included in the following section. No contracts were awarded during this fiscal year; however, 5 contracts were continued for the support of the Animal Models program. Program staff attended site visits and scientific meetings, and some program activity can be reported for BPAP.

II. ANM: Models Subprogram Report

This Subprogram is primarily aimed at the development and support of studies on comparative aging processes in vertabrates. The Subprogram supports studies to identify, define, characterize, and evaluate vertebrate model systems for the study of normal comparative aging changes, age-associated pathologic lesions that may stimulate or replicate aging processes, and environmental effects on survival and aging. It includes the study of functional and morphologic changes of age, and the survey and actuarial analyses of this information. The Subprogram develops criteria for the selection, development, definition, and environmental requirements of species needed for research on aging and establishes programs to make available and provide selected species and strains for research on aging.

The requirements for the maintenance of animals for aging research are more stringent and costly than for other research since these animals must be able to survive to old age. These animals must be maintained free of disease to allow the development of such colonies. The Subprogram must anticipate late occurring age specific pathological and degenerative changes. Therefore, the Animal Models Subprogram must support further studies on the biological characteristics of a variety of species and strains over their life-span.

A list of the contracts supported by the Animal Models Subprogram in FY 1980 are listed below.

TITLE	NUMBER	VENDOR
"Maintenance of a Long-Term Aged Rat Colony"	NO1-AG-0-2101	Charles River Breeding Lab.
"Survey of Animal Models for Research on Aging"	NO1-AG-7-2118	National Academy of Sciences, Institute of Laboratory Animals
"Acquisition, Maintenance, and Distribution of a Colony of Barrier Reared, Age, Retired Breeder Sprague-Dawley Rats"	NO1-AG-7-2127	Charles River Breeding Lab.
"Development of a Colony of Multigenotypic Aged Aged Mouse Strains"	NO1-AG-2128	Charles River Breeding Lab.
"Laboratory Rat Pilot Studies on Inbred Strains and Selected Hybrid Crosses"	NO1-AG-2135	Charles River Breeding Lab.
"Aging Monkey Tissue and Organ Resource"	NO1-AG-2854	Washington State University

II. END: Subprogram Report

The endocrinology subprogram of the Biophysiology and Pathobiology of Aging Program supports research on age-associated alterations in endocrine functions. This includes studies which examine 1) the effects of aging on the biosynthesis and secretion of both steriod and polypeptide hormones 2) age associated changes in receptor function 3) the response of target tissues and 4) the interdependent action of various hormone systems. This broad approach is necessitated by the fact that different components of the endocrine system exhibit variable responses to aging.

The male and female sex hormones represent a hormonal system which exhibits marked changes with aging, and the NIA is currently supporting a number of research projects which are attempting to deliniate these changes and their consequences. It is well known that estrogen production by the ovaries ceases in postmenopausal women. It is also known that estrogen can still be produced by non-ovarian tissues in these women. A recent study has found that both aging and obesity are associated with an increased estrogen production from a plasma steriod, androstenedione, by extraglandular tissues. The results of this study indicate that adipose tissue may be a major site for the formation of both estrone and estrogen and that the availability of plasma androstenedione may be important in determining the amount of these hormones which are formed in extraglandular tissues.

Another problem which appears to be influenced by the changing sex hormone profile in aging men is the widespread occurance of prostatic hypertrophy. In an NIA supported study it has been found that dihydrotestosterone accumulates in excessive amounts in the hyperplastic prostate. In experiments carried out on aging dogs it was found that another steriod hormone, 17-B- estradiol, had a stimulatory effect on the development of prostatic hypertrophy also. Thus the accumulation of dihydrotestosterone in the aging prostate and the stimulatory effect exerted by 17-B estradiol may represent possible mechanisms for the pathogenesis of prosthetic hyperplaysia in aging males.

The endocrinology program is currently supporting: 18 Traditional Research Project Grants; 2 Program Project Grants; 8 New Investigator Research Awards; 3 PostDoctoral Individual National Research Service Awards.

This subprogram does not have a project officer specifically assigned to it. Consequently, very little program activity has resulted. It is very important to have more programming in this subprogram and additional staff is planned for FY 81.

II: EXR: Subprogram Report

The goal of this subprogram is to support research which will provide new information and understanding of the role that exercise, or the lack of it, may have on the health and quality of life of the elderly. It is anticipated that this knowledge will provide direction for the establishment of a variety of programs to improve the health and quality of life of the elderly.

Specifically, the program supports research that includes: 1) integrative studies which address the important functional relationships between nutrition and exercise, endocrine modifications during exercise, changes in the cardiovascular system and the effects of these changes in the aging brain; 2) benefits of acute versus chronic exercise for the elderly; 3) optimal exercises for the maintenance of strength, proprioception, mobility, and flexibility of bones, muscles, tendons, and joints of the elderly; 4) capacity of the elderly to adapt to chronic exercise and its role in preventive and rehabilitative medicine as well as specific exercises to achieve adaptations in the elderly, e.g., cardiovascular and respiratory benefits that accrue to the elderly from exercise; 5) the effects of exercise on age-associated changes in tissue metabolism, the endocrine response to exercise and endocrine regulation of metabolism in the elderly; and 6) psychological and social benefits of exercise for the elderly.

EXR is currently funding: 7 Traditional Research Project Grants; 1 Exploratory/Developmental Grant; 1 Post-Doctoral Individual National Research Service Award and; 1 Institutional National Research Service Award. EXR has a great deal of potential which is not realized because of the lack of staff.

II. NUT: Subprogram Report

The ultimate goal of the nutrition program is the understanding of the multi-faceted effect that nutrition has upon the aging process. It is anticipated that this understanding will provide directions for the improvement of the health and general quality of life of the elderly. More specifically, the nutrition program encourages and supports research into 1) the relationship among aging, nutritional status, dietary intake and health status of the elderly; 2) the effects of specific diseases on nutritional status, interactions of nutrients with therapeutic agents, surgical procedures or preventative regimens; 3) the effects of aging on nutrient utilization, digestion, absorption, and metabolism; 4) the factors which may regulate changes in lean body mass, regulation of metabolic processes and disease susceptibility; 5) the effects of diet on modifying immune, endocrine, and metabolic processes; 6) the effects of deficiencies of essential nutrients, vitamins, minerals and trace elements; 7) the effects of nutrition and age-related mental deterioration and loss of neural function, particularly senile dementia, including the decline in sensory sensations of taste, smell, motor coordination and cognition.

Although dietary restriction has been shown to increse the longevity of living organisms, the mechanisms underlying this effect are unknown. It is probable that prolonged dietary restriction may have a direct effect on the synthesis of RNA and protein as aging occurs. Experiments were designed by Dr. Richardson, at the Illinois State University, to study the effect of two dietary regimens on the synthesis of RNA and protein during the life span of rats. His preliminary data suggest that in rats which were fed 60% of an ad libitum diet, the cell free protein synthetic activity of testicular tissue decreased 50% between 3-30 months of age. Although, these studies are in their preliminary stages it is hoped that they will provide some additional insight into the effect of dietary restriction on the metabolic activity of aging tissue. Dr. Vernon Young, at the Massachusetts Institute of Technology, is studying age-related changes in protein and amino-acid metabolism in the liver of aging humans. Preliminary data suggest a lower rate of synthesis of albumin in elderly subjects as compared to young adults. It was additionally observed that those younger individuals who had a lower level of ingestion of protein also had a reduced synthesis of albumin. In contrast, both elderly subjects and young adults demonstrated a similar rate of synthesis of glycine. Individuals in both age groups who ingested a decreased amount of protein also exhibited a reduced synthesis of glycine.

Some scientists feel that, in addition to what we eat, when we eat it may also be important. Dr. Halberg, at the University of Minnesota, is attempting to demonstrate that the effect of restricted diet on life-span may also be induced by an alteration of circadian rhythmn. Mice, with a different circadian rhythmn, will be fed a restricted as well as an ad libitum diet. This research is currently in progress and it may

provide information concerning the effect of eating habits on the aging process. A clinically oriented study is being performed by Dr. Griminger at the Rutgers University. These studies, still in their preliminary stages, have been designed to determine whether mice subjected to specific trauma such as surgery may have specific nutrient requirements. In another clinically oriented study, Dr. Goodwin, at the University of New Mexico, will study the question of the significance of poor nutrition in the elderly. He will determine the nutritional status of 250 healthy elderly through the use of a clinical examination, an examination of their diet and clinical biochemical assays. It is planned that these subjects will be followed over a five year period in order to determine the consequences of subclinical malnutrition, the relation between nutritional status and depressed immune response, and the effect of major life events on the nutritional status of the elderly.

In addition to these research programs, the nutrition program is currently supporting: 16 Traditional Research Project Grants: 1 Program Project Grant: 1 New Investigator Research Award and: 1 Post-Doctoral Individual National Research Service Award. This subprogram does not have a program officer specifically assigned to it. Consequently, this very important program has not received the attention it requires. A new program officer Dr. Elizabeth McGuire, will be joining the Institute and will have specific responsibilities for the Nutrition program.

II. GER: SUBPROGRAM REPORT

The Geriatric Medicine Academic Award Program was initiated in this Subprogram for the development and improvement of curricula in Geriatric Medicine in medical colleges and other appropriate institutions. This award provides support to individual faculty members for their educational development and for the implementation of the curriculum in Geriatric Medicine. More specifically, the Geriatric Medicine Award supports programs which: 1) encourage the development of a quality curriculum in geriatrics that will attract outstanding candidates for aging research and medical practice; 2) ensure superior learning opportunities in geriatric medicine; 3) help to develop the career of the promising young faculty whose interests and training are in geriatric medicine; 4) develop superior faculty who have major commitment and possess educational skills for teaching geriatric medicine and; 5) facilitate the interchange of educational ideas and methods among awardees and institutions.

Currently, this subprogram is supporting 18 Geriatric Medicine Academic Awards, 11 of which are in their first year. The vigor that is apparently brought to these programs by the various principal investigators, e.g., Dr. Patrick Irvine of the University of Minnesota, gives support for high expectations from these awards. The remaining 7 awards are in their second year. A prime example of the effectiveness of this award may be the program at Harvard Medical School. Although this program, directed by Dr. Rowe, was started in July of 1979, there have been significant accomplishments. The previously existing Committee on Geriatrics has been replaced by the Division on Aging and has been placed administratively in the office of the Dean for Medical Services of the Harvard Medical School. This Division has multi-disciplinary memberships drawn from several departments in the pre-clinical and clinical branches of the Faculty of Medicine as well as representatives from the School of Dental Medicine, Public Health, Kennedy School of Government, and Harvard College. Accomplishment due primarily to this academic award include: 1) incorporation of specific information on Gerontology and Geriatrics in a program designed for all first year medical and dental students; 2) the presentation to first year students, of a series of three integrated, two and one-half hour sessions revolving about clinical problems in geriatrics; 3) the development of three elective courses for second year medical students; and 4) the inclusion of substantial didactic and clinical geriatric information in a course required by all second year medical students. Additionally, the division of aging has initiated a series of seminars in gerontology and geriatrics and has established a program of stipends for student research projects in geriatrics and gerontology. Twelve students are currently being supported by this award and are involved in a wide variety of age-related research projects.

The Geriatric Medicine Subprogram is a very active one as indicated by the support of 18 new awards during the past two years. This not only

reflects significant program activity but also reflects the need in the scientific geriatric community for the support that this award affords. It is anticipated that program activity will expand during the next fiscal year as it holds the promise for a long-term beneficial impact upon gerontology and geriatric medicine.

II. SEN: Subprogram Report

This subprogram is designed to establish a data base in the following areas of investigation:

1) neurochemistry: biochemical, morphologic and pharmacologic studies of the pathology of the chronic dementias and other neurologic disorders.

2) cerebral circulation and metabolism: application of new in vivo methods to examine local brain metabolism in man and evaluate specific pharmacotherapeutic regimens.

3) neuroendocrinology; studies of the role of brain peptides and hormones in regulating a complex variety of (physical brain and psychological) functions.

4) neuroplasticity: studies on the reestablishment of neural connections in relation to possible restoration of certain loss functions, such as learning and memory.

5) genetics: studies on the role and mode of inheritance of organic brain disorders (by pedigree analysis).

To attain the above objectives, the subprogram will continue to support the formulation of new theories and the development of novel perspectives, methods, and instrumentation for the study of the aging nervous system. The long-range goal of the program is to facilitate the translation of basic scientific information into practical applications for the treatment and care of the elderly with neurological disorders.

Senile Dementia of the Alzheimer Type (SDAT) is a chronic degenerative disease seen predominently in the elderly. It is characterized by neuropathologic alterations which consist of neurofibrillary tangles in the neurons of the frontal and temporal cortex and of the hippocampus. SDAT is of special interest to this subprogram, and there is encouraging research into the understanding of its cause, prevention and control. Dr. Daniel Perl of the University of Vermont in Burlington has generated data on the aluminium content of a large number of hippocampal neurons. Neurons with neurofibrillary tangles were shown to have aluminium in the nuclear region, whereas few of the nontangled neurons had intranuclear aluminium. Although aluminium was not detected in the neurons from the hippocampus of elderly, nondemented subjects, it was observed in the nuclear region of neurofibrillary tangles of extremely elderly, non-demented controls. These observations require additional research to determine if a casual relationship exists between the presence of aluminium in the neurofibrillary tangles and SDAT.

Previous work had shown major deficiencies in enzymes related to the syntheses and degradation of acetylcholine in cerebral cortical tissue from autopsied SDAT patients. Dr. Peter Davies at the Albert Einstein College of Medicine has reported that there was a less marked reduction in activity of choline acetyltransferase and acetylcholine esterase in normal human brain tissue. Their studies additionally demonstrated a positive correlation between the reduced choline acetyltransferase and a psychological evaluation in cases with demonstratable pathological features of SDAT. This study is significant in that it identified a major deficiency which may give insight into an effective therapy.

Memory loss in the elderly and its association with senile dementia was the subject of investigations by Dr. Schwartz at the University of Pennsylvania. He has hypothesized that lexical loss frequently seen in dementia is qualitatively different from amnesic aphasia which results from a focal brain insult. Data on demented and aphasic subjects has been generated using a full semantic test battery. Preliminary data suggested that within the general population of demented patients there exists a subset who show pronounced and early disruption of language function. This impairment in language function was characterized by a form of word finding loss. Their data suggests that the mechanism for this word finding loss in demented patients is different from that which is responsible for word finding deficits in aphasics. Thus SDAT is being subjected to a more analytic analyses and has implications for the care and treatment of those aged with senile dementia.

The understanding of the sleep/wake disturbance which accompanies the process of aging is the subject of several research programs being supported by this subprogram. Dr. Webb of the University of Florida has been measuring the sleep characteristics of 50 to 60 year old persons. The objectives of this study are to determine the relationships between electrophysiological measures of sleep and cognitive and personality measures. These studies, currently in progress, have indicated that electroencephalographic measurements show objective evidence of increased awakenings. These studies may prove useful for the differentiation between a behavioral and a physiological etiology of sleep disorders.

The neurosciences program is also supporting research into the area of sensory modifications in the elderly. During FY 80, Dr. Bartoshuk of the John B. Pierce Foundation reported on studies of the effective aging on the thresholds and magnitude estimates produced by taste stimuli. His studies indicated that the function of taste is not necessarily lost with age. It may be possible that ne apparent loss in taste intensity is not organic in origin but may be a age-related behavioral modification which results in an alteration of perceived taste.

The neuroscience program is currently supporting; thirty-six traditional research project grants, four exploratory/developmental grants, four new investigator research wards, nine program project grants, ten post-doctoral individual National Research Service awards, five Research

Career Development Awards, two Clinical Investigator Awards, and four Institutional National Research Service Awards.

The neuroscience program is one of the largest and most active in BRCM. This is due primarly to the attention given to this program by two successive program directors, Dr. Zaven Khachaturian and Dr. Bernard Wortman. It is anticipated that with continued attention this program will continue to be productive and provide important information to the understanding of the neurobiologial changes associated with the aging process.

II. PHS/PTH: Subprogram Report

This subprogram was designed to support biomedical research on age-related physiologic changes and the pathology that may result from these changes. Since physiologic functions change with age, a knowledge of the mechanisms by which these changes occur is necessary for the generation of programs which would ameliorate age-related physiologic deficiencies. For example 1) the increased occurrence of hypothermic and hyperthermic deaths in older individuals emphasizes the deficiencies in the knowledge of thermoregulatory functions and capacities in humans, and 2) with the steady decline in renal hemodynamics during aging, intrarenal adaptations of function must occur to allow the maintenance of homeostatis.

The pathology/physiology subprograms currently fund: 8 Traditional research project grants; 1 program project grant; 3 exploratory/developmental grants; 2 New Investigator Research Awards and 1 Institutional National Research Service Award.

These subprograms are underdeveloped due to the absence of attention and activity.

IV. BRCM: Research Contract Summaries for FY 1980

Summaries are provided for contracts managed out of CEL, IMM and ANM. No other BRCM subprograms supported contracts.

IV. CEL: Research Contract Summaries for FY 1980

Contract Number: NO-AG-0-2100

Contract Title: Selection, Production, Characterization and Distribution of Genetically Marked Cells for Aging Research

Contractor: Institute of Medical Research, Camden, New Jersey
Principal Investigator: Dr. Arthur E. Greene

Money Allocated: $207,980.

Objectives:

1. Provide standard, highly-characterized, lines of normal human diploid fibroblast-like cells.

2. Provide banked and characterized, genetically marked or mutant cells identified as possessing features of value in probing mechanism of aging.

3. Provide standard, characterized, lines of tissue-specific cell types.

4. Contribute to the development of the field of cellular aging theory, concepts, and techniques, through consultation and workshops, particularily in the areas of somatic cell genetics and cytogenetics.

5. Limited cytogenetic screening for aging research.

Significance to Aging Research:

Events at the cellular level are probably major determinants of the expression of longevity and senescence at the level of the organism. The techniques of cell-culture enable investigation of cellular events consequent to aging independent of the complexity of the whole organism. Such *in vitro* studies are conducted on cells as they age in the cell culture environment, and also, on cells derived from humans and experimental animals of different ages. Most such studies to date are on fibroblast-like cells. Advances in cell culture technologies show considerable promise that an increasing number of tissue-specific cell types, that is differentiated cells in culture, will become available to the gerontologist for comparative studies of cellular aging. The contractor is to provide leadership in the acquisition, banking and distribution of differentiated cell lines valuable to the gerontologists. The contracted resource also supports studies on genetic mechanisms of cellular aging by supplying various genetically marked cell-lines.

Contract Progress:

Cell-lines acquisition and distribution:

The NIA-Cell-Line Repository has established 397 cell lines. These include the female and male fetal lung fibroblasts (IMR-90 and IMR-91); fibroblasts obtained from skin biopsies of normal individuals of various ages (this includes 97 human skin fibroblasts from Baltimore Longitudinal Study), as well as newborn and fetal materials; fibroblasts from individuals who exhibit features resembling premature aging, such as progeria, Werner's syndrome, Alzeheimer's disease and abnormal DNA repair such as Bloom's Syndrome, ataxia telagiectasia, Fanconi's anemia and Xeroderma pigmentosum. Cells that maintain a differentiated function in culture are also banked and these include endothelial smooth muscle, renal epithelium and endometrium cells. In this latter category both human and animal cells are included. All frozen cells are pre-screened for Mycoplasma, bacteria, yeast, and mold contamination. Karyotyping and selectd isozyme studies and species identification are also carried out, as necessary.

The two highly characterized cell lines are IMR-90 and IMR-91. The characterization studies include chromosome banding, frequencies of chromosomal abnormalities during culture lifespan, cell kinetics, sister-chromatid exchanges during culture lifespan, isozyme analysis, HLA typing and a variety of growth parameters. These cell lines have been developed and stored in large quantity at early passage and at intervals throughout their lifespan with eventual aim of replacing the existing stock of WI-38 cell-line as it is exhausted. The IMR-90 cell has found extensive usage not only in gerontology, but by the biomedical research community, in general. During the past year there have been 198 requests for IMR-90/91 starter cultures, compared to 160 requests for other cell lines.

There have been considerable interest in the possibility of using IMR-90 and IMR-91 as substrates for vaccine production. Two commercial companies and the U.S. Army have been making trial vaccines with IMR-90. Feedback from these trials has indicated that the cells are satisfactory for this use. Bureau of Biologics, NIH, is also interested in IMR-91 as a vaccine substrate. The possibility of obtaining a patent of IMR-90 or IMR-91 for vaccine production was discussed with the Bureau of Biologics, but this action seems unlikely.

Cytogenetics Service:

In addition to the cell line repository the present contract provides a limited karyology service to provide limited cytogenetic analysis to NIA grantees who do not have expertise in cytogenetics or do not have ready access to cytogenetic service at their institution. The karyology service is designed to handle basic determination of chromosome number, banded karyotypes, chromosome breakage frequencies, sister chromatid exchange frequencies, replication kinetics, etc., which are relatively short term in scope. The service has been used for the cytogenetic characterization

of IMR-90 and IMR-91 as well as a limited cytogenetic characterization of new lines as needed. During the past year 40 cultures were cytogenetically analyzed.

Computerization: In November 1977, IMR leased an IBM System 32 computer and retained the services of a systems-analyst-programmer to design and install management programs for the NIGMS and NIA Repositories. The computer is now being utilized in all aspects of the cell repository, including receiving, distribution, cataloging, ampule storage, data feedback, etc. While the various data files programmed into the computer are functional, some backlog data from the early years of the repository has not yet been entered. Early in 1980 the storage capacity of the IBM System 32 has been reached, and a switch is now made to the larger IBM System 34 computer containing four times the memory capability of the System 32, in addition to increased speed of operation and dual input capability.

Workshop: No workshops were held in conjunction with the aging repository in 1979-80. The aging repository staff has organized and conducted five workshops since 1974. These have resulted in the publication of five books. The workshops were: 1) Regulation of Cell Proliferation and Differentiation; 2) Senescence: Dominant or Recessive in Somatic Cell Crosses? 3) DNA Repair Processes and Cellular Senescence; 4) Mycoplasma Infection of Cell Cultures; 5) The Use of Differentiated Cells in Culture for Aging Research. These workshops have addressed themselves to the uses of genetically marked cells for aging research and an attempt to focus the efforts of additional disciplines on aging research.

The NIA Cell-Line Repository has achieved significant contribution to the cellular aging research community, a contribution expected to increase as the diversity of cell types in culture expands.

Publications and Presentations

W.W. Nichols, Genetic effects of mycoplasma, In: MYCOPLASMA INFECTION OF CELL CULTURES, Vol. 3, Cellular Senescence and Somatic Cell Genetics, edt. G. McGarrity, D. Murphy and W.W. Nichols, Plenum Press". N.Y. 1978, pp. 151-157.

In vitro anaphase and metaphase preparations in mutation testing, W.W. Nichols, R.C. Miller and C. bradt, In: HANDBOOK OF MUTAGENICITY TEST PROCEDURES, ed. B.J. Kilbey, M. Legator, W.W. Nichols and C. Ramel,, Elsevier Scientific Pub. Co., 1977, pp. 225-233.

Cytogenetic comparison of diploid human fibroblast and epithelioid cell lines, R.C. Miller, W.W. Nichols, J. Pottash and M.M. Aronson, Exptl. Cell Res. 110: 63-73, 1977.

Induction of sister chromatid exchanges by transformation with SV40 virus, W.W. Nichols, C.I. Bradt, L.H. Toji, M. Godley and M. Segawa. Cancer Res. 38:960-964, 1978.

Banded karyotypes of three whales: Mesoplodon europaeus, M. Carlhubbsi and Balaenoptera acutorostrata. U. Arnason, K. Benirschke, J.G. Mean and W.W. Nichols. Hereditas 87:189-200, 1977.

R.C. Miller, Acute and long term cytogenetic effects of childhood cancer chemotherapy and radiotheapy. R.B. Hill, W.W. Nichols and A.T. Meadows. Cancer Res. 38:3241-3256, 1978.

J. Nove, J.B. Little, R.R. Weichselbaum, W.W. Nichols and E. Hoffman, Retinoblastoma, chromosome 13, and in vitro cellular radiosensitivity. Cytogenetics & Cell Genetics 24:176-184, 1979.

Short-term tests for carcinogens and mutagens. M. Hollstein, J. McCann, F.A. Angelosanto and W.W. Nichols. Mutation Research 65:133-226, 1979.

Interstitial deletion of chromosome 13 and associated congenital anomalies, W.W. Nichols, R.C. Miller, E. Hoffman, D. Albert, R.R. Weichselbaum, J. Nove and J.B. Little. Human Genetics 52:169-173, 1979.

Retinoblastoma: an in vitro approach. J. Nove, R.R. Weichselbaum, W.W. Nichols, D. Albert and J.B. Little. International Ophthalmology Clinics: Advances Regarding the Pathogenesis and Treatment of Ocular Tumors, Little, Brown & Co. (in press).

Homogenously staining regions at the same chromosomal location in two different childhood genetic tumors. G. Balaban-Malenbaum, R.C. Miller, W.W. Nichols and F. Gilbert. Amer. J. Hum, Genetics 31, No. 6, 87A, 1979.

The role of genetic factors and therapy in cancer susceptibility. W.W. Nichols. Presented, American Society of Human Genetics 30th Annual Meeting, Minneapolis, October 3-6, 1979.

Application of cytogenetic monitoring to hereditary disease. R.C. Miller, Presented, Workshop on Fundamentals of Population Monitoring (DHEW), Honolulu, July 18-20, 1979.

In vitro cytogenetics, W.W. Nichols. Presented, Symposium, Principles and Practices of Genetic Toxicology, University of Texas Medical Branch, Galveston, October 21-26, 1979.

Books edited

DIFFERENTIATED CELLS IN AGING RESEARCH. International Review of Cytology, Supplement 10, ed. W.W. Nichols and D.G. Murphy. Assoc. eds., L.H. Toji. L.J. Jacobs and R.C. Miller. Academic Press 1979.

CONCEPTS OF THE STRUCTURE AND FUNCTION OF DNA, CHROMATIN AND CHROMOSOMES, Year Book Medical Publishers, Chicago, London, 1979. A.S. Dion, ed.; D.C. Farwell, L.J. Jacobs, W.W. Nichols, L.H. Toji, Assoc. Eds.

Competitive Renewal of the Contract:

In Janaury 1980, the NIA renewed its cell-line repository contract at IMR. The present collection will be expanded to include a variety of differentiated cell types when cryogenic storage does not interfere with the viability and sufficient stability of differentiated functions can be measured. Some of the proposal work continuation during the contract year 1980-1981 includes:

a) Completion of large freeze of IMR-91S (skin) at population doublings 10, 15, 20, 30 and 40, and characterization of the cell line.

b) Expansion of the cell cultures received from the Longitudinal Study at the Gerontology Research Center.

c) Continued acquisition of skin biopsies, cell cultures and peripheral bloods from patients and their family members who exhibit features resembling premature sging such as progeria, Werner's syndrome, Alzheimer's disease, Rothman syndrome, Mullibrey nanism, etc.

d) Investigate with the Project Officer the possibility of acquiring, culturing and banking human and other mammalian normal differentiated cell types.

e) Cytogenetic characteristics of specific cells cultured in the Aging Repository will be undertaken when necessary as described in the Workscope of the 1980 contract, as well as the limited cytogenetic investigations for investigators in aging research that do not have this capability.

f) Research projects to improve the performance of the Aging Repository will continue with the approval of the Project Officer.

Contract Number: NO1-AG-9-2113

Contract Title: Caenorhabditis Genetics Center

Contractor: Curators of the University of Missouri-Columbia, Columbia, Missoiuri.
Principal Investigator: Dr. Donald L. Riddle

Objectives:

1. Acquire, maintain and distribute wild-type and mutant strains of C. elegans for biomedical research.

2. Determine that the nematodes for distribution are free from contamination and conform to visible phenotype description.

3. Conduct yearly survival tests on a limited number of frozen strains, and test potentially advantageous new methods for strain storage and distribution.

4. Maintain and distribute the genetic map, nomenclative information and related data.

Significance to Aging Research:

This resource is in support of all scientists qualified to study and/or are training to study, Caenorhabditis elegans as a model organism in biomedical research. This policy appreciates that the entire range of research being conducted on C. elegans, mostly very fundamental molecular genetics and developmental biology, is of immeasurable value to gerontology. It is this same community from which the NIA seeks to stimulate grant applications in aging based on an existing foundation in embryology, growth and development.

The Caenorhabditis Genetic Center (CGC) is of special significance to gerontologists. C. elegans manifests typical life span phenomena of species, specific longevity, post-reproductive onset of senescence, followed by morbidity and death. It is a simple organism and it has a short lifespan. It is rapidly becoming genetically characterized, and is cultured with relative ease. C. elegans offers promise of being among the organisms offering the earliest insights into mechanisms of longevity and senescence. The CGC should greatly facilitate acquisition of this knowledge.

Contract Progress:

Announcements of the existence and function of the CGC have appeared in Nature (Vol. 284:514, 10 April 1980), Genetics (Vol. 93, September 1979 issue), Molecular and General Genetics (MGG 177:724, March, 1980) and the C. elegans Newsletter (January 1980) in addition to the notice which appeared in the NIH Guide for Grants and Contracts. In addition to announcements in professional publications, new stories have been carried

in several university publications, local newspapers and on two television stations. This local publicity has made CGC existence known to others in the University community.

In response to the announcements in the NIH Guide and in Nature several requests for nematodes, information and advice were received. Five investigators have been added to the mailing list, for a total of 95. The mailing list represents the current CGC clientele and will be used for distribution of bibliographic updates as well as for solicitation of strains, and genetic data.

Twelve separate requests for strains have been filled. There were an additional ten requests (not listed) for information only. Requested strains have been mailed out within 2-5 days after the request has been received by the CGC. Since the CGC was established, 7 requests for strains not yet acquired have been received.

The January, 1980 CGC Bibliography has been revised and updated as a consequence of library research as well as the response received from the 90 investigators who were recipients of the original bibliography. The list of 420 references has been changed in the following ways: (1) 28 additions, including new publications and a few references overlooked in our computer search; (2) 16 deletions of citations not directly concerning Caenorhabditis species; (3) 11 corrections to citations; and (4) 3 references to abstracts were replaced by references to full papers (same topic-same authors). The policy will be to include references to abstracts only when full papers have not been published. Of the 430 references currently listed, 320 now are represented in the reprint file. The reprint collection emphasized the more recent papers, including all publication on genetics. CGC will continue to request reprints routinely as new publications appear.

CGC received two requests for the bibliography from investigators who had read the notice in the NIH Guide, and were considering Caenorhabditis as a model system for aging studies.

During this reporting period CGC has acquired 46 strains representing 25 genes and 10 chromosomal rearrangement. We now have reference alleles of more than 230 genes. All strains received thus far have been verified by phenotype, freed of microbial contaminants if necessary, and subsequently frozen.

Based on the compilation of map data, and on a systematic search of reprint collection, CGC generated a list of 145 strains yet to be acquired. This list includes reference alleles for 50 new genes not yet represented in our collection. CGC has prepared a standard for investigators to supply information accompanying the strains they send. Since the requests were mailed only in the past few days, none of the 145 strains has been received as yet. CGC expects them to arrive over the next 1-2 months.

The contractor's general policy wil be to acquire non-reference strains which have been described in some detail in published literature, in addition to reference alleles for each mapped gene. This is why the 145 requests represent only 50 new mapped loci.

Overall organization of the CGC is on schedule. The contractor has been providing services promptly on request. The only aspect of the project which has not progressed as predicted is strain acquisition, CGC originally estimated that its laboratory collection would have to be supplemented with about 150 strains to complete an up-to-date CGC collection. They have acquired, verified and frozen about 140 strains already. However, after a comprehensive literature review, CGC discovered another 145 strains to be acquired, in spite of the fact that their acquisitions policy is reasonably conservative. Thus, this aspect of the project will require approximately twice the effort originally projected. CGC's entire strain collection (850 strains) will be about 20% larger than originally projected. The task of record-keeping and data compilation also will be increased proportionately beyond the original estimate.

Contract Number: N01-AG-8-2117

Contract Title: National Institute on Aging Mycoplasma Contamination Testing Service

Contractor: Institute for Medical Research, Camden, New Jersey
Principal Investigator: Dr. Gerald J. McGarrity

Money Allocated: $56,000

Objectives:

1. The detection, prevention, and control of Mycoplasma infection as a service, primarily to NIA grantees and the NIA Intramural Program.

2. Provide consultation to laboratories having chronic problems with mycoplasmas and other micro-organisms.

3. Distribute a quarterly newsletter on quality control of cell cultures.

Significance to Aging Research:

The NIA encourages the use of cell-culture technologies to pursue knowledge of genetic, molecular and cellular mechanisms of human aging. Investigators working with cultured cells must exercise extreme caution to avoid microbial contamination of those cultures. Gross microbial infections due to bacteria or fungus usually are obvious and rapidly destroy the contaminated culture. It is the more subtle infections, as with Mycoplasma, which may go undetected. Mycoplasmas, the smallest known free-living organisms, are common contaminants of cultured cells. They are difficult to detect, and while presence may not destroy the host cell-culture, it often alters the metabolism and function of the cultured cells. Unlike bacteria, mycoplasma lack cell walls and are resistant to many common antibiotics. Mycoplasma contamination is of particular concern in gerontological research in which it is often necessary to maintain cell-cultures for a relatively long period of time.

Contract Progress:

During the second year of this three year contract 497 specimens were submitted to the NIA mycoplasma Testing Service (MTS). Twenty-two infected with mycoplasmas (4.4%). Species isolated were: M. hyorhinis, M. arginini, M. orale, A. laidlawii and M. Pneumoniae. Twenty-seven eligible laboratories submitted specimens, 11 more than the previous year. An informal survey of the types of specimens submitted this year indicated a greater diversity compared to the first when primarily

human fibroblasts were assayed. The precise type of cell culture submitted as not always indicated, but this year lymphocytes, endothelial cells, human-human hybrids, mouse-human hybrids, and smooth muscle cell cultures were submitted in addition to fibroblasts.

The circulation of the communication, "Tissue Culture Quality Control" has been significantly increased. The present circulation is approximately 320 for the quarterly publication.

One of the objectives of this contract was to work in close collaboration with the NIA Cell Repository housed at this institute. This has also been achieved. Mycoplasma testing was performed on 78 cell cultures submitted by the Repository from January 1, 1979 until March 31, 1980. These cultures originated in 21 additional different laboratories.

Two articles that were partially supported by NIA were published during the past year. These were: "Factors influencing microbiological detection of cell culture mycoplasma" and "Comparative studies between microbiological culture and uptake of uridine/uracil to detect mycoplasmal infection of cell cultures." Two additional articles are in press. These are: "Detection of Mycoplasma hyorhinis infection in cell repository cultures" (Cytogenetics and Cell Genetics) and "Mycoplasmal infection of lymphocyte cultures: infection with M. Salivarium (In Vitro)." The principal investigator will serve as convener of an invited interest session at the International meeting on Mycoplasmology, to be held in Custer, South Dakota in September, 1980 entitled "Detection of Mycoplasma Infection in Differentiated Cell Cultures."

The quality control procedures used by the contractor are continuously reviewed. The microbiological media are tested with each assay by the ability to promote colonial growth of known infected cultures. In addition, new lots of horse serum, yeast extract, base media, Noble agar and other media components are tested to determine growth promotion. This is done by use of stock ampules of M. arginini frozen in liquid nitrogen. This culture contains 5.5x10 CFU.ml of an uncloned cell culture isolate of M. arginini. Ampules are rapidly thawed to 37°C, serially diluted in media prepared with the component under assay and plated. New media that have a 2 log decrease or more in #CFU/ml are rejected; media that show a 1-2 log decrease are retested. If the same decrease occurs, the new lot is rejected.

During the past year, several quality control problems have been detected. These have included yeast extract (several lots); poor growth promotion with commercially prepared Ham s F12 and RPMI-1640 cell culture media, poor cell attachment with commercially prepared plastic Leighton tubes were detected before they were used in routine assays. The poor growth promotion by the cell culture media was finally detected by the commercial supplier, but only 4-6 months after the media was in general use at the Institute and after the Institute brought the problem to the attention of the supplier and began its own investigation.

A number of in-depth consultations on the quality control in cell cultures have been given to NIA grantees and contractors. Records are not kept on the quality control in cell cultures. The topics included prevention, detection techniques, effects of mycoplasmas on cell cultures.

Publications:

1 McGarrity, Gerald J. Sarama, Judi, and Vanaman, Veronica. "Factors influencing Microbiological Assay of Cell-Culture Mycoplasmas". In Vitro, Vol.15, No. 2, 1979.

2 McGarrity, Gerald, J., Vanaman, Veronica, and Sarama, Judi. "Comparative Studies Between Microbiological Culture and Uptake of Uridine/Uracil to Detect Mycoplasmal Infection of Cell Cultures". Experimental Cell Research, Vol. 121, 1979.

IV. IMM: Research Contract Summary for FY 1980

Contract NO1 AG-9-2100 was awarded on November 15, 1978 to the University of Alabama. Dr. Ray Hiramoto was the Principal Investigator of a contract to study the "Longitudinal Study of Immune Function in Thymic Hormone Treated Mice". The contract was awarded for a period of three years at a total estimated cost of $384,255. During the first contract year an advisory group met with NIA to review the requirement for the satisfactory preparation of four known thymic hormones. The group indicated that refinements in the workscope would significantly improve and expand the scientific information to be produced. The recommended changes involved the collection of data using the planned technical protocol but with more animals and more hormone preparations. Consequently, a justification for noncompetitive procurement (JNCP) was requested during FY80. The JNCP request for $99,000 and an extension to November 1982, is in the final stages of approval. The contract involves two studies on the effect of four thymic hormone preparations on the immunological status of three strains of mice. The work was divided into a longitudinal and cross-sectional study. In the longitudinal study, mice two months old would be treated with the hormone preparations for life and monitored every three months for immunological competence. In the cross-sectional study, mice of different ages would be treated intensivly for three weeks and evaluated for a variety of of immunological functions and pathological conditions. The longitudinal studies were started in September 1979. The mice were treated with different thymic hormone preparations at two months of age and a number of immunological functions were monitored every three months thereafter. As of June 30, 1980 four data points were available on the C57/Bl mice, three on the CFW mice and two on the A/JN mice. Since this is a long term study involving the lifespan of the animals under study, the data currently available on young mice are not yet amenable to analysis.

IV. ANM: Research Contract Summary

Project Title: Maintenance of a Long-Term Aged Rat Colony

I.D. Number: N01-AG-0-2101

FY Begun: FY 1977

Objective: The objectives of this contract are to support the maintenance and distribution of a large colony of Fischer 344 rats which are used extensively for aging research.

Approach/Methodology: Not Applicable

Major Findings: This new two year contract has recently been awarded to Charles River Breeding Laboratory for the continued maintenance and distribution of Fischer 344 rats. Plans are being completed for the evaluation of this contract with recommendations for future award. It is anticipated that the contract will be openly completed at the end of these two years.

Significance to Aging Research: This colony (originally Contract Number N01-AG-3-2725) has provided the backbone support over the past several years for aging research which used the rat model. At the present time a large amount of data has been collected on this rat model and it thus serves as a very important standard by which to compare results obtained with other animals.

Principal Investigator/Organization: Charles River Breeding Laboratories

Staff Member Responsible: Dr. Bruce Maurer

Actual and Projected Costs:

FY 1980 - 879,756
FY 1981 - 1,275,949

Publications: None

Project Title: Acquisition, Maintenance, and Distribution of a Colony of Barrier Reared, Age, Retired Breeder Sprague-Dawley Rats

I.D. Number: N01-AG-7-2127

FY Begun: FY 1980

Objective: This contract was primarily designed to provide a resource of a commonly used laboratory rat which could be used for comparison and evaluation of intra- and interspecies data. Prior to the acquisition of this colony, the Fischer 344 was the rat principally used in aging research. A standing colony of 3020 animals ages 6 to 30 months, currently exists. Added monthly to this colony are 250 six month retired breeders.

Approach/Methodology: Not Applicable

Major Findings: This contract is scheduled to expire in December 1980. A Sources Sought Announcement seeking sources which can meet or exceed the current contract specifications has been issued. Based on the result of this process a new contract will be awarded. This contract will be evaluated during the upcoming year to determine other possibilities for maintaining this colony of rats. Recommendations could include the open competition of a small colony which will help to meet the increasing demand for this strain of rat.

Significance to Aging Research: The study of the aging process require availability of a variety of species and strains of animals in large enough quantity to accommodate the ever-increasing demand for well-defined research animals.

Principal Investigator/Organization: Charles River Breeding Laboratory

Staff Member Responsible: Dr. Bruce Maurer

Actual and Projected Costs: FY 1981 $145,000

Publications: None

Project Title: Development of a Colony of Multigenotypic Aged Mouse Strains

I.D. Number: N01-AG-7-2128

FY Begun: FY 1977

Objective: To develop, define, maintain, and distribute commonly used strains of laboratory mice necessary to provide model systems for research in the growing fields of immunology, neurobiology, pharmacology, nutrition, endocrinology, genetics, and psychology.

Approach/Methodology: Not applicable

Major Findings: The current developmental contract, which will expire in October, 1980, has progressed very well. At the present time the colony contains approximately 70,000 mice of twelve different genotypes and ranging in age from 3 to 30 months. This contract will be extended to allow for the recompetition process to be completed, and is scheduled to expire in January 1981. A Sources Sought Announcement has recently been issued seeking sources which are capable of providing these animals at the current contract requirements. It is anticipated that no sources other than the present contractor will be able to meet the requirements of that solicitation; therefore, the recompetition process for this contract will be continued based upon the results of this process.

Significance to Aging Research: This contract will provide strains of mice to be used for research on aging. This represents a significant increase in the number of mouse strains currently available and therefore provides a much broader base for comparative research. This colony was initially developed in response to investigator's requests that a larger number of mouse genotypes be made available. This contract is deemed very important for continued progress.

Principal Investigator/Organization: Charles River Breeding Laboratory

Staff Member Responsible: Dr. Bruce Maurer

Actual and Projected Costs: FY 81 $ 1,316,573

Publications: None

Project Title: Laboratory Rat Pilot Studies on Inbred Strains and Selected Hybrid Crosses (In a Defined Environment)

I.D. Number: N01-AG-6-2135

FY Begun: FY 1976

Objective: 1. To develop a rat model for aging research that provides a broad gene pool with maximum genetic control.
2. Develop an F-1 hybrid rat strain free of pathological conditions seen in currently available inbred strains and outbred strains.
3. To develop a F-1 hybrid rat strain which is as close as possible in its aging pattern to outbred stocks but with the uniformity and predictable pathological and biological characteristics of inbred strains.

Major Findings: This contract has recently been extended through December 31, 1980, to allow the animals remaining in the colony to live out their normal life-span and thus to provide more complete data on life-span. During this six month period a panel of experts will be convened to examine the data already collected on the three hybrid rat strains and to provide the NIA with an evaluation of which strains would be most suitable for further development. Since NIA owns the breeding stock for the hybrid animals, it will be possible to open up the developmental phase of this colony to competition.

Significance to Aging Research: At the present time the Fischer 344 represents the best rat model currently available for aging research. This contract will hopefully lead to the introduction of at least one, and as many as three new strains of rat. This is an important development for the continued progress of aging research since the new strains will likely prove more suitable for certain types of research than the currently available Fischer 344.

Principal Investigator/Organization: Charles River Breeding Laboratory

Staff Member Responsible: Dr. Bruce Maurer

Actual and Projected Costs: FY 1980 $ 24,259

Publications: None

Project Title: Aging Monkey Tissue and Organ Resource

I. D. Number: NO1 AG-5-2854

Publications: None

FY Begun: FY 1975

Objective: This contract provides for funds to be provided for post mortem evaluations to be performed on old Rhesus monkeys in a colony at the University of Washington.

Approach/Methodology: Not Applicable

Major Findings: This contract was awarded in June 1975 and covered six Rhesus monkeys maintained at the University of Washington Primate Center. Since the initial date two animals have expired and were subjected to complete post mortem evaluation as called for in the contract. Plans are under way for studies in the areas of behavioral sciences and neuroscience. After these studies are complete, the remaining four animals will be sacrificed and tissues, organs, etc, will be distributed to investigators in the field of aging to perform a variety of studies, to obtain information about longevity and physiological changes with aging in non-human primates. It is anticipated that this information will then be compiled into a publication which will serve as a valuable resource to investigators in the field of aging research.

Significance to Aging Research: Non-human primates represent an important source on which a wide variety of mechanisms related to aging can be studied. The number of aged non-human primates available for research is limited and this resource is costly. Therefore, the NIA has entered into this contract to provide for complete post mortem evaluations of six old Rhesus monkeys maintained at the University of Washington.

Principal Investigator/Organization: Washington State University

Staff Member Responsible: Dr. Carol Letendre

Actual and Projected Costs: $12,312 Total Costs

Publications: None

Epidemiology, Demography, and Biometry Program

I. Major Program Area Summary Statement for FY 1980

During the year, the EDB has carried out an active scientific program but, perhaps, the major accomplishment has been committing of substantial intellectual and economic resources into our on-going program. The basic administrative divisions remain Population and Clinical Research Analysis and Population Dynamics and Research Analysis. Since the EDB has not yet become an official program, these divisions do not have the branch status which would be appropriate. Our research is conducted primarily by EDB staff supplemented and augmented by research contracts and interagency agreements, professional services contracts, and numerous working arrangements with other Government and non-governmental agencies. Our budget for this year, pending final quarter funding will be between $3,500,000. and $5,000,000.

The professional staff consists of Jacob A. Brody, M.D., Epidemiologist (Associate Director NIA); Joan Cornoni-Huntley, Ph.D., Senior Research Epidemiologist, (Expert Appointment which expires in April 1981); Douglas A. Parker, Ph.D., Sociologist, (IPA whose appointment will terminate in July 1981); Lon R. White, M.D., M.P.H., Medical Epidemiologist; Yuko Palesch, M.S., Research Statistician; and Dennis Cosmatos, M.S., Research Statistician. In addition, Mary Farmer, M.D., who received one of thirteen PHS Epidemiology Fellowships elected to join our staff. She will spend the first of her 3 years with us getting her M.P.H. from The Johns Hopkins University School of Hygiene and Public Health. During the year Clifford H. Patrick, Ph.D., Research Demographer, left EDB to become Chief of the Prevention, Education, and Manpower Branch in the Division of Lung Diseases, NHLBI. Our lack of high level expertise in demography and economics will pose progressively more severe programatic problems.

In June, the EDB was moved from Building 31 to the Federal Building. This move permitted the entire EDB staff to share contiguous space for the first time.

II. Program Reports

A major accomplishment was the issuing of three contracts for "Establishment of Populations for Epidemiologic Studies of the Aged" to Yale University, University of Iowa, and the Harvard Neighborhood Health Center associated with the Peter Bent Brigham Hospital. These will provide major population laboratories for studies of elderly populations integrating medical, socioeconomic, and behavioral aspects of health. These undertakings will provide data on a wide variety of subjects of great interest and importance to NIA. Durng this year, our efforts to study the health effects of nutrition through following up on the HANES I Survey proceeded and a successful feasibility study was completed. This invaluable data source should provide outcome information related to numerous questions concerning diet and nutrition, blood chemistries, and other physical and social aspects covered in the HANES I Survey. Our response rate for the feasibility study was in excess of 85 percent. This suggests that the project will produuce valuable and reliable data.

Through a reimbursable agreement with NHLBI, we will conduct a 4 year feasibility study for a case-control, randomized drug trial for treatment of benign systolic hypertension of the elderly. This study has now commenced.

Senile dementia studies have been expanded. We are attempting to document the prevalence of senile dementia and other mental diseases through an interagency agreement with NIMH using their epidemiologic catchment area in New Haven (Yale University). This study has encountered many difficulties almost exclusively related to OMB clearance and to a hiatus during which the 1980 Census was conducted. Rather than belabor the tremendous burden in time and energy imposed upon us in the forms clearance process, let us state that our problems seem to have been solved and the surveys should be proceeding well by the end of this fiscal year. Data should be available within 2 years on prevalence in a community. As a second approach to the senile dementia problem we have issued an RFC to study the natural history of senile dementia among noninstitutionalized individuals. This will be a 5 year case control study in which we will emphasize the use of non institutionalized populations to determine the full extent of the natural history of senile dementia. Great emphasis will be placed on sequential interviews and neurologic and psychologic testing done over time. Progression of cognitive loss will receive special attention. We are also supporting minor efforts in studying the possible familiar association of senile dementia with Down's Syndrome and also trying to work out a field instrument which will classify senile dementia and evaluate the stage and type of disease.

We have been attempting to establish studies of the last days of life in order to develop realistic information for physicians and the patients on the basic events associated with dying. For example, we hope to determine who dies peacefully in his sleep, who dies in great pain, who dies in the presence of his family, who dies after long illness with full awareness of his impending demise, and who dies suddenly and with no warning. Attempts to conduct this study through other agencies using established populations have not been successful. We are, therefore, planning to conduct this study in several phases. The first will be a retrospective study using death certificates as our source of index persons rather than an open population. We have prepared an RFC which may be funded this fiscal year to accomplish this.

We are trying to identify researchable issues for NIA in the area of long term or continuing care. We have spent many hours in conferences and committees at all levels of Government and are also attempting to conceptualize a meaningful conference on this subject at NIH. We have been set back in these efforts because of lack of staff and the departure of Dr. Ludwig who had assumed principle responsibility for this effort. We are, however, in contact with many of the respected workers in this complex field and believe a successful program can be developed.

We are actively participating in studies of alcohol problems and aging. Dr. Brody serves as Vice-President of the Blue Ribbon Study Commission on Alcoholism and Aging. In addition we are utilizing data collected in a large survey in Detroit relating to occupational conditions, health problems, and alcohol use among employed women and men. Of great importance is our study of the effects of alcohol use for different durations and at different life stages on cognitive functioning with advancing age.

We are studying the effects of climate on age-specific mortality. We have established a data bank which contains mortality (by age and cause) and selected climate by day as well as place.

We have conducted several studies of the use of estrogen in post-menopausal women. These data show a decline in the use of estrogen by postmenopausal women in recent years. We are currently extending this aproach to investigate the use of other drugs by the elderly, particularly the digitalis glycosides, antihypertensives, coronary vasodilators, soporifics, antidepressants, and major and minor tranquilizers.

Our study of cohort mortality for cardiovascular diseases revealed surprising and important results which suggest that the age-specific rate of cardiovascular mortality among females has been declining steadily over a 25 year period. For males, however, there was a sudden change in the slope of the decline in cardiovascular mortality rates for all cohorts around 1965. This suggests that an environmental or exogenous factor at that time exerted the principal effect on male

cardiovascular mortality since the phenomenon was observed in all age groups only following that date. The male rate even at the lower level still is higher than the female rate. Our continuation of this study involves a prediction of deferred mortality by an increase in mortality among the older male cohorts; of particular interest will be whether or not the "payback" is expressed mostly in cardiovascular deaths or in deaths due to all causes.

Through complicated funding mechanisms, we have established a macroeconomic demographic growth model of the United States at the President's Commission on Pension Policy. This permits the answering of scientific questions concerning the impact of the economy on the elderly and the effect of the elderly on the labor markets and the national economy. In the future, we hope to use this model to determine the affects of health costs. The President's Commission on Pension Policy ceases to exist in March 1981. At that time, the macromodel which is already on the NIH computer will become and EDB contract. This poses an acute problem since we have no demographer or economist and could not conceivably utilize this magnificant resource to anything approaching its potential.

We have several interagency agreements with the Bureau of Census and have received one lengthy report on social indicators and utilization of health care facilities. A major publication in the Special Studies Series entitled Social and Economic Characteristics of the Older Population, 1978, also resulted, to a large extent, through our input.

In collaboration with the Social and Behavioral Research Program, we have transferred funds through an interagency agreement to AoA to support the National Aging Data Archive at the University of Michigan. This archive will be broadened and made directly useful and relevant to NIA interests.

III. Research Grants Summary for FY 1980

EDB has no Research Grants to report for FY 1980.

IV. Research Contracts Summary for FY 1980

Contracts were initiated in 1978 and the first was awarded in June of 1980 for the "Establishement of Populations for Epidemilogic Studies of the Aged" (NIH-AG-79-09) to Yale University ($242,762.), The University of Iowa ($198,921.), and the Peter Bent Brigham Hospital ($293,421.), for a total of $735,104. 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. In each area more than 3,000 individuals over age 65 will be included. The project shall include cross-sectional and prospective studies in a carefully defined and accessable population using standard field and analytical techniques. Yearly surveillance of the population will be included. 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 system. As these problems involve the complex interaction of the social and physical environment, it is important to have studies representing the real conditions in a community population. Within obvious logistical constraints the population will be available for specific studies to the NIA scientific community.

In March 1979, RFP No. NIH-AG-79-36 was issued requesting proposals to incorporate age structure into an existing macroecomomic model of the U.S. We had estimated such an effort to take 3 years and cost approximately $450,000. Of the 6 proposals which arrived, the evaluation committee found 5 to be technically acceptable; 4 of the 5 estimated the cost to be between $300,000 and $500,000 for the 3 year effort while one, Wharton Econometrics, estimated a cost of $969,000. Following the April closing date for the RFP, several events lead to the decision to join the President's Commission on Pension Policy (PCPP) in funding a proposal they received to do many of the analyses which our proposal requested.

We have entered into professional services contracts with:

North Carolina Pharmaceutical Research Foundation, University of North Carolina at Chapel Hill at a cost of $8,732.00 to provide a report which will include models of medication use by the elderly.

Columbia University College of Physicians and Surgeons, Institute of Human Nutrition at a cost of $1,000.00 to review current literature on nutritional factors associated with aging and provide a report on the "Nutritional Status of the Elderly."

University of Washington School of Public Health and Community Medicine Department of Epidemiology, Seattle at a cost of $9,979.00 to carry out and deliver five copies of an analysis of the relationship between Down's Syndrome and Alzheimer's Disease.

Health Research Inc., Albany, New York at a cost of $9,967.00 to prepare a report on diagnostic criteria and decision-making procedures appropriate to a community-wide epidemiologic study of Alzheimer's Senile Dementia.

University of Miami, Coral Gables, Florida, at a cost of $7,291.00 to produce a document entitled "Specifications for a 1980 U.S. Census Elderly Public Use Sample for Aging Research."

University of Delaware, Newark, Delaware, at a cost of $9,972.94 to deliver 12 copies of an analysis of migration patterns of demographic segments of the elderly population of the U.S.

The University of Texas Health Science Center at Houston, School of Public Health, at a cost of $9,800.00 to deliver 12 copies of an evaluation of demographic models of mortality and longevity of the Spanish surname and the General population of Texas.

Texas A&M Research Foundation, College Station, Texas at a cost of $4,996.00 to deliver 12 copies of an evaluation of the health research potential of the Aging Research Information System (ARIS).

University of Illinois, Champaign, Illinois at a cost of $3,826.38 to design and devleop a data set "Housing and Living Arrangements of the Elderly U.S. Population, 1946-1979."

Dr. Kenneth C. Land (non-competitive procurement--to the director of the best known and most respected social indicators research project in the U.S.) at a cost of $3,184.00 to prepare a report entitled "The Design of Time Series Indicators of Health Status, Health Care and Biomedical Research on Aging."

Duke University Center for Demographic Studies, Durham, North Carolina, at a cost of $9,500.00 to examine the survival patterns of blacks, whites, males, and females by age groups and to determine such factors as the age-specific and cause-specific mortality.

Columbia University in the city of New York, Department of Economics at a cost of $4,413.00 to provide an analysis of the 1972-73 Consumer Expenditure Survey of the U.S. Bureau of Labor Statistics.

V. Intramural Project Summaries for FY 1980

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG01050-01 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

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

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA

Other:

Yuko Y. Palesch, M.S.
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

President's Commission on Pension Policy

LAB/BRANCH
PDRA

SECTION
EDB

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 (200 words or less - underline keywords)

Age structure is being incorporated into a macroecomonic demographic growth model of the U.S. The impact of aging on demand and supply of goods and services and the interaction of these on labor force participation is being analyzed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: To determine the impact of aging on the U.S. economy and the impact of economic changes on the elderly segment of the population.

Methods Employed: The Hudson-Jorgenson macroeconomic growth model of the U.S. is being combined with a demographic projection model to examine age and sex group labor force participation, income, and total output. This model is a computerized system of mathematical equations used to project economic and demographic variables and currently set up on the NIH 370 computer. The projections will be made to the year 2050.

Current Status: Aggregate economic and demographic interactions are amenable to mathematical modeling. A model has been developed and is almost to a stage to provide quantitative results.

Significance to Biomedical Research and the Program of the Institute: Age structure to the year 2050 and estimated medical costs are planned as future significant outputs in the model. The projects will aid all NIA programs to judge the size and economic conditions of the elderly population. From this, estimates of the health problems of the elderly can be made.

Proposed Course: Continuing, if possible.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG01090-01 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Demographic Characteristics of Older Populations in the U.S.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Yuko Y. Palesch, M.S.
Research Statistician, EDBP, NIA

Other: Jacob Siegel
Demographer, Bureau of Census

COOPERATING UNITS (if any)
Bureau of the Census

LAB/BRANCH
PDRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

Using the data from the Census Bureau, the project identifies the socioeconomic and demographic characteristics of the elderly population in the U.S., and the figures are updated annually. It covers population by age and sex, mortality and survival information, living arrangements, and other socioeconomic characteristics regarding the older populations.

PHS-6040
(Rev. 10-76)

Objectives: To utilize census data to identify the socioeconomic and demographic differentials among the aging population of the U.S. over time. To examine mortality trends as they relate to population projections for the different age groups. To identify data resources available from the Census Bureau for aging research.

Methods Employed: Standard demographic methodology will be utilized to analyze census data from the 1970 Census and the recent current population surveys.

Major Findings:

Significance to Biomedical Research and to NIA: The data suggest the impact of selective biomedical research which is age-specific. The census analysis will indicate the magnitude of the lederly population and the growth over the next century in the elderly.

Proposed Course: Continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 01100-02 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Demographic Patterns of Mortality

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA

OTHERS:
Yuko Y. Palesch, M.S.
Research Statistican, EDBP, NIA

COOPERATING UNITS (if any)

Center for Demographic Studies
Duke University

LAB/BRANCH
PDRA

SECTION
Epidemiology, Demography, and Biometry

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

Patterns of mortality of males and females of each race and age are being analyzed by major causes of death using standard demographic methodology and vital statistics of the U.S. major conditions afflicting the elderly are being identified by analysis of associated conditions present at time of death.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: To examine the survival patterns of blacks, whites, males, and females by age groups and to determine such factors as the age-specific and cause-specific mortality.

Methods Employed: Examine the vital statistics using standard demographic methodology in analyzing cause-specific mortality by age and cohort.

Major Findings: The underlining causes of deaths understate the significance of selected diseases for the elderly because of the tendency for multiple diseases to be present in the aged. There are significant variations in impact of major causes when associated causes, e.g. diabetics, are taken into account, especially by age, race, and sex.

Significance to Biomedical Research and the Program of the Institute: Research will indicate the major conditions afflicting the elderly at time of death and suggest associated diseases which deserve further research. The results will suggest areas of aging research deserving greater emphasis and new initiatives.

Proposed Course: Completed in FY 1980

Publications: K.G. Manton, C.H. Patrick, and E. Stallard, The Impact of Elimination of Major Causes of Death on the Saved Population, International Journal of Epidemiology, June 1980.

Public Health Reports (accepted for publication)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG01120-01 EDBP

PERIOD COVERED

October 1, 1979 - September 30, 1980

TITLE OF PROJECT (80 characters or less)

Survey of Awards to Retirees, Dependents and Survivors (SARDS)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Yuko Y. Palesch, M.S.
Research Statistician, EDBP, NIA

Other: Alan Fox
Social Security Administration

COOPERATING UNITS (if any)

Social Security Administration

LAB/BRANCH

PDRA

SECTION

EDBP

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project will plan and implement the SARDS with SSA. The survey is done to determine the socioeconomic status of retirees who receive social security benefits,

PHS-6040
(Rev. 10-76)

Objectives: To determine the socioeconomic status of demographic groups and retirees receiving social security benefits. The health status of the retirees will be ascertained as well as any disabilities and diseases which might have been employment related.

Methods Employed: A fiedd study using survey instruments will be utilized to solicit responses from the retirees receiving social security benefits. Analysis will be standard demographic, epidemiologic and statistical methodology.

Major Findings:

Significance to Biomedical Research and to NIA: The survey will indicate the health problems of those people who survive into retirement and the characteristics of retirees by demographic and socioeconomic status. The study will indicate the demographic and socioeconomic status, the health problems and the overall living conditions of the elderly retirees.

Proposed Course:

NIA will be contributing funds for the Survey for FY81.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 01150-02 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mortality Follow-Back Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Yuko Y. Palesch
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PDRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

This project is designed to determine the demographic, social, and economic characteristics of surviving members of cohorts relative to the deceased members. This information will be used to model and project future aged populations based on cohort characteristics. The study suggests that additional factors must be analyzed in order to determine the quantitative impacts of the environment on the health of the elderly.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: To determine the characteristics of surviving cohorts over time and cross sectionally to survey survivors of deceased persons to determine the status of the deceased in their last days of life, and determine differences between survivors and nonsurvivors in demographic and socioeconomic factors.

Methods Employed: A survey of survivors of persons recently deceased will be carried out by NCHS Mortality Branch. The instrument will be designed by NCHS, NIA and other participating agencies using standard field methodologies. As a prelude, Census data and vital statistics on cohort characteristics will be examined in a simulation model being developed.

Major Findings: Differences in socioeconomic factors among cohorts exist and can be modelled. A theoretical model is being developed to use in simulations of population cohort behavior.

Significance to Biomedical Research and the Program of the Institute: Factors in cohort survival will be identified which should influence the direction of biomedical research towards specific diseases in this study. The information gathered on the last days of life of the deceased and their socioeconomic and demographic characteristics will aid in further NIA research.

Proposed Course: Continuing

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG01161-01 EDBP

PERIOD COVERED
October 1, 1979 - September 1980

TITLE OF PROJECT (80 characters or less)

Length of Stay and Outcomes of Nursing Home Patients: 1977 NNHS

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Yuko Y. Palesch, M.S.
Research Statistician, EDBP, NIA

Other: Korbin Liu, Ph.D.
Demographer, HCFA

COOPERATING UNITS (if any)

Health Care Financing Administration

LAB/BRANCH
PDRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

Using the 1977 National Nursing Home Survey, this project determines the relative effects of patient, payment and facility characteristics on the length of stay (LOS), and also estimates the probabilities of being discharged alive or dead at specific intervals after admission given the various biomedical and socioeconomic characteristics of the patients

PHS-6040
(Rev. 10-76)

Objectives: To determine the relative effects of patient, payment and facility characteristics on the length of stay (LOS). Also, to determine the probabilities of being discharged alive or dead at specific intervals after admission.

Methods Employed: The 1977 National Nursing Home Survey is examined with respect to our objectives. Regression analysis will be employed to determine what variables affect LOS. Life table analysis method will be utilized to estimate probabilities of discharge.

Major Findings:

Significance to Biomedical Research and to NIA: Given a means of estimating when a patient can be discharged, the administrators and managers of nursing homes will be able to plan its policies and operations for their facilities. This will have great impact on long term care, especially with expected increase in the elderly population.

Proposed course: Continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 01190-02 EDBP.

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Environmental Impact on the Elderly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA

OTHERS:
Yuko Y. Palesch, M.S.
Research Statistician, EDBP, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
PDRA

SECTION
Epidemiology, Demography, and Biometry

INSTITUTE AND LOCATION
NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

An analysis of the environmental impact of pollutants generated in electric energy production was undertaken using county level data on type of facility and mortality rates of the elderly. No significant impact was found in the preliminary analysis of data from coal, oil, gas, and nuclear counties.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: To determine the impact on the elderly of alternative energy generation capacity in the U.S.

Methods Employed: Standard biostatistical methods utilizing county level vital statistics on mortality of those over age 65 and county level energy generating capacity were utilized in a linear regression framework.

Major Findings: Alternative energy generating capacity by county has no apparent statistical relationship to mortality of the elderly. Numerous confounding factors were not included in this preliminary study which must be taken account of in future studies in order to statistically test the hypotheses of no effect.

Significance to Biomedical Research and the Program of the Institute: The hypotheses was to test the significance of energy related pollutants on health, many of which are being studied in the laboratory by other NIH Institutes. A positive finding would suggest certain specific pollutants may be injurous to health especially to the elderly and require additional research into mechanisms and prevention. The study indicates that additional factors must be considered and analyzed in order to appreciate the impact of the environment on the health of the elderly. Many of these factors such as migration, occupation, diet, and leisure activities are being addressed by grantees.

Proposed Course: Completed in FY 1980.

Publications: C. H. Patrick and Y.Y. Palesch, Determining Health Impacts of Power Plants: The Elderly as an Example Study Population, Review of Industrial Management, 18(2), 9-24.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02010-02 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Follow-up of Health and Nutrition Examination Survey I (HANES I)

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist
OTHERS:
Jacob A. Brody, M.D.
Associate Director

Dennis Cosmatos, M.D. (Hyg.)
Research Statistician

COOPERATING UNITS (if any)
National Center for Health Statistics, Division of Analysis

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, Behtesda, MD

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 (200 words or less - underline keywords)

The purpose of this project is to design and complete a follow-up of persons examined in the HANES I to study how factors previously measured on these subjects 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 services and behavorial and social status plus some physical measurements as blood pressure, height, and weight.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: The purpose of this project is to provide analysis of NCHS Data to answer specific hypotheses related to the elderly. The research problems being investigated are:

1. The relationship between osteoporosis and the existence of flouride in drinking water
2. Estimate of the incidence of stroke by studying changes in reported paralysis or limited activity due to stroke
3. Family Studies
4. Lean Body Mass
5. Changes in the incidence of accidents

Methods Employed: The analyses will include a clear statement of the problem; identification of the appropriate data sets; determination of the best statistical methods for analysis; prepare work tapes; program and process the data analysis, interpret the results and generate further hypotheses.

Major Findings: Analysis in Progress

Significance to Biomedical Research and the Program of the Institute: Obtaining estimates on incidence, prevalence, change in these statistics and associations for the U.S. population about problems such as osteoporosis, stroke, accidents plus national estimates concerning social functions are a contribution to biomedical research.

Continually questions are generated and information is needed pertaining to problems of the aged. This funded mechanism to obtain answers from within the National Data System is extremely useful.

Proposed Course: Reimbursable Agreement - continuing

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02031-01 EDBP

PERIOD COVERED
October 1, 1979, Sept. 30, 1980

TITLE OF PROJECT (80 characters or less)
The Impact of Living Arrangement and Family Structure on the Health of the Elderly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist , EDBP, NIA

COOPERATING UNITS (if any)

Division of Analysis, NCHS

LAB/BRANCH
PCRA

SECTION
EDBP

INSTITUTE AND LOCATION
NIA/Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study health, social, and economic problems of the elderly related to different aspects of residential living and family composition. In particular, families including elderly persons who are functionally disabled will be compared with other types of families with and without elderly persons.

PHS-6040
(Rev. 10-76)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02064-01 EDBP

PERIOD COVERED
October 1, 1979, to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Trend in Drug Use Among the Elderly Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

Others:

Fred Eckel, M.S.
North Carolina Pharmaceutical Research Foundation

Dennis Cosmatos, M.S.
Satistician (Health), EDBP, NIA

COOPERATING UNITS (if any)

North Carolina Pharmaceutical Research Foundation (NCPRF)
University of North Carolina at Chapel Hill

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry

INSTITUTE AND LOCATION
NIA, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05 (NIA)	.05 (NIA)	.05(NIA)

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In conjunction with the North Carolina Pharmaceutical Research Foundation, NIA is conducting a study of drug usage by the elderly.

The Medicaid records for years 1973 thru 1976 will be the primary data source for this study. Questions on self medication, medical care factors, drug cost, and drug problems related to the elderly will be addressed.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: This study aims at identifying several aspects of drug usage in the elderly. These will include quantity and quality of drugs used, self medication, relationships of demographic factors and medical factors to drug usage, identification of the prescribers and dispensers of the drugs, relationships between drug cost and drug usage, drug related problems seen in the elderly, and problems related to the use of specific drugs in the treatment of several chronic conditions.

Methods Employed: The Medicaid Data Records for years 1973 thru 1976 will be the primary data source which will be used in addressing the questions presented above. Staff from the NCPRF have developed the data set so it is in a form which allows immediate application to pharmeceutical research.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: As a group which is more prone to illness, the elderly are therefore more subject to drug treatments. A detailed profile of drug usage may suggest cases of drug interaction, overusage, and underusage. Estimates of drug efficacy for specific conditions may also be feasible if the usage patterns identified by this study are compared to morbidity and mortality data. This will be the first study of this type to focus on the health aspects of drug usage in this group as a primary concern with marketing and cost aspects secondary.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 02090-02 EDBP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

General Analysis of National Center for Health Statistics Data Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:

Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP,NIA

OTHERS:

Dennis Cosmatos, M.S. (Hyg.)
Research Statistician, NIA

Jennifer Madans, Ph.D.
NCHS

Joel Klienman, Ph.D.
NCHS

COOPERATING UNITS (if any)

National Center for Health Statistics, Division of Analysis

LAB/BRANCH

PCRA

SECTION

Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION

NIA, Bethesda, MD

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 (200 words or less - underline keywords)

This contract allows the National Institute on Aging to work cooperatively with the National Center for Health Statistics in conducting analyses of appropriate data sets as the need for such analyses arises. By working in this joint manner pertinent questions can be answered more efficiently and the level of research is likely to be of higher quality than if either institute worked alone.

At the present time the research topics include: 1) the relationships between the existance of flouride in drinking water and osteoporosis, 2) changes in incidence of stroke, 3) analysis of Family Data, 4) differences in lean body mass between the sexes and 5) mortality rates from accidents.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: During the years 1970 to 1975, the National Center for Health Statistics conducted the Health and Nutritional Examination Survey (HANES I). This was the first and probably the largest indepth national survey of health and nutrition ever conducted. A cohort of about 28,000 people were selected to be used for estimating the prevalence of various conditions and the distribution of physical, psychological, and biochemical characteristics for the entire U.S. population.

In order to maximize the utility of this baseline data, the individuals originally in the sample can be followed over time and the conditions which have developed since the initial survey can be identified. The ability to identify the antecedent and consequence relationships by this method is important in analyzing the factors related to problems of aging. Those factors that exist in one point in time are observed as predictors of health status at a later time.

Most of the problems associated with aging are a consequence of long-term and low dosage exposure to a combination of environmental, dietary, social, and demographic factors. By following this cohort over time, this study aims at identifying the levels and combinations of these factors that are associated with specific health conditions.

Methods Employed: The proposed full scale project is to complete a follow-up study on those individuals who were 25 years and older at the time of their participation in the initial HANES survey.

A feasibility study will be conducted in order to estimate the ability to find and interview the persons who had previously participated in the survey, and to estimate the cooperation that can be expected in the follow-up survey from the respondents and their medical care providers.

A pretest will then be administered to those who participated in the HANES I pretest, in order to perfect and finalize the questions to be used on the actual HANES I Follow-up Survey.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: The HANES I Follow-up Survey will provide national estimates of risk to certain health conditions for a representative sample of the United States population, and allow relationships to be drawn between these estimates and nutritional, social, demographic, and behavorial characteristics of the population at two points in time.

It is of primary importance for the National Institute on Aging to completely understand the epidemiology of the problems related to aging. The surveys conducted by the National Center for Health Statistics give baseline information on a representative sample of the U.S. population. This extensive amount of information can be used to identify the pertinent associations related to health outcomes of individuals in the older age groups. This analysis would act as a screening of data in the physical, psychological, social areas as well as the utilization of health care to identify and define appropriate hypotheses for further investigations. Through the information obtained by these follow-up studies, in-depth research can be designed to answer specific questions of interest to the National Institute on Aging.

Proposed Course: Reimbursable agreement continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG03061-01 EDBP

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cohort Mortality Patterns for Heart Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Clifford H. Patrick, Ph.D.
Research Demographer, EDBP, NIA

Other: Yuko Y. Palesch, M.S.
Research Statistician, EDBP, NIA

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

Manning Feinleib, M.D.
Div. of Heart and Vascular Diseases, NHLBI

COOPERATING UNITS (if any)

NHCBI

LAB/BRANCH

PDRA

SECTION

EDBP

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

This project examines the past mortality trends from heart disease by 5-year birth cohorts born between 1886-1910. Using loglinear regression analysis, future trends in heart disease mortality are projected for each cohort.

PHS-6040
(Rev. 10-76)

Objectives: Examine the past trend in death rate form Disease of Heart and create a mathematical model which would permit projection of future mortality patterns from heart diseases.

Methods Employed: We used the mortality statistics from the Vital Health Statistic Volumnes of NCHS and set up cohort mortality patterns. We applied a loglanier regression analysis to the data.

Major Findings: For white females, the heart disease mortality rate declined at every age with each succeeding cohort and this trend is expected to continue. However, for white males, a break in pattern occured during 1960-1965 where the HD mortality rates began to decline substantially with each succeeding cohort. We see some kind of period effect in this decline.

Significance to Biomedical Research and the Program of the Institute: Heart Disease is the #1 cause of death for overall as well as aging population. Evaluation of past trends would give some insight to future direction of HD mortality.

Proposed Course: Completed in FY80

Publications:

C.H. Patrick, Y.Y. Palesch, and J.A. Brody: Cohort Death Rates from Heart Disease: Past and Future Trends (Abstract), CVD Epidemiology Newsletter, No. 28, Feb. 1980, 31.

C.H. Patrick, Y.Y. Palesch, M. Feinleib, and J.A. Brody: Cohort Death Rates from Heart Disease: Past and Future Trends, Submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG03070-01 EDBP

PERIOD COVERED

October 1, 1979 to September 1980

TITLE OF PROJECT (80 characters or less)

Projection of Cohort Mortality Patterns for Major Causes of Death

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Yuko Y. Palesch, M.S
Research Statistician, EDBP, NIA

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

COOPERATING UNITS (if any)

LAB/BRANCH

PDRA

SECTION

EDBP

INSTITUTE AND LOCATION

NIA, NIH, Bethesda, MD 20205

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 (200 words or less - underline keywords)

Using the cohort analysis method, the project examines the past mortality trends for major causes as well as all causes to create a mathematical model to predict future trends in mortality from these causes. It is anticipated that the overall mortality will increase due to the aging population surviving even longer with current decline in CHD mortality.

PHS-6040
(Rev. 10-76)

Objectives: Evaluate the trends in mortality rates of the elderly population by specific diseases (5 leading causes) since 1900. Project future cause-specific mortality patterns among the elderly, particularly among the 85+ group.

Methods Employed: Make computerized file of mortality rates by cause, age, sex, and race. Utilize curve fitting methods to project future patterns of mortality.

Major Findings:

Significance to Biomedical Research and to NIA: We may see some changing directions in the current cohort mortality trends in leading causes due to the aging population. This discovery will have various demographic, socioeconomic impact on the health care system of the U.S.

Proposed Course: Continuing.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 04010-02 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Catchment Area Study of Senile Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

OTHERS:
William Eaton, Ph.D.
NIMH

Ben Locke
NIMH

COOPERATING UNITS (if any)

National Institute of Mental Health
Center for Epidemiologic Studies

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05 (NIA)	.05 (NIA)	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In conjunction with the National Institute of Mental Health, 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 follow-up of all subjects. A questionnaire has been developed by the investigators.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: The Division of Biometry and Epidemiology, National Institute of Mental Health (NIMH) is in the process of investigating the occurrence of mental illness in an adult community population. An award has been made to Yale University to survey a sample of the New Haven, Connecticut adult population for this purpose. The National Institute on Aging (NIA) is specifically interested in defining the occurrence of senile dementia in persons over 65 years of age. Since this requires a larger sample population, the study population that has been developed by Yale University for NIMH will be expanded. In the development of the descriptive epidemiology of the prevalence of senile dementia in a community, the NIA shall work with the staff of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS).

Methods Employed: In addition to the household sample of 3000 in the original ECA Research design for the New Haven site, persons 65 years of age and older shall be selected from households for interviews such that 2,000 additional persons 65 and over are included in the sample. The household sample as referred to throughout the design specifications will thus consist of 3,000 completed interviews representing the adult (over 18) population in the catchment area, and an additional 2,000 completed interviews representing the aged (65 and over).

A questionnaire developed by the investigators will be administered to all persons in the sample and a follow-up of each individual will be included in the study.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: As more people age more people will develop senile dementia. Prevalence among those over 65 years of age with severe progressive disease has been found in different studies to range from 1 to 9 percent and up to 15 or 20 percent for milder forms. No epidemiologic pattern for senile dementia has been discerned by race, sex, geographic, occupation, environmental or infectious exposures. This project will attempt to provide epidemiologic patterns for epidemiologic clues.

Proposed Course:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 04011-02 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Senile Dementia: Natural History in a Noninstitutionalized Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

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

Elizabeth Parker, Ph.D.
NIAAA

COOPERATING UNITS (if any)

National Institute on Alcohol Abuse and Alcoholism

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry Program

INSTITUTE AND LOCATION
NIA, Bethesda, MD

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 (200 words or less - underline keywords)

Epidemiologic studies of senile dementia are needed in order to determine if the disease occurs in specific patterns by age, sex, race, or other demographic and social characteristics and if such patterns would provide etiologic clues. This project will characterize persons within a community based, non-institutionalized sample who appear to have senile dementia and/or depression or to be at high risk of having these disorders. This sample will be compared with an unaffected group. Both samples will be followed over a three year period to discover risk factors and etiologic events.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: This project will seek to determine if there are specific patterns or entities of senile dementia. An epidemiologic study of these conditions will be completed to define etiology and preventive measures.

Within a community-based non-institutionalized sample of persons felt to have depression and/or senile dementia or to be at high risk for having these disorders will be characterized and followed over three years. This sample will be compared with an unaffected group in order to discover risk factors and etiologic events.

Methods Employed: From a free living population of a defined community, a sample of persons will be identified as positive for senile dementia and depression by a screening instrument. These persons shall be evaluated by a complete clinical, indepth assessment. In addition, a representative sample of persons who appear to be normal or negative in regard to depression and/or senile dementia according to their response on the screening questionnaire. Both cases and controls will be followed for 3 years with contacts every 6 months to determine outcome of senile dementia and depression.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: Little is known about the occurrence of senile dementia and depression in persons over 65 years of age. Etiologic mechanisms and risk factors are unknown; the natural history of these illnesses is obscure. Senile dementia and depression are apparently the most common disorders which primarily affect mental abilities in the elderly. Moreover, especially early in the illness, the two disorders may be indistinguishable. This project will attempt to provide a better definition of these illnesses and identify associated factors.

Proposed Course: Project being planned and designed.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-04013-01-EDBP

PERIOD COVERED
October 1, 1979, September 30, 1980

TITLE OF PROJECT (80 characters or less)

Relationship between Down's Syndrome and Alzheimer's Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Joan Cornoni-Huntley
Senior Research Epidemiologist, EDB, NIA

Others:

RYK H. Ward, M.D.
University at Washington
Deptartment of Epidemiology

Dennis Cosmatos
Statistician (Health), EDB, NIA

COOPERATING UNITS (if any)
University of Washington, Seattle
School of Public Health and Community Welfare
Department of Epidemiology

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry

INSTITUTE AND LOCATION
NIA, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.05(NIA)	.05(NIA)	.05(NIA)

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is to investigate the prevalence of Alzheimer's Disease in relatives of individuals with Down's Syndrome. Patterns of familial aggregation of these diseases will be compared to their genetic models. The study will focus on 200 probands is higher in prevalence than expected.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: There has been extensive speculation about the possible relationship between Alzheimer's Disease and Down's Syndrome. Several previous studies have demonstrated an excessive prevalence of Alzheimer's Disease, Down's Syndrome, leukemia and related disorders in the families of probands diagnosed to have Alzheimer's Disease. It is speculated that a common genetic diathesis underlay both disorders. Among the many important questions raised by these studies are the following: Is there an increased prevalence of Alzheimer's Disease in relatives of individuals with Down's Syndrome; and, is the pattern of familial aggregation of these diseases consistent with a genetic model? An affirmative answer could be required to provide positive evidence for the hypothesis of a common genetic diathesis.

Methods Employed: In order to answer these questions in an epidemiologically sound manner, a study has been designed which has probands cross matched for Alzheimer's Disease and Down's Syndrome as well as having an adequate group of control probands. This required study of the family members of the following four groups of probands, using a standardized procedure:

1) probands who have a child with Down's Syndrome
2) probands with Alzheimer's Disease
3) probands with a non-dementing neurological disorder (e.g. Parkinson's)
4) probands representing a random sample of the population

All probands being age and sex matched and information gathered about the prevalence of possible Alzheimer's Disease, and Down's Syndrome and related disorders in first degree relatives.

This study will use data collected on 200 probands all of whom have at least one child with Down's Syndrome. Data on these increased prevalence of Alzheimer's Disease is suggested.

Major Findings: Work in progress

Significance to Biomedical Research and the Program of the Institute: The relationship between Alzheimer's Disease and Down's Syndrome has been examined by numerous investigators. This, however, is the first study to undertake an active genetic-epidemiological approach to the relationship of these diseases. The identification of both diseases as being governed by the same genetic mechanism will lead the way to a better understanding of the conditions and eventually a better understanding of the underlying etiology of the diseases.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 04014-01 EDBP

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Epidemiologic Studies of Alzheimer's Senile Dementia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
Joan Cornoni-Huntley, Ph.D.
Senior Research Epidemiologist, EDBP, NIA

OTHERS:
Peter Greenwald, M.D.
Health Research, Inc.

Dennis Cosmatos, M.S.
Statistician, EDBP, NIA

COOPERATING UNITS (if any)
Health Research, Inc.
New York State Department of Health
Division of Epidemiology

LAB/BRANCH

SECTION
Epidemiology, Demography, and Biometry

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 (200 words or less - underline keywords)

This project will aim at the development of instruments, diagnostic criteria, and procedures necessary to conduct a planned, geographically based epidemiologic study of Alzheimer's Senile Dementia. With the help of staff from Health Research, Inc., methods to identify patients with senile dementia of the Alzheimer type (SDAT) will be developed. Examination of the criteria most critical in the diagnosis of SDAT patients will be made. The reliability and validity of the procedures developed will be determined.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: The National Institute on Aging, Epidemiology, Demography, and Biometry Program will work with members of Health Research, Inc., the New York State Department of Health in the development of methods to establish a diagnosis of Senile Dementia of the Alzheimer-type (SDAT) in a general research setting. These methods will attempt to resolve the differences between the "ideal" approach that is feasible only in a university hospital and realities of a community-wide study. These methods can be used in a variety of settings, including nursing homes, adult care homes, and acute care facilities as well as in the patient's home and in the community. There will be an examination to determine which criteria should be assessed in making the diagnosis of Alzheimer's Senile Dementia (e.g., memory, abstract thinking, personality) and develop a procedure to translate diagnostic criteria into a final statement of diagnosis. Methods for checking the reliability of the diagnosis will be developed. The methods will include a plan for efficient use of clinicians in making and validating the diagnosis in a large group of patients.

Methods Employed: An expanded and current literature review of Alzheimer's Senile Dementia; mental status tests and related topics will be reviewed. Experienced investigators will be contracted and their input and opinions solicited in the areas of diagnostic methods and mental status tests. Methods being used in current epidemiologic research studies of Alzheimer's Senile Dementia will be reviewed and a set of diagnostic criteria and a protocol will be developed for diagnosis of Alzheimer's Senile Dementia in community-based epidemiologic study. The protocol will be pilot tested for feasibility and reliability.

Major Findings: Work in progress.

Significance to Biomedical Research and the Program of the Institute: In any epidemiologic study, it is understood that the identification of the cases are of paramount importance. Any associations detected between the condition of interest and a secondary factor, is contingent on the accurate identification of the subjects with the test condition. This project will review all procedures presently employed for the identification of SDAT cases and compile a single comprehensive diagnostic procedure high in reliability and validity, to be standardly applied by researchers in this area.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-0-6000-01-EDBP

PERIOD COVERED
October 1, 1979, September 30, 1980.

TITLE OF PROJECT (80 characters or less)
Climatic Effects on Morbidity/Mortality in the Elderly

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dennis Cosmatos, M.S.
Statistician (Health), EDB, NIA

COOPERATING UNITS (if any)
National Climatic Center, NOAA

LAB/BRANCH
PCRA

SECTION
Epidemiology, Demography, and Biometry

INSTITUTE AND LOCATION
NIA, Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20 (NIA)	.20 (NIA)	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 beween 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 is more likely will be investigated in greater detail. Concomitant information on socioeconomic status and geographic location of the cases will also be considered.

PHS-6040
(Rev. 10-76)

PROJECT DESCRIPTION:

Objectives: The basic aim of this project is to investigate responses to temperature extremes by people in different age groups, incorporating concomitant information on socioeconomic status, and geographic location. A basis hypothesis of older people living in relatively warmer climates with lower socioeconomic status being more susceptible to climatic extremes, will be adressed. Identification of the climatic conditions which effect the various age groups most will also be attempted. A variety of climatic variables and their interactions will be used in the study.

Methods: Daily climatic data will be purchased from the National Climatic Center and linked with detail mortality data collected by the National Center for Health Statistics. Since day of death has only been coded on the mortality tapes for years 1962 through 1966 and 1972 through the present, this study at daily mortality is limited to these dates. Initially, only the most recent group will be examined. As the study proceeds, both groups will be examined and monthly analyses on the intermedia the years will be performed.

Major Findings: Work in progress

Significance to Biomedical Research and the Program of the Institute: Most studies of this type fail to examine responses to climatic variables across separate age groups. This study plans to incorporate age as a significant variable and concentrate on these at that variables extreme values (i.e. the very old) in examining responses to climatic extremes. Factors such as financial conditions and geographic location are also, logically, variables which must be considered. Many previous studies fail to do so. It is expected that several new areas of interest will develop as the project progresses.

Proposed Course: Continuing

GERONTOLOGY RESEARCH CENTER

Report of the Intramural Research Program

Despite the loss of several key scientific and administrative staff and hiring restrictions imposed during the last half of the year, the Gerontology Research Center had a most successful year as the focal point for intramural research within the NIA.

Significant expansion of several research programs took place this year and efforts to improve the Center's physical plant and services commenced.

Rapidly gaining a reputation as a regional center for behavioral medicine, the Laboratory of Behavioral Sciences established a continence clinic for people over age 65. This clinic, operated in cooperation with Baltimore City Hospitals, will be used to investigate whether patients with urinary and fecal incontinence can use operant conditioning techniques to become continent.

During this year also, the Laboratory of Neurosciences entered the clinical research realm with the initiation of a project to systematically assess age changes in cerebral metabolism in neurologically normal human subjects using F^{18}-deoxyglucose positron emission tomography. In the near future, as the staffing picture brightens similar studies will be extended to neurologically impaired patients.

Scientists in the Clinical Physiology Branch made an interesting discovery about the efficacy of digoxin. This widely prescribed drug is useful during the acute stages of congestive heart failure where it helps improve the heart's pumping capacity. However, GRC investigators conducted double blind cross-over studies which showed that digoxin use after a patient's condition has stabilized is ineffective in further improving the individual's condition and, indeed, its continued use may lead to toxicity in many patients.

The women's program of the Baltimore Longitudinal Study of Aging continued to expand, now numbering over 250 women as compared to 650 active men. The continued growth in size of the women's cohort has been limited by the number of personnel available to provide the requisite testing procedures for both the male and female volunteer groups.

The research productivity of Center investigators is evident it seems, from the fact that 73 scientific papers were published in the first six months of calendar year 1980. In addition, GRC staff were invited to present papers or to chair sessions at international congresses or symposia in 10 foreign countries and in 22 of the 50 states.

In Baltimore, the latest research advances of a multidisciplinary nature were shared with the GRC community of scholars during 36 weekly research seminars held during the academic year. These stimulating seminars featured distinguished investigators from across the United States as well as from four foreign countries. An innovation in the all important cross-fertilization of ideas within the Center was the initiation this year of twice monthly GRC Director's Seminars. Seventeen of these useful internal seminars were held which provided the opportunity to highlight the work of various laboratories, particularly that done by young Staff Fellows, Clinical Associates and Visiting Fellows receiving aging research training at the Baltimore facility.

Major attention this year was given to improving and repairing the 12-year-old GRC building. The complete replacement of the Center's original roof began and plans are in the works to construct new kennel facilities to expand the canine colony. A building maintenance contract was negotiated which will provide improved maintenance services. Other contracts in negotiation or out on bids will cover security, roads and grounds upkeep, and heating, air conditioning and ventilation systems. The latter contract will include provisions to replace older equipment during the coming year. Needed interior renovation of the building will be undertaken soon with the help of the NIH Division of Engineering Services.

In order to support the Laboratory of Neurosciences' implementation of crucial clinical research on brain diseases and related conditions which impact heavily on the older population, considerable procurement of equipment and instrumentation took place. Major items either in place or ordered so far include: an electroencephalograph with sophisticated multi-channel recording accessories; a gas chromatograph for pharmacokinetic research; microforges to make micro tools used for single cell studies; and a fluorescence microscope.

The Technical Development Section began replacing the original GRC computer system while, at the same time, maintaining crucial data processing services.

Center support for investigators involved in morphological studies was bolstered this year with the acquisition of the latest in transmission electron microscopes and freeze fracture apparatus for use in the Experimental Morphology Section.

The Center's successful year is a tribute to its staff, many of whom wore several hats as the result of staff vacancies and hiring restrictions. Even with their extra duties and unexpected emergencies they performed outstanding services in the research laboratories, administrative areas, and in research support facilities.

Among those honored for their dedication, either by receiving awards or special honors were: Mr. Wayne French, supervisor, Animal Resources Facility, winner of the NIH Merit Award for his exemplary performance in managing the GRC animal colonies; Dr. Reubin Andres, Clinical Director and Chief of both the Clinical Physiology Branch and Metabolism Section, voted President-elect of the Gerontological Society; Dr. Bernard T. Engel, Chief, Laboratory of Behavioral Sciences, elected to a similar post with the Biofeedback Society and winner of the Pavlovian Society's Award for his meritorious contributions to behavioral research; and, Dr. Nathan W. Shock, NIH Scientist Emeritus and former GRC Director, winner of the first Gerontological Society Brookdale Award for Biological and Clinical Research in Aging, as well as a special NIA award for his efforts leading to establishment of the Institute.

Office of the Director

Technical Development Section

The Technical Development Section continued to provide maintenance and development services to support scientific programs of the Center. Several new instruments were developed during the year.

Every GRC program received support from the Section's Mechanical Shop. Examples of this support include: (1) A combination microscope and micro manipulator platform for the Comparative Nutrition Section; (2) Rat mazes, learning chambers and a grip strength instrument for the Learning and Problem Solving Section; (3) An exercise bicycle for the Psychophysiology Section; and, a Hemoscan electrode support for the Inorganic Biochemistry Section. In addition, several building maintenance functions were transferred to the Shop this past year.

The Section is in the process of replacing the original GRC computer system with one that will provide a multi-user disc-based operation and the capability to run large statistical packages. The new system has been defined, justified and ordered. Many of the interfaces used with the old system were duplicated this year for use with our second system, thus allowing for continuous data processing services to be available for ongoing research programs during the changeover period.

New microprocessor computer systems were implemented to meet instrumentation needs of Center staff. This relatively new technique allows moderately complicated instrumentation to be designed and fabricated quickly. The following systems were completed and installed in FY 1980: (1) A system that will deliver a multiexponential infusion rate--Laboratory of Neurosciences.

(2) A camera controller--Clinical Immunology Section; (3) A concept problem solving computer--Learning and Problem Solving Section; and, (4) An oxygen-dissociation data collector and analyzer--Inorganic Biochemistry Section.

Several other systems are in various stages of development, including: (1) Digital recording and control of exercise ECG testing--Human Performance Section; (2) A multi-processor system to maintain several monkeys on operant conditioning schedules simultaneously--Psychophysiology Section; (3) Multi-channel event times--Intermediary Metabolism Section; and, (4) A temperature rate controller--Inorganic Biochemistry Section.

Animal Resources Facility

This past year, the Animal Resources Facility underwent its first on site reinspection by the American Association for Accreditation of Laboratory Animal Care. By demonstrating our compliance with the Association's standards, the Animal Resources Facility maintained its' full accreditation status. The Facility also became an Institutional Member with the National Capitol Area Branch of the American Association for Laboratory Animal Science.

Several new methods were designed and implemented to increase the capacity and efficiency of the Facility. The Wistar Rat Colony was redesigned and new equipment ordered to enable the colony to be maintained under barrier conditions, rather than a conventional system. A new washroom is being constructed to convert the Isolation Area into a Quarantine Area. Additional dog kennels are being planned for on the roof of the GRC, and will house 500 beagles. The addition of new cages in the Primate Holding Area, has tripled holding space for Rhesus monkeys.

This past year also marked the first potentially devastating epidemic ever to invade the colonies. An intracellular parasite, murine Mycoplasma pulmonis, was discovered during routine virus screening. This lethal organism was held in check and finally eradicated by the strict isolation and husbandry measures enforced upon detection of the organism. Both the Aging Rat and Mouse Colonies were unaffected, as were most long term experimental animals.

Surgical and anaesthesia skills and techniques were supplied by this section to aid in over 50 different surgical procedures. A prefabricated cranial platform, developed by this section, is being used during electrode implantations in monkeys. The use of this platform has cut surgical procedure times in half.

Monthly inventories show that in the Open Animal Colony, care and housing were provided for 18,500 mice, 1,300 rats, 50 rabbits, 10 monkeys, and 5 cats. Throughout the year, the Animal Resources Facility received, maintained or issued 18,000 mice, 3,635 rats, 810 rabbits, 20 dogs, 23 primates, and 22 cats.

There are over 15,000 C57BL/6J Mice in the Aging Mouse Colony. Approximately 1,000 weanlings are added to the colony monthly. A total of 1,357 mice were issued from this colony; 232 were 1-11-months-old, 510 were 12-23-months-old and 615 were 24-months-old or older.

The Aging Wistar Rat Colony is being maintained at 12,000 animals. There were 9,370 weanling rats introduced into the colony. A total of 3,502 rats were issued; 2,059 were 1-11-months-old, 784 were 12-23-months-old, 659 were 24-months-old or older.

Information Activities

The GRC Information Office actively served its many publics while widening the scope of its activities to meet the increasing information needs of the internal community of scientists and the general public. The Office's outreach educational program expanded with presentations at national meetings, regional workshops, local colleges, senior centers, health fairs, and Federal job opportunity events.

Last winter, the Office took a proactive stance, in cooperation with local health authorities, to correct misleading energy guidelines advertised by the Baltimore Gas and Electric Company that could have put many customers at risk of experiencing hypothermia. The successful campaign involved letters to the editors of area newspapers, to the company, and an interview on TV.

This year, NIA established a Geriatric Continence Clinic in cooperation with Baltimore City Hospitals. Seeking patient referrals, the Laboratory of Behavioral Sciences sought guidance from the Information Office. Information was passed on to local agencies, papers, and newsletters which resulted in several aritcles and a number of calls and referrals to the clinic.

The addition of a new editorial assistant, also an excellent writer, enabled the Office to increase its information output and has already paid off with several interesting articles prepared for JAMA's "From the NIH" and the NIH RECORD.

Special programs were arranged for visiting groups such as those from the Gettysburg College Senior Scholars Seminar, National Geriatrics Society, and the Maryland Academy of Sciences Junior Science Seminar.

During the year, the GRC conducted over 50 briefings and tours for more than 400 individuals or groups. Visitors came from China, Costa Rica, England, Egypt, France, Japan, Romania, Scotland, and Switzerland. Nationally, visitors came from as far away as Oregon and California including a delegation from the Eisenhower Medical Center seeking guidance in the development of a gerontology center in Palm Springs. Other visitors included Sen. Mathias, staff from Rep. Mikulski's Office, Catholic University professors of nursing, staff of the USDA-Tufts Nutrition Center, and representatives of the Long-Term Care Division, Health Care Financing Administration.

Cooperation with the media proved productive as CBS-TV's "February Magazine" featured an excellent segment on the Baltimore Longitudinal Study of Aging; NBC-TV's "Nightly News" used Center footage during a five-part series on aging aired last fall, as did PBS-TV's "Here's to your Health (August 1980) and BBC-TV's Horizon series (December 1979).

Center staff were featured on WBAL-Radio and TV, WMAR-TV, and WITH Radio, all in Baltimore; WTTG-TV and WGTS Radio (D.C./ Atlanta); WIND Radio (Chicago); and WXYZ Radio (Detroit). Assistance was also provided to "Good Morning America, ABC-TV; "The Human Body," CBS-TV; "Today Show," NBC-TV; KQED-TV (San Francisco); and RAI-TV (Italy).

Articles on NIA/GRC programs ran in a three part Family Health aging series; The Providence Sunday Journal (3); New Republic; Smithsonian Magazine; Baltimore Sunday Sun; Washington Post; Baltimore Evening Sun; Family Circle; Baltimore News-American; Lady's Circle; Baltimore Magazine; NCSC's Senior Citizen News; and Baltimore County Senior Digest (3).

Information staff responsible for referencing aging literature indexed over 10,000 titles during the year for "Current Publications in Gerontology and Geriatrics" published bi-monthly in the Journal of Gerontology, and for use in updating the classified bibliography books. They also responded in superior fashion to numerous reference requests from professionals and the public, including Congress. The excellent performance of this small unit earned them a group award this year.

In other activities, the Communications Officer keynoted a seminar on aging at the National Easter Seal Society's 1979 annual meeting, made presentations before two regional workshops on aging, as well as to students from six area colleges, two senior centers, and one nursing home. Audiences for these presentations totaled over 450 persons. Information personnel ran the annual CFC campaign at GRC; coordinated and helped prepare the annual and other recurring reports; helped conduct several orientation programs for new NIA staff; served on the NIH Handicapped Committee and GRC Library Committee; and, worked closely with the NIH EEO Advisory Committee.

For example staff participated in a Federal job opportunities fair held during Baltimore's Hispanic Festival (August 1980).

Library Services

The GRC Library continued to serve scientists and investigators as a research library and to search for ways to improve services. Even with the departure of the Librarian early in the year, the one full-time employee, with the help of students and part-time workers, succeeded in completing the cataloguing of the core collection of aging literature. The classification and cataloguing of all other holdings in the Library is progressing.

During the year, more than 1,500 new acquisitions were processed and catalogued. Reciprocal interlibrary loan programs were developed and a total of 3,000 such loans were requested by staff with the Library successfully filling 95 percent of these requests.

Reference services were provided to patrons from the NIA headquarters, GRC, and from the private sector. Some 300 online and offline searches were made this year.

Photocopying services for investigators were provided through contracts with private sector and Federal libraries. Arrangements were approved by the NIH to have journals for binding picked up directly from the GRC Library. This new system has already saved considerable time and man hours for the small library staff.

Besides serving as a research library, the Library has been visited by numerous groups, students, and others particularly interested in the aging collection, and is open during normal hours for the public to use its collection on the premises.

In Fiscal Year 1981, the Library projects implementing the Ohio College Library Center, Inc. services.

Comparative Nutrition Section

One study in this section attempts to establish whether aging is accompanied by the formation of selective errors in the genetic code. In one experiment carried out this year, the specific activities of aldolase of cytosols isolated from livers of 30-31-month-old mice were significantly lower (40%) than those isolated from 2.5- or 7.5-month-old female animals. This age decrement results from structural changes in the aldolase molecule as indicated by a 30% decrement in specific activity of the purified enzyme. Again, no significant difference was seen in the specific activity of the purified preparations from younger age groups.

There were no differences in the degree of purification since in 2.5-, 7.5- and 31-month animals the enzyme was purified 34, 34, and 39 times, respectively. Induction of aldolase in young and old animals by feeding a high fructose-no fat diet for two days resulted in a two-fold increase in specific activity of the cytosol. The specific activity of the enzyme purified from these cytoscls did not differ as a function of age and was equal to that observed in normal young animals. The data suggest that the age-associated decrease in the specific activity of purified aldolase from old animals must result from post-translational modifications.

In the same study, ingestion of certain apparently nutritionally acceptable salt mixtures and low protein levels shortened life span. All animals fed a 4% protein diet containing modified Rogers-Harpers mineral mix died before 9.5 months of age, whereas, only 15% of animals fed the 24% protein diet with this mix died by this age. The 50% mortality of the latter animals did not occur until at least 25 months of age comparing favorably with the 24 months reported by Jackson Laboratory. Intermittently fed animals ate approximately 30% less than controls with 40% of their intake being ingested within the first three hours of feeding, and 60% during the remainder of the 24 hour period.

In a second study, weanling female Fisher 344 rats were fed diets containing 4, 8, 12, or 24% protein for 14 months prior to determination of serum levels of triiodothyronine (T_3), thyroxine (T_4), prolactin, corticosterone, aldosterone, creatinine, and cholesterol. Levels of all the variables examined were similar in rats fed the 8, 12 and 24% protein diets. Rats fed the 4% protein diets had markedly higher T_3 and T_4 levels, lower corticosterone and aldosterone levels, and similar creatinine and cholesterol levels as compared to the other three groups.

Another investigation examined the effect of age on absorption of fat-soluble and water soluble vitamins. Female Wistar rats, aged 6, 12 and 24 months received orally administered radioactive forms of vitamins A and D, then were sacrificed 18 hours later. A second group of the same ages were also fed radioactive B_{12} and niacin and sacrificed 16 hours later. In both experiments, the esophagus, stomach, small intestinal wall, contents of the small intestine, cecum, colon, and collected feces were assayed for isotope. Liver and plasma levels of the administered vitamins were determined at sacrifice. Results showed that absorption of each of the vitamins was unaffected by age. Plasma and liver levels of the isotope at sacrifice also were not related to age of the animals.

An investigation of the age-dependent stability of gene control mechanisms continues in which the approach is to examine the stability of gene repression by determining whether specific genes, thought to be normally repressed, remain so with increasing age. The study to date has examined α and β-globin, casein, α-fetoprotein genes and the set of genes making up the c-type endogenous mouse leukemia virus (MuLV) as well as the mouse mammary tumor virus (MMTV) in the livers and brains of C57BL/6J and AKR mouse strains.

Evidence for age-dependent derepression of these genes was found with the exception of casein which remained repressed. More detailed studies of the two endogenous c-type viruses showed both undergo a qualitative type of gene derepression (more different types of genes being derepressed with increasing age). Most of the viral RNA is not transferred to the cytoplasm but, instead, appears to remain in the nucleus where it cannot be translated.

The search continues for age-dependent alterations in the physicochemical structure of chromatin via a search for a specific non-histone chromatin protein, the T_3-hormone receptor. This receptor protein is essential for proper control of several specific liver enzymes and, potentially, can be related to general physiological processes which change with age.

Study results, using liver tissue from Sprague Dawley rats of ages 8 to 32 months, showed a slight but statistically significant decrease in the number of T_3 binding sites but no change in binding affinity. Old hypophysectomized animals have been shown by other laboratories to undergo some rejuvenation-like changes, and in this study, using EM analysis, some evidence for regeneration of the kidney glomerular region was found. However, the old hypophysectomized animals showed no return of T_3 receptor levels in the liver comparable to young animals. The effects of T_3 administration to C57BL/6J mice on the number of nucleoli and ribosomal RNA gene dosage was also investigated and, although nucleoli number increased up to 50%, no effect on ribosomal gene dosage was evident.

In studies of superoxide dismutase (SOD), using phenylmethylsulfonyl fluoride and leupeptin (a specific proteolytic inhibitor of the lysosomal enzyme, cathepsin B), it was found that most of the age-related decline in SOD activity in mice, reported elsewhere, can be eliminated. However, the correlation found between SOD specific activity per specific metabolic rate and maximum life span potential in primate species was not affected by the use of the enzymatic inhibitors. Genetic linkage and functional correlation between the various histocompatibility loci in man and mouse are suggested by mutations in these loci affecting longevity, susceptibility to disease, immunological competence, and levels of SOD, catalase, aryl hydrocarbon hydroxylase, interferon, cAMP, and DNA repair.

Experimental Morphology Section

This section assesses qualitative and quantitative modifications which occur as the result of normal or experimentally induced aging. Such modifications are related to the functional attributes of the system under scrutiny. The modern techniques employed by scientists using the section's resources include scanning and transmission electron microscopy, freeze fracture, and high resolution autoradiography.

Research initiated this year includes: a collaborative study with NINCDS investigators on aggregating factor; freeze fracture studies of programmed cell death using nematodes; a project investigating ectopic osteogenesis ultrastructure; and a study of brush border ultrastructure alterations with ischemia.

The Section also provided consultative and technical services for research initiated in other Center laboratories, offered instruction in the use of microscopy or other equipment to scientists, as well as helped in the interpretation of experimental results for investigators.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00101-04-SCN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Relation between Nutritional State and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. H. Barrows	Chief	SCN, OSD, NIA
	G. C. Kokkonen	Chemist	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	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The specific activities of aldolase of cytosols isolated from the livers of 30-31 month old female mice were significantly lower (40%) than those isolated from 2.5 or 7.5 month old female animals. This age decrement results from structural changes in the aldolase molecule as indicated by a 30% decrement in the specific activity of the purified enzyme. Induction of aldolase in young and old animals by feeding a high fructose-no fat diet for two days resulted in a two-fold increase in the specific activity of the cytosol. The specific activity of the enzyme purified from these cytosols did not differ as a function of age and was equal to that observed in normal young animals. These data suggested that the age-associated decrease in the specific activity of purified aldolase from old animals must result from post-translational modifications. The ingestion of certain apparently nutritionally acceptable salt mixtures and low protein levels results in a shortening of life span. Intermittently fed animals ate approximately 30% less than control animals with 40% of their intake being ingested within the first three hours of feeding and 60% during the remainder of the 24 hour period.

PHS-6040
(Rev. 10-76)

Project Description: Z01-AG-00101-04-SCN

Objectives: Attempts were made to establish whether aging is accompanied by the formation of selective errors in the genetic code which may occur with age and or use. In addition efforts were made to evaluate different salt mixtures on mortality.

Methods Employed: The activities of aldolase and the protein contents of the cytosols isolated from the livers of C57BL/6J female mice were determined at various ages in animals fed either Purina Laboratory Chow, or a high fructose-no fat diet. Aldolase from the livers of the animals was purified by ion-exchange chromatography on a phosphocellulose column using a linear salt gradient followed by substrate elution.

Body weights and food intakes were established for male and female $B_6D_2F_1/J$ mice fed diets containing either a 24% protein (ad libitum or intermittently) or 4% protein (ad libitum). Two different salt mixtures were used in some studies. One was USP XIV which had been used in the past in this laboratory. The second was Rogers-Harper which contained no aluminum or fluorine but did contain molybdenum, selenium, and zinc. In addition, the amounts of chromium, manganese, and zinc were supplemented so as to agree with the AIN recommended diet. Synthetic diets consisting of this modified Rogers-Harper mineral mix and either 24, or 4% protein were than fed ad libitum to $B_6D_2F_1/J$ male and female mice.

Major Findings: The specific activities (enzyme activity/mg. protein) of aldolase of cytosols isolated from the livers of 30-31 month old female mice were significantly lower (40%) than those isolated from 2.5 or 7.5 month old female animals. No significant difference was observed between the latter two age groups. This age decrement was due to a decrease in enzymatic activity since the protein content of the cytosols was not age-dependent.

Seventy-five per cent of this age decrement results from structural changes in the aldolase molecule as indicated by a 30% decrement in the specific activity of the purified enzyme. It is assumed that the remaining 25% decrease results from decreased enzymatic synthesis. Again no significant difference was observed in the purified preparations of the younger age groups. There were no age differences observed in the degree of purification since in 2.5, 7.5, and 31 month old animals the enzyme was purified 34, 34, and 39 times respectively.

Induction of aldolase in young and old animals by feeding a high fructose-no fat diet for two days resulted in a two-fold increase in the specific activity of the cytosol. The specific activity of the enzyme purified from these cytosols did not differ as a function of age and was equal to that observed in normal young animals.

During a 48 hour period, variations in enzyme activities were previously observed in the livers of intermittently fed mice (24% protein diet fed ad libitum for 24 hrs. on Monday, Wednesday and 8 hrs. on Friday). Since these variations may be related to the time of feeding, the food intake patterns of male $B_6D_2F_1/J$ mice were determined in 6 and 12 month old animals. In addition, food intakes were measured in animals fed ad libitum either a 24 or 4% protein

diet. Results indicated that low-protein ad libitum fed animals ate approximately 7% less food than the ad libitum fed controls. Intermittently fed animals are approximately 30% less than control animals with 40% of their intake being ingested within the first three hours of feeding; 10% during the following three hours; and 50% during the remainder of the 24 hour period. During the weekend fast, from Friday 3 p. m. until Monday 9 a. m., these 30 gram animals experienced a 26% loss in body weight, of which approximately 75% was regained during the first 24 hours of feeding. During the two 24 hour fast and re-feeding cycles during the remainder of the week approximately 20% of their body weight was lost and regained. Thus, these animals are apparently experiencing extreme body weight variations during this period of time. However, it should be pointed out that during a 24 hour eating period, the animals are ingesting 7.0 grams of food or approximately 20% of their body weight. At present, it is difficult to know the degree to which these fluctuations in body weight really represent concommitant changes in tissue mass. Nevertheless the body weights of the intermittently fed animals are 30% smaller than those of the controls.

All animals fed the 4% protein diet containing modified Rogers-Harpers mineral mix died before 9.5 months of age whereas only 15% of the animals fed the 24% protein diet containing this mix were dead by this time. The 50% mortality of these latter animals was found to be at least 25 months of age which compares well with 24 months reported by Jackson Laboratory.

Significance to Biomedical Research and the Program of the Institute: In the previous report it was pointed out that the decrease in specific activity of purified aldolase of rats (15%) was considerably less than that reported in the literature for the mouse (50%). The data of this report agree with these findings however the decrement in purified mouse aldolase (30%) was less than earlier reports. Earlier reports found in the literature regarding this age difference were criticized since 2.5 month old animals were compared with 31 month old animals. Such data do not identify growth changes from senescent changes. The present report which employed 7.5 month old animals as well as the 2.5 month old animals clearly indicate that the age decrement is associated with senescence. Most importantly however is the fact that the activity of the purified induced aldolase isolated from old mice was equal to that of young induced as well as young normal animals. These data imply that induction does not result in a structural change in the enzyme from young animals and therefore newly synthesized enzyme in old animals is essentially structurally the same as that in young animals. Therefore the age-associated decrease in the specific activity of purified aldolase from old animals must result from post-translational modification. Although this has been proposed in recent reviews the data of the present report is the first direct in vivo evidence to substantiate this proposal. In addition, it had been proposed that the age changes in purified enzyme resulted from a greater proteolytic activity in old preparations than young during enzyme purification. However, the data of the present report is not in agreement since there were no age-related differences in the degree of purification.

Although intermittent feeding has been used as a dietary manipulation in aging

studies for approximately forty years, no data are available on the food consumption of individual animals subjected to this dietary regimen. As would be expected, the fluctuations in enzymatic activities seem to be related to the time of feeding. In addition the 30% decrease in food consumption agrees with the 35% decrease previously obtained by others on multiple housed animals whose life span was increased.

Recently the American Institute of Nutrition published a report recommending standards for experimental diets to be used in nutritional studies on rats and mice. In addition, interest has continued to increase concerning the effects of selenium and other trace minerals on the relationship between nutrition and aging. Therefore it was decided to change our present experimental diets containing mineral mix (USP XIV) to that of a modified Rogers-Harper mineral mix. The data clearly indicate a marked shortening of the life span of the animals fed the 4% but not the 24% diet containing this latter mineral mix. This finding was clearly due to the modified Rogers-Harper mineral mix since two previous studies from this section indicated an increase in life span of the animals fed the 4% as compared to the 24% protein diet containing USP XIV mineral mix. It is interesting to note that this decreased life span was not associated with acute toxicity but rather took approximately half of the normal life span of these animals to develop. The other point of interest is that a relationship must exist between the mineral mix and the level of protein since animals fed the 24% protein level experienced a normal life span.

Proposed Course: 1) Identify the post-translational modification of aldolase by the use of biochemical reagents specific for the following functional groups: epsilon amino group of lisine, imidazole ring of histidine, carboxy terminal tyrosine, and sulfhydryl. 2) Other studies will establish the time sequence of the conversion of normal aldolase to the modified enzyme following induction in old animals. 3) Since the age-related modification of aldolase of mouse liver is not the result of primary structural changes which result from transcriptional errors, other enzymes will be examined in the nematode. Many age-associated structural changes in enzymes isolated from this organism have been reported.

Publications:

Johnson, J. E. and Barrows, C. H.: Effects of Age and Dietary Restriction on the Kidney Glomeruli of Mice: Observations by Scanning Electron Microscopy. The Anatomical Record 196: 145-151 (1980).

Barrows, C. H.: Life Span and Nutrition. Contemporary Nutrition in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00141-03-SCN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Effect of Aging on Vitamin Absorption

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B.B. Fleming	Staff Fellow	SCN, OSD, NIA
	C. H. Barrows	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:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project was to examine the effect of age on absorption of fat-soluble and water-soluble vitamins. Female Wistar rats, aged 6, 12, and 24 months were orally administered radioactive forms of vitamins A and D and were sacrificed 18 hours later. A second group of 6, 12, and 24 month old rats were also orally administered radioactive B_{12} and niacin and sacrificed 16 hours later. In both experiments, the esophagus, stomach, small intestinal wall, contents of the small intestine, cecum, colon, and collected feces were assayed for isotope. Liver and plasma levels of the administered vitamins were also determined at sacrifice. Absorption of each of the vitamins was unaffected by age. Plasma and liver levels of the isotope at sacrifice were also not related to the age of the animals.

PHS-6040
(Rev. 10-76)

Objectives: The objective of this project was to examine the effect of age on absorption of the fat-soluble and water-soluble vitamins. Absorption of vitamins A and D (fat-soluble) and vitamin B_{12} and niacin (water-soluble) was examined in rats of three ages.

Methods Employed: Female Wistar rats, aged 6, 12, and 24 months were fasted for 24 hours and then dosed via stomach tube with radioactive forms of the vitamins. One group was given 13 ug of vitamin A and 12 ug of vitamin D and sacrificed 18 hours after dosing. A second group was dosed with 2.0 mg of niacin and 0.54 ug of B_{12} and sacrificed 16 hours after dosing. The esophagus, stomach, small intestinal wall, contents of the small intestine, cecum, colon, and collected feces were assayed for isotope. The concentration and total amount of isotope in the liver and the concentration of isotope in the plasma were also determined.

Major Findings: 1) The Fat-Soluble Vitamins: Absorption of vitamin A during the 18 hour period following dosing was similar in the 6, 12, and 24 month old rats with 32, 33, and 25 percent of the administered dose recovered in the intestinal tract and feces of the respective groups. Plasma and liver levels of the vitamins were also similar among the three age groups.

A total of 47, 47, and 49 percent of the administered dose of vitamin D was recovered in the intestinal tract and feces of the 6, 12, and 24 month old rats at the end of the 18 hour period following dosing. Liver and plasma levels of the isotope also did not differ among the groups.

2) The Water-Soluble Vitamins: Recovery of B_{12} in the intestinal tract and feces 16 hours after dosing was independent of the age of the rats. Eighty-three, 80, and 73 percent of the dose of B_{12} was recovered in the intestinal tract and feces of the 6, 12, and 24 month old animals. Liver and plasma levels of the isotope were also similar among the three groups.

Absorption of niacin was not related to the age of the animals. The recovery of the dose in the intestinal tract and feces averaged 18, 17, and 20 percent in the 6, 12, and 24 month old rats. Liver and plasma levels of the isotope were similar among the three groups.

Significance to Biomedical Research and the Program of the Institute: Absorption of vitamins A, D, B_{12}, and niacin did not differ among the 6, 12, and 24 month old female Wistar rats. These data suggest that the reduced levels of some vitamins reported in elderly individuals are not due to absorption but are the result of other factors such as reduced intake. The large reserve absorptive capacity of the small intestinal mucosa appeared sufficient to compensate for any age-related changes in absorptive capacity of the intestinal mucosa when physiological levels of a group of fat and water soluble vitamins were administered.

Proposed Course: Significant differences in vitamin absorption do not appear to occur as a result of age in rats. Therefore, this line of investigation will not be pursued. However, very intriguing questions regarding the

relationship of specific vitamins and longevity have been raised by such studies as Chope's of a population group in San Mateo, California and Schlenker's study of a population group in Michigan. These studies have shown an association between the intake of specific nutrients, such as vitamin A, and mortality. Studies with Turbatrix aceti are currently being conducted which will explore the influence of the vitamins on longevity in this model system.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00143-01-SCN

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Effect of Protein Intake on Serum Hormones, Creatinine, and Cholesterol

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. B. Fleming	Staff Fellow	SCN, OSD, NIA
Other:	C. H. Barrows	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:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study was to examine the effect of dietary protein intake on serum levels of triiodothyronine (T_3), thyroxine (T_4), prolactin, corticosterone, aldosterone, creatinine, and cholesterol in female Fischer 344 rats. Weanling rats were fed diets containing 4, 8, 12 or 24% protein for 14 months prior to the determination of serum hormones, creatinine, and cholesterol levels. Levels of all the variables examined were similar in rats fed the 8, 12, and 24 percent protein diets. Rats fed the 4 percent protein diets had markedly higher T_4 and T_3 levels, lower corticosterone and aldosterone levels and similar creatinine and cholesterol levels compared to the other three groups.

GRC/OSD-18

Project Description: Z01-AG-00143-01-SCN

Objectives: Restricted protein intake has been shown to increase the life span of both rats and mice. It has been theorized that restricted protein intake delays development of the neuroendocrine axis and changes in hormone levels leading to altered activity of certain genes resulting in impaired growth, delayed sexual maturation, and reduced rate of change of developmental life-shortening pathology. The objective of this study was to compare serum hormone levels, creatinine levels, and cholesterol levels of rats fed diets containing different levels of protein.

Methods Employed: Weanling, female Fischer 344 rats were fed diets containing 4, 8, 12, and 24 percent protein for 14 months. Rats were sacrificed and plasma levels of thyroxine (T_4), triiodothyronine (T_3), prolactin, corticosterone, aldosterone, creatinine, and cholesterol were determined.

Major Findings: Hormone, creatinine, and cholesterol levels of the four dietary groups are presented in Table 1. Levels of all the variables examined were similar in rats fed the 8, 12, and 24 percent protein diets. The rats fed the 4 percent diet had markedly higher T_3 and T_4 levels and lower corticosterone and aldosterone levels than the other three groups. Prolactin levels of the 4% group did not appear to differ from the other dietary groups. Creatinine and cholesterol levels of the 4 percent group were similar to the other three groups. Statistical comparisons of the 4 percent group with the other groups were not possible. The small size of the rats in the 4 percent group and thus the small amount of blood collected made it necessary to pool blood samples from this group.

Table 1 Hormone, Creatinine and Cholesterol Levels of Rats Fed Four Dietary Protein Levels [1]

Group	T_3 ng/dl	T_4 ug/dl	Prolactin ng/ml	Corticosterone ug/dl
		Mean ± SEM		
4% (pooled groups) (n=5/group) [2]	173.0	5.9	9.3	7.1
	190.0	5.6	22.3	19.4
8%	91.1 ± 3.2(12)	3.0 ± 0.1(9)	10.6 ± 1.3(10)	31.9 ± 6.5 (10)
12%	91.6 ± 6.1(14)	2.7 ± 0.1(13)	15.8 ± 2.5(10)	40.4 ± 6.1(14)
24%	87.6 ± 4.8(14)	2.5 ± 0.1(11)	17.4 ± 3.7(11)	30.3 ± 7.6(11)

Group	Aldosterone ng/100 ml	Creatinine mg/dl	Cholesterol mg/dl
		Mean ± SEM	
4% (pooled groups) (n=5/group) [2]	2.0	0.2	58
	6.4	0.4	71
8%	11.2 ± 2.0(9)	0.5 ± 0.1(12)	74.4 ± 4.6(11)
12%	9.5 ± 1.6(10)	0.5 ± 0.1(11)	78.2 ± 7.6(11)
24%	11.1 ± 1.9(11)	0.3 ± 0.1(13)	69.5 ± 4.0(12)

[1] No statistical differences between 8, 12, and 24% groups for any of the variables.

[2] Number in parentheses is sample size

Significance to Biomedical Research and the Program of the Institute: The life span of rats and mice can be significantly increased by restricted protein intake although the mechanism responsible for this effect is unknown. Finch has theorized that restricted protein intake results in increased life span as a result of delayed development of the neuroendocrine axis. In this study, hormone levels of rats fed the 4 percent diet differed from those of rats fed the 8, 12, and 24 percent protein diets (T_3 and T_4 were increased while aldosterone and corticosterone were decreased). Therefore, although these data do not suggest a general delay in neuroendocrine development resulting in decreases in all hormone levels, there does appear to be a selective difference in hormone levels between the 4 percent group and other groups. Although baseline levels of the hormones in the 8, 12, and 24 percent dietary groups were not affected by protein intake, it is possible that lower protein diets may not be adequate for maintenance of homeostasis in stressed animals.

Proposed Course: A study is currently underway which will expand this investigation of the effect of protein intake on the neuroendocrine status of rats. Male Fischer 344 rats are being fed diets containing 4, 8, 12, 24, or 48 percent protein. Many American diets contain very high protein levels, therefore, it is of practical interest to examine the effect of high protein intake on these selected variables. Baseline and stressed levels of corticosterone, aldosterone, T_3 and T_4 will be determined at various times during the feeding trials. The spleen, thymus, adrenals, and testes will be weighed at sacrifice.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00102-04-SCN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Mammalian Biochemistry
I. Stability of Gene Control Mechanism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:. R. Cutler	Research Chemist	SCN, NIA	
R. Dean	Staff Fellow	SCN, NIA	
K. Kator	Visiting Fellow	SCN, NIA	(DOD 12-1-79)

COOPERATING UNITS (if any)
Pediatric Oncology, DTC, NCI; Department of Medicine, Yale University School of Medicine; Mayo Clinic Found.; Department of Cell Biology, Baylor College of Medicine; Cancer Research Unit, McEachern Laboratory, University of Alberta.

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:
1.2	0.9	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued our investigation of the age-dependent stability of gene control mechanisms. Our approach has been to examine the stability of gene repression by determining if specific genes which are thought to be normally repressed do indeed remain repressed with increased age of an animal. We have examined α and β-globin, casein, α-fetoprotein genes and the set of genes making up the c-type endogenous mouse leukemia virus (MuLV) and the mouse mammary tumor virus (MMTV) in liver and brain of C57BL/6J and AKR mouse strains. Evidence for age-dependent derepression of these genes was found with the exception of casein, which remained repressed. More detailed studies were undertaken on the two endogenous c-type viruses, where both were found to undergo a qualitative type of gene derepression (more different types of genes being derepressed with increasing age). Most of the viral RNA is not transferred to the cytoplasm, but instead appears to remain in the nucleus where it cannot be translated.

GRC/OSD-22

Objective: The general long-term objective of our research program is to determine the genetic/biochemical basis of the human's unique innate long period of general health maintenance. Past evolutionary, genetic and biochemical studies of this and other laboratories have indicated the existance of primary health maintenance processes which are likely to be considerably less complex than the aging processes they govern. These primary health maintenance processes are identified as longevity determinant mechanisms, and a common set are thought to exist in all mammalian species.

Through a biochemical and genetic comparative experimental approach using mammalian species having different innate potentials of longevity, we hope to identify and study these hypothetical longevity determinant mechanisms.

Two broad classes of longevity determinant mechanisms have been postulated. One concerns the potentially harmful side-effects of metabolism and other biochemical processes held common in most cells of an organism and present at all ages. Examples are the metabolism of oxygen producing the hydroxyl radical and hydrogen peroxide. Another is the peroxidation of lipid membranes. Protective enzymes such as superoxide dismutase or glutathione peroxidase would be predicted to be in higher concentrations in longer lived species if such protective processes have played an important role in the relatively recent evolutionary increase in mammalian lifespan potentials. Recent data from our laboratory indicates a correlation between the levels of superoxide dismutase per specific metabolic rate with maximum lifespan potential for a number of primate species. These data strongly suggest that such longevity determinants actually exist. In addition, the maximum lifespan potential correlation of DNA repair and the activation of precarcinogens further supports the existance of longevity determinant mechanisms.

The second class of longevity determinants concerns the long-term harmful consequences of biological processes associated with development. Examples are the various hormones related to maturation and growth. Postponement or retardation of general developmental rate would be an effective and simple means to avoid their effects, and there is some evidence that this means did occur in the evolution of longer lifespan in the primate species. Evidence for the general deleterious effect of the steroid hormones, and in particular the adrenal glucocorticoids, is accumulating but as yet no known biochemical process which might serve to protect cells is known. The adrenal steroid dehydroepiandrosterone (DHEA) may be such a protective agent against the developmental processes associated with sexual maturation. Humans appear to have by far the highest concentrations in the plasma, and other primates and non-primate species appear to have lesser amounts roughly in proportion to their lifespan potentials. Moreover, DHEA levels in the plasma increase dramatically during puberty and then fall with increasing age.

As in the past, our present work continues to investigate the evolutionary aspects of human longevity by reviewing and organizing the existing literature on the comparative aspects of physiology, biochemistry and molecular biology of primates and their age-dependent dysfunctions and diseases. Our laboratory work is continuing on three fronts, whose projects are entitled: I. Stability of Gene Control Mechanism, II. Physico-chemical Characteristics of Chromatin, and III. Species-dependent Levels of Genetic Repair and Protective Processes. The remainder of this report concerns the first project.

We are investigating the possibility that the unique differentiated state characteristic of each cell in an organism is inherently unstable and has a finite duration related to the maximum lifespan potential of the species. The age-dependent drift from the optimum state of differentation could be a

result of mutagenic and/or epigenetic-like processes and the rate of drift governed by longevity determinant mechanisms, both of the continuous and developmentally-linked types. Our present experimental aim is to examine if genes normally in the repressed state become derepressed with increasing age.

Methods Employed: The general method used to investigate improper specific gene expression is to search for the presence of RNA transcribed by this gene in cells where this gene would not normally be expected to be expressed. The RNA is detected by using a radioactive complementary DNA (cDNA) probe. The cDNA probe is obtained by isolating pure messenger RNA known to code for a specific gene product and then using this RNA as a template with reverse transcriptase enzyme to synthesize the radioactive cDNA probe. RNA is then extracted from the nuclei and/or cytoplasmic fractions of certain tissues and the presence of RNA complementary to the cDNA probe is determined by DNA·RNA hybridization techniques.

Major Findings: To date we have examined α and β -globin, casein, α -fetoprotein genes and the set of genes making up the c-type endogenous mouse leukemia virus (MuLV) and the mouse mammary tumor virus (MMTV) in the liver and brain of C57BL/6J and AKR mouse strains. In general, an age-dependent increase in expression of these genes was found, except for the casein gene which remained repressed in both liver and brain tissues.

RNA·cDNA hybridizations were used to investigate RNA sequences in AKR mouse tissues (thymus, brain and liver) corresponding to murine leukemia virus (MuLV) and globin cDNA. Similarly, RNA sequences in C57BL/6J mouse tissues (brain and liver) corresponding to mouse mammary tumor virus (MMTV) and casein cDNA were studied. Globin gene expression in AKR mice (2 to 10 months) was low, with globin RNA/total RNA ratios of between 2×10^{-6} and 3×10^{-5}. Within range, most tissue fractions were found to decline in the amount of globin RNA present with age. MuLV expression in AKR mouse thymus showed the expected increase with age. MuLV RNA/total RNA ratios increased from about 1×10^{-6} to 2×10^{-5} (nuclear fraction) and from 1×10^{-6} to 1.5×10^{-5} (cytoplasmic fraction). The liver nuclear fraction also increased in ratio from 1×10^{-6} to 9×10^{-6}. All other tissue fractions showed little or no change with age. In C57BL/6J mouse tissues, no significant age-dependent change in casein gene expression was found. This was also true for MMTV, except in liver nuclear RNA an increase with age in the amount of MMTV genome transcribed of about 4-fold was observed. These results, in accord with previous results, indicate age-dependent derepression of c-type viruses occurs in particular mouse tissues.

The nucleotide sequence complexity of murine leukemia virus (MuLV)-related RNA has been measured by RNA-complementary DNA hybridization analysis in nuclear and cytoplasmic RNA isolated from liver and brain of low-leukemia-strain C57BL/6J mice of different ages. In these two tissues, an approximate 1.5- to 2-fold increase in the complexity of steady-state nuclear MuLV-related RNA sequences was observed as a function of age. Maximum complexity was observed with nuclear RNA extracts from old mice and corresponded to roughly 70 to 75% of the total MuLV genome. In contrast to the age-related increase in complexity of nuclear MuLV-related sequences, a consistent 30 to 40% of the total MuLV genome was detected in liver and brain steady-state cytoplasmic RNA, irrespective of animal age. These data suggest that control mechanisms

regulating the transcription and/or stabilization of nuclear RNA transcripts of endogenous mouse MuLV-related genomes become less stringent with animal age even in low-tumor mouse strains. The data also support the existence of independent posttranscriptional mechanisms which prevent accumulation of these MuLV-related transcripts in steady-state cytoplasmic RNA and which do not seem to be as subject to the relaxation of stringency as a function of age.

Significance to Biomedical Research and Program of the Institute: These findings so far indicate that all structural genes do not undergo an age-dependent derepression and that, instead, this derepression is likely to be highly specific, not only to the type of structural gene involved but also to the type of tissue, cell fraction (nuclei or cytoplasmic) and strain or species of animal. In contrast to structural genes, however, an age-dependent derepression of endogenous c-type viruses related to cancer and other diseases may be universal. These results, if confirmed by more extensive sutdies which are underway, could lead to the identification of an important genetic alteration of cells which may underlie many of the mammalian aging processes.

Proposed Course of the Project: Studies will concentrate on the α-fetoprotein in mouse liver and brain tissues and on globin gene expression in human cells (WI-38) as a function of passage number.

Our long-term proposed course will be to determine (1) the generality of age-dependent relaxed gene expression in terms of tissues and number of different genes affected, (2) if a correlation exists between aging rate of a species and the rate of loss of proper gene control, (3) the molecular mechanisms leading to a loss of gene control and (4) to identify the biological processes that may govern the age-dependent stability of gene expression. These latter two objectives are the projects of the second and third parts of our research program.

Publications:

Cutler, R.G.: Evolution and genetics of human longevity. to appear in: Viidik, A. (ed.) Lectures on Gerontology, Academic Press, London.

Cutler, R.G.: Lifespan extension. in press: McGaugh, J. (ed.) Biology, Behavior, and Aging, Natl. Acad. Sci., Washington, D.C.

Florine, D.L., Ono, T., Cutler, R.G., Getz, M.J.: Regulation of endogenous murine leukemia virus-related nuclear and cytoplasmic RNA complexity in C57BL/6J mice of increasing age. Cancer Res. 40:519-523, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00103-04-SCN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Mammalian Biochemistry
II. Characterization of Chromatin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Cutler	Research Chemist	SCN, NIA
Other:	J. Johnson	Staff Fellow	SCN, NIA

COOPERATING UNITS (if any)
Laboratory of Nutrition and Endocrinology, NIAMD.

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:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have continued our search for age-dependent alterations in the physico-chemical structure of chromatin. Our approach has been to investigate a specific non-histone chromatin protein, the T_3-hormone receptor. This receptor protein is known to be essential for proper control of several specific liver enzymes and can be related potentially to general physiological processes known to change with age. Results using liver tissue from Sprague Dawley rats of ages 8 to 32 months show a slight but statistically significant decrease in the number of T_3 binding sites but no change in binding affinity. Old hypophysectomized animals have been shown by other laboratories to undergo some rejuvenation-like changes, and we have also found some evidence for the regeneration of the kidney glomerular region according to electron microscopy analysis. However, the old hypophysectomized animals do not show a return of T_3 receptor levels in the liver to that of young animals. The effects of T_3 administration to C57BL/6J mice on the number of nucleoli and ribosomal RNA gene dosage has been investigated. Although nucleoli number was increased up to 50%, no effect on ribosomal gene dosage was evident.

GRC/OSD-26

Project Description:

Objectives: This project is designed to investigate whether the genetic apparatus of cells remains intact throughout the lifespan of the animal or if significant physico-chemical or mutational alterations occur that could lead to the improper function of the cell and possibly to the aging of the organism. The specific objective of these studies is to search for alterations at the chromatin level which might help explain the possible dedifferentiation processes that have been found to occur with increased age in mouse brain and liver tissues.

We have chosen to investigate the non-histone chromatin proteins which bind specifically to the T_3 hormone. In addition, it is known that tissue response to T_3 hormone is reduced with age and that many physiological aspects of aging appear similar to a hypothyroid condition. In particular, it has recently been shown by other laboratories that maximum response of the hepatic enzymes, α-glycerophosphate dehydrogenase and malic enzyme decreases significantly with increasing age. We therefore have a system where a specific protein on chromatin in a particular cell type (hepatocyte) can be related to enzymatic and general physiological changes already known to occur with age.

We are also interested in studying the effects of hypophysectomized animals, which have been reported to regain their age-dependent loss of T_3 hormone responsiveness and appear to be rejuvenated in a number of physiologican and immunological parameters. In collaboration with Dr. John Johnson, we examined for evidence of possible kidney rejuvenation in hypophysectomized animals. Furthermore, because it has been reported that T_3 hormone can induce increased rRNA gene dosage number in tissue culture cells, we investigated, in collaboration with Dr. James Gaubatz, the in vivo effects of T_3 administration on rRNA gene dosage in normal intact mice.

Methods Employed: Major techniques employed in these studies are the T_3 hormone receptor assays developed by Dr. Donald Gruol. In this assay the interaction of T_3 with highly soluble, expanded rat liver chromatin is measured. Bound hormone and free hormone are separated by the adsorption of the hormone-nucleoprotein complex onto hydroxylapitite. This procedure has been found to satisfy several important criteria for a successful assay: (1) the binding capacity is stable throughout the time required to reach equilibrium, (2) the ratio of specific-to nonspecific binding is at least 20:1, (3) the amount of bound hormone is directly proportional to the quantity of chromatin used, (4) the hormone and its analogs display a range of affinities for the binding site and (5) the binding occurs at a limited number of sites, over a free hormone concentration range which is similar to the hormone concentrations found in vivo.

A second assay used whole nuclei and was developed after the procedure described by Dr. John Baxter. Here, the nuclei were incubated with the T_3 hormone and free T_3 was separated from bound T_3 by a series of differential centrifugation steps.

Both techniques were employed using both purified whole chromatin preparations and whole purified preparations of nuclei and were found to give similar results.

Techniques used in the electron microscopy of the kidney tissue are described in this annual report by Dr. John Johnson. Measurement of rRNA gene

dosage was by a standard technique, involving extraction and purification of DNA from control and T_3-treated C57BL/6J mice and the DNA·RNA hybridization of this DNA with purified, radioactively-labeled rRNA.

Major Findings: An age-dependent accumulation of damage at the genetic level or epigenetic-like changes could lead to altered gene expression and subsequently to altered function, similar to that seen during aging. Although previous studies of chromatin have indicated various types of age-dependent alterations, few have dealt with a specific constituent of known physiological function. The receptor protein for thyroid hormone is an integral part of the chromatin structure. We used ^{125}I-labeled T_3 as a specific probe to determine if any age-dependent changes could be found in the T_3 receptor proteins of Sprague-Dawley rat liver chromatin. In addition, old 6-month post-operative hypophysectomized rats were used to determine if the recovery of T_3 sensitivity and other physiological functions in these animals was accompanied by a T_3 receptor protein change. An in vitro assay using nuclei and a similar assay using chromatin were employed. Results with 7 young and 7 old animals indicate about a 20% decrease in T_3 binding capacity with increasing age (9 months to 28 months) using either the chromatin or nuclei preparations. No difference was found between 7 young and 8 old hypophysectomized animals.

The kidney glomeruli of 9 month old intact, 23 month old intact, and 23 month old hypophysectomized female rats were examined by scanning and transmission electron microscopy. With increasing age, the glomeruli increased in diameter and more podocyte microvilli were found. Hypophysectomy (4 months before sacrifice) reduced these values to levels approaching values seen in the 9 month intact control. Transmission electron microscopy revealed that increased numbers of podocytes and endothelial cells had cytoplasmic dense bodies with advancing age. Granular cytoplasmic material, seen in podocytes, did not alter in frequency with age. Hypophysectomy reduced the number of cells containing the dense bodies as well as the number of podocytes containing granular material. Basal laminar thickness, while increasing with age, was unaffected by hypophysectomy. The results show that hypophysectomy can return some structural age-related changes to values seen in younger subjects. The effects are similar in some regards to the effects of dietary restriction.

The ability of 3,3',5-triiodo-L-thyronine (triiodothyronine) to induce nucleolar formation in mouse liver cells has been compared with the rRNA dosage (number of rRNA genes per genome) of the same cells. The number of nucleoli per nucleus was determined as a function of time after a single injection of triiodothyronine (hormone-treated) of solvent minus triiodothyronine (sham-operated). Increased numbers of nucleoli were detected as early as two hours after hormone administration. Both sham-operated and hormone-treated mice exhibited peak increases by 4 hours in nucleolar numbers of 50% and 23%, respectively, over control values. The higher nucleolar number was sustained for an extended time period (66 hours) in hormone-treated mice, but significantly declined below control values after 17 hours in sham-operated mice. At even later times (128 hours), the nucleolar counts of both experimental animals approached control values.

No increase in nuclear DNA content was detected between control and hormone-treated mice. Control, sham-operated, and hormone-treated mice had the same rRNA gene dosage, which was approximately 165 rRNA genes per hepatic nuclei. Thus, no correlation between rRNA gene dosage and nucleolar number

was observed. The results suggest that the number of nucleoli found in mouse liver cells may be directly related to the metabolic state of the cell. Furthermore, the time of appearance of increased nucleolar number is one of the earliest changes yet detected in triiodothyronine stimulation.

Significance to Biomedical Research and Program of the Institute: Much of the aging process could theoretically be explained by an age-dependent alteration of the genetic apparatus of cells. It is therefore essential to determine if chromatin (the major genetic constituent of cells) is altered with age, and if so, if this alteration is reversible. In terms of the T_3 receptor protein, although less receptor appears to be available for binding in older animals, this decrease may not be reversible by hypophysectomy. On the other hand, hypophysectomy does appear to be able to reverse a number of physiological as well as morphological parameters. Determination of what types of aging are indeed reversible could prove important in developing new types of medical care for the elderly.

Proposed Course of Project: We plan to concentrate now on relating the observed age-dependent decrease in T_3 hormone receptors to the age-dependent decrease in the induction of specific enzymes in the liver and the possible recovery of this decrease in hypophysectomized animals. Through these efforts we hope to determine what effects chromatin alterations may have on the biological response of a cell and to the animal's general physiological condition.

Publications:

Johnson, J.E. and Cutler, R.G.: Effects of hypophysectomy on age-related changes in the rat kidney glomerulus: Observations by scanning and transmission electron microscopy. Mech. Ageing & Develop. in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00105-03-SCN

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Comparative Mammalian Biochemistry
III. Levels of Repair/Protective Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Cutler	Research Chemist	SCN, NIA
	K. Kator	Visiting Fellow	SCN, NIA (DOD 12-1-79)

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:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigations are continuing on superoxide dismutase (SOD), where we have been studying the possible influence of proteolytic enzymes on our measurements. Using phenylmethylsulfonyl fluoride and leupeptin (a specific proteolytic inhibitor of the lysosomal enzyme, cathespin B), we find that most of the age-related decline in SOD activity in mice, reported by other laboratories, can be eliminated. However, the correlation we have found between SOD specific activity per specific metabolic rate and maximum lifespan potential in primate species is not affected by the use of the enzymatic inhibitors. Genetic linkage and functional correlation between the various histocompatibility loci in man and mouse are suggested by mutations in these loci affecting longevity, susceptibility to disease, immunological competence, and levels of SOD, catalase, aryl hydrocarbon hydroxylase, interferon, cAMP and DNA repair. Preliminary evidence indicates that agents known to induce higher levels of SOD (such as paraquat, streptonigrin and dinitrophenol) also reduce the effects of mitomycin C induction of sister chromatin exchange in tissue culture cells and the life-shortening effects of x-rays in mice.

GRC/OSD-30

Project Description:

Objectives: This project represents a search for genetic repair and protective processes which might act to govern the time-dependent stability of gene expression, and particularly those processes which may play an important role in general maintenance of health in man. The approach used is to compare levels of potential genetic repair and protective processes with the maximum lifespan potentials of different mammalian species, with emphasis being placed on the primate species. To date we have investigated the selective degradation of abnormal proteins and the levels of superoxide dismutase, catalase, and guanylate cyclase in both primate and rodent species.

Methods Employed: Superoxide dismutase assays were similar, as reported in previous annual report. The major difference was the use of the proteolytic inhibitors, phenylmethylsulfonyl fluoride and leupeptin, as described by Petell & Lebherz (J. Biol. Chem. 254:8179-8184, 1979).

HTC cells were employed for studying the effects of superoxide dismutase inducing agents on sister chromatid exchange frequencies. Mice were injected intraperitoneally with these agents for about 5 days before exposure to x-ray irradiation. Radiation was varied over a range of doses from 100 to 1000 rads.

Major findings: Superoxide dismutase was measured as a function of age in liver and brain tissues of C57BL/6J males, wild Mus musculus males, and Peromyscus maniculatus males. For brain, little if any changes were found, but for liver an age-dependent decrease of about 30% was evident. This was similar to previous reports in the literature. However, the addition of either or both of the proteolytic inhibitors in the preparation of the enzyme extractions, as described by Petell & Lebherz, eliminated at least two-thirds of the age-dependent decrease of superoxide dismutase activity found in liver. Thus, it appears that for brain and liver in these animals, the activity of superoxide dismutase is essentially constant with age. Clearly, measurement of any enzyme activity should be made with serious consideration of the use of such proteolytic inhibitors. Point checks using these proteolytic inhibitors in primate tissues, however, showed no significant difference, apparently because only young animals were used in these studies. Thus, our report on the correlation of superoxide dismutase per specific metabolic rate with maximum lifespan potential in primate species remains unchanged.

Many aspects of aging could be a result of dedifferentiation processes. Species having different aging rates would accordingly be postulated to have different innate abilities in maintaining proper cellular differentiation. Possible differentiation-state stabilizing systems are: mixed-function oxidation detoxification, anti-oxidation, DNA repair, immunological, intracellular catabolism and interferon. Genetic mapping by other laboratories using congenic mouse strains indicates a role for the major histocompatibility complex (MHC) in governing tissue levels of superoxide dismutase, aryl hydrocarbon hydroxylase (Ah), catalase and cAMP. On analyzing these reports, we found a correlation of these four parameters with lifespan of mice. R. Walford previously reported a MHC correlation with DNA repair and lifespan. Susceptibility to a number of diseases often associated with aging is also related to specific HLA marker profiles. A correlation of superoxide dismutase, catalase and guanylate cyclase levels with maximum lifespan in primates was found by us.

Maintenance of proper differentiation concerns distinguishing self from non-self, and so biological recognition mechanisms may be a common underlying property for this purpose. Thus, the MHC loci may represent a genetic region with a more global life maintenance function than previously recognized.

Our study with chemical agents known to induce the internal generation of the superoxide radical $O_2^{-\cdot}$ has shown that such agents also induce sister chromatid exchange, indicating that the $O_2^{-\cdot}$ can initiate genetic damage. These $O_2^{-\cdot}$ -inducing agents also are known to increase the endogenous levels of superoxide dismutase. In this regard, we found that low levels of paraquat, streptonigrin or dinitrophenol reduce the ability of mitomycin C to induce sister chromatid exchange. Thus, it appears that genetic protection against mutagenic agents might be induced by chemical agents. To test this concept further we injected mice with dinitrophenol over a 5-day period. This procedure results in a two-fold increase of SOD in the liver and lung tissue. Preliminary results now suggest that these animals are more resistant to x-ray radiation, as evidenced in their ability to survive for about twice the length of time as compared to saline-injected controls.

Significance to Biomedical Research and Program of the Institute: These studies support the concept that an increase in the level of activity of a common set of repair and protective processes in the mammalian species may play an important role in governing their different aging rates. It is also possible that the processes being studied could act to protect the genetic apparatus and thus be important in stabilizing the differentiated state of the cell. If MLP and health maintenance of primates are governed in part by the level of expression of a commonly held set of genetic repair and protective processes, then this may offer a basis to further enhance man's innate resistance to chemical and radiation hazards. In addition, when toxicity data derived from short-lived species such as rat is used to determine safe levels for man, the possible higher constitutive levels of genetic repair and protective processes in man may need to be considered.

Proposed Course of the Project: Further studies will continue measuring the levels and studying the mechanism of control of superoxide dismutase in rodents and primate species. In particular, we plan to examine both the cytoplasmic and mitochondrial levels of SOD, instead of looking only at total activity, as we had in the past.

A descriptive study of dehydroepiandrosterone (DHEA) is planned with the collaboration of the Orentreich Foundation. Although DHEA is known to fall rapidly with age in human, little is known about its levels with age in experimental animals used at the GRC for aging studies. We will measure DHEA and DHEA-sulfate levels by radioimmunoassay as a function of age in C57BL/6J, wild-type Mus musculus and Peromyscus maniculatus and the GRC Wistar rat. Collaboration has been arranged with Drs. E. Masaro and R. Walford, measuring DHEA and DHEA-sulfate levels as a function of dietary restriction, in intermittent fasting and in hypophysectomized animals. These latter experiments are designed to study the possible relation between lifespan extension by dietary restriction and intermittent fasting and plasma DHEA and DHEA-sulfate levels. We are also collecting plasma samples from primate species to determine how plasma levels of DHEA and DHEA-sulfate in the young adult correspond to their innate maximum lifespan potential.

A primate tissue culture bank is now being established with the assistance of Dr. James Regan of Oak Ridge National Laboratory and Dr. Ted Brown of Cornell University Medical School. These cells represent mostly skin

fibroblast strains of low passage number and are taken from a number of species with a wide range of maximum lifespan potentials. Future use of these primate cell cultures will be in determining relative metabolic activation of precarcinogens and ultraviolet light sensitivity as a function of maximum lifespan potential. Previous work reported by Dr. A. Schwartz showed an inverse correlation of activation of precarcinogens with maximum lifespan potential, but he did not investigate whether such a correlation exists with the primate species. Moreover, although a correlation has been reported between the extent of UV repair and maximum lifespan potential in primate species, it is not known how the extent of UV repair correlates with degree of UV sensitivity in terms of survival.

Publications:

Cutler, R.G.: Lifespan extension. in press: McGaugh, J. (ed.) Biology, Behavior, and Aging, Natl. Acad. Sci., Washington, D.C.

Tolmasoff, J.M.,Ono, T., Cutler, R.G.: Superoxide dismutase: correlation with lifespan and specific metabolic rate in primate species. Proc. Natl. Acad. Sci. USA 77:277-2781, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00151-01-OSD

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Aggregation Factor Visualized on Aggregating Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	EMS, OSD, NIA
	Thomas Reese	Chief, Functional Neuroanatomy	LNNS, NINCDS

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Scientific Director

SECTION
Experimental Morphology Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sponge aggregation factor (AF), the large glycoprotein complex involved in species specific aggregation is the first aggregation factor to be visualized on the surface of aggregating cells. 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. It is a model of specificity of cellular interactions.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Aggregation factor (AF) is a 18 x 10^6 Dalton glycoprotein which species-specifically causes marine sponge (Microciona prolifera) cells aggregate to cause physiological adhesion. This aggregation factor is the only purified huge glycoprotein for which a biological assay exists and the sponge aggregation system which is most dramatically dependent upon one factor for cellular cohesion.

It is a fibrous macromolecule with a circular filament for a backbone and about sixteen filaments radiating outwards in a sunburst configuration. These experiments are to discover where on the macromolecule are the sites which bind to cells and where are the sites which bind to other AF macromolecules. They are also designed to show where on the surface of the sponge cell the factor binds and if there are specialized zones of adhesion.

Methods Employed: Microciona prolifera, freshly collected by the Supply Department of the Marine Biological Laboratory, Woods Hole, MA, were dissociated by soaking in calcium and magnesium-free sea water, a procedure which liberates aggregation factor (AF) into the supernatant. AF was further purified by differential centrifugation and chromatography.

Cells returned to complete sea water in a gyratory shaker aggregate upon the addition of AF.

Dissociated cells, aggregating cells, and purified factor were rapidly frozen, freeze fractured and replicated or freeze substituted, stained en bloc with $HFCl_4$, embedded and sectioned for transmission electron microscopy.

Major Findings: Aggregation factor was visualized by transmission electron microscopy on the surface of aggregating sponge cells.

Dissociation of cells was achieved by divalent cation depletion, which extracts AF. The surface of these cells appear remarkably smooth. However, surfaces of aggregating cells to which an excess (to be certain of having enough to visualize) of purified AF and 10^{-2} M $CaCl_2$ were returned have a filamentous network over much of their surface.

In sections and replicas at fortunate angles, the distinctive filamentous configuration of AF, a circular backbone with about 16 arms radiating outward can be seen. The circular backbone is sandwiched between adjacent cells with some arms extending to one cell and some extending to the other. Arms can also contact other AF macromolecules.

These experiments show that:

1. AF macromolecules bind to the cell surface during aggregation.

2. At a given site a single AF sunburst complex may suffice to link adjacent cells but many such linkages exist between cells, and

3. Sites in the chains of AF bind it to cells.

Current efforts are to complete the experiments by careful examination of stereo pairs of replicas and sections of cells aggregating in the minimal concentration of AF. This painstaking analysis at the minimal concentration of AF is necessary to demonstrate that all AF which visualized is actually involved in adhesion.

Significance to Biomedical Research and Program of the Institute: Cellular surface specificity, which allows cells to recognize their appropriate place in an assemblage of cells, is essential to proper morphogenesis and histogenesis, for example, the awesome specificity of neural synapses. Such surface specificity of cells is no less important to adult functioning of cells and tissues and their misfunctions in cancer and injuries.

Proposed Course of the Project: This project will be terminated after analysis is completed and publication of results.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00152-01-OSD

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Ectopic Osteogenesis Ultrastructure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	OSD, NIA
Other:	James T. Irving	IPA	LCMB, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Scientific Director, NIA

SECTION
Experimental Morphology

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 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 (200 words or less - underline keywords)

Osteogenesis induced by subcutaneous implantation of bone extracts in rodents follows the ultrastructural pattern expected for normal bone formation and produces fully mineralized bone.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objective is to assess the normality of osteogenesis during ectopic bone formation, utilizing conventional microscopical methods.

Methods Employed: Ectopic bone was induced by subcutaneous implants of bone or tooth powders in Wistar NIA rats. Induced bone was excised for microscopy at various time intervals after implantation until stages where the extra cellular matrix was too mineralized to cut. Standard fixation by immersion into glutaraldehyde-formaldehyde in cacodylate buffer and post fixation in osmium tetroxide and embedding for sections for electron microscopy were employed.

Major Findings: As far as we have been able to ascertain, ectopic bone formation in the rodent proceeds indistinguishably from normal bone formation, once the osteoblasts have differentiated sufficiently to be recognizable as osteoblasts. The source of the cells which become osteoblasts has yet to be determined.

Significance to Biomedical Research and Program of the Institute: Results thus far suggest that the mechanisms involved in the formation of ectopic bone, induced by powdered bone or other promoters, are no different from those in normal osteogenesis. This suggests the possibility of inducing and controlling new bone formation in situations where bone mass is deficient, e.g., osteoporosis.

Proposed Course of the Project: Cytological examinations will be made periodically as new developments in induction of ectopic bone occur. If appropriate labelling can be achieved, the identification of the stem cells may be possible. Once they are identified, regulation of their recruitment to form bone may be more precisely studied.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00153-01-OSD

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Brush Border Ultrastructural Alterations with Ischemia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Susie Humphreys	Sr. Staff Fellow	OSD, NIA
Other:	Uzi Reiss	Visiting Fellow	LMA, GRC, NIA
	Bertram Sacktor	Chief, LMA	LMA, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Scientific Director, NIA

SECTION
Experimental Morphology

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.4	.6

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Regeneration of the brush border of the proximal kidney tubule after isolateral ischemia has been monitored by transmission electron microscopy. The brush border takes longer to reform than does restoration of full activity of three enzymes (alkaline phosphatase, maltase, and γ-glutamyltranspeptidase) used as biochemical markers of brush border integrity.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objective is to give a structural basis to the biochemical findings of Dr. U. Reiss and Dr. B. Sacktor in the Laboratory of Molecular Aging. Biochemical extracts of isolated brush border of the proximal tubule show that activity of alkaline phosphatase, maltase, and γ-glutamyl-transpeptidase decline after isolateral kidney ischemia. Morphologic examination was performed to determine possible explanations for these declines in activities and to discern relative roles of *de novo* synthesis and reorganization of the brush border in the reformation of brush border and how this reformation might vary with age.

Methods Employed: Kidneys were removed from rats after experimental manipulations of unilateral renal artery occlusion and blood reflow and were put into cold saline. Portions of the cortex and outer medulla of the mid-kidney were excised and fixed by standard immersion fixation in glutaraldehyde-formaldehyde in cacodylate buffer, postfixation in osmium tetroxide, and were then processed for transmission electron microscopy.

Major Findings: Our findings paralleled those of Venkatachalam, et al. (Kidney Intern. 14, 31-49, 1978) except for the delayed recovery after our longer (1 hour as opposed to their 25 minutes) ischemia. The tubules collapse and show degenerative changes. The microvilli swell, coalesce, and lose parallel order. The microvilli were largely internalized, although some were sloughed off into the lumen along with blebs of cytoplasmic material.

Other cytoplasmic changes included mitochondrial damage, cytoplasmic swelling and formation of large vesicles. In addition to the luminal vesicles and infoldings characteristic of S_1 and S_2 regions of the proximal tubule, relatively translucent vesicles, usually considered lysosomes, are present in cells of all regions of the proximal tubule. After 3 hours reflow following 1 hour ischemia, these are dense and contain membrane elements in the cells which appear to be recovering in S_1 and S_2. The basement membrane remained grossly intact during all the experiments.

The lumens are completely impacted with sloughed material and dying cells until about 3 hours after reflow. Then the S_1 and S_2 portions are partially cleared but the S_3 portion, so disorganized that it is difficult to recognize, remains largely impacted.

No zonula occludens junctions are observed in ischemically damaged cells. The zonula adherens junctions between the lateral plasma membranes at the level of the base of microvilli appear to widen during ischemia. These junctional changes, if not artifacts of tissue processing, may be of interest if they are involved in the known depression of sodium transport during ischemia.

The primary question of the source of the new brush border has not been answered. Most, by far, of the brush border is coiled and internalized. However, there is no obvious realignment of this membrane and extrusion. The microvilli of regenerating cells have exaggerated dense tips beneath the cell membrane where the actin filaments terminate. These exaggerated densities have not been found on the control, nonischemic kidneys, and may be characteristic of de novo microvillar formation.

Some cells in S_1 and S_2 have an essentially normal brush border after 30 minutes reflow, but others lack the border after 3 hours. Biochemical markers, alkaline phosphatase, maltase, and γ-glutamyltranspeptidase, reached full activity by 30 minutes. Thus, the complete structural integrity is not necessary for full activity of three enzymes characteristic of the brush border.

The major difference between mature adult and aging rats is that recovery is slower in the aging rats.

Significance to Biomedical Research and Program of the Institute: If the reformation of the brush border which occurs in the convoluted proximal tubule is really de novo synthesis, then it offers a system to study how profound the differences in brush border of young and old rats are. All that is known now is that three transport enzymes differ in form in young and old animals.

Proposed Course: The morphological data will be further analyzed and cytochemical assays for alkaline phosphatase will be performed on pellets of brush border enzymes to partially check the purity of the brush border preparations.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00154-01-OSD

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Programmed Cell Death

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Susie Humphreys Sr. Staff Fellow OSD, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Office of the Scientific Director, NIA

SECTION
Experimental Morphology

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 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 (200 words or less - underline keywords)

Programmed cell death in the ventral nerve cord of the nematode Caenorhabditis elegans is being studied by light and electron microscopy to learn the pattern of steps involved in death. These steps may indicate how the control for death is mediated and give some insight into the regulation of the process.

GRC/OSD-42

PHS-6040
(Rev. 10-76)

Project Description:

Objective: Study of programmed cell death, often an essential part of normal embryonic development, has two goals: (1) to understand the regulation of normal cell death which benefits the organism to better understand the abnormal cell death occurring in disease and by accident which does not benefit the organism, and (2) to develop a possible model for the nature of degenerative changes which lead to the death of an organism.

Methods and Questions: While embryonic programmed cell death occurs widely in invertebrates and vertebrates, including humans, only in the nematode Caenorhabditis elegans has cell death been described for a particular cell identifiable by its position in the organism (Sulston and Horvitz, Dev. Biol. 56: 110-156, 1977). Elsewhere, only populations of cells can be described, some of which die and some of which do not die.

Development of living worms will be observed in Nomarski optics and staged. Appropriate stages before cell death will be selected and processed for electron microscopy to determine the first evidence of impending death. Is it signaled by a lesion in a particular organelle?

If the death is programmed the mechanism of the program may be indicated by the sequence of predeath changes. These changes may differ from those of a cell "murdered" by injury. The question of mechanism has been examined in other systems, but because of the confusion inherent in populational events, and perhaps because of multiple causes and pathways of cell death, there is no agreement.

If it is possible to microinject into or onto the tiny neurons fated to die, it is then possible to attempt to prevent death with growth factors or repair enzymes.

Characteristics of the cell surface are of significance because the surface interacts with other cells and hormones. The surface will be studied by freeze fracture and deep etching. If we are technically able, lectins and receptors will be labelled with ferritin, microinjected, and observed in replicas and in the scanning electron microscope. Quantitative and qualitative changes will be noted and compared to neurons which do not die.

Currently C. elegans are being cultured, basic anatomy learned, and microinjection methods explored.

Significance to Biomedical Research and Program of the Institute: The basis of normal cell death is being explored as an example of time-dependent regulation and as a model of the nature of aging.

Proposed Course of the Project: Exploration of culturing and microinjection methods will be continued.

As the following summary will show, this has been a productive scientific year for this laboratory. However, a personnel freeze has prevented any growth or development of two major programs. The recently founded section on Stress and Coping is seriously undercapitalized and needs to supplement its scientific staff if it is to continue progress toward meeting its mission. Research programs in Behavioral Medicine exist both in the Stress and Coping Section and in the Psychophysiology Section. In fact, the Psychophysiology Section is a leader in research in Behavioral Medicine throughout the world. However, progress in Behavioral Medicine research has been greatly limited because of the inability of LBS to obtain authority to recruit a permanent staff physician due to the freeze. This constraint greatly limits the kind of research questions which can be pursued. Two staff fellows and a National Research Service Award postdoctoral fellow, all recruited last year, joined the Laboratory staff during the current year; and the visiting foreign fellow in the Stress and Coping Section left. Collaborative research continues to represent a substantial component of the Laboratory programs. Several divisions of Baltimore City Hospitals including Cardiology, Dermatology, Gastro-intestinal, Psychiatry, and Urology; the Department of Medicine of Columbia Medical Plan; the John F. Kennedy Institute of Johns Hopkins University School of Medicine; and guest workers from the Department of Human Development at the University of Maryland and from Crownsville State Hospital are involved in active collaborative programs.

One of the major goals of this Laboratory is to apply research findings to develop behavioral procedures for ameliorating problems of the aged. In its role as a leading research center in the field of Behavioral Medicine, this Laboratory has applied behavioral principles to treat patients with urinary or fecal incontinence. Incontinence is a major factor in a family's decision to move an elderly relative into a nursing home. This year a geriatric continence clinic was established to evaluate behavioral treatment of fecal or urinary incontinence in men and women over 65. Development of such procedures depends upon a knowledge base, and much of the Laboratory program is designed to contribute to that prerequisite pool of research knowledge in many areas. These studies include: (1) behavioral procedures for modifying reflex cardiovascular responses; (2) understanding neurochemical relationships with behavior and how these are affected by aging; (3) the effects of aging on electrophysiological phenomena in skin; (4) voluntary control of blood pressure; (5) the effects of hypertension on cognitive performance and on measures of personality; (6) the effects of aging on reasoning performance; (7) the role of personality in the well-being of the elderly and personal adjustment to aging; (8) the stability of personality with aging; (9) the effects of dietary manipulations and exercise on behavior, physiology, and longevity; and (10) the effects of age on the flexibility of autonomic response patterns. Important results in all of these areas were found during this year.

Several experiments have shown that behavioral procedures (operant cardiac conditioning) can modify reflex cardiovascular responses such as baroreceptor sensitivity or cardiac work during physical exercise, or elicit cardiovascular responses such as the heart-rate increase during electrical stimulation of the brain. The general principle which these experiments seem to underscore is that behavioral adjustments to specific stimuli can override reflex cardiovascular responses under a variety of conditions, all of which have in common that the well-being of the animal is threatened, i.e., the behavioral reinforcer is shock avoidance. Studies with humans have shown that normal men and women exercising at 50% of maximal heart rate or at 70% of maximal heart rate can be operantly conditioned to attenuate the tachycardia of exercise.

Two physiological response systems which change with age are the dopamine neurotransmitter system of the brain and the sudomotor system. A series of experiments in this Laboratory have shown that there are age differences in the dopaminergic system of the brain. This system is important because one major component of it, the nigrostriatal system, is involved in various age-related motor disorders such as Parkinson's disease. Experiments carried out this year have suggested that the age-related deficit in dopamine in the nigrostriatum of the rat may be in the synaptic release or in the post-synaptic metabolism of the transmitter rather than in the production of dopamine. Other experiments indicate that a decline in dopamine receptors also may contribute to the age differences. However, other experiments suggest that old rats can proliferate receptors following nigrostriatal lesions, indicating that they retain a degree of plasticity in the face of deficit. To overcome technical difficulties precluding direct measurement of sweat gland activity in man, an experimental model was developed in this Laboratory. A series of experiments with this model has suggested that, as individuals grow older, the electrical resistance of their sweat glands increases. This aging effect is reflected in higher electrical skin resistance and less negative electrical skin potential.

High blood pressure is an important health concern which seems to be related to age. An extensive field study of 127 patients with mild, borderline high blood pressure was initiated to evaluate the behavioral interventions of biofeedback and relaxation. During the past two years, this study has shown that: 1) it is feasible to enlist these patients in a study that requires them to monitor their pressures daily (only 2% of a series of 224 patients expressed fear about monitoring their pressures); 2) systolic and diastolic pressures are usually highest in the afternoon; systolic is lowest in the morning and diastolic is lowest in the evening; 3) women have a wider range of systolic pressure during the day than do men; 4) both systolic and diastolic pressures show habituation over at least two months of daily monitoring; 5) various indices of systolic pressure (level and variability) are correlated with age, but this is not so for diastolic pressure; 6) subjects will participate in a self-administered, behavioral treatment program; and their abilities to control their pressures improve with practice. Hypertension has long been considered of importance in the decline of cognitive abilities among aging individuals. More recently some investigators have suggested that hypertension may affect personality. Longitudinal data from

the BLSA were analyzed to provide evidence about these issues. Little, if any, longitudinal change in intellectual function was attributable to a history of blood pressure elevations. Among a sample of over 70 year old men tested six times over a ten year span, there was no evidence of accelerated decline among hypertensives. Although individuals who are informed that they are hypertensive may react with temporary anxiety, depression or somatic concern, longitudinal results also demonstrated that hypertension has no enduring effect on personality.

From the time the first longitudinal results of intellectual performance showed that the "classic" decline (based on cross-sectional studies) portrayed in textbooks of developmental psychology did not describe what happens to individuals as they age, some investigators questioned whether cognitive performance declined with age at all. Previous cross-sectional analyses of problem-solving performance of men in the BLSA indicated age differences across the entire adult life span. Longitudinal data, however, showed mean declines over six years only for the men who were in their seventies when measured initially. Regression procedures were developed in this Laboratory to estimate age changes for groups of men born during the same period. These estimates of change avoid some of the problems of attrition which may have reduced or obscured declines in the longitudinal measures of change. Only the oldest birth cohort, who were in their seventies at the beginning of the study in 1967, showed substantial estimates of decline in performance, corroborating the longitudinal findings. This is further evidence that, when memory demands are reduced by allowing problem solvers to review information they have elicited to solve a problem, reasoning performance declines only late in life. Age-related decline late in life has been found previously in other areas of cognitive performance (such as learning and memory) in this Laboratory.

Subjective well-being is an important component of personal adjustment to aging, and much of the literature in gerontology has been devoted to understanding well-being among the elderly. Recently, investigators in this Laboratory have formulated and successfully tested a model which relates aspects of personality to subjective well-being. Extension of the personality-well-being model to personal adjustment to aging ("successful aging") again showed significant links between extroversion and neuroticism with longitudinal reports of adjustment and positive attitudes in various life areas (e.g. Health, Friends, Work, Economic Security, Family, Happiness, and Usefulness.) Individuals higher in subjective well-being and adjustment to aging were lower in neurotic traits and higher in extroverted traits.
The issue of constancy or change in personality over the adult portion of the life span has become a major focus of attention for gerontologists. Longitudinal data from the men in the BLSA previously showed either small or no changes at all in personality traits in mean level or in relative standing of individuals over time. Current findings indicate that the structure of personality dimensions is quite similar for men of different ages. Moreover, when measures obtained six year after the initial measures and again twelve years later were factor analyzed, the structure of personality did not change longitudinally. All of these findings are further evidence of the stability of personality throughout adult life. Openness to experience is a broad personality dimension which is hypothesized by investigators in this Laboratory

to influence degree of defensiveness and choice of coping styles. Analysis of sentence completion responses of open and closed men showed that openness is associated with developmental maturity. Further, open individuals showed more flexibility in their conception of rules and of social roles, more complexity in their cognitive judgments, and a greater use of humor. These observed characteristics suggest that open and closed men use different strategies in dealing with stress.

Diet and exercise are thought to be important behavioral determinants of morbidity and mortality. One project in this Laboratory explores the effects of diet and exercise on performance and survival in rodents. Recently, investigators found that rats with access to food every other day lived longer than rats with food available continuously. That is what happened when the alternate fasting procedure began early in life (1.5 months). This year, it was found that access to food every other day increased longevity even when the procedure was initiated later in life (10 and 18.5 months), although the effect was diminished substantially for the 10 month animals and even more for the 18.5 month group. Voluntary exercise, which had been shown in this Laboratory to increase longevity for animals with access to food at all times, did not affect longevity further for animals fed every other day. Exercise, however, did delay the terminal drop in weight preceding death, an index of the general condition of these animals.

Individual specificity, the tendency of an individual to respond physiologically in a similar pattern to all stimuli, is used in this Laboratory to determine whether there are age differences in flexibility of autonomic nervous system functioning. If an individual's response pattern is consistent regardless of the stimulus, that inflexible response pattern often will be inappropriate to the demands of a situation. Results indicated that individual specificity increases with age; that is, the autonomic response patterns of older men are less flexible than the response patterns of younger men.

This has been another highly productive year for the scientific programs of this Laboratory. Progress has been made at the descriptive level determining what happens behaviorally to individuals as they age, at the level of understanding processes and mechanisms of age changes, and at the level of developing procedures to improve the functioning of patients and the elderly. If projected plans to establish a clinical research facility to test various behavioral treatment procedures in elderly subjects come to fruition, we can expect to make major advances in future years to mollify the infirmities of late life which prevent so many older persons from enjoying their last years.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00061-18 LBS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Behavioral Genetics and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	Donald Ingram	Staff Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	.65	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to determine behavioral differences throughout the lifespan for populations of mice which differ in genetic structure, and which also differ in longevity. The present topics of interest concern the relations of growth rate, maximal body weight and longevity for mice fed low levels of dietary protein and for groups fed adequate dietary protein, in addition to studies of aging and voluntary wheel exercise, and ethanol preference.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The principal objectives of this project are: (1) to determine group differences for behavioral traits and longevity among inbred strains of mice; (2) to determine heritability (degree of genetic determination vs. degree of environmental determination), mode of inheritance (e.g., overdominant, dominant, intermediate, or recessive), and number of segregating units (gene blocks) controlling a particular trait (e.g., longevity); and (3) to examine relative behavioral differences among mouse strains as aging progresses. Other objectives include determining the influence of diet (e.g., protein available) on behavioral traits, growth, and longevity; and identifying single-gene influences upon behavioral traits, growth, and longevity.

Methods Employed: Inbred mice (C57BL/6J and A/J) of a high degree of homozygosity are maintained under uniform environmental conditions. The animals are tested behaviorally during one period of their life span, viz, when mature, mature-old, or aged. Aged is defined as the 50% mortality point for groups maintained throughout their life span. Statistically reliable techniques have been developed to determine behaviors relevant to natural selection such as exploration, general activity level, emotionality, simple or complex problem solving ability and taste preference. The use of segregating F_2 hybrid groups allows an estimate of the mode of inheritance, e.g., dominant or intermediate, and the number of gene blocks or segregating units controlling behavioral traits or life span. For studies in which protein intake is varied for groups of inbred and hybrid mice, isocaloric synthetic diets with 4% (low protein), 26% (control), and 48% (high protein) casein are used. Numerous kinds of mutant mice are maintained on the C57BL/6J background at the Jackson Memorial Laboratories, Bar Harbor, Maine. Our work has concentrated on the albino, beige, yellow and obese mutations. In addition, inbred and hybrid mice are used to test the hypothesis that protein malnourishment increases longevity due to slowing of the rate of growth. Studies of behavioral differences as a function of dietary protein and stage of development are also in progress.

Major Findings:

A. Inbred mice (A/J and C57BL/6J) and hybrid mice (F_1, A/J C57BL/6J) were maintained on low, normal, or high protein diets throughout the life span (N=180). Longevity was found to be inversely related to level of protein. The data will be reported in detail next year.

B. Obese and yellow mutations and littermate controls were started on periodic fasting (fed every other day) at maturity (20 weeks old). The effect of this procedure was to increase longevity of each group (total N = 40). For obese mice, the increment (above the longevity of ad libitum fed mice) was 31.3%. Yellow mice obtained an increment in longevity of 47.5% over ad libitum fed yellow mice. The magnitude of the increments was partly dependent on body weight. Obese mice maintained a high level of body weight over a long time interval, remaining obese until late in the life span; and this group had the smallest increment in life span. Therefore maintenance of high body weight may adversely affect life-span. Mean life span in months and percentage increment in the life span are given in Table 1 for the six

groups. The effect of periodic fasting was to increase the life span for all three genetic groups, F (1,84) = 50.52, $p < .001$. Differences among groups were also statistically significant, F (2,84) = 46.59, $p < .001$, but the interaction of diet and group only approached statistical significance, F (2,84) = 2.36, $p = .10$.

Significance to Bio-Medical Research and Program for the Institute: The study of the genetics of behavior and longevity allows an assessment of: (1) the mode of inheritance (i.e., dominant, intermediate, etc.) for the factor studied;(2) the relative importance of hereditary and environmental factors; and (3) the number of genes or gene blocks which control the factor studied. Lack of adequate dietary protein is a condition which affects a large proportion of the world population. This project attempts to determine the effect of diet (such as different proportions of protein in the total diet) during particular stages of the life span upon behavior and longevity for animal populations which differ in genetic constitution. Studies of single gene mutant animals are important because they allow the assessment of a specific genetic locus for physiological or behavioral factors.

Proposed Course of Project: Other inbred strains and F_1 hybrid groups are being studied to determine the generality of mode of inheritance of behavioral factors. Cross-sectional and longitudinal studies of mouse behavior will continue with various mouse strains. The longevity of inbred and hybrid groups are also being determined. A special effort will be made to construct a behavioral battery of tests to predict lifespan in the C57BL/6J strain. Experiments with low, normal, and high protein diets should determine: (1) the effect of varying protein diets upon behavior at maturity after access to these diets during various stages of development, and (2) the immediate effects upon behavior of a diet of low, normal or high protein for young or aged mice. Collaboration will also be sought to determine the physiological and pathological changes affected by these dietary manipulations.

Collaborative effort will also be made to determine the neurochemical correlates of age-related behavioral change.

Publications: None

Table 1

Mean Life Span in Months for Mutants and Littermates and percentage increment in the life span for groups fed every other day

	ob	y	Littermates
		Life Span	
Ad Libitum	13.27	20.01	23.66
(n)	(13)	(24)	(13)
Fed Every Other Day (EOD)	17.44	28.52	33.81
(n)	(10)	(10)	(20)
		% Increment	
	31.3%	42.5%	42.8%

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG 00062-07 LBS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Daydreaming and Aging: Normative and Experimental

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard M. Giambra	Research Psychologist	LBS GRC NIA
Other:	Bernadette Cider		Towson State Univ.
	Eric Klinger	Prof. of Psychology	University of Minnesota
	Beth Stone	Psychologist	Macquarie Univ. Australia
	Reginald Quilter	Electronics Tech.	LBS GRC NIA

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning & Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.9	.1

CHECK APPROPRIATE BOX(ES)

☒(a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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) racial, religious, ethnic, and socio-economic influences on daydreaming, (b) the relation of sexual daydreaming and sexual activity to the menopause, (c) the relation of daydreaming incidence to tasks of varying vigilance requirements, and (d) the dimensions of ongoing thoughts on a life-span sample.

PHS-6040
(Rev. 10-76)

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, various races, 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 28 scales 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 occurrence 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 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 continous 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.

In the thought sampling task each person carrys an alarm device (a beeper) which emits a soft tone at random intervals with a mean of 40 minutes. Each time the beeper sounds the person stops what he or she is doing, records the last thought which occurred when the beeper sounded and rates that thought on 24 scales of a Thought Sampling Questionnaire. The beeper is carried with the person during day-to-day normal activities. Each person provides about 150 thought samples.

Major Findings: I. Religious, ethnic, and socio-economic effects on daydreaming.
Data from more than 1800 people who responded to the Imaginal Processes Inventory and to a biographical questionnaire were analyzed to determine unconfounded differences due to religious, ethnic, and socio-economic differences. Inferential statistical analyses were carried out to determine reliable differences and are summarized below.

Daydreaming

Simple demographic effects. The belief in daydreaming as acceptable adult behavior was found to be lowest in the lowest income group, highest in the highest group and intermediate in the $]5,000 to $29,999 range. The positive or pleasant aspects of daydreaming were more likely in those with at least some post-graduate education. The temporal setting of daydreaming was affected by Residency and Religion. Day-dreaming of the personal future is higher for those from cities of 20,000 or more; it is also lowest in Catholics, next higher in Protes-tants and highest in other non-Jewish religious groups. The content of daydreams was systematically influenced by Highest Degree Level, Education in Years, and Socio-Economic Status. Day-dreams which deal directly or indirectly with the solution of problems, difficulties, and "puzzles" are more likely among those with a Master's degree and/or more years of education. Daydreams dealing with a negative self-image and feeling of guilt are more likely in those who describe themselves as Middle Class or lower than those who describe themselves as Upper-Middle-Class or higher. The pattern which emerged is sketchy but suggests that income, educational experience, or in general socio-economic level is directly related to a mode or view which sees daydreaming as positive, pleasant, and useful and inversely related to having daydreams which are negative, frightening, and perhaps nonuseful.

The sex interaction. Males who describe themselves as Middle-Class and below show a reduced tendency to daydream of the future when compared with females and with the Upper-Middle-Class and above group of males or females. Males had a high tendency to daydream of the personal future regardless of the level of the highest degree; females showed the same tendency only for those with a high school diploma or a Bachelor's degree. Females with a Master's degree had a much higher tendency to daydream about the personal future than those females with a lower degree level and a somewhat higher tendency than males at all degree levels. Females with a Master's degree also showed a much greater tendency, though still quantitatively low, to have hostile daydreams than males with that degree or any group with a lower degree. Daydreams with negative self-image and guilt showed a tendency to decrease with increasing income for females while no pattern was evident for males; all means were at a low absolute level. The pattern suggested here is that women with the ambition or drive to have attained a Master's degree have more daydreams of their own future and more hostile daydreams while showing a reduction in negative self-image daydreams, when contrasted with other women and with men.

The age interaction. Among those 17-44 years of age, Middle-Class individuals showed more imagery in daydreaming than Upper-Middle-Class while for those 45-92 years of age the opposite was true. Daydreaming about one's own personal future was less likley for Protestants and Catholics as they become older. Those describing themselves as atheists, agnostics, non-denominational Christians, and members of non-Western religions maintained their same highest tendency to daydream of the personal future for all age groups. Daydreaming of the general future did not decrease for Protestants as the age of the group increased. The "other" religious category had the

greatest likelihood of bizarre-improbable daydream content for the 17-23 year olds and the least likelihood for the 65-92 year olds when compared with Catholics or Protestants. Bizarre and improbable daydream content goes down with age for those with Master's degrees, is not related to age for those with Bachelor's degrees, and increases with age for those with a high school diploma. Daydreams with hostility in them drop with age of group for incomes in the $15,000 to $29,999 range. For those with incomes of under $15,000 and over $30,000 hostile daydreams increase in the 24-44 year old group with the under $10,000, 24-44 year olds having the highest level of hostile daydreaming among age-income groups. No pattern seems to be suggested by these easily described age-demographic variable interactions.

Daydreaming, it seems, is affected by one's categorization within the demographic variables investigated. Coherence among the significant effects is sparse. Most usefully the coherence of the results provide a source of hypotheses which may be tested by "small" specifically designed experiments. Least usefully, the sparse coherence suggests that, in a sample of the kind investigated, the effect of these demographic variables, when properly controlled for age and sex effects, is minimal.

Attentional Processes

Simple demographic effects. Boredom is lower and attentiveness higher for those with an M.A. or M.S. degree when compared with those with a high school diploma or a B.A. or B.S. degree. Likewise Upper-Middle-Class and above reported lower boredom than Middle-Class and below. Mindwandering is reported as less by those with income of $30,000 or more. The pattern suggested here is one of greater attentiveness and concentration in those at the higher levels of education, social status and economic success. These people also characterize their overall life style as more interesting and less boring.

People with degrees in the arts characterize their mental processes as faster than those with degrees in the sciences or only from high school. Those with an M.A. or M.S. had fewer periods in which their mind was blank. These results suggest that those with post-graduate degrees in the arts have conscious minds which are more likely occupied with a fast changing stream of thoughts than are post-graduate science degrees or high school graduates.

The sex interaction. No patterns were suggested from the obtained significant Sex by Demographic Variable Interactions.

The age interaction. Mindwandering was reported as decreasing with age for those with a Master's degree but somewhat increasing with age for those with lesser degrees. Task distractability with competing external stimuli showed the same age relationship as mindwandering after age 44 with regard to highest degree and years of education. There is a suggested relationship here among educational experience, age cohort and self-control or mental discipline or singlemindedness.

Curiosity

Simple demographic effects. Curiosity sbout the intimate-private lives of people was lowest in those with a high school diploma, highest in those

with degrees in the sciences, and intermediate in those with degrees in the arts. Intimate-private curiosity also increased with number of years of education. Curiosity about things or mechanical objects was lowest in those with an arts degree, highest in those with a science degree, and intermediate in those with a high school diploma. Atheists, agnostics, non-denominational Christians, and members of non-Western religions reported higher curiosity about things than Catholics or Protestants who were equal in such curiosity. What is suggested by these results is that those with science degrees who, perhaps, are also less likely to belong to an organized religion, have the highest level of curiosity regardless of the nature of that curiosity.

The sex interaction. Curiosity about people in a general or social way was higher for women than men. Men with a high school education were highest in general-social interpersonal curiosity while women were lowest. For women either an arts degree or a more advanced degree resulted in greater general-social interpersonal curiosity. These results suggest that education tends to increase male-female differences in general-social curiosity about people.

The age interaction. No patterns were evident from the two significant demographic variable by age interactions.

II. Interrelations among daydreaming characteristics, health, and estrogen in pre-menopausal, menopausal, and post-menopausal women. A prior study of sex differences in daydreaming (see 1976-77 annual report) found a large drop in likelihood of sexual daydreaming in women at age 50 years as compared with women 35 to 49 years. Since age 50 is the menopausal transition period for most women, it was decided to investigate this potential relationship more closely. Information on daydreaming, menopausal state, health, demographic variables, and drug usage has been collected on 476 women 40 to 60 years of age. This information has been coded and punched. It is currently being analyzed statistically.

III. Vigilance and daydreaming incidence.
The vigilance task described earlier is being used to measure directly the incidence of daydreaming and mindwandering for people across the life-span. A life-span sample is being used since the self-reports of subjects, based on the Imaginal Processes Inventory, suggest that with increasing age that 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, about 35 longitudinal participants have participated. They ranged in age from 31 to 79 years old. Analyses based upon this small sample indicated that daydreaming and mindwandering does seem to drop after age 60. Data collection continues.

The Mackworth Clock vigilance task described earlier will also be used in a longitudinal study of subjects who participated in the identical study in 1963. Measures of reaction time and galvanic skin response will also be taken. These same subjects will receive the first vigilance task and thus

will allow correlations to be made among the present tasks as well as with tasks performed 17 years ago.

IV. Dimensions of the stream of thought across the life-span.
In a small study of adults 17-30 years of age, one of the cooperating investigators, using the thought sampling procedure described earlier, has been able to find consistencies in both the number and kind of dimensions which characterize the stream of thought. A study involving the life-span has begun using the thought sampling technique. The purpose of this study is to determine the extent to which the stream of thought may be different at different ages.

V. A longitudinal study of daydreaming and related mental activity.
Starting in January, 1979, men in the Baltimore Longitudinal Study of Aging again began completing the IPI. Some of these men have not taken the IPI before but most took it 5-6 years ago. This data collection will continue until the entire BLSA population of men has been completely tested or retested. In addition 17-23 year old subjects are being recruited from area colleges and universities. As women enter the study, they also will be tested and a limited number will be retested.

VI. Australian-American differences in daydreaming, attentional processes, and curiosity. Using responses to the Imaginal Processes Inventory with an Australian sample, comparisons were possible on cultural differences in daydreaming, attentional processes and curiosity. The Austrialian sample consisted of students of Macquarie University; there were 17-23 year old males and 17-44 year old females.

A Nationality X Sex analysis of variance (ANOVA) for 17-23 year olds and a Nationality X Age ANOVA for 24-44 year old females was performed on each of the 33 factors of the IPI. The results of these ANOVAs in terms of significant effects are presented. For clarity of exposition, this description of significant effects omits any mention of probability of Type I error.

For females, the belief Daydreaming as Acceptable Adult Behavior was significantly greater for Americans than for Australians. Daydreaming Frequency, while not significantly affected by Nationality or its interactions in the 17-23 year old ANOVA and the 24-44 Females ANOVA, was significantly affected by Nationality in an additional ANOVA of females 17-44 years old; American women 17-44 years showed somewhat more daydreaming frequency than Australian women. For women 24-44 years, the Nationality X Age interaction effect was significant for the factor measuring Repetitive Absorption in a Daydream; from age 24-34 years Americans had greater values while from 35-44 years Australians had greater values.

Positive or Pleasant aspects of Daydreaming was significantly greater among Australians 17-23 years old than among American 17-23 years old; no nationality difference was noted for women after age 23 years. With Negative or Frightening Aspects of Daydreaming, Nationality significantly interacted with Sex for 17-23 year olds, and with Age for 24-44 year old women. Among 17-23 year olds, American men had greater means than Australian men, but

American women were equal to Australian Women. Among women, from age 24 to 39 years the greater means for Americans steadily approached the mean values of Australians; Australian women showed a large increase in Negative or Frightening Aspects of Daydreaming in the 40-44 year olds.

Daydreaming Imagery: Visual-Auditory was significantly effected by the Age X Nationality interaction; visual-auditory daydreaming imagery showed a steady drop with age for Americans while Australians showed no consistent age trend. An Age X Nationality interaction also was found for Auditory Imagery: Non-voice sounds; Americans had greater means than Australians from 24-39 years with both nationalities showing an age drop. At the 40-44 year old age group American women continued to drop in non-voice auditory imagery but Australian women dramatically rose to the highest level of all age groups studied.

Among 17-23 year olds, Australians had significantly greater mean values on factors measuring sexual and bizarre-improbable daydreaming content and significantly smaller mean values on factors measuring problem solving and criminal daring content. Among 24-44 year old women there was a significant Age X Nationality effect on factors measuring Achievement-Oriented Daydreams and Bizarre-Improbable Daydreams. For both types of daydreams, Australians had: (a) Smaller means than Americans age 24-29 yrs, (b) greater means than Americans age 35-39 years and (c) essentially equal means age 40-44 years. With 30-34 year olds, Achievement-Oriented Daydream mean values for Australians was greater than for Americans and for Bizarre-Improbable Daydreams the mean for Americans was greater than for Australians.

With regard to the temporal orientation of daydreams, Australians had significantly greater means than Americans on the factor The Present in Daydreams for 24-44 year old females and for 17-23 year olds of both sexes. Also for 17-23 year olds, Daydreams of the Personal Future was significantly effected by Nationality; Australians had smaller means than Americans.

Nationality differences were found on Mindwandering, Mentation Rate and Unblank (Occupied) Mind Likelihood among the 17-23 year olds; among 24-44 year old women only Mentation was significantly effected by Nationality. Australians had greater values on Mindwandering and Mentation Rate and had smaller values on Unblank Mind Likelihood.

Among 17-23 year olds, Australians showed a significantly lower mean on the factor measuring Uninteresting-Boring Lifestyle. Australian 17-23 year old Females showed a lower mean than Americans on the Factor measuring the Need to Do Risky Things; little Nationality difference occurred for males.

Impersonal-Mechanical Curiosity had a significantly smaller mean for 17-23 year old Australians than for 17-23 year old Americans. Interpersonal Curiosity: General-Social had a significantly greater mean for 24-44 year old female Australians when compared with 24-44 year old American women.

Nightdreaming Frequency, as recalled, was significantly lower for Australians than for Americans, for 17-23 year olds and for females 24-44 years old.

Significnance to Bio-Medical Research and Program of the Institute: The study of daydreaming is fundamentally a study of thought processes. In order to understand fully the thought processes of man, the total spectrum of those processes meeds to be examined. In addition, it is important to know how this wide spectrum is affected by aging. Thus the study of daydreaming in adults, along with other variables, such as differences in age, socio-economic status, attitudes, etc., may help us understand the fundamental processes which underlie all these behaviors.

Proposed Course of Project
Since many of the present experiments are in the early portions of the 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.

Publication:
Giambra, L.M. A factor analysis of the items of the Imaginal Processes Inventory. Journal of Clinical Psychology, 1980, 36, 383-409.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00063-13 LBS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)

Learned Modification of Visceral Function in Animals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Joseph	Senior Staff Fellow	LBS, GRC, NIA
Other:	B. T. Engel	Chief, LBS	LBS, GRC, NIA
	S. P. Tzankoff	Senior Staff Fellow	CPB, GRC, NIA
	G. S. Roth	Research Chemist	CPB, GRC, NIA
	C. Filburn	Research Chemist	LMA, GRC, NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Psychophysiology

INSTITUTE AND LOCATION
GRC, NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.95	1.70	2.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 reflex adjustments of the cardiovascular system can be modified by instrumental cardiovascular conditioning (ICC). Thus far, three procedures have been utilized. In the first, electrical stimulation is administered to localized CNS cardioacceleratory-pressor or cardiodeceleratory-depressor sites, e.g., hypothalamus, and analyses are made to determine if the animal can learn to alter their effects; in a second procedure animals are trained to perform on a food-reinforced exercise task and the ability of the animal to control heart rate during combined exercise and ICC sessions is examined; in a third, pharmacological agents which produce pressor or depressor responses are given during ICC and/or during control periods and baroreflex sensitivity is examined. In other experiments we examine age-related changes in pre- and post-synaptic portions of nigrostriatal pathways of the rat using behavioral and biochemical analytical methods.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A-C. To determine the neural mechanisms involved in cardiovascular conditioning in monkeys.

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

Methods Employed: A. There have been a great number of experiments in which electrical stimulation of the brain (ESB) has been delivered to various CNS structures and subsequent cardiovascular changes examined. However, most of these studies have been carried out in infraprimates. In the few studies which have been done in primates the animal was anesthetized and unable to interact with its environment. Therefore, there was no chance to observe the possible interrelationships among ESB, cardiovascular changes and behavior of the animal. The present experiments were designed both to examine these relationships and to carry out a thorough mapping of the cardiovascular control areas in the infrahuman primate. Three monkeys were operantly conditioned to speed and to slow heart rate (HR) in sessions comprised of a 512 sec baseline period and a 2048 sec testing period. Three types of sessions were examined: "speeding sessions" (F) during which the animal was operantly trained to speed its heart, slowing sessions (S) during which the animal was trained to slow HR, and no feedback (NF) sessions in which the animal was not required to speed or to slow HR. It simply sat in a closed booth throughout a 512 sec baseline period and a 2048 sec "training" period. Information on speeding and slowing was provided by lights. A red light indicated that the animal was to slow HR and a 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 128 sec duration. Following training, an acrylic platform containing holes drilled to pre-specified anterior-posterior and lateral coordinates approximately corresponding to those of the anterior hypothalamus (AH), posterior hypothalamus (PH), subthalamic nucleus (SN), and the striate cortex (SC) was stereotoxically mounted on the monkey's skull and cemented in place under aseptic conditions. Following a 1 week recovery period, the animal was given .5 mg/kg of phencyclidine, i.m., and electrodes were then passed through the skull and lowered by hand until a suitable (see below) cardiovascular effect was obtained. Stimulation was delivered and the cardiovascular changes observed by examining BP and HR tracings on a polygraph. 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. Dose response curves were carried out in the fully awake animal and intensities were adjusted to produce a 10-20 BPM change in HR. Intensities 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 except that each segment and the baselines were shortened to 64 sec and 512 sec respectively. Stimulation for each session was delivered on alternate segments. Only one area was studied within a set of 3 sessions (S, F, NF). At present only accelerator-pressor areas have been examined. Two areas were examined/day on alternate days; data were analyzed by examining changes from baseline during each stimulation and non stimulation segment.

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 either 4 or 6 kg, depending upon the size of the animal, in order to receive a 300 mg food pellet. The monkey was trained to receive a reinforcement on a fixed ratio schedule of reinforcement: 1 pellet for each 12 lever pulls (FR-12). HR, SBP, DBP, and number of lever pulls and O_2 consumption were measured during each session. Once stable exercise rates were achieved, each animal was trained as previously described (A) to slow its HR. HR, SBP, DBP, and O_2 consumption were measured during these sessions as well. Once the 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 previously mentioned were recorded.

C. There is an abundance of evidence that the cardiovascular (CV) system is modulated by neurogenic reflexes. However, the emphasis on reflex modulation of CV function has fostered a lack of appreciation of the extent to which behavioral events also can modulate CV function and even can inhibit reflexes. One example of this reflex inhibition is the case of baroreceptor inhibition during exercise. It is the purpose of this series of experiments to show that baroreceptor sensitivity also can be modified in experiments in which animals are operantly conditioned to slow or speed HR. Two monkeys were trained to control HR as previously described. After they were fully trained each animal had a catheter chronically implanted in the cephalic vein with its tip in the subclavian vein. This cannula permitted us to inject phenylephrine or nitroglycerin to raise and to lower BP respectively during F, S, or NF sessions. HR, SBP and DBP were monitored on a beat-to-beat basis as previously described. Each animal received 60 baroreceptor tests with each drug for a total of 120 tests. Twenty tests were given under each of the three experimental conditions (S, F, NF). All 60 of the tests with phenylephrine were completed before any of the nitroglycerine tests were initiated. Baroreceptor sensitivity was determined for each drug by measuring the linear rate of change of heart period (in ms.) during the drug induced blood pressure rise. For phenylephrine the sensitivity (ms/mm Hg) was measured over 6 to 8 consecutive heart beats; for nitroglycerin sensitivity was measured over 12 to 15 consecutive heart beats.

D. It has been reported previously that a surgical lesion in the substantia nigra of young rats results in a substantial decline in the level of dopamine (DA) in the caudate nucleus on the lesioned side. The caudate

nucleus has been shown to be very important in the control of movement. An imbalance in the neurotransmitters between the lesioned and unlesioned nigrostriatal pathways produces a directional dominance in an animal's movements such that after injection of certain drugs such as apomorphine (a DA receptor agonist) or amphetamine (which promotes the release of DA) animals can be induced to turn in circles. These rotations can be accurately measured with a device called a rotometer. Numerous studies have shown that: (a) amount of depletion; (b) DA receptor activity; (c) development of denervation receptor hypersensitivity; and (d) presynaptic release events can be gauged by the strength and directionality of the rotational behavior. This behavior seems to be almost exclusively modulated by the nigrostriatal system and by dopaminergic-cholinergic interactions within this system. In the present experiments lesions were produced unilaterally (left side) in the substantia nigras of young (4-6 mo) and old (25-29 mo) male and female rats following several prelesion baseline measures of rotational behavior. Following a 7-10 day recovery period rotations to a 2 mg dose of amphetamine were examined. Animals exhibiting fewer than 75 rotations in 30 min were discarded from the study. In one series of experiments the rotational behavioral method was used to examine what pharmacological agents might be utilized to overcome deficits in turning behavior seen in old animals. Old and young animals were given 1 mg/kg of amphetamine on alternate days (3 doses/wk) for 40-50 days after surgery. Very stable response rates were achieved with this method. They were then given 3 graded doses of L-DOPA alone (2,3,5 mg/kg) on alternate days. Following these initial tests the animals were given 5 mg/kg dose of L-DOPA 1 hr prior to receiving a 1 mg/kg dose of amphetamine and tested for 1/2 hour. The L-DOPA-amphetamine combinations were replicated 3 times in each animal. Dopamine levels were assayed in the left and right striata from these animals to determine the efficacy of the lesion. A parallel group of lesioned old and young animals was used to assess striatal dopamine levels 1 hr after an L-DOPA injection. Dopa decarboxylase and tyrosine hydroxylase were assessed in still another group of old and young animals.

E. In a second series of experiments development of post-synaptic hypersensitivity was investigated in lesioned young and old male and female rats in order to determine if differences existed in the proliferation of differential dopamine (DA) receptors as a function of age. Following a recovery period (7-10 days), the lesioned animals were given an initial test with amphetamine and non turners were discarded. After a 30 day waiting period, the animals were tested for the development of striatal DA receptor supersensitivity with the DA agonist lergotril. Three weeks later they were sacrificed and DA receptor activity was analyzed by measuring the amount of [3H] spiroperidol binding and DA stimulated adenyl cyclase activity in the striata from the lesioned and unlesioned hemispheres.

F. In a third series of experiments we have begun to inject small quantities of DA releasing agents (e.g., amphetamine (10 ug)) and agonists (dopamine (50 ug)) into the striata through chronically implanted cannulas in rats unilaterally lesioned in the left substantia nigra. Rotational behavior is examined following these injections.

G. Finally, in a forth series of experiments, nialamide, a monoamine oxidase inhibitor, was peripherally administered at various doses, and several different stereotypic behaviors postulated to be related to striatal DA activity (e.g., gnawing, sniffing, rearing, grooming) were examined. Additionally, open field locomotor activity, and emotionality also were assessed in order to determine if there were any age-related differences in these behaviors following nialamide.

Major Findings: A. Thus far the effects of ESB on HR and BP during S, F, and NF have been analyzed in three monkeys from 4 brain regions: (I) lenticular nucleus, globus pallidus, ventromedial tegmentum; (II) anterior or dorsomedial hypothalamus; (III) posterior or lateral hypothalamus; (IV) subthalamic nucleus. A fourth monkey died before completion of the study and only 2 brain areas were examined in it (I,III). Results showed that there were significant interactions between experimental conditions (S,F,NF) and ESB when changes in HR (ΔHR), SBP, and DBP from baseline to each stimulation (ST) and non stimulation ($\overline{ST}$) segment was analyzed using a 2 (ST; $\overline{ST}$) by a 3 (S,F,NF) mixed model analysis of variance: (a) For each animal during slow sessions Δ HR was significantly attenuated when ESB was given in regions I or III but not when regions II or IV were stimulated; (b) During speeding Δ HR either was enhanced or was similar to Δ HR during NF but this varied among animals and regions; (c) BP was not reliably altered during ST--i.e., there were no consistent effects across animals. Thus, these experiments show that animals can alter the effect of ESB through behavioral conditioning and that the degree of alteration can be very great. Results for a typical animal are shown below for Δ HR in beats/min. Note that the scores in this table are changes from baseline. Thus, ST effects are superimposed on pre-existing performance levels. Compare ST and $\overline{ST}$ during S or F with ST and $\overline{ST}$ during NF.

	S		NF		F	
REGION	ST	$\overline{ST}$	ST	$\overline{ST}$	ST	$\overline{ST}$
I	-32.3	-33.8	1.1	-7.3	20.5	13.5
II	-3.9	-21.2	51.1	29.8	42.0	26.4
III	-14.1	-14.1	-1.0	-10.8	22.5	8.6
IV	11.0	-10.3	23.0	9.6	29.8	14.0

B. Two animals have been fully tested so far. However, only the results for one animal will be discussed since the second animal performed in a way which rendered its results uninterpretable. Table 1 presents the results. During exercise sessions the monkey increased its HR from a baseline rate of 130.9 BPM to a rate of 199.4 BPM (68.5 BPM). During sessions when exercise was combined with operantly conditioned cardiac slowing the baseline HR and the change in HR was comparable to the exercise only sessions. Likewise, the change in O_2 consumption during exercise and combined exercise and HR slowing also were similar (69.8 ml/min and 70.6 ml/min, respectively). The change in SBP was significantly greater during exercise (21.8 mm Hg) than during combined exercise and slowing (17.6 mm Hg). Thus, the change in the product of HR and SBP--which is an index of left ventricular work--rate-pressure product (RPP)--was greater during exercise sessions than it was during the combined sessions (1693.7 mm Hg-BPM and 1269.1 mm Hg-BPM respectively). The major

difference was that the animal worked much more during the combined sessions (48.9 bar pulls/min) than in the exercise only sessions (31.6 bar pulls/min). Thus, the animal worked about 55% more, but did only about 75% of the cardiac work while it was slowing its HR. Other analyses showed that the rate at which the animal increased its cardiac work (RPP) per unit physical work (bar pulls) was significantly less during the combined procedure (97.6 mm Hg BPM/bar pull) than during the exercise only procedure (130.6 mm Hg BPM/bar pull). These data suggest that at least some aspects of what has been called physical conditioning can be explained as behavioral conditioning. Note the relationship of this experiment to comparable experiments in our human clinical research program (Learned modification of visceral function in man, subsections B and C).

Table 1

	HR (BPM)		SBP (mm Hg)		O_2 consumptions (ml/min)		Work
	Base	Exercise	Base	Exercise	Base	Exercise	pulls/min
Exercise	130.9	199.4	96.4	118.2	50.6	121.2	31.6
Exercise + HR slowing	129.2	197.3	113.9	131.5	68.7	138.5	48.9

C. The tables below show the major findings of this study. The two animals tested thus far have responded with identical patterns. During slowing baroreceptor sensitivity was similar to the control condition when phenylephrine was injected, but was significantly inhibited during nitroglycerin injection (Table 1, I). During speeding baroreceptor sensitivity was significantly inhibited during both drug conditions (Table 1, II). Since SBP reactivity was not attenuated during either of the experimental conditions relative to the control condition (Table 2), the experimental effect clearly influenced the cardiac response.

Table 1

Cardiovascular adjustments to baroreceptor stimulation during operant cardiac conditioning (ms/mmHg)

I. Nitroglycerin

Animal	Doseage	Condition			F-ratio‡
		Slow	Speed	Control	
1	100 ug/kg	1.15	0.96	1.81	3.85*
2	200 ug/kg	0.23	1.28	2.56	5.90**

II. Phenylephrine

Animal	Doseage	Condition			F-ratio‡
		Slow	Speed	Control	
1	20 ug/kg	1.52	0.91	1.69	6.10**
2	25 ug/kg	2.45	0.21	2.64	11.07**

‡ df = 2,57; *$p<.05$; **$p<.01$

Table 2

Systolic blood pressure ranges during baroreceptor sensitivity testing.

I. Nitroglycerin

Animal	Condition			F-ratio‡
	Slow	Speed	Control	
1	24.6	29.6	23.4	7.41**
2	19.3	27.2	17.7	6.13**

II. Phenylephrine

Animal	Condition			F-ratio‡
	Slow	Speed	Control	
1	38.4	37.2	32.7	3.17*
2	24.4	21.2	23.0	0.93

‡df = 2,57; *p<.05; **p<.01

The results of these experiments show that in both cases where the animals had to override the baroreceptor reflexes in order to escape electric shock they did so. Thus, it would seem that conditions which threaten the well being of the animal will produce behaviorally mediated adjustments in CV reflexes, and a learned response will take precedence over a reflex.

D-G. The studies reported in this program are inter-related and will be reported together. They have concentrated on trying to determine the striatal locus (presynaptic or postsynaptic or both) of the age-related deficits previously observed in rotational behavior following amphetamine administration. In the intitial experiment L-DOPA was administered prior to amphetamine in order to see if a precursor of DA would raise DA levels in the old animal and subsequently enhance rotational behavioral response strength. Results showed that young rats increased their left/right turn ratios by 50% while old rats showed a 23% decline in responding. This finding contributed to a significant statistical interaction between age and the presence or absence of L-DOPA pretreatment ($F(1,27) = 26.79$ $p<.001$) ($\bar{X}$ L/R young amphetamine alone 100 $\pm$ 8.0, $\bar{X}$ amphetamine + L-DOPA 200 $\pm$ 10; $\bar{X}$ old 62 $\pm$ 7.7, $\bar{X}$ = 55 $\pm$ 7.8). This suggests that at least part of the behavioral deficit seen in the senescent animal may be presynaptic, possibly in the synthesis of DA. Analyses of striatal DA levels in young and old animals given saline or L-DOPA prior to sacrifice, confirmed these hypotheses by showing that L-DOPA stimulated young animals exhibited higher DA levels than young animals not given L-DOPA prior to sacrifice. Old animals showed the same DA levels whether they were stimulated with L-DOPA or not ($\bar{X}$ young unstimulated = 12.16 ug/g DA $\pm$ .85; $\bar{X}$ old = 19.99 $\pm$ 3.20; $\bar{X}$ young stimulated = 25.22 $\pm$ .62; old 19.67 $\pm$ 3.01). These findings indicate that there was some loss of ability of sensecent animals to synthesize DA from the exogenously administered L-DOPA. However, the DA levels in the unlesioned striata of the old

animals were extremely high relative to the DA levels in the unlesioned striata of the young animals when these groups were not given L-DOPA before sacrifice (see above) suggesting that there is not normally a deficit per se in DA levels in the striatum of the senescent rat. This was further supported by subsequent biochemical analyses of tyrosine hydroxylase and dopa decarboxylase, two enzymes important in the synthesis of DA. Neither enzyme was found to be depleted to any appreciable degree in the old animal. Thus, the presynaptic deficit may be in the release or metabolism of DA. There is also some indication of receptor involvement in these behavioral deficits in old animals since a DA receptor binding assay with [3H] haloperidol indicated a 33% loss in striatal DA receptor number with senescence. A subsequent experiment in which DA was injected directly into the striata of young and old rats showed that the young animals exhibited significantly higher rotational behavior strength than the old animals ($\bar{X}$ young 25.4 $\pm$ 4.9 old = 10.8 $\pm$ 1.64 $t(18) = 2.84$ $p<.02$). Thus, at this point we can say that although more experiments are needed, the data suggest that the response deficit in the old animal may be associated with a decline of DA receptors in the striatum as well as with some defect in DA release or metabolism.

Interestingly, the old animal can exhibit some plasticity in the face of such deficits, since one set of experiments recently completed have shown that similar denervation hypersensitivity concomitant with striatal DA receptor proliferation following lesions of the substantia nigra takes place in both old and young animals. The lesions in these animals were all chemically (6-OHDA) produced in the left substantia nigra. Therefore, following the 30 day recovery period, the animals turned contralaterally to the lesion (right) following lergotril administration. A behavioral index of right/left turns was computed. No age-related differences in this index were seen. Three weeks after these tests the animals were sacrificed and ratios of adenyl cyclase activity and [3H] spiroperidol specific binding in the lesioned left striatum relative to the unlesioned right striatum were computed (L/R). These values are given in Table 1.

Table 1
Left/Right Striatal Ratios for 3[H] Spiroperidol Binding and DA Stimulated Adenylate Cyclase Activity

	N	[3H] Spiroperidol Specific Binding	100 uM AC	5 uM AC	1 uM AC
*Mature males	6	1.37 $\pm$ 0.12	1.28 $\pm$ 0.05	1.78 $\pm$ 0.18	2.48 $\pm$ 0.60
Senescent male	6	1.48 $\pm$ 0.07	1.48 $\pm$ 0.07	1.78 $\pm$ 0.22	2.05 $\pm$ 0.18
Mature females	4	1.51 $\pm$ 0.26	1.25 $\pm$ 0.07	1.56 $\pm$ 0.27	2.00 $\pm$ 0.38
Senescent females	5	1.44 $\pm$ 0.19	1.14 $\pm$ 0.14	1.42 $\pm$ 0.34	2.00 $\pm$ 0.63

*Values represent means $\pm$ standard error for numbers of experiment indicated.
+The [3H] spiroperidol concentration which gave the highest amount of specific binding was used to calculate these ratios.

As can be seen there are no systematic age or sex differences in these ratios, indicating that old animals had not lost the ability to proliferate receptors

following lesions--"Plasticity in the face of a deficit." We also showed in this same experiment that rotational behavior is more highly correlated with DA receptors linked to [3H] spiroperidol specific binding than to those linked to adenyl cyclase.

Finally, we have begun to examine the effect of monoamine oxidase inhibition (with nialamide) on many dopamine-mediated stereotypic behaviors in young and old rats. Early results have shown that nialamide reduced these behaviors (e.g. gnawing, sniffing, locomotor activity) in a dose dependent manner in both age groups. However, more reduction was seen in one behavior, rearing, in the young group following nialamide than in the old group. Rearing is a behavior that is very strongly mediated by dopamine. The fact that it is not depressed following inhibition of monoamine oxidase in the old animal suggests that rearing is so low that it cannot be lowered any further with nialamide administration. At present we cannot yet explain the paradoxical effect that monoamine oxidase inhibition is having on rearing. Several possibilities are presently being explored.

Significance to Bio-medical Research and the Program of the Institute: There is growing evidence that the central nervous system provides a high degree of plasticity for peripheral CV and motor functions. Thus, responses which ordinarily occur in one way under one set of conditions, may occur in another way under another set of conditions. For example animals which can learn to modulate their CV responses during such behaviors as predation or evading or escaping predators should have selective advantages over conspecific animals not so well endowed. Thus, learning is an important process which can operate through the nervous system to modulate peripheral function. A second process which may actually reduce plasticity and response capabilities is aging. Until we understand fully how these processes operate, and under what conditions they will express themselves, we will never be able to explain behavior in normal or diseased individuals, or in subjects of any age. Furthermore, when we understand the principles which underlie neurally mediated plasticity, we should be able to evolve strategies which will enable subjects to compensate, at least in part, for the infirmities associated with the disorders of adult life. For example, the neural systems that we are now studying in the primate projects deal with the integration of somato-motor and cardiovascular responses. It is well-known that both of these systems become less adaptable with age; but what is not known is the extent to which this diminished adaptability can be modified by experience. Moreover, we believe that the experiments involving the interaction of ESB and behavior during ICC, and the experiments concerned with instrumental modification of HR during exercise together with those concerned with alterations in baroreflex sensitivity lend themselves well to delineation of the contribution that experience makes in modifying neural events. Another example of the relevance of this project to the assessment of age-dependent neural changes in plasticity comes from the work on the nigrostriatal system. It is well-known that neuromuscular disorders of late adult life such as Parkinson's Disease are associated with various changes in function and structure of the nigrostriatal system. It is our goal to characterize these age-related declines and the extent to which they can be ameliorated.

Proposed Course of the Project: A,B,C. Data collection is continuing on the effects of stimulation of various anterior hypothalamic, thalamic, striatal and posterior hypothalamic pressor, depressor cardioacceleratory and decelleratory areas during the three training procedures, i.e., fast, slow and no feedback, in order to further characterize the effects thus far observed. In later experiments attempts will be made to place electrodes more posteriorly in brain stem sites to determine if stimulation of these areas can be altered during ICC. Additionally, efforts will be made to determine the vagal and sympathetic contribution to these changes through the use of various peripheral adrenergic and cholinergic agonists and antagonists. Central neurohormonal characterization of these extralimbic and limbic sites will be made by the central injection of various pharmacologic agents into chronic indwelling cannulae implanted in these areas. The exercise experiments are also progressing. Additional animals will be taught to speed HR during exercise and the combined effect of these conditions observed. Flow probes will be placed around the aortas of these animals so that cardiac stroke volume as well as the other measures can be taken during combined and uncombined conditioning and exercise sessions. In future research we also shall try to determine whether attenuation of the baroreceptor reflex which occurs during operant HR conditioning is due to the modulation at the receptor site, the afferent limb of the reflex or the efferent limb of the reflex.

D-G. So far the project has concentrated on the dopamine system. Further investigations will examine turning behavior after modifications on serotonergic, noradrenergic, and cholinergic systems. We will continue to analyze the effects of intracerebral administration of the test drugs on rotation in young and old animals, and we will begin to look at synaptic events such as release, uptake and turnover in striatal slices from young and old animals.

Publications:

Gottlieb, S.H. and Engel, B.T.: Autonomic interactions in the control of heart rate in the monkey. Psychophysiology, 16: 528-536, 1979.

Joseph, J.A., Boggan, W., and Powell, D.A.: Adrenal demedullation and peripheral 6-OHDA administration in the rabbit: Effects on body weight, general activity and cardiovascular responsivity. Pharmacol. Biochem. Behav., 10: 875-881, 1979.

Joseph, J.A. and Engel, B.T.: Nervous control of the heart and cardiovascular system. In Weisfeldt, M.L. (Ed.): Aging Heart (Aging, Vol. 12). New York, Raven Press, 1980, pp. 101-115.

Joseph, J.A. and Powell, B.A.: Peripheral 6-Hydroxydopamine administration in the rabbit (oryctolagus cuniculus): Affects on Pavlovian conditioning. J. Comp. Physiol. Psychol., in press.

Joseph, J.A., Filburn, C., Tzankoff, S.P., and Engel, B.T.: Age-related neostriatal alterations in the rat: Failure of L-DOPA to alter behavior. Parmacol. Biochem. Behav., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00064-19 LBS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Problem Solving and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PIs:	David Arenberg,	Chief, Learning & Problem Solving Section	LBS GRC NIA
	Leonard M. Giambra	Research Psychologist	LBS GRC NIA

Others: None

COOPERATING UNITS (if any)

Baltimore City Hospitals
Jan Sinnott, NRSA postdoctoral fellowship (sponsored by David Arenberg)

LAB/BRANCH

Gerontology Research Center - LBS

SECTION

Learning and Problem Solving Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	.5	1.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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. 10-76)

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: All of the three experiments in progress are long-term aging studies. Experiments IV and V are longitudinal studies; and in Experiment XI each subject solves more than 100 problems and a model is constructed which describes and predicts how he or she solves a wide variety of concept-learning problems.

Experiment V is a longitudinal study of concept identification. In this study, subjects select instances (examples) sequentially and are informed whether each selection is a positive example of the concept or a negative example. On the basis of the information elicited by these selections and their classification, the specific attributes of the concept can be identified. This procedure provides a measure of information gain for each selection. Ordinarily when subjects select instances, logically equivalent selections do not always result in equivalent information gains, and fortuitous selections can alter drastically the difficulty of a problem. A technique was developed which provides equivalent information gains for logically equivalent selections and avoids fortuitous gains in information. This technique also has two other important characteristics: (1) different types of problems which are logically equivalent can be constructed and compared, e.g., conjunctive concepts in which two attributes must both be present can be matched with two-attribute disjunctive concepts in which either attribute can be present; and (2) initial information gain can be manipulated because the experimenter can minimize or maximize the information gain for each selection. Experiment V was designed to study age differences and age changes for six different types of problems determined by the number of attributes in the concept (one or two), and for two-attribute problems, the concept rule (conjunctive and disjunctive) and the amount of initial information gain.

Major Findings: Experiment V is the longitudinal study of concept problem solving and age. Previous analyses of number of problems solved correctly have shown: (1) substantial cross-sectional age differences; (2) longitudinal age changes in six years only late in life; and (3) estimates of age changes within birth cohorts (based on regression procedures developed in this Section) only for the earliest born (oldest) cohort. During this year, analyses were carried out to determine: (1) whether specific types of problems accounted for the relationship between age and magnitude of change in number of problems solved correctly; and (2) whether a relationship between

age and magnitude of change would be found for measures of effectiveness for correct solutions in each of the twelve problems.

In order to determine whether specific types of problems accounted for the relationship between age and magnitude of change in number of problems solved correctly, the following subsets of four problems were analyzed: (1) all four problems with one-attribute solutions; (2) the four conjunctive problems; (3) the four disjunctive problems; (4) the four two-attribute problems with high initial information; (5) the four two-attribute problems with low initial information. Each subset of four problems was based on the 327 men for whom correctness measures were available for all twelve problems at both times of measurement. The four one-attribute problems are the easiest. Although the mean number of problems solved correctly decreased virtually monotonically over the seven decade age groups (from the twenties to the eighties) both at first testing and at second testing six (or more) years later, for each age group the means were nearly the same at the two times of measurement. There was almost no change in means for any age group for the four one-attribute problems. The four conjunctive problems also showed a monotonic decrease in means over the seven age groups at both times of measurement. Except for the youngest group who improved, the means for each age group were about the same at the two times of measurement. Similarly, the four disjunctive problems showed monotonic (or nearly monotonic) decreases with age at both times. Although the largest mean declines were found for the men initially in their sixties and seventies, the correlation between magnitude of change and age was only .05. The four high-initial-information problems also showed monotonic (or nearly monotonic) decreases with age at both times. The mean changes also decreased with age. The three youngest groups improved, and the largest mean improvement was found for the youngest group. The oldest groups declined with the largest decline found for the oldest group. The correlation between age and magnitude of change was -.09 which was similar to the correlation for all twelve problems. The four low-initial information problems showed nearly monotonic decreases in means with age at both times, but the first and second means for each age group were nearly the same. Although these were the most difficult problems, there was no relation between age and magnitude of change.

These problems were designed to provide measures of effectiveness in reaching a solution for all problems solved correctly. For each of the twelve problems, those participants who solved the problem correctly both times were included in the analysis for that problem. For all twelve problems, mean effectiveness measures decreased monotonically (or nearly monotonically) at both times. Furthermore, for eleven of the twelve problems, the correlation between age and magnitude of change was negative. For seven problems, the negative correlations were larger than the correlations for number of problems correct. Although the correlations were low between age and magnitude of change both for number of problems solved correctly and for measures of effectiveness in problems solved correctly, the consistency of the correlations indicates that younger men tend to improve and older men tend to decline in performance over a six-year period. The mean declines are probably conservative, especially for the effectiveness measures; for those measures, only participants who solved a particular problem correctly both

times were included. Even when all current information is available for review (as it is in all of these problems), thereby reducing the memory load, the elderly who solved problems correctly solved less effectively than the younger men and declined in effectiveness over the six-years between measures.

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 for the longitudinal study of concept problem solving (Experiment V) has been initiated with women and will continue for both men and women. Data collection for Experiment IV, another longitudinal study of reasoning performance, will also continue. Models which describe and predict how an individual solves concept-learning problems continue to be constructed for participants in Experiment XI. In the two longitudinal studies, all current information is available for review to minimize memory components of the task. In Experiment XI, the role of memory is very important in each individual's model. Although it is unlikely that new studies of reasoning will be initiated with the current staff, a new study of memory for logically related sentences (Experiment XXXVI, Project Z01 AG 00065-20)in this Laboratory will provide information about how memory performance of young and old adults is affected when inferences can be used to enhance memory.

Publication:

Giambra, L.M., and Arenberg, D. Problem solving, concept learning, and aging. In L. Poon, et al. (Eds.): Aging in the 1980's: Selected contemporary issues in the psychology of aging. American Psychological Ass'n., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00065-20 LBS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Verbal Learning and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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

Others: None

COOPERATING UNITS (if any)

Elizabeth A. Robertson-Tchabo — Baltimore City Hospitals, University of Maryland

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning and Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	.4	1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The primary purposes of this project on aging are to identify measures of verbal 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. 10-76)

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: Experiment XXXIII is a recently initiated longitudinal study which includes immediate free recall, delayed memory, dichotic listening, forward digit span, simple reaction time, and four levels of choice reaction time. These procedures (somewhat modified) were included in Experiment XXXI of this project. In immediate free recall, twelve familiar words are presented visually at a rate of one per second, and the task is to recall as many words as possible (in any order and without time limits) at the end of the presentation. Two delayed memory procedures are included. In delayed recall, the task is to recall as many words of the free-recall list as possible after an interpolated task (digit span). In delayed recognition, the twelve words in a free-recall list mixed with 12 other familiar words are presented in random order after the interpolated task; the recognition task is to identify which words are from the list and which are not. In dichotic listening, two different digits are presented simultaneously, one to each ear; the task is to identify the two stimuli. In forward digit span, lists of three to nine digits are presented at a rate of one digit per second, and the task is to respond with all the digits in a list in the order presented. In simple reaction time, a zero appears on a screen 15 times during a 90 second period, and the task is to press a button as fast as possible for each stimulus. In the four choice-reaction procedures, 90 digits appear at a rate of one per second, and 15 of these digits are target stimuli which are signals to press the button as fast as possible. The four rules for responding are: (1) a specific digit; (2) any odd (or even) digit; (3) any even digit which immediately follows an odd digit (or vice versa); and (4) any even digit which immediately follows an even digit, and any odd digit which immediately follows an odd digit. Current analyses are cross-sectional correlations between performance and age for 78 to 80 women.

Experiment XXXVI is an age study of memory for sentences which are logically related (e.g., "Jane is taller than Beth," "Beth is taller than Ann," "Jane is taller than Ann"). The study is designed to determine whether memory performance of educationally active young and old adults is affected differentially by linguistic integration based on inferences. Two acquisition conditions, one devised to provide high linguistic integration and the other to provide low linguistic integration, are included. There are six parts to the experimental procedure: (1) acquisition; (2) recall of names; (3) ordering of names: (4) recognition of sentences; (5) recall of information; and (6) inference.

(1) During acquisition, ten different sentences (of which five are repeated twice) are presented on a screen, one at a time. The sentences involve six names and their relative heights; each sentence compares two names (e.g., "Jane is taller than Beth").

(2) Recall of names determines how many of the six names are recalled.
(3) Ordering of names determines how well the ordering has been mastered (the names are provided during this task).
(4) In recognition, the ten different acquisition sentences are intermixed with five consistent sentences and six contradictory sentences, and the task is to decide for each sentence whether it had been seen during acquisition or not. A confidence rating is made for each judgment.
(5) In recall, 15 sentences in the form "Jane is shorter than Beth" are presented. About half are consistent with the acquisition information and about half are contradictory. The task is to decide for each sentence whether it is true or false. A confidence rating is made for each judgment.
(6) The inference task involves another set of 15 sentences in the form "Jane is shorter than Beth." The task is to decide whether each sentence is true or false; but unlike the recall task (which requires remembering the acquisition information), during inference the basic information necessary for ordering is provided.

Major Findings: Experiment XXXIII is the longitudinal study of several cognitive and response-time measures. Each measure was repeated; therefore, there are two correlations with age for each:

(a)	immediate free recall	-.52 and -.55
(b)	delayed recall	-.52 and -.61
(c)	delayed recognition (hits)	-.11 and -.28
(d)	dichotic pairs	-.17 and -.07
(e)	digit span	-.32 and -.25
(f)	simple reaction time	.43 and .40
(g)	choice reaction time (1)	.47 and .42
(h)	choice reaction time (2)	.31 and .39
(i)	choice reaction time (3)	.47 and .46
(j)	choice reaction time (4)	.26 and .25
(k)	choice 3--errors	.42 and .30
(l)	choice 4--errors	.36 and .32

Experiment XXXVI is a new age study of memory for logically related sentences. Apparatus and materials have been prepared, and data collection is about to begin.

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 verbal learning 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 is expected to be completed for Experiment XXXVI, the new study of memory for related sentences. Further studies of memory for sentences or text will depend upon availability of staff.

Publications:

Poon, L. W., Fozard, J. L., Cermak, L. S., Arenberg, D., & Thompson, L. W. (Eds.). New directions in memory and aging: Proceedings of the George Talland memorial conference. Hillsdale, N.J.: Erlbaum, 1980.

Arenberg, D. Comments on the processes which account for memory declines with age. In L. W. Poon, J. L. Fozard, L. S. Cermak, D. Arenberg, & L. W. Thompson (Eds.). New directions in memory and aging: Proceedings of the George Talland memorial conference. Hillsdale, N. J.: Erlbaum, 1980.

Arenberg, D. Localization of decline and the role of attention in memory. In L. W. Poon, J. L. Fozard, L. S. Cermak, D. Arenberg, & L. W. Thompson (Eds.). New directions in memory and aging: Proceedings of the George Talland memorial conference. Hillsdale, N.J.: Erlbaum, 1980.

Robertson-Tchabo, E. A., & Arenberg, D. Age differences and age changes in cognitive performance: New "old" perspectives. In R. L. Sprott (Ed.), Age, learning ability, and intelligence. New York: Van Nostrand Reinhold, In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00066-19 LBS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Perceptual Retention and Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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

COOPERATING UNITS (if any)

Elizabeth A. Robertson-Tchabo, University of Maryland
Baltimore City Hospital
Don Reynolds, Crownsville State Hospital

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.7	.2	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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; and (3) to investigate processes of interference and perception. Current studies include nonverbal memory and a visual analog of the dichotic-listening procedure.

PHS-6040
(Rev. 10-76)

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 non-verbal 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. The current analyses include regression procedures to estimate age changes within birth cohorts and to estimate nonmaturational effects over time within age groups.

Experiment XII is a study of visual information processing, stemming directly from the results of Experiment X. In Experiment X, several subjects responded sequentially (by visual fields rather than by simultaneous pairs) indicating sequential processing of visual information. Previous research in this laboratory showed that many (but by no means all) subjects typically responded sequentially in a dichotic-listening task. Experiment XII was designed to determine whether there are people who typically respond sequentially regardless of sensory modality. In other words, do some people process information sequentially for both visual and auditory inputs and others simultaneously for both modalities? In addition to digits visually presented with a tachistoscope, auditory digits are presented dichotically (one of each simultaneous pair to each ear). In both modalities, each presentation consists of four different digits: two simultaneous digits followed by two more simultaneous digits. There are two samples; each consists of 32 men and women between 18 and 30 years of age.

Major Findings: Experiment VII is the longitudinal study of memory for designs (Benton). Previous analyses of the data for men showed that substantial mean age decrements were found only for groups initially in their seventies over a six-year period and initially in their sixties over a twelve-year period. Regression procedures recently developed in this Section were applied to all first-time measures during the first sixteen years of the study to obtain estimates of age changes within birth cohorts and estimates of nonmaturational effects over time within age groups.

When participants are grouped by time of birth and, for each birth cohort, performance is plotted on calender time, the slope of the best-fit time is an estimate of age change for that cohort. Similarly when participants are grouped by age and, for each age group, performance is plotted on calendar time, the slope of the best-fit line is an estimate of nonmaturational effects for that age group.

The birth-cohort slopes were:

1877-1884	.49	1917-1924	.12
1885-1892	.35	1925-1932	.14
1893-1900	.17	1933-1940	.04
1901-1908	.35	1941-1948	-.03
1909-1916	.20		

The rank-order correlation (Spearman rho) between period of birth and slope was .92; the earlier born (oldest), the larger the estimate of age change. These slopes for birth cohorts were statistically different.

The age-group slopes were:

76-83	.05	44-51	.05
68-75	.17	36-43	.07
60-67	.01	28-35	.02
52-59	.18	20-27	-.01

These slopes were not (statistically) significantly different; the common age-group slope was .074. Over the first sixteen years of the study, the estimate of the nonmaturational time-of-measurement effect was .074 errors per year, and the estimate was similar for all age groups.

If this common age-group slope of .074 is assumed to reflect secular effects (or sampling), then it can be compared statistically with the birth cohort slopes to assess whether the estimates of age change could be accounted for by nonmaturational time-of-measurement effects. These comparisons indicated that for four (1877-1884, 1885-1892, 1901-1908, 1909-1916) of the five earliest born cohorts, the estimates of age changes could not be accounted for by the nonmaturational effects. These results are consistent with the longitudinal analyses of individual changes; memory for designs declines with age late in life.

Experiment XII is a study of both visual and auditory information processing. The results were rather surprising. Almost all of the 32 young adults responded sequentially to the dichotic stimuli; as a result, it was not possible to determine whether individuals who respond sequentially to stimuli in one modality also respond sequentially to stimuli in another modality. Previous research in this Section (and in another laboratory) had indicated that a substantial minority of subjects did not respond sequentially to dichotic stimuli. A replication of the current study was carried out to determine whether the first result was a quirk of sampling; but again, almost all of the additional 32 young adults responded sequentially.

One major difference between the previous studies referred to and the current studies was the number of stimuli. In the previous studies, only a few stimuli had been presented at each list length. In the current studies, only one list length was used (each list consisted of two simultaneous digits followed by two more simultaneous digits), but 35 lists were presented.

Inspection of the early lists indicated that some subjects did, in fact, respond nonsequentially to a few early lists but soon switched to consistent sequential responding. Apparently true nonsequential responders are rare, but seem more numerous when only a few lists are used because some individuals do not respond sequentially immediately.

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. It is the purpose of this project to explore the generality of the age-interference hypothesis for non-verbal memory and perception. It is important, both for theoretical and applied reasons, to identify those conditions which are especially interfering for the old. In addition, response slowing is a general behavior which pervades many aspects of information processing; another purpose of this project is to improve our understanding of information processing and how it changes with age.

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.

Publication:

Robertson-Tchabo, E.A., Arenberg, D., and Costa, P.T.,Jr.: Temperamental predictors of longitudinal change in performance on the Benton Revised Visual Retention Test among seventy year old men: An exploratory study. In Hoffmeister, F., and Müller, C. (Eds.): Brain Function in Old Age, Proceedings of Bayer Symposium VII. Springer-Verlag, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00067-13 LBS

PERIOD COVERED
October 1, 1979 through September 30, 1980

TITLE OF PROJECT (80 characters or less)
Learned Modification of Visceral Function in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. T. Engel	Chief, LBS	LBS, GRC, NIA
Other:	K. Gaarder	Research Physician	LBS, GRC, NIA
	M. Garwood	Staff Fellow	LBS, GRC, NIA
	M. Glasgow	IPA Fellow	LBS, GRC, NIA
	A. Perski	Visiting Fellow	LBS, GRC, NIA
	E. Lakatta	Chief, Cardiovascular Section	CPB, GRC, NIA
	S. Gottlieb	Cardiologist	Balto. City Hospital
	W. Whitehead	Guest Worker	LBS, GRC, NIA
	M. Schuster	Gastroenterologist	Balto. City Hospital
	S. P. Tzankoff	Senior Staff Fellow	CPB, GRC, NIA
	W. F. Baile	Staff Physician	Balto. City Hospital
	P. T. Costa	Chief, Stress & Coping Sec.	LBS, GRC, NIA
	D. Brinker	Dir. Cardiac Catheterization Lab.	Johns Hopkins Hospital

COOPERATING UNITS (if any)
Columbia Medical Plan
Baltimore City Hospitals
The Johns Hopkins University School of Medicine

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Psychophysiology

INSTITUTE AND LOCATION
GRC, NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.25	4.5	1.75

CHECK APPROPRIATE BOX(ES)

☑ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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; a second study investigates heart rate biofeedback in patients with angina pectoris; another study investigates the physiological mechanisms of heart rate control during exercise in normal man; one study investigates bowel function in normal man and patients with irritable bowel syndrome; another study is designed to learn whether patients with histories of meningomyelocele and/or urinary and fecal incontinence can be trained to become continent; one study investigates age differences in autonomic response patterns; one study investigates age differences in electrodermal activity; one study investigates whether subjects can perceive contractions of their stomachs and whether this perception enables them to control gastric motility.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: A. To evaluate the clinical effectiveness of relaxation and biofeedback in the control of blood pressure (BP) in patients with borderline or mild hypertension.

B. To determine if patients with angina pectoris can learn to slow heart rate (HR) and can transfer this skill to an exercise stress test.

C. To identify the mechanisms underlying learned, voluntary control of HR during exercise in normal man.

D. To determine whether patients with histories of meningomyelocele and fecal incontinence can become continent.

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

F. To describe age differences in individual patterns of autonomic nervous system response, and to determine if, with increasing age, there is a decline in autonomic nervous system adaptability.

G. To describe age differences in the peripheral sudomotor system by studying age differences in the electrical properties of the skin (electrodermal activity).

H. To determine whether some subjects can perceive contractions of their stomachs, and if so, whether this perception enables them to control gastric motility.

I. To determine the relationship between the prevalence of self-recorded episodes of angina pectoris to the prevalence of anginal episodes inferred by a 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 Columbia Medical Plan. All had been diagnosed as suffering from high BP. Study patients were limited to those receiving no antihypertensive medication but having clinically-determined diastolic pressures (DBP) $\geq$ 90 mm Hg, or to those patients receiving diuretic therapy (only) for their hypertension regardless of their clinically-determined BP. One hundred fifty-eight patients participated in this multiphasic, controlled study. Phase 1 was one month long and served as a baseline phase. All patients recorded their blood pressures 9 times/day (3 times upon awakening; 3 times during the middle of the day; 3 times during the evening). In addition, patients had their pressures taken approximately once weekly by a health professional. In phase 2, patients were assigned to one of three behavioral

treatment groups: self administered relaxation, self administered BP biofeedback, or control. Assignment to behavioral groups in phase 2 was such that the three groups were matched on baseline BP, and such that approximately equal numbers of medically treated and untreated patients were in each group. Phase 2 was 3 months long. Phase 3 was similar to Phase 2 in duration and in nature of the treatments: control patients continued as controls; relaxation patients were subdivided into two groups--group A continued to relax, group B was trained in the feedback condition; and feedback patients from Phase 2 were divided into two groups similarly to the relaxation patients. In phase 4, control patients received six months of therapy (relaxation followed by feedback); patients on diuretic medication had their diuretics withdrawn if their DBP $\leq$ 86 mm Hg, and they were followed as before; patients who received only one therapy (relaxation or feedback) were offered the other treatment; patients who received both therapies were monitored for an additional 6 months. All patients in phases 2-4 continued to record their BP daily and were asked to have their BP taken weekly by a health professional.

B. A group of 12 male patients experiencing angina pectoris in association with medically verified coronary artery disease are being studied. All patients are referred from the outpatient service of Baltimore City Hospitals. Each patient is given an exercise stress test to determine the level of HR, systolic blood pressure (SBP), and the product of HR and SBP, the rate-pressure product (RPP), at which he experiences pain. Then he is trained during 25 sessions to attenuate his HR while exercising on a bicycle ergometer. Following such training he will again receive an exercise stress test to determine whether the training in the laboratory improved his performance on the stress test. Half of the group are control patients and exercise on the bicycle without feedback training. They also receive pre- and post-exercise stress tests. Finally, the control patients receive training in HR control as did the experimental patients. In addition, follow-up procedures enable the assessment of the carry-over effect of training on daily routines.

C. Two studies were done with normal, healthy subjects to assess the ability of these subjects to attenuate HR while exercising. In Study I, ten, young, physically untrained subjects exercised on a bicycle ergometer at about 50% of maximal HR. Five subjects were in the experimental group and five were in a control group. The experimental subjects received feedback to slow HR while exercising during five sessions, the control subjects merely exercised for five sessions. Following training, the experimental subjects exercised for two additional sessions without feedback to determine whether they could transfer the skill that they had learned; the control subjects were trained for five additional sessions with feedback. In study II, ten, young, physically conditioned subjects (trained athletes) were studied in an experiment which was similar to Study I, but with the following additions: 1) All subjects received maximal exercise stress tests before and after training--the experimental subjects received two stress tests, one before training and one after training; the control subjects received three stress tests, one before entering the control exercise period, one after the

control period, and the third after HR training; 2) During the stress tests serum levels of epinephrine, nor-epinephrine and blood lactate were measured, and oxygen consumption was measured; 3) Catecholamine levels in the serum and lactate levels as well as oxygen consumption were measured during specified experimental sessions.

In Study III, which has just started, 15 racing bicyclists are divided in three groups of five subjects each. One group practices HR increase during five exercise sessions. The second group is engaged in five HR slowing sessions while the third group serves as a control and just exercises. HR, systolic blood pressure (SBP), and oxygen consumption will be measured during each session.

D. This project is a continuation of a project reported last year. An additional 10 patients aged 5-15 with fecal incontinence secondary to meningomyelocele have been entered into the study.

Diagnostic evaluation and biofeedback training were accomplished using a rectal tube as follows: The patient was placed in the right lateral position and a hollow cylinder 1.2 cm in diameter to which two doughnut-shaped balloons were tied was inserted into the anal canal so that one balloon was surrounded by the external anal sphincter and the other was surrounded by the internal sphincter. A third balloon 5 cm in length was inserted through the cylinder into the rectum and was used to distend the rectum. Momentary rectal distensions from 5 ml to 60 ml of air in increments of 5-10 ml were injected and immediately withdrawn in the rectal balloon.

In addition to measuring the pressure change in the external sphincter balloon caused by external anal sphincter contraction, the electromyographic activity of the external sphincter and the adjacent gluteal muscles were measured during training sessions. Parents completed daily records at home of number and type of incontinent episodes and number of appropriate toiletings.

Training consisted of 3 phases: Phase 1 was a diagnostic procedure which assessed the ability of the patient to contract the external anal sphincter prior to training, and the minimum volume of rectal distension which the patient could perceive. In Phase 2 the patient was taught to emit an appropriate contraction of the external anal sphincter in the absence of rectal distension by providing visual feedback of the amplitude of his response and verbal praise. In Phase 3 the patient was instructed to contract the external sphincter whenever he perceived distension of the rectum by balloon. Beginning with the lowest level of rectal distension which the subject could perceive, the volume of rectal distension was gradually increased as the subject acquired the ability to overcome the dilation of the lower anal canal by voluntarily contracting the external anal sphincter. In addition to verbal praise, marbles exchangeable for toys were given for appropriate responses to children under age 10.

Each training session was 45-60 min long and consisted of approximately 25

training trials. Training sessions were scheduled at two-week intervals, and training was continued until the patient became continent or ceased progress.

E. Patient flow will be described separately for fecal incontinence and urinary incontinence.

For patients with fecal incontinence a physical evaluation and history will be done by a gastroenterologist to identify, for exclusion patients with inflamatory bowel disease, tumors, or fistulas. This examination will include laboratory studies for parasites or occult blood in the stool and a barium enema to evaluate diverticulosis. Physical examination will be followed by a rectal motility study to evaluate the following: (1) strength of the external anal sphincter muscle, (2) ability to contract 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 investigators will explain 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 will consist 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 will be given another appointment for 4 weeks after initial testing, and will be advised that a research assistant will arrange a visit at the patient's home. The research assistant will 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 will be classified into one of three groups with disposition as follows: (1) if no longer incontinent, patients will be 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 will 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 will be given such training. Biofeedback training will be similar to the rectal motility study except that the patient will receive visual feedback and verbal praise to help him learn to contract the external anal sphincter with adequate strength when he senses rectal distension. Between biweekly visits during biofeedback training patients will be instructed to contract and relax

the external anal sphincter several times each day to strengthen this muscle.

The active treatment phase will last 8 weeks during which time patients in the second and third category above (behavior modification and biofeedback) will come to the laboratory once every two weeks for evaluation and/or further training. Home records of incontinent episodes will continue to be kept. At the end of the treatment phase patients will be scheduled for follow-up at 6 and 12 months. At these times the enema retention tests and the rectal motility study will be repeated.

Patients with urinary incontinence (involuntary loss of urine during waking, occurring at least 2/week) will be given a physical examination and a medical history will be taken by a urologist to identify, for exclusion patients with bladder infection, bladder outlet obstruction, detrussor hyporeflexia, and paradoxical (overflow) incontinence. Examination will include urinalysis, cystometry, cystoscopy if clinically indicated, and for males, a prostate examination. A urologist will carry 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 (up to 350 ml) 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 investigators will explain to the patient and his family 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 will consist in having the patient go to the toilet and attempt urination every four hours. Various behavioral strategies such as turning on a faucet or tapping or pressing on the abdomen to assist micturation will be suggested. The patient will be given another appointment for 4 weeks, and will be advised that a research assistant will telephone to arrange to visit the patient's home. The research assistant will visit 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 Beck depression inventory.

At the second laboratory visit patients will be grouped in the same manner as fecally incontinent patients: (1) If no longer incontinent, patients will be 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 will continue in the habit training program but with the addition of explicit rewards (rewards to be 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, will be 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 will be given biofeedback training to inhibit bladder contractions during bladder filling and to contract the distal urethral sphincter. This will be similar to the cystometrogram except that the patient will be given visual feedback and verbal instructions during bladder filling. Between biweekly visits, during biofeedback training patients will be instructed to contract and relax the pelvic floor muscles several times each day to strengthen these muscles, and they will be instructed to practice stopping the stream during every micturation.

The active treatment phase will last 8 weeks followed by follow-up visits at 6 months and 12 months. Cystometrograms will be repeated, and ability to inhibit micturation will be retested at these follow-up visits. Home records of the frequency of incontinence will be kept throughout the 8 weeks of active treatment.

F. Test of the hypothesis that the ANS of the aged is functioning differently than that of young adults necessitates age comparisons on the interrelationships among ANS response measures. Comparison of age groups on the correlations between ANS response measures is inadequate because the ANS of young adults does not show a uniform response, and correlations among ANS response measures across individuals often are non-significant. The pattern of intra-individual ANS responses thus needs to be investigated. The concept of individual specificity is a means by which to do this. Individual specificity refers to the tendency of an individual to emit the same hierarchy of responses to all stimuli. For example, hypertensives have been reported to have a greater tendency than normotensives to respond to all stimuli with a pressor response. Comparisons of age groups on individual specificity reflects age differences in the adequacy of ANS function because if an individual's psychophysiological response pattern is consistent across stimuli, his response pattern often will be inappropriate to the demands of the situation. Fifteen subjects in each decade from 20 to 80 years were tested. Each subject received the following stimuli which were presented in counterbalanced order for 30 seconds: (1) cold pressor, (2) mental arithmetic, (3) time estimation, (4) incongruous slide, and (5) exercise. These stimuli were selected because they are effective (i.e., elicit responses) and diverse, the appropriate stimuli by which to assess specificity. Physiological response measures were breathing rate, skin potential, digital blood flow, heart rate, and systolic and diastolic blood pressure. Intra-class correlations were computed to assess individual specificity.

G. Previous research in this laboratory has shown that there are age differences in two types of electrodermal activity--skin potential level and skin resistance level. With increasing age skin potential level becomes less negative and skin resistance level becomes higher. These age differences could reflect several peripheral physiological mechanisms since surface recordings reflect a composite of the underlying structures. It previously has been demonstrated in this laboratory that it is possible to separate the physiological components of electrodermal activity by recording under certain conditions. If the subject is allowed to relax and his resting potential

meets certain criteria, the recording primarily reflects epidermal events. This has been verified by comparison of recordings from palmar and forearm sites (the forearm has markedly fewer sweat glands than the palm). If a stimulus is then presented and sampling occurs before any response recovery, the change primarily reflects changes in the sweat duct. The procedure has been validated by recording with different electrolyte mediums. Electrodermal activity of young (20 to 39 years), middle-aged (40 to 59 years), and old (60 to 79 years) men was compared using the procedure. The stimulus was mental arithmetic.

H. Twenty normal young adults were recruited from a university medical center. They were taught to swallow a nasogastric tube during one session and then were tested for ability to perceive stomach contractions during 6 sessions. The test involved turning on a signal light at the peak of a stomach contraction or 12 seconds after the peak of a stomach contraction, and requiring the subject to judge whether or not the light coincided with the stomach contraction. Signal detection methodology was used to analyze perceptual sensitivity. A similar test was used to determine the ability of the same subjects to perceive their heart beats. After the perception test subjects were tested for ability to voluntarily increase and decrease gastric motility. Subsequently 10 of the subjects were given biofeedback training to increase their voluntary control of gastric motility.

I. One hundred consecutive adult male and female patients who are under treatment by their private physicians for chest pain, and who are scheduled for coronary angiography at the Johns Hopkins Hospital cardiovascular laboratories because of the chest pain, will be contacted by a team member shortly after their scheduling (usually 2-3 weeks prior to their catheterization). Their names will be obtained from a schedule book at the catheterization laboratory. They will be telephoned, the existence of the study explained, and their participation solicited. A visit to the hospital will be requested to meet with one of the team members. If distance or other circumstances such as poor health preclude this, arrangements will be made for a member of the investigative team to visit the patient at his home or arrangements will be made to mail the package to the patient. At this time data relating to socio-economic factors will be obtained as well as information relating to the character and duration of the angina. General information concerning the patient's history of cardiovascular disease also will be taken, and the subject will be asked to fill out the Cornell Medical Index and the 30 item Guilford-Zimmerman Temperment Survey Emotional Stability Scale. This interview should last no more than forty-five minutes. The patient will then be given copies of an anginal questionnaire and franked, addressed envelopes. He will be asked to complete one form for each anginal attack he experiences. Patients will be instructed to complete this form retrospectively, after they have treated their angina in their usual fashion (e.g., nitroglycerine, rest) and the episode has subsided. Furthermore, they will be instructed to follow any other advice given by their physician even if it prevents the completion of the questionnaire at that time. This 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 work-up upon admission for angiography. In this regard the patient's referring physician will be contacted by mail and asked to complete a questionnaire regarding the nature of his patient's angina. The house officer immediately responsible for the patient's care while in the hospital will do the same. Patients also will be given copies of the Profile of Mood States and will be asked to complete a form once a day usually around dinner time. Patients will be interviewed again in the hospital in order to determine whether, in the weeks prior to catheterization, they had changed their activity pattern or other aspects of their behavior. Patients will be asked to continue record keeping after discharge from the hospital for a period of approximately two weeks. If surgery is performed, an attempt to collect post-surgical data also will be made. Data will be 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 and house-officers analyses,

(4) analysis of anginal patterns post-angiography and pre-angiography will be made.

Major Findings: Two hundred twenty-four patients were contacted. Of these, 58 refused to participate and 39 withdrew during the baseline phase. Thus, 127 patients completed phase 1 (baseline), 96 completed phase 2, and 90 completed all three study phases. Reasons given for discontinuing participation--e.g., "I'm too busy"--reflect the likelihood that the demands placed upon the participants were quite burdensome. Only 2.2% (2 of 92) of the medically treated patients expressed contentment with continued diuretic therapy, and 2.3% (5 of 224) either refused to participate or withdrew during the baseline because they found self-monitoring of BP to be frightening. Among those who refused to participate, more were female and/or advanced in age.

Major conclusions resulting from the analysis of baseline data are the following: 1) Extensive, self-monitoring of BP is feasible and practical; 2) SBP rises throughout the day but is highest in the afternoon; 3) DBP falls from morning to evening but is highest in the afternoon; 4) intra-daily range of SBP but not DBP is higher among women than among men; 5) both SBP and DBP fall during a one month period of self-monitoring and regular clinical monitoring; 6) standard deviations and ranges of self-determined BP are highly intercorrelated, however, changes in professionally measured BP are

poorly correlated with these indices of BP lability; 7) SBP levels, rates of decline throughout the baseline period and lability indices are correlated with age, but comparable measures of DBP are not correlated with age. Some implications of these baseline data for the treatment of hypertension in the elderly are: that over-treatment due to over-estimation of BP is less likely if home-recorded BP are obtained using a comprehensive protocol; that a differential taxonomy of hypertension based upon patterns during the day might reveal important age-related differences in the natural history of high BP.

Major findings resulting from the second phase of the study are the following:

1) Both SBP and DBP continued to fall through the sixteenth week of self- and clinical monitoring as indicated both by negative slopes and by BP changes which are significantly different from zero.

2) Analyses of variance on the SBP and DBP levels during baseline (Phase 1) and during the first 35 days (Phase 2A) and the last 35 days (Phase 2B) of Phase 2 revealed the following:

a) During Phases 1 and 2A medicated, non-medicated, and combined groups all showed significant time-of-day effects on SBP and DBP levels, but there were no differences among the Control, Relaxation, or Feedback groups and no group X time of day interactions.

b) Analysis of Phase 2B data gave results which were similar to those above except that significant differences in DBP levels were observed between behavioral treatment groups of non-medicated patients and in the combined group.

c) Feedback and relaxation groups combined were compared with the control patients. There were significant phase and time-of-day effects for both SBP and DBP; but significant behavioral treatment effects were present for DBP only.

3) All medication groupings showed significant time of day effects on lability. Analysis of variance on the changes in standard deviations of SBP and DBP from Phase 1 to Phase 2A, Phase 1 to Phase 2B, and Phase 2A to Phase 2B, shows that both BP lability indices decreased with time in the study for all groups. This decrease in lability also is seen in the ranges and standard deviations of the ranges in Phases 1, 2A, and 2B.

4) Analysis of changes in BP as a result of feedback or relaxation practice revealed that both treatments were effective in lowering BP. Furthermore, since the number of times the behavioral treatments were practiced did not differ significantly between behavioral treatment groups nor from phase to phase, but the extent to which BP was lowered during practice improved with time; it is clear that these patients were continuing to practice their respective techniques and that their skills at lowering BP were improving with practice.

5) Both SBP and DBP measured professionally were observed to decline with time during the study.

While analysis of data collected during Phase 3 and subsequent phases is still in progress, early findings from Phase 3 are the following:

1. Professionally measured BP were significantly lower at the end of Phase 3; the most significant effect having been achieved by the group receiving feedback followed by relaxation. The average decrease in BP from Phase 1 to Phase 3 was -5.1/-4.2 as measured professionally (-12.2/-9.6 for the afternoon pressures of this group).

2. Self-determined BP also decreased significantly from Phase 1 to Phase 3B (last 35 days of Phase 3). Both SBP and DBP decreased most in the group receiving feedback followed by relaxation.

B. Five experimental and two control patients have completed the study. Because the study is not finished, no meaningful analyses of the findings are possible. However, it can be reported that the patients had no difficulty in acquiring HR control during biofeedback sessions, and that this skill can be transferred to the conditions of maximal stress test.

C. In association with the feedback training the experimental subjects in Study I were able to significantly attenuate the exercise-induced increase in HR by about 20% relative to the control subjects. This same effect was seen in the control subjects when they underwent feedback training. Neither group showed a significant alteration in SBP relative to the control condition. Thus, feedback training led to a specific effect on HR with no effect on SBP. Consequently, the product of HR and SBP, rate pressure product (RPP), which is an index of left ventricular work, also was significantly attenuated during training. Finally, the experimental subjects participated in two, additional post-training sessions without feedback during which they showed that they were able to maintain the skill that they had learned, i.e., they continued to attenuate their HRs with no change in SBP.

Study II has demonstrated that physically conditioned subjects can attenuate the exercise-induced tachycardia during feedback training, even when the exercise load is heavy, about 70% of maximal HR. The effect was present for the experimental subjects and for the control subjects during feedback training as well. SBP was similar in the control and feedback conditions so that RPP followed HR and also was attenuated. Oxygen consumption as well as catecholamines did not show a consistent pattern of changes.

D. Nine of 10 patients in the current series are still in training. Therefore, outcome cannot be specified. However, in working with a younger group of patients we have identified behavioral problems which limit the effectiveness of biofeedback training with preschool-aged children and have tentatively identified solutions. One problem was that young children were not especially concerned about their incontinence and quickly lost interest in the biofeedback display. Providing marbles as tokens for appropriate

responses during training sessions and later allowing the child to exchange the marbles for toys has increased compliance with the training procedures. A second problem was that young children lacked the necessary skills for toileting themselves as well as the skills required to postpone evacuation. Giving the children training in these skills (i.e., toilet training) appropriate to their physical abilities has helped younger children to achieve continence.

E. Up to the present time three patients with fecal incontinence have been entered into the study, of whom one has finished training. This patient is now continent.

At present four patients have been entered into the study of urinary incontinence, but none have finished training. These four patients include two with stress incontinence, one with neurogenic bladder, and one with no specific diagnosis.

F. Results show that consistent with hypothesis there was a significant increase in individual specificity with increasing age. That is, there was greater tendency for older people (as compared to younger people) to have a consistent pattern of response across stimuli.

G. Age differences in the electrical activity of the skin can be accounted for by changes in sweat duct resistance. There were no age differences in either the resting epidermal potential or resting epidermal resistance. There were significant age differences in skin potential level and computed sweat duct resistance during stimulus presentation. With increasing age skin potential level became less negative. There was a marked increase with increasing age in the derived measure of sweat duct resistance. The absence of age differences in the derived measure of sweat gland potential suggests that age differences in electrodermal activity can be accounted for by one mechanism--sweat duct resistance. Additionally, the magnitude of age difference in electrodermal activity is dependent on recording site. Much larger age differences are obtained when the recording site is the medial phalanx than when the thenar or hypothenar eminence is used.

H. Ten of 20 subjects were able to discriminate their stomach contractions at better than chance levels. Perceptual sensitivity scores ranged from 0 (50% correct) to 1.15 (78% correct), and men were significantly better than women at discriminating both stomach contractions and heart beats. The perception of stomach contractions was correlated with the perception of heart beats across subjects ($r = .51$), suggesting that there is a generalized tendency for subjects to be aware of visceral sensations.

Initially subjects showed little ability to control gastric motility, and their ability to control motility was unrelated to their ability to perceive stomach contractions. With feedback training subjects acquired an ability to increase motility significantly above resting levels but could not decrease motility below resting levels. However, the amount of learned control which was shown was not correlated with ability to perceive stomach contractions.

I. This project has just begun. No results are available yet.

Proposed Course of Project: A. This project is nearing completion as most patients have finished their follow-up phases. A manuscript about the baseline has been submitted for publication, and others will be submitted to appropriate medical journals as the data analyses continue.

B. The project will continue until 12 patients complete all phases of the study.

C. Study I has been published. Study II is being prepared for publication. Study III has just begun. Further experiments in this project will be designed to determine in greater detail the physiological and behavioral mechanisms underlying the role of the central nervous system in the cardiovascular adjustments to exercise.

D. An additional 30 patients will be studied. The outcome of biofeedback training and maintenance of treatment effects will be compared on such parameters as sensitivity for perceiving distension, sensitivity for perceiving the response of squeezing the sphincter, age, intelligence, and level of lesion.

E. The project will continue to be run as an outpatient project until approximately 20 patients have completed training for fecal incontinence and approximately 20 have completed training for urinary incontinence. We will then move the program to a nursing home and operate it on an inpatient basis until similar numbers of patients have been studied.

F. The second test (test-retest interval of two years) is now in progress. A third test is projected at ten years.

G. Further work delineating the mechanisms of electrodermal activity will be conducted. Although research to date indicates that age differences in static electrodermal activity are almost totally sweat duct related, there may be important age differences in dynamic electrodermal activity.

H. This project is completed.

I. If the results show that there are psychological correlates of angina, it should be possible to improve medical management of these patients through the use of appropriate behavioral strategies. If behavioral or psychological procedures improve the diagnosis of those patients who will benefit from bypass surgery, it should be possible to incorporate these procedures into the diagnostic assessment of patients.

Significance to Bio-Medical Research and Program for the Institute:
A. Hypertension research has been hindered by the lack of a reliable measure of BP and of a method for adequately determining baseline BP. This program makes a significant contribution toward both of these goals by providing reliable measurements which can be used for a baseline. This is

especially important in the evaluation of hypertension in the elderly, where a strong iatrogenic effect can lead to differences between office BP readings and home BP readings which can lead to faulty diagnosis or treatment. The behavioral treatment of hypertension is a current topic of great interest, and a well-done study will settle many questions. A behavioral treatment is especially relevant to the elderly because they respond less reliably to medication, and because systolic hypertension is apt to be aggravated by increases in cardiac output which may be especially amenable to behavioral treatment.

B. Angina pectoris is a serious disease whose likelihood increases with advancing age. Furthermore, it is a disease which greatly limits the quality of life. Any technique which can improve the ability of such patients to perform in their daily lives will have considerable value and biofeedback might be a good supplementary therapy during the rehabilitation process.

C. Cardiovascular adjustments to physical conditioning are attributable to processes mediated through the nervous system as well as mechanical adjustments which take place in the heart, blood vessels and in working muscles. Very little is known about the way in which the neurally-mediated effects occur, or about the way in which these effects are determined by behavioral mechanisms. This research will be fundamental in understanding these processes.

D. Meningomyelocele affects 0.1% of births, and approximately 40% of patients with meningomyelocele have fecal incontinence. Therefore, there is a large population of patients for whom these procedures have relevance. Additionally, we should be able to apply these procedures to adults with incontinence secondary to other types of CNS injury, and some of the behavioral training procedures which we are developing for preschool-aged children may be applicable to the treatment of senile older patients with fecal incontinence.

E. Most incontinence occurs in young children and in old people. The prevalence of incontinence in residents of nursing homes is 10-25%, and the prevalence of urinary incontinence in nursing homes 13-48%. Incontinence is at best an obstacle to normal social interaction, and is often the deciding factor in whether to institutionalize an older person as opposed to caring for him at home.

F. Diseases of the autonomic nervous system (e.g. hypertension, coronary artery disease) predominate in old age. The life history of the individual is likely involved in the development of these disease processes. Autonomic specificity is one aspect of an individual's life history--the development of an individual's autonomic nervous system response to different situations. The older person's tendency to respond consistently to all stimuli (individual specificity) reflects a decline in autonomic nervous system adaptability. In studying a person's pattern of autonomic nervous system response over time it may be possible to determine the significance of this maladaptive response pattern. For example, a lifetime pattern of an overreactive pressor

response may be involved in hypertension. The study of individual specificity enables these proposed relationships to be investigated.

G. Adequacy of function of the peripheral sudomotor system is crucial for older individuals. There is a reduction in sweat gland response which impairs the older person's ability to regulate temperature. The morphological changes in the epidermis present not only a cosmetic problem but also may be related to a loss in the skin's protective function. This research program is involved in developing adequate measures of sudomotor function so that age-related alterations and their significance can be investigated in man.

H. 1) This study confirms and extends earlier observations on the relationship of heart beat perception to heart rate control. The theory whereby biofeedback training is explained as learning how to discriminate and correctly label sensations associated with visceral responses has been shown to be invalid. 2) These data have implications for the use of biofeeedback to treat psychophysiological disorders in old people and other age groups. Specifically, the data suggest that incentives are more important to such learning than is discrimination training. 3) The techniques which were developed for measuring perception can be used to investigate the hypothesis that older people may be more aware of somatic sensations and that this increased awareness may be responsible in part for the age-related increase in the incidence of psychosomatic disorders, and of the age-related increase in individual response specificity which this laboratory also is investigating.

I. Angina pectoris is a serious, age-related illness which affects about 5 million Americans with about 150,000 to 200,000 new cases each year. Well in excess of 100,000 coronary artery by-pass operations were done last year. Many patients who are screened for surgery are found unsuitable after coronary artery angiography reveals no significant atherosclerosis. Since angiography is a procedure which has a relatively high risk, any means of identifying patients who can not be operated before they are subjected to angiography would be valuable. Furthermore, any technique which improves medical management of these patients is sorely needed since current methods are of limited value.

Publications:

Baile, W.F., Brinker, J.A., Wachspress, J.D., and Engel, B.T.: Signouts against medical advice from a coronary care unit. J Behav. Med. 2: 85-92, 1979.

Capriotti, R., Garwood, M., and Engel, B.T.: Skin potential level: Age and recording site interactions. J. Gerontol., in press.

Perski, A, and Engel, B.T.: The role of behavioral conditioning in the cardiovascular adjustment to exercise. Biofeedback Self-Regualtion. 5: 91-104, 1980.

Rubin, S.A., Quilter, R., and Battagin, R.: An accurate and rapid inflation device for pneumatic cuffs. Am. J. Physiol: Heart Circ. Physiol. 3: H740-H742, 1978.

Whitehead, W.E., and Drescher, V.M.: Perception of gastric contractions and self-control of gastric motility. Psychophysiology, in press.

Whitehead, W.E., Engel, B.T., and Schuster, M.M. Irritable bowel syndrome: Physiological and psychological differences between diarrhea-predominent and constipation-predominant patients. Dig. Dis. Sci., in press.

Whitehead, W.E., Fedoravicious, A.S., Blackwell, B., and Wooley, S.: Psychosomatic symptoms as learned responses. In McNamara, J.R. (Ed.): Behavioral Approaches in Medicine: Application and Analysis. New York, Plenum, 1973, pp. 65-99.

Whitehead, W.E., and Parker, L.: Behavioral treatment of urinary and fecal incontinence in children. In Coates, T.J. (Ed.): Behavioral medicine: A practical handbook, in press.

Whitehead, W.E., and Schuster, M.M.: Psychological management of irritable bowel syndrome. Pract. Gastroenterol. 3: 32-43, 1979.

Whitehead, W.E., and Schuster, M.M.: Therapeutic application of biofeedback in digestive diseases. In Berk, J.E. (Ed.): Developments in digestive diseases, Vol. 3. Washington Square, Pa., Lea and Febiger, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00068-18 LBS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Operant Performance, Memory and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
OTHERS:	Donald Ingram	Staff Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Laboratory of Behavioral Sciences

SECTION
Learning & Problem Solving

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.65	.45	.20

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major purpose of this project is to analyze complex maze learning of young and aged animals, and to determine techniques which act to improve learning ability. Another goal of this project is to determine age differences in operant performance for young and aged rats or mice, and to determine factors which may act to improve performance, and also improve retention of, the learned responses.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of one phase of this project are: (1) to study age differences in motor performance during operant responding; and (2) to develop operant techniques which improve the retention of learned responses in aged animals. The general objectives of the other phase of this project are: (1) to analyze complex maze learning of young and aged animals; and (2) to determine variables which may act to enhance or retard maze learning ability.

Methods Employed: Operant conditioning performance and retention studies have used 2-bar test boxes in which hungry animals are trained to press one bar to obtain a food reward while the alternate bar remains neutral. By increasing the complexity of the task (using two bars rather than one), it is possible to make a finer analysis of performance and the retention process. We are studying performance and retention as a function of reward schedule, and we are particularly interested in the partial reinforcement effect. The retention of partially rewarded responses is vastly greater than responses continuously rewarded; and analysis of this phenomenon will provide information regarding the general retention process.

A complex 14-unit multiple-T maze also is utilized. This maze has been shown to be a highly reliable test of learning, and it has been used in many major studies of aging. Additional mazes of 6-units are being developed to study mastery of consecutive problems by young and aged rats. These mazes will be used to analyze aging effects in short-term and long-term memory and to determine aging effects in interference, both proactive and r[illegible]ive.

Major Findings: 1. A study has been completed of operant retention after training on fixed ratio (FR) or fixed interval (FI) schedules of reward. Rats were given a series of training trials (bar pressing for milk-sucrose rewards) on continuous reward, FR 25, FI 30", and FI 90". After training, a series of 12 one hour retention trials with no reward (extinction) were given; then 6 one hour retention trials with secondary reward were given (the secondary reward was the smell of fresh milk placed in the training chamber, but now inaccessible). These 18 one hour retention trials were given daily over 18 consecutive days starting the day following the end of training. Within each trial, measures of bar pressing (reward bar and neutral bar) were taken for each of three 20-min. intervals. Separate analyses of variance are being computed for reward bar total responses, and percentage responses on the reward bar for trials 1-12 and trials 13-18, [within-trial-interval (3), level (2, low vs. high), and schedule type (2, response contingent, FR vs. time contingent, FI]. Complete analyses will be presented next year, but one major result is apparent. All groups significantly increased the number of reward bar responses when a secondary reward stimulus was given on trial 13 (comparing the first 20 minutes of trial 13 with the first 20 minutes of trial 12). CRF, FI 30", and FI 90" schedules increased level of responses from 300 to 400 percent; but the FR 25 group obtained complete spontaneous recovery, increasing reward bar responses by a factor of 5100 percent, a unique finding of great theoretical importance. Fixed ratio schedules of reward coupled with appropriate secondary reward systems may act to sustain the behavior of aged or inactive animals.

Significance to Bio-Medical Research and the Program of the Institute: Learning and/or memory deficits represent a major problem among the aged human population. Major behavioral techniques to reduce performance deficits obtained for aged animals have been studied in our laboratory. This project may facilitate research with man by identifying optimal conditions for learning and for retention of learned responses.

Proposed Course of Project: Further studies are in progress to determine the nature of the partial reinforcement effect in relation to: (a) time contingent vs. response contingent partial reinforcement; and (b) massed vs. distributed extinction trials. Other studies will examine age differences in operant performance as a function of response effortfulness. Maze studies will concentrate upon the effects of central nervous system stimulants on behavioral rigidity within the maze for old rats. We have also initiated preliminary studies of memory in aged rats to develop a model system to study neurohumoral correlates of memory.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00069-15 LBS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Exercise, General Activity Level and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Charles L. Goodrick	Research Psychologist	LBS GRC NIA
	Donald Ingram	Staff Fellow	LBS GRC NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Learning and Problem Solving

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.15	.65	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is to determine the effect of voluntary wheel exercise upon behavior for animals tested in lifespan developmental research, and in addition to consider the effect of voluntary wheel exercise upon longevity. Another goal of this research is to increase the period of vigorous activity during later stages in the lifespan of the rat or mouse.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The general objectives are: (1) to determine methods for increasing vigorous physical activity of lower animals during late stages in the life span; (2) to examine behavioral and longevity differences among animals which differ in physical activity level; and (3) to determine the physiological mechanisms underlying differences in activity.

Methods Employed: Wistar rats or various strains of mice are placed in standard activity wheels and allowed access to free voluntary exercise. Hungry animals also may be rewarded with food for running. A technique to control body weight experimentally and to increase voluntary exercise utilizes feeding of animals on a periodic schedule, such as every other day. Other studies utilize inbred, hybrid, and mutant mice or species which differ in activity level due to different genetic constitutions (See Project Z01 AG 00061-15 LBS Behavioral Genetics and aging).

Major Findings:

A. Last year we completed testing of groups of rats started on modified diets and/or exercise when 1.5 months old. The four groups were fed ad libitum (AL) or every other day (EOD) and were allowed wheel exercise or placed in standard cages (2 X 2 design; AL-cage, AL-wheel, EOD-cage; EOD-wheel). This year we have completed four groups started at 10 months and four groups started at 18.5 months to determine the effect of diet (EOD) feeding and exercise on life span when the conditions were initiated later in the life span. The four conditions for the groups started when 10 months of age and the groups started when 18.5 months old were comparable with the four conditions for the groups started at 1.5 months: AL-cage, EOD-cage, AL-wheel, EOD-wheel. All twelve groups are listed in Table 1, which also gives the number of animals tested under each condition. The major new findings concern the effects of diet as aging progresses. Rats on a periodic fasting feeding schedule lived significantly longer than ad libitum fed rats regardless of when (during the life span) the fasting was initiated ($p < .05$ for all comparisons). However, the increment in the life span decreased as a function of increasing age of initiation of the fasting procedures. This effect is clearly shown through the percentage increments in life span (due to periodic fasting) given in Table 1. An increment in life span due to exercise was obtained for the ad libitum rats allowed exercise from 1.5 months of age, but not when allowed exercise at 10 or 18.5 months of age. At the older starting ages the major effect of exercise was to maintain health and vigor (as indexed by delay in terminal drop in body weight preceding death) relative to rats not allowed exercise.

B. A project has been completed to determine the self-selection of protein during early growth. Male and female rats were allowed to select 4%, 26%, or 48% protein (low, normal, or high levels of protein) from 6 to 18 weeks of age, the period of maximal body weight increment. Rats were individually housed in either cages or activity wheels, so that the experimental design was 2 X 2 (male or female and cage or wheel exercise). Each rat (6 per group, N = 24) was allowed simultaneous access to three isocaloric diets which differed in protein percentage, and were isolated from each other. Weekly measures were obtained of water intake, food intake at each

of the three levels of protein, body weight, and wheel activity (for those rats in wheels).

The major results were:

1. Increment in body weight was less for females than for males, F (1,20) = 78.65, $p < .01$, and increment in body weight was less for rats allowed wheel exercise than for rats maintained in cages. For both of these main effects, group differences decreased significantly over time (weeks X sex, interaction, F (11,220) = 2.68, $p < .01$; weeks X exercise, interaction, F (11,220) = 9.34, $p < .01$).

2. Food intake per unit body (FI/BW) was greater for female than male rats, F (1,20) = 60.11, $p < .01$. Food intake per unit weight was greater initially for rats in cages than for rats in wheels, but during all later trials rats allowed exercise ate more food per unit body weight than rats in cages. The interaction of weeks and exercise was also highly statistically significant, F (11,220) = 5.53, $p < .01$.

3. The percentage of protein ingested was calculated from the measurements of protein ingested at each of the three concentrations. Rats allowed voluntary exercise selected a higher percentage of protein than rats maintained in cages F (1,20) = 5.27, $p < .05$. These data, of importance in terms of our understanding of growth in relation to dietary self-selection and exercise, will form the basis for comparisons with groups of aged rats tested under the same conditions.

Significance to Bio-Medical Research and the Program of the Institute: One of the consistent findings of gerontological research is the decline in general activity level of old animals compared with young animals. It is important to determine whether experimental techniques may be developed to increase quantity of activity (e.g., wheel activity) and/or quality of activity (e.g., increased exploration behavior or greater response variability) for old and senescent animals. It is also important to examine the role of heredity and diet with respect to voluntary exercise throughout the entire lifespan and the effect of exercise and diet upon behavioral decrements associated with advanced old age. The knowledge and utilization of factors which change base activity levels of aged animals may result in the development of methods which can increase the productive later years of aged humans.

Proposed Course of Project: The rat studies of wheel exercise will continue to determine the effects of voluntary exercise upon longevity for paired rats and to determine the amount of voluntary exercise during advanced old age. Additional studies will determine the effect of food restriction (periodic fasting) upon voluntary wheel exercise and longevity for groups started late in the life span. Control groups will be added to obtain data with respect to normal longevity. In addition, collaboration with other investigators will be sought to determine physiological and pathological changes resulting from manipulations of diet and exercise.

Studies of wheel exercise periodicity of young and aged mice will be continued. Periodicity patterns of mice will be examined throughout old age. Additional studies will determine the level of voluntary activity for young and aged mice which are allowed voluntary control of lighting conditions within the home environment. Studies of the effect of dietary protein changes late in the life span upon voluntary wheel exercise and longevity will continue. Nutritional and behavioral correlates of lifespan will be studied using self-selection of dietary protein content.

Publication:

Goodrick, C. Effects of long-term voluntary wheel exercise on male and female Wistar rats. I. Longevity, Body Weight, and Metabolic rate. Gerontology, 1980, 26, 22-33.

Goodrick, C. Dietary factors affecting rats used in aging research: A reply. Journal of Gerontology, 1980, 35, 442-443.

Table 1a

Mean life spans and standard errors as a function of age of initiation of voluntary exercise or periodic fasting.

	Starting Age in Months		
Ad Libitum	1.5	10.0	18.5
Cages	18.7 ± .50 N = 58	19.3 ± .48 N = 18	21.9 ± .45 N = 24
Wheels	24.7 ± .69 N = 40	19.3 ± 1.03 N = 18	22.3 ± .55 N = 24
Periodic Fasting (EOD)			
Cages	31.8 ± .94 N = 24	25.9 ± 1.58 N = 12	24.4 ± .67 N = 24
Wheels	27.2 ± 1.13 N = 24	26.7 ± 1.24 N = 12	26.2 ± 1.09 N = 24

Table 1b

Percent increment in the life span of periodically fasted rats (compared with the appropriate ad libitum control group) as a function of age and voluntary exercise.

	1.5	10.0	18.5
Cages	70.8%	34.1%	11.4%
Wheels	10.1%	38.3%	17.4%

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00075-02 LBS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Stress, Coping and Personality in Aging Men and Women

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Paul T. Costa, Jr.	Chief, Section on Stress and Coping	LBS	GRC	NIA	
Other:	Robert R. McCrae	Senior Staff Fellow	LBS	GRC	NIA	
	John DeFigueiredo	Senior Staff Fellow	LBS	GRC	NIA	(7/80 EOD)
	Eleonore Lehr	Visiting Fellow	LBS	GRC	NIA	(5/80 Resigned)

COOPERATING UNITS (if any)

1. Clinical Physiology Branch
2. Department of Dermatology, Baltimore City Hospitals
3. Section on Learning and Problem Solving

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Stress and Coping

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.7	2.2	2.50

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the effects of stresses, coping processes, and enduring personality dispositions on psychological and health outcomes. One study investigates sex differences in stress, coping, and personality; a second examines the influence of daily moods and stresses on clinical conditions; a third measures personality through interviews and projective tests; a fourth utilizes existing longitudinal data to examine age related constancy or changes in personality and personal adjustment; the fifth examines health perceptions as a function of personality, age, and biomedical status; the sixth investigates individual differences in personality and cognitive performance over the adult life span.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The program of the Section on Stress and 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 determinants of all three of these categories of variables. Personality is defined as the organization of mechanisms for adaptation or coping, ad 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 needs 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 determine the nature and extent of sex differences in life stress, personality, and subjective well-being.

B. To determine prospectively whether emotional states or stressful events influence clinical conditions such as psoriasis and angina pectoris.

C. To assess adult personality through a variety of non-self-report methods.

D. To describe maturational change or constancy in personality, well-being, and to determine the relations between personality and subjective well-being or personal adjustment to aging.

E. To identify the determinants of perceptions of health or medical complaints.

F. To analyze maturational changes in cognitive performance in relation to other psychological and medical variables.

Methods Employed

A. A major effort of this section has been the recruitment of a longitudinal sample based on the Baltimore Longitudinal Study of Aging (BLSA). Beginning with the active participants in the BLSA, and excluding a 10% hold-out sample for comparative purposes at the time of a longitudinal retest, additional subjects were recruited from the spouses of BLSA participants and from a group of no-longer active BLSA participants who had agreed to consider requests for questionnaire participation. Of the 1374 persons initially asked to participate, 48 were deceased or at an unknown address; 67 were long-time BLSA failures; and no response was received from 174 others. Of the remainder, 451 (or 78%) of the men and 322 (or 64%) of the women agreed to join the study. Total sample size is thus 773. A series of questionnaires were mailed out, including: 1) a modification of the Holmes-Rahe schedule of life events. In this version, subjects were asked to check

events which had occured to them in the past year, and to rate on a five-point scale the degree of stressfulness of the event. Subjects were also asked to rate events which they anticipated would occur in the coming year. Finally, the list of life events was expanded to include such potential stresses of later life as loss of hearing and vision. 2) A well-being assessment sheet. This instrument included the Bradburn Affect Balance Scale, the Andrews and Withy D-T Scale, and items measuring satisfaction and dissatisfaction with fourteen areas of life. 3) The Profile of Mood States (POMS). This was administered under two instructional conditions--how you have felt the past week vs. right now; and before and after filling out the battery of tests. 4) The Eysenck Personality Inventory, Form A.1; and 5) A Daily Events checklist which asked participants to rate the pleasantness or unpleasantness of routine events, and also check if they had recently experienced them.

B. A group of 27 psoriatic patients were compared with three non-psoriatic control groups in terms of stressful events, personality, and mood. Control groups were spouses (N = 14), patients' siblings (N = 11), and clinic controls (N = 12) who have skin diseases unrelated to stress. All subjects completed an initial battery of personality and well-being measures and a history of recent life events. Patients, spouses and siblings completed a second questionnaire at the end of 7 weeks which included personality and measures of self-perceived health; patients and spouses completed a re-test of first battery tests at the end of the project to allow assessment of re-test stability or change. On alternate days, both patients and spouses filled out checklists of moods, states, or daily events. Finally, the patients were examined at Baltimore City Hospitals every two weeks in order to measure any changes in extent or severity of psoriasis. The design permits retrospective and prospective analysis of the effect of stress on subsequent exacerbation of psoriasis, as well as an examination of the effects of psoriasis on subjective well-being and the possible mediating effects of personality and moods.

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 other methods of assessment are being employed. Interviews and projective tests are being administered to BLSA participants on a six-year cycle. Interviews which will be video-tape recorded for later re-scoring, intensively explore life stresses associated with family, friends, careers, and leisure activities. Inquiries also will be made concerning the participant's methods and strategies used to cope with stress. Data from the interview are quantified by use of the California Q-Sort, which is completed by the interviewer and a second rater who has seen video-tapes of the interview. On another visit to the GRC, participants are 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, and cards from the Thematic Apperception Test (TAT); and the Projective Assessment and Aging Method (PAAM), a projective test designed for use with older subjects. A number of reliable coding systems exist by which to quantify data from the TAT. Data from all these

sources will be analyzed in conjunction with objective data in classifying processes of coping, adaptation, and successful aging.

D. (1) In recent years, the issue of constancy or change in personality through the adult portion of the lifespan has become a major focus of attention. The stability position has been receiving support from longitudinal studies that this Section has been conducting which show that the relative standing of individuals on personality dimensions is fairly constant over time. However, test-retest correlational methods by which the stability model can be tested, are incapable of demonstrating ordered change. In contrast, longitudinal analyses of the mean levels of personality variables might give evidence of either constancy or ordered change (e.g., maturational decline). Recent longitudinal studies of mean levels using standard measures, large samples, and extensive time intervals have concurred in finding either small changes or no changes at all in mean levels of personality traits. A third way in which meaningful change in personality can occur across the lifespan involves reorganization in the relations among traits; a change which can be assessed through factor analysis. Qualitative changes in the organization of personality might be manifest as changes in the number or composition of factors or dimensions of personality traits. Changing factor structure with age would have methodological as well as theoretical implications for gerontologists. If major differences were found in the factor structure of standard personality tests in older samples, the use and interpretation of the tests in these groups could be challenged. A few studies have reported age-related differences in factor structure; but they have been cross-sectional in nature. A recent study re-examined the question of age-related differences in the structure of personality, cross-sectionally by comparing the factor structure of the GZTS scales in different cohorts of BLSA men, and longitudinally, by comparing the first with the second and third administrations, six and twelve years later. In an attempt to determine whether cultural changes affect the organization of personality variables, the study also compared the factor structure of the GZTS for men who first completed the test in the ten years before and after July, 1968. Subjects for the study were 769 male participants in the BLSA, aged 17 to 97 (mean = 49.8) at the time of the first administration. Second administration data were obtained from 346 men aged 25 to 91 (mean = 57.6 years); third administration data were from 171 men aged 33 to 86 (mean = 61.9 years). Cross-sectional comparisons are based on first administration data from three age groups: Young (17-44, mean age = 34.4, N = 314); Middle (45-59, mean age = 51.6, N = 242); and Old (60-97, mean age = 70.4, N = 213). To assess possible structural differences due to times-of-measurement effects, the sample was divided into two groups: 455 men (age range = 17-83, mean = 52.1) before July, 1968 and 314 men after that date (age range = 18-96, mean = 45.6). In order to avoid possible confounding with practice effects, these analyses were limited to first administration data. All factor analyses were restricted to subjects with valid scores on all ten GZTS scales.

D. (2) Subjective well-being seems to be the most appropriate criterion for personal adjustment to aging, and much of the literature on gerontology has been devoted to the conceptualization, measurement, and prediction of well-being. A central concern is to determine if the relations between personality and subjective well-being can be generalized to other measures and samples of adults. Previous research has frequently shown an association

between personality variables and happiness. Investigators in this section have recently interpreted this literature (Costa & McCrae, 1980) and hypothesized that two sets of traits, which can be identified as facets of the domains of neuroticism and extraversion are related to well-being. Using measures of these dimensions of personality taken from three standard personality inventories, concurrent correlations were found with Bradburn's affect balance scale and with measures of personal security, hopelessness and a life satisfaction index covering satisfaction in nine areas of life. Ten-year predictive relations between personality measures and the affect balance scores were also significant. A study completed during the period covered by this report used a different measure, the Guilford-Zimmerman Temperament Survey, GZTS, to operationalize personality, and the Chicago Attitude Inventory (CAI) to operationalize subjective well-being as personal adjustment to aging. Subjects for the study were 557 men aged 17 to 97 at the time of the first administration of the CAI as participants in the BLSA. Subjects who remained in the study for the twelve-year follow-up period tended to be psychologically better adjusted than those who dropped out.

Measures. The eight sections of the CAI each consists of 7 items, to which the respondent marked "Agree", "Disagree", or "?". Items were coded -1 (poor attitude), 0 (?), or +1 (good attitude) and summed to form eight scales, after the original scoring method (Cavan et al., 1949). The CAI was supplemented by two global items taken from the Activities section of "Your Activities and Attitudes" questionnaire. Items L6 (assessment of life) and L8 (satisfaction with accomplishments) along with eight CAI scales were added to create a summary variable. The measures of personality were orthogonal factor scores from the GZTS: neuroticism factor scores were composed of reflected scores on the emotional stability, objectivity, friendliness, personal relations, and masculinity scales; extraversion was defined by general activity, ascendence, and sociability scores; while a third factor, predicted to be unrelated to CAI scores (and overall index), was made up of restraint and thoughtfulness scores.

Analyses. In the first series of analyses, Pearson correlations were calculated between the CAI scales and the contemporaneous GZTS personality factors. Subjects were selected who had taken the GZTS within two years of their first CAI. Analyses were conducted for two subsamples: 418 men aged 18 to 49 (mean = 36.6 years) and 391 men aged 50 to 97 (mean = 64.3 years). In other analyses, predictive relations between personality and adjustment were sought by correlating the GZTS neuroticism and extraversion factor scores with scales from the second and third administration of the CAI.

E. (1) Longitudinal analysis of somatic complaints. Previous research has shown that both age and neuroticism affect total scores on self-report health inventories; research conducted by the Stress & Coping Section concerns the influence of these two factors on reports of specific health problems. Six-and twelve-year longitudinal analyses of the physical health sections (A-L) of the Cornell Medical Index (CMI) reported last year were supplemented with cross- and time-sequential analyses. Subjects, aged 17-97, were taken from a group of male participants in the BLSA.

Analyses. Four sets of repeated measures analyses of variance (ANOVA) were conducted, using age and neuroticism as classifying variables. In all analyses, subjects were classified as Young (20-44), Middle (45-51), or Old (57+) on the basis of their age at first administration. Cross-sequential (N = 551) and time-sequential (N = 637) analyses were also conducted on first administration data. Since recruitment into the BLSA was continuous, we contrasted two successive intervals of testing (1958-1963 vs. 1964-1969) rather than two distinct time points, and thus only approximate a true cross-sequential design. Birth cohorts were defined in six-year intervals (from 1896-1901 to 1926-1931) to match the six-year period between times of testing. Age groups were defined in six-year intervals from 26-31 to 68-73. In addition to the series of analyses above, supplementary multivariate categorical analyses were performed. Because of low endorsement frequencies, distributions for most of the CMI section scores were skewed. Although ANOVA is a robust technique, relatively insensitive to departures from normality, there was some concern that results might be distorted. The Grizzle-Starmer-Koch (GSK) approach to the multivariate analysis of categorical data was applied to the item endorsement data. The GSK approach essentially involves the construction of test statistics for hypotheses involving functions of the observed proportions which are directed at the relationships under investigation and the estimation of corresponding model parameters via weighted least squares computations. Any compounded function of the observed proportions which can be formulated as a sequence of the following transformations of the data vector--linear, logarithmic, exponential, or the addition of a vector of constants--can be analyzed within this general framework. The funcat procedure algorithm of SAS produces minimum modified chi-square statistics which are obtained by partitioning the sums of squares as in ANOVA.

E. (2) Hypertension, Personality, and Somatic Complaints. Hypertension is often classified as a psychsomatic illness on the premise that psychological factors form one of the major determinants of the disease. Certain personality variables (e.g., repressed rage) have traditionally been thought to be among the determinants of hypertension. In recent years, however, critics have noted a number of problems with the methodology used in earlier studies, and better controlled research has generally failed to find an association between personality and hypertension. As an alternative, it has been proposed that evidence suggests that hypertension patients become more anxious, depressed, or concerned with bodily symptoms, although this may be an effect of knowledge of their condition or of treatment rather than of the hypertension itself, and it may result in only a temporary change. In an attempt to answer these kinds of questions, data on systolic and diastolic blood pressure (SBP, DBP), the Cornell Medical Index (CMI) and the Guilford-Zimmerman Temperament Survey (GZTS) were examined among more than 700 male subjects in the BLSA. Data were from participants who entered the study from late 1958 through 1978. At the time of first administration of the GZTS, age ranged from 17 to 97 (N = 769, mean = 49.8 years). Second administration data were obtained from over 500 men aged 25 to 91. The smaller sample size is due to the varying number of years in the study as well as death and withdrawal. A number of subjects (N = 101) were on medication for hypertension either upon entry into the study or at the succeeding four visits. Because being on medication may increase somatic concern while

decreasing blood pressure, including those subjects would obscure any real positive association between those two variables. Thus, these subjects were omitted from all analyses. No other screening was done in the major analyses to be reported. Blood pressure recordings are known to be unstable or variable. In order to provide a more stable estimate of these variables, four SBP and four DBP readings were used. These were taken over a two-day period as part of a basal metabolism rate procedure by the nursing staff. Each subject was given the standard GZTS and CMI instructions individually and completed the questionnaire during the remainder of his first or second three-day visit to the GRC. Subjects were re-administered the GZTS every six years. Because of complications in scheduling, a few subjects took the test two years in succession. To maintain consistency of the time interval, longitudinal analyses are limited to subjects who took their second GZTS 4 to 12 years after their first medical examination. The CMI was re-administered on the fifth visit; longitudinal analyses again are limited to re-tests taken 4 to 12 years after the first blood pressure readings. The average predictive intervals were 7.4 years for the GZTS and 6.5 years for the CMI.

F. An extensive literature on aging and cognitive functioning has documented that declines in performance are to be expected in a number of areas, including speed of reaction and short-term memory. Since elevated blood pressure is a frequent accompaniment of age, it has been suggested that these cognitive declines may be caused at least in part by hypertension. Investigators in this Section recently examined new longitudinal data on this question of whether blood pressure or hypertension influences intellectual performance. Researchers have pointed out that longitudinal studies are required if the long term effects of hypertension on performance are to be assessed. The present study used the Army Alpha Test, a general intelligence test with both "fluid" and "cystallized" components, which has been given to BLSA subjects over a twenty-year period. The test is administered individually under standard timed conditions. After scores are determined, the test is returned to the subject and he is given an opportunity to finish the test at leisure. Two sets of scores are thus obtained; a timed and an untimed set. All subjects were given Form A of the Alpha on their visit. Thereafter, subjects were treated differently according to age. On their fifth visit to the GRC, after an interval of from 4 to 8 years, subjects under age 70 were given a parallel Form B of the Army Alpha, in order to avoid practice effects; subjects over 70 are given retests of Form A at each annual visit. Thus, two sets of longitudinal data are available for analyses: (1) Re-test data after four-to-eight years on Form B for 350 men initially aged 17-65, and (2) data from six administrations of Form A spanning five to eleven years for 51 men initially aged 66-84. An extensive series of analyses was carried out to examine the relations of blood pressure and Army Alpha intelligence performance among men under 65, and for a smaller group of men over 65. In the first set of analyses, subjects were classified into three age groups: 20-39, 40-49, and 50-65. They were cross-classified as low, average, or high in systolic and then diastolic blood pressure using three different sets of blood pressure recordings to operationalize blood pressure groups: (1) average of 4 basals, (2) average of right and left casual sitting pressures taken by a physician at the subject's first visit

(120/80 = low; 140/90 = high), (3) averaged right and left casual blood pressure from the second visit, using the same cutoffs. The fifty-one men were identified who had taken the Alpha (Form A) on six occasions, varied in age from 66 to 84 at first administration and from 74 to 93 at sixth administration. All were free of definite cerebrovascular disease. Physicians' second visit casual blood pressure, which were used as the basis for these analyses, ranged from 98 to 186 for systolic and 55 to 115 for diastolic.

Major Findings:

A. A series of t-tests were conducted on well-being measures to determine if there were sex differences. No differences were found in the Bradburn Affect Balance Scale, total satisfaction or total dissatisfaction. Women were slightly, but significantly, lower on the D-T scale, a single item measure of assessment of life in general. With regard to specific areas, women expressed greater satisfaction with their friends, and less satisfaction with appearances, than did men. No differences were found for the other twelve areas of life, including work, leisure, marriage and government. Table 1 shows the correlation of specific areas with Affect Balance and the D-T scale.

	ABS		D-T Scale	
	Men	Women	Men	Women
House	22	17	39	32
City	17	22	33	20
Government	11	06	18	03*
Work	43	42	41	34
Leisure	38	43	49	42
Appearance	28	26	34	22
Sex	29	26	41	36
Health	30	30	37	26
Marriage	23	28	43	49
Family	28	26	44	45
Financial	29	20	33	26
Friends	35	41	46	41
Self Respect	42	41	52	36*
Faith	21	23	34	19*
Total	48	54	66	59

N = 388 Men; N = 274 Women. * = significant difference ($p < .05$).

For both men and women, satisfaction with work, leisure, marriage, family friends, and self-respect were strongly related to overall well-being; satisfaction with city, religious faith, and government were less related. Statistical comparison of pairs of correlations for men and women showed that no differences were found with regard to correlations with the Affect Balance Scale. However, satisfaction with governement, self-respect, and religious faith were more strongly related to the D-T scale for men than women. This may mean that these areas are more salient in determining overall valuation of life for men than for women. Analysis of the Eysenck Personality Inventory showed no sex differences on extraversion or on the lie

scale, which measures a tendency to falsify scores. However, consistent with previous literature, a strong effect, $F(1,691) = 52.60$, $p < .001$, for sex was noted in the neuroticism scale. Women scored almost three points higher than men. Whether this difference represents a greater willingness to admit neurotic tendencies or a higher incidence of such tendencies among women is not yet clear. In this sample, most life events are experienced with equal frequency by men and women. Only three of 56 events showed a different distribution for men than women. A raise at work was reported by 28% of the men but only 22% of the women; and 7% of the men but only 3% of the women retired. (These figures do not take into account the proportion of men and women actually in the work force the preceding year.) On the other hand, 6% of the women began a new school, while only 2% of the men did so.

B. After the first three months of the study, it became apparent that psoriatic flare-ups occured with a much lower frequency than had been anticipated at the beginning of the study. In order to obtain a sufficient number of cases for analysis, subjects were asked to continue filling out daily forms and having dermatological examinations. Twenty-three subjects remained in the study for at least three months; of these, 16 remained for the full six month period of data collection. Analysis of the data has recently begun; to date the main finding is confirmation that flare-ups occur only rarely, at least in a population under continuous observation. Using a change of 10% of total skin area involved as the provisional criterion, only three subjects showed a flare during the course of the study. However, when changes in particular parts of the body are considered separately, twelve subjects appeared to have had a flare during the study period.

C. To date, 19 subjects (including five women) have been interviewed, and Q-sort ratings have been made by the interviewer for 12 of them. Fifty men and 14 women have been given the Embedded Figures Test and the TAT-PAAM. Of these, 38 men and 10 women have been given the Holtzman Inkblot Test, and 20 of these protocols have been completely scored for a set of 22 standard variables. Eighteen of the subjects are over 70 years old.

D. (1) Both principal components and principal axes factor analyses were examined. Three factors had eigenvalues greater than unity, and accounted for similar amounts of variance in all eight analyses: 28.3% to 30.3% for the first factor; 20.8% to 22.6% for the second and 11.8% to 13.7% for the third. After varimax rotation, comparison of the two methods of factoring showed highly similar results. Only the principal components solutions are discussed, since these results are somewhat clearer. The first factor, labeled Emotional Health by Guilford, had consistent loadings (definers) from Emotional Stability, Objectivity, Friendliness, Personal Relations, and Masculinity scales across all eight analyses (three administrations, three age groups, and two times-of-measurement). The second factor, Social Extraversion, showed a pattern of General Activity, Ascendance, and Sociability in all eight analyses. The third factor, Thinking Introversion (Guilford), was clearly composed of Restraint and Thoughtfulness across all eight analyses, this factor also showed a small contribution from low Masculinity in some cases. It is clear from these results that the major definers were the same at each time and in each age group. Small variations in loadings did occur, but they did not show a clear direction or pattern across longitu-

dinal and cross-sectional analyses. To quantify these impressions of invariance, coefficients of factor congruence (Wrigley & Neuhaus, 1955) were calculated between corresponding factors for administrations, age groups, and times-of-measurement.

Coefficients of Congruence

	Factors		
	I	II	III
Administrations			
First vs. Second	.98	.99	.98
First vs. Third	.99	.99	.83
Second vs. Third	.99	.98	.91
Age Groups			
Young vs. Middle	.99	.99	.95
Young vs. Old	.99	.98	.95
Middle vs. Old	.99	.99	.95
Times-of-Measurement			
Pre vs. Post - 1968	.99	.99	.93

All coefficients were above .98 for the first two factors; for the third and smallest factor, they ranged from .83 to .98. Additional analyses were conducted on data from second administration for subjects aged 25 to 45 (N = 60), 46 to 59 (N = 154), and (60 to 91 (N = 132); and on data from the third administration for subjects aged 33 to 62, (N = 84) and 63 to 86 (N = 87). In all five analyses, these factors had eigenvalues above 1.0, and despite small sample sizes, generally similar structures were observed. These are reflected in congruence coefficients (when compared with full first administration solution) ranging from . 96 to .99 for the Neuroticism factor, .91 to .99 for the Extraversion factor, and .58 to .98 for the Thinking Introversion factor.

D. (2) Alpha internal consistency coefficients for the eight CAI scales were: Health (.61), Friends (.49), Work (.19), Economic Security (.43), Religion (.72), Usefulness (.41), Happiness (.61), and Family (.53). Concurrent correlation coefficients for neuroticism and extraversion factor scores of adjustment and positive attitudes were significant for almost all areas of life for both younger and older age groups. Only positive attitudes toward religion showed a pattern of independence from neuroticism and extraversion. As an omnibus test for the relation between GZTS factors and CAI variables, canonical correlations were computed. These were .52, $p < .001$, for the younger group, and .49, $p < .001$, for the older group. Longitudinal analyses (predictive relations) supported the cross-sectional relations. Over an interval of from 2 to 10 years, all measures of personal adjustment except religion were predicted by low neuroticism factor scores, and all but economic security were predicted by high extraversion scores. Over the even larger temporal interval of from 10 to 17 years, low neuroticism significantly predicted feelings of usefulness, happiness and positive attitudes toward family; and extraversion significantly predicted these three attitudes, the friends scale, and the global items of assessment of life and satisfaction with accomplishments.

E. (1) Mean level changes over the first interval showed significant age effects on only two sections (Sensory Systems and Genito-Urinary) which were

modest, with less than one-quarter item increase in six years. Effects for neuroticism were significant on all twelve sections and somewhat larger, than age effects with total scores about four items (42%) higher for unstable than for stable subjects. Two significant age-group-by-time interactions were replicated. Cardiovascular complaints accelerated with age, showing the greatest increase among the oldest subjects. Poor health habits declined, but primarily in the old (56+) and middle (45-51) groups, with little or no improvement in the young group. As a check on the analysis of variance results, multivariate categorical analyses were performed on dichotomized section scores to parallel the cross- and time-sequential analyses. Scores for Sensory Systems, Digestive, Neurological, Miscellaneous Diseases, and Health Habits were classified as "none or one" vs. "two or more" endorsements; scores on all other sections were dichotomized as "none" vs. "one or more" endorsements. Times-of-measurement and birth cohorts (as defined in the cross-sequential analyses) were used as factors in one set of analyses, and times-of-measurement and age groups (as defined in the time-sequential analyses) were factors in the second. Dichotomized section scores were treated as responses. In the first set of categorical analyses, significant ($p < .05$) main effects were found for Sensory Systems, Cardiovascular, Genito-Urinary and Miscellaneous Diseases. Significant effects for cohort/aging were found for Sensory Systems, Cardiovascular, and Genito-Urinary sections. In the second set of analyses corresponding to the time-sequential analysis of variance, no significant effects were observed for the time/cohort. Aging/cohort effects were found for Sensory Systems, Genito-Urinary, Miscellaneous Diseases and Health Habits. Each main effect in the categorical analysis was found in the ANOVA, although ANOVA suggested some effects not replicated or found by categorical analysis. The greater sensitivity of the ANOVA procedure may be due to the additional information available from using scores as a continuous variable. In any case, the general pattern of results is similar from the two sets of analyses.

E. (2) The table below shows the intercorrelation of the SPB and DBP readings. The internal consistency (coefficient alpha) of the average systolic and diastolic pressures were .92 and .86 ($N = 761$) respectively.

	Systolic (N = 763-829)			
	First Day		Second Day	
Reading	1	2	3	4
First Day				
Reading 1		.93	.72	.73
Reading 2			.70	.72
Second Day				
Reading 3				.92
Reading 4				

	Diastolic (N = 760-828)			
			First Day	
Reading 1		.86	.51	.50
Reading 2			.54	.53
Second Day				
Reading 3				.83

Average systolic pressure ranged from 89 to 189 (mean = 119.0, s.d. = 14.8). Average diastolic pressure ranged from 45 to 113 (mean = 75.2, s.d. = 8.9). About 9% of the sample (74 men) had systolic pressure above 140; about 6% (51 men) had diastolic pressure above 90. The correlation between age and SBP is .56 and .38 with DBP. When partial correlations of personality (10 GZTS scores) and physical and psychiatric scores (14 CMI scores) were computed with average systolic and diastolic pressure (the effects of age partialled out), none of the 24 correlations was statistically significant. Given the reliability of both personality and averaged blood pressure measures, and the size and nature of the sample, this is rather clear evidence of the independence or lack of association between personality and blood pressure. The data further showed that reliable, averaged measurements of blood pressure are also relatively stable. Between the first and fifth medical examinations (from 4 to 8 years) the stability coefficient for average systolic pressure was .66 ($N = 288$, $p < .001$); for average diastolic pressure .50 ($N = 290$, $p < .001$). Average pressures at first measurement were correlated with GZTS and CMI scores collected 4 to 12 years later. Of 24 cross-lagged correlations, again with age statistically controlled, only four were significant. Diastolic pressure was only correlated (negatively) with Personal Relations scale of the GZTS ($r = -.12$). Systolic pressure was also correlated with poorer Personal Relations ($r = -.15$) and with increased Ascendance ($r = .11$) and Sociability ($r = .12$). These correlations were only marginally significant and are probably best interpreted as chance findings. Further analyses attempted to examine patterned combinations of traits. Using scales F (Friendly vs. hostile), R (Restrained vs. impulsive), A (Ascendant vs. submissive), and M (Masculine vs. feminine), six hypotheses were tested: that higher average pressure might be found in individuals who were: (1) hostile but restrained, (2) ascendant but restrained, (3) masculine but restrained, (4) hostile but submissive, (5) masculine but submissive, (6) hostile but feminine. Neither main effects nor interactions proved significant when age was used as a covariate. Other analyses were also conducted. Scatterplots of average pressure by each GZTS and CMI variable were examined to detect a possible curvilinear relationship. None was observed. In order to eliminate possible artifacts introduced by illness or other drugs, the previous contemporaneous and predictive analyses were redone using physicians' readings of blood pressure on a subsample of drug- and disease-free Ss. Three of the 24 contemporaneous correlations were significant with age covaried, but none of these accounted for as much as 1% of the variance. Finally, an attempt was made to address the conception of hypertension as a disease process. For these analyses, hypertension was defined as average systolic pressure of 150 to 189 mm Hg. Hypotension was defined as systolic pressures from 89 to 100 mm Hg or diastolic pressures from 45 to 60 mm Hg. A normotensive group was also included with systolic pressure at 109-120 mm Hg and diastolic pressure at 74-85 mm Hg. Analyses of covariance with age as the covariate showed no effects for hypertension classification on any of the personality scales or the CMI sums.

F. In the first set of analyses, each of the eight timed subtests and the total Alpha scores were examined for six (systolic or diastolic by Basal, First Casual, or Second Casual) definitions of blood pressure classification. Age group and time were also used as classifying variables. Fifty-four F

ratios were computed in which blood pressure was a main effect, and 162 F ratios in which it was a part of an interaction term. One main effect was significant ($p < .05$). There were no significant interactions with age group, although there was one significant ($p < .05$) interaction with time and six significant ($p < .05$) age x time x blood pressure interactions. One of these was found for the Second Casual pressure on both systolic and diastolic pressures; another was found for both First and Second Casual but not Basal pressures. The other interactions were not replicated. By chance, if these were independent we would expect 8 (5%) of the 162 interactions terms to be significant at the .05 level. We found seven. There is no evidence in these data for any effect of hypertension (within the present BP ranges) on Army Alpha performance among men under 65. Analysis of the untimed sub-tests and total showed 3 of 48 main effects, and 5 of 144 interaction terms were significant. Again chance seems to be a compelling explanation. When analyses were restricted to 117 men free from drug therapy and major illnesses other than hypertension, a few effects were replicated across different operationalizations of blood pressure groups, and showed poorer performance for individuals with higher levels of blood pressure. Unlike previous findings reported by some other investigators, no age-by-blood pressure interactions were observed, nor was there any evidence that hypertensive individuals declined more in intellectual performance than normotensives over the retest interval. In the second set of analyses, 51 men aged 66-84 and retested on five occasions within an 11 year interval were classified into two levels of blood pressure and age. Repeated measures analysis of variance using six levels of the repeated factor showed only one sub-test, "Practical Judgment", where hypertensive subjects had consistently poorer performance. As in the analyses of men under 65, men over 65 do not appear to change in cognitive performance as a function of elevated blood pressures.

Significance to Biomedical Research and the Programs of the Institute:

A-C. 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 of the 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, on the developmental antecedents of Openness to Experience, and on the determinants of well-being and successful aging contribute directly to the mission of this institute.

D. (1) The invariance of factor structures in the GZTS in the BLSA sample is clearly evident. Definers of factors stand out in each case from the

marginal elements which show slight variations from one analysis to another. Despite aging, attrition, and possible practice effects, the same pattern is seen at each administration. From a practical point of view, gerontologists who employ personality measures should be encouraged by evidence that certain standard personality measures, at least, maintain the important psychometric property of factorial validity when used in older populations.

D. (2) Theorists have drawn attention to the host of distinct influences which are presumed to operate in determining levels and varieties of well-being. In general, regardless of their theoretical rationales, measures of well-being, morale, or life satisfaction tend to operate in the same way. One of the important ways in which they operate similarly is in relation to personality variables: individuals higher in subjective well-being appear to be lower in neuroticism and higher in extraversion. Considerable research has been devoted to efforts to understand the determinants of life satisfaction, morale, or well-being in the elderly. Social interaction, and related concepts like social support and networks, have often been identified as correlates of life satisfaction, but some investigators have concluded that quantitative variables such as scope or frequency of interaction are poor predictors of well-being and that a broader understanding of adjustment to the process of aging will ultimately be found in the quality of the interactional experience. One interpretation of the association of extraversion and neuroticism with well-being fits this prescription for research on the quality of interactional experience. Extraverts characteristically show warm, expressive interactions, while the personal relations of individuals higher in neuroticism are often characterized by hostility or self-consciousness. But the implications of these findings have seldom been recognized. The domains of neuroticism and extraversion have a host of behavioral correlates, and one or the other of these personality dimensions may be the "third variable" which accounts substantially for correlations between morale or life satisfaction and such variables as perceived health, marital status, activity level, or the use of a confidant.

E. (1) Age has a selective effect on physical complaints, while neuroticism appears to produce a more general and diffuse effect on physical complaints. These findings contradict the stereotype of aging people as hypochondriacs or "crocks", but are consistent with other studies. This does not mean that there are no elderly hypochondriacs; however, the proportion of such people is no higher in old age than in youth or middle age. At any age, excessive complaints are associated with neuroticism, or poor psychological adjustment which is unrelated to age. Clinicians or researchers who employ self-reports of health or illness should be aware of the pervasive role of neuroticism. Many researchers have suggested that self-ratings of health can provide valid cost-effective measures of health assessment. But to the extent that self-ratings of health share the same determinants as measures of somatic complaints, such ratings will be determined by both objective health and neuroticism. Research concerned with the influence of physical health on morale, illness, behavior or adjustment should either use objective measures of health or supplement self-ratings with measures of neuroticism in order to control for its effects.

E. (2) Individuals who are informed that they are hypertensive often react by temporary anxiety, depression, or somatic concern - understandable responses in the circumstances. It appears that these reactions are indeed temporary, with no permanent or long-term effects, as shown by the lack of predictive relations between blood pressure and personality and perceived health scores taken several years later. The independence of blood pressure from enduring personality dispositions certainly does not mean that physicians and psychologists have nothing further to offer each other in this area. It means, rather, that the focus should shift from etiology to treatment.

F. The two studies reported here both suggest that elevated blood pressure levels have little if any effect on intellectual performance for men in the age range from 17 to 88. These conclusions, however, must be tempered by a number of qualifications and limitations in the present study. Most importantly, the levels of elevation of blood pressure seen in the BLSA group is relatively modest; there are no cases of untreated, severe hypertension. It may well be the case that the deleterious effects of hypertension on cognition only manifest themselves in extreme cases. While the present results cannot rule out that possibility, they do speak to the much more common problems of borderline and moderate hypertension. Recent research has indicated that even small chronic elevations of blood pressure result in an increased risk of mortality from coronary heart disease; but a similar claim does not seem to be justified with regard to the danger of intellectual impairment. If exercise of cognitive skills restores or prevents the adverse effects of hypertension, then the negative findings of the present study would not preclude the possibility that even moderate levels of hypertension might be a cause of intellectual decline among the less gifted. At present, evidence from a number of sources points to the conclusion that hypertension is more likely to be detrimental than beneficial if it has any direct effect on cognitive capacity at all. There are sound medical grounds for attempting to reduce hypertension and nothing in the cognitive performance literature would contest that approach.

Proposed Course of the Project:

A. Sex differences in stress, coping, and personality will remain a major concern in on-going and future research. In addition to routine analysis of all data by sex, plans are being made for the inclusion of questionnaires specifically designed to measure stresses of particular relevance to women. The influence of potentially stressful events will be assessed by comparing the well-being levels of groups who have experienced different events. The presence of adaptation level effects will be assessed by comparing the perceived stressfulness of daily events in different age, socio-economic status, and life events groups.

B. Analysis of the data on stress and psoriasis will be completed this year. If predictive relations are found between stress, personality, and psoriasis, the study can be replicated on a larger sample, and possible interventions to modify stressfulness of events will be considered.

C. Data collection will continue. It is anticipated that the rate of interviewing subjects will increase, and that the second Q-sort rating of video-taped interviews will be begun. Typed transcription of TAT-PAAM protocols will be made preparatory to scoring. Plans for the incorporation of computer-assisted testing devices are being made; this would allow both controlled administration and immediate scoring of tests.

C. 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 in 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. Specific plans include an analysis of the effects of retirement and widowhood on subjective well-being.

E. (1) Future research will examine personality and perceived health as predictors of symptomatic and asymptomatic Coronary Heart Disease groups among a sample of BLSA males who have been followed up to 20 years. Subjects will be classified as unconfirmed angina, angina with corroborating EKG evidence, or asymptomatic (EKG evidence but no anginal complaints during followup). Using an unselected (non-clinic) sample, prospective design, objective personality assessment and extensive follow-up intervals, the proposed course of research will determine whether personality affects the presentation and perhaps the experience of anginal symptoms.

E. (2) Among the questions in the hypertension and personality field which appear useful to pursue are: (i) What kinds of cognitive and behavioral treatments are effective in lowering blood pressure? (ii) Which types of intervention are most effective for individuals characterized by different personality traits? (iii) How can information on personality be used in the treatment of distress-related medical complaints among hypertensives? (iv) How do personality-related differences in the utilization of medical care affect programs for the prevention of hypertension? (v) What personality factors affect compliance or non-compliance with prescribed medical treatment? The role of traits in the major personality domains of neuroticism, extraversion, and openness should be investigated in relation to treatment of hypertension. These projects will involve collaboration with other investigators and are dependent on personnel and resources to implement them.

F. Further analyses of the Army Alpha are planned, particularly longitudinal studies of changes in cognitive performance over an age span from 17 to 97. Analyses planned include: comparison of initial scores of those who returned for retest with those of dropouts (from whatever causse); speed vs.

power, or determination of which sub-tests are most heavily influenced by the time constraints of the regular administrations; also, use of untimed as well as timed test scores in repeated measures analyses will allow an interpretation of the extent to which declines in test performance with age are a function of speed only, or speed plus some more basic intellectual functions. Finally, future analyses will examine the relations of age and personality dimension, specifically neuroticism, extraversion, and openness to experience with cognitive ability as measured by the Alpha. In particular, one hypothesis to be tested is that subjects who are more open to experience continue to learn and thus maintain intellectual performance longer than subjects closed to experience.

Publications:

Costa, P. T., Jr., & McCrae, R. R. Influence of extraversion and neuroticism on subjective well-being: Happy and unhappy people. Journal of Personality and Social Psychology, 1980, 38, 668-678.

Costa, P. T., Jr, McCrae, R. R., & Arenberg, D. Enduring dispositions in adult males. Journal of Personality and Social Psychology, 1980, 38, 793-800.

McCrae, R. R., Costa, P. T., & Arenberg, D. Constancy of adult personality structure in males: Longitudinal, cross-sectional, and times-of-measurement analyses. Journal of Gerontology, in press.

Costa, P. T., Jr, & McCrae, R. R. Somatic complaints in males as a function of age and neuroticism: A longitudinal analysis. Journal of Behavioral Medicine, in press.

Costa, P. T., Jr., McCrae, R. R., & Norris, A. H. Personal adjustment to aging: Longitudinal prediction from neuroticism and extraversion. Journal of Gerontology, in press.

Costa, P. T., Jr., McCrae, R. R., Andres, R., & Tobin, J. D. Hypertension, somatic complaints, and personality. In M. F. Elias & D. Streeten (Eds.), Effects of hypertension on behavior. Beech-Hill Publishers, in press.

Costa, P. T., Jr., & Shock, N. W. New longitudinal data on the question of whether hypertension influences intellectual performance. In M. F. Elias & D. Streeten (Eds.), Effects of hypertension on behavior. Beech-Hill Publishers, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00076-01 LBS

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Openness to Experience and Coping Styles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert R. McCrae	Senior Staff Fellow	LBS	GRC	NIA
Other:	Paul T. Costa, Jr.	Chief, Section on Stress and Coping	LBS	GRC	NIA

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Behavioral Sciences

SECTION

Stress and Coping

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project is concerned with the personality dimension of openness to experience and its relation to coping styles. One study investigates alternative techniques for the measurement of openness, including self-report, spouse ratings, sentence completion, and interviewer rating; a second study explores the relations between openness to experience and styles of coping and defense; a third study is concerned with the developmental antecedents of open dispositions in adulthood.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Openness to experience is a broad dimension of personality which comprises a number of traits (including dogmatism, rigidity, openness to feeling, 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 often 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, new and standard measures of coping and defense mechanisms will be used so that the relation between openness and degrees of defense and styles of coping can be determined. In order to investigate the antecedents of openness to experience, research will be done on the developmental history of the individuals who, in adulthood, are characterized as relatively open or closed.

Specific project objectives:
A. To determine the correspondence between assessments of openness derived from self-reports, spouse reports, sentence completions, and interviewer ratings.

B. To investigate the relations between coping and defense mechanisms and the disposition of openness to experience.

C. To examine parent-child relations and other developmental variables as antecedents or consequences of openness.

Methods Employed: A. The Experience Inventory, a 48-item scale measuring openness in the area of phantasy, esthetics, feelings, actions, ideas, and values, was administered to a sample of male volunteers, participants in an on-going longitudinal study (The Normative Aging Study, Boston, Massachusetts). On 230 men, responses to Loevinger's Washington University Sentence Completion Test were also available. Sentence completions of the 45 highest and 45 lowest scorers on the Experience Inventory (EI) were examined independently by two judges, blind to the EI scores. This procedure allowed an assessment of the degree to which openness, as measured on a self-report scale, would be expressed in a projective test. In another phase of this study, an expanded version of the EI was administered to participants in the BLSA and their spouses (N = 773) as part of the NEO Inventory. In order to compare self-reports with spouse ratings, a parallel form of the test requiring spouses to rate their husbands or wives on the same items will be administered to both members of the couples who have agreed to participate in the study. In this version of the test, items have been re-written in the third person in order to provide a rating instrument which is as directly comparable to the self-report scale in content as possible. Finally, a subset of subjects, who are being interviewed and video-taped, are being rated by two judges on each of six facets of openness. Comparison of these interviewer ratings with self-reports will be made.

B. A series of instruments designed to measure styles of coping and defense will be administered to a sample of 773 men and women, participants in the BLSA and their spouses. Included will be the Marlowe-Crowne SD scale, which has recently been interpreted as a measure of defensiveness; the Ihilevich and Gleser Defense Mechanism Inventory, which consists of a series of vignettes to which subjects are asked to choose a response they might make; the Ways of Coping inventory of Lazarus and Cohen; and a Coping Self-Interview developed by the PI (R.R. McCrae). On the basis of their previous response to a life events questionnaire, subjects will be asked to complete the Ways of Coping questionnaire with regard to an event they experienced within the past year classified as a threat, loss or challenge. A review of the literature suggested fifty specific defensive reactions or coping strategies which can be used. On the Coping Self-Interview, subjects are asked to think of a threat they have faced in the past year, and to determine, for each of the fifty mechanisms described, whether they experienced the reaction or used the strategy; and, if so, whether it seemed to help solve the problem, and whether it made them feel better. Similar questions are asked with regard to a "loss" event and a "challenge" event. Subjects who are interviewed are rated by the interviewers on the use of these fifty mechanisms also. Data on coping styles will be analyzed in conjunction with information on the personality dimension of openness to experience.

C. The Parent-Child Questionnaire, which asks subjects to describe retrospectively the relationship they had with their parents, was administered to 773 BLSA participants and their spouses. This instrument yields scores on the dimension of Love vs. Rejection, Casual vs. Demanding, and Attention. Subjects who perceive their parents as rejecting and demanding are hypothesized to be more closed as adults. In addition, information on some early experiences including death or absence of parent or divorce can be obtained from this questionnaire. Several personality theories would suggest that children growing up in broken homes should be more closed to experience as adults. Another approach to understanding the development of openness is provided by correlation with the Loevinger Sentence Completion Test, which measures level of ego development in adolescents and adults. The relation between ego level and openness was examined in a sample of 230 male volunteers who were participants in the Normative Aging Study (Boston, Massachusetts).

Major Findings:

A. Crosstabulation of judges' rating of openness based on sentence completion responses with total Experience Inventory scores are shown in the table below.

	First Set (N= 40)				Second Set (N= 50)			
	First Judge[a]		Second Judge[b]		First Judge[c]		Second Judge[d]	
	Open	Closed	Open	Closed	Open	Closed	Open	Closed
EI Total Openness								
Upper 20%	15	2	14	3	23	5	23	5
Lower 20%	6	17	5	18	5	17	8	14

[a] $\chi^2=12.75$, $p< .001$; [b] $\chi^2=12.07$, $p< .001$; [c] $\chi^2=15.32$, $p< .001$; [d] $\chi^2=9.10$, $p< .01$.

In two separate samples, two judges were able independently to discriminate between the responses of open and closed men. The correlation between combined judges' ratings and the self-report criterion was $r = .66$, $N = 90$, $p < .001$. This demonstrates that openness is expressed in projective as well as self-report methods. The fact that nine months separated the administration of the two measures supports the argument that openness to experience is an enduring disposition. Administration of the expanded Experience Inventory to the BLSA has been completed, and responses have thus far been received from 636 subjects. In the interviewer rating phase of this study 20 subjects have been interviewed and rated for openness to experience by the interviewer.

B. Since this study had just begun, there are no findings to date.

C. The correlation between total openness and ego level was $r = .23$, $N = 230$, $p < .001$. When corrected for differences in verbal fluency, this correlation rose to .31. This suggests that openness may contribute to the attainment of higher levels of ego development. However, cross-sectional results showed no increase in ego level with age, even among open men. Thus, the developmental consequences of openness may be restricted or limited to the period of pre-adulthood.

Significance to Biomedical Research and the Programs of the Institute:
Successful adaptation to aging requires the constant use of coping and defensive processes. Medical researchers have been increasingly attentive to the role of individual 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 disposition 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.

Proposed Course of the Project:
A. As a part of a larger spouse-rating questionnaire, a rating version of the expanded Experience Inventory will be sent to 205 couples who have agreed to participate in this project, and who have already completed the self-report version of the EI. In addition to correlations between self-report and spouse-rating measures of openness, this design will allow analyses of the extent to which married couples are similar with regard to openness, and whether individuals tend to over- or under-report their own openness in comparison with the perceptions of their husband or wife. Exploration of highly discrepant vs. highly concordant cases will also be made. In future analyses, spouse ratings obtained in this study can be used as an alternative measure of personality free from the possible biases of self-report instruments. Interviews and

personality ratings will continue, with the expectation that an additional 40 subjects will be rated in the coming year.

B. Analysis of the coping and defense battery will be used to answer the questions: 1) Are there individual differences in coping styles that can be detected across several different instruments? 2) Are coping processes determined by the demands of the situation, the characteristics of the individual, or both? 3) Are open individuals less defensive, and more mature in their choice of coping strategies? 4) Are particular kinds of coping used more frequently or more successfully by open people? Longitudinal readministration of these instruments now planned will provide data on changes in coping styles with age among open and closed men and women.

C. Analyses of parent-child relationship variables with self-report and spouse-report ratings of openness to experience will be conducted. Additional evidence on the developmental antecedents of Openness to Experience will be sought by examining data obtained in a biographical questionnaire to determine whether childhood environments or later life events (e.g., "off-time" transitions, occupational shifts, military experience) influence present levels of openness.

Publications:

McCrae, R. R., and Costa, P. T., Jr. Openness to experience in Loevinger's sentence completion test: Dispositional contributions to developmental models of personality. Journal of Personality and Social Psychology, in press.

NIA Annual Report
October 1, 1979 through September 30,1980
Gerontology Research Center
Laboratory of Cellular and Molecular Biology

The Laboratory of Cellular and Molecular Biology brings together various research projects that are related through a common interest in the processes of genetic information transfer and the genetic basis for the biological aging process. The Section on Inorganic Biochemistry is concerned with fundamental studies at the molecular level of biological macromolecules 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 Section on Macromolecules is concerned with the interaction of cells with synthetic macromolecules. Age changes on the cell surface are probed with specially designed macromolecules, and potential polymeric drugs are synthesized. The Section on Cellular Aging and Genetics has been concerned with cell replication and repair of DNA damage as a function of aging. The activities of this section are presently curtailed, since the Section Head, Dr. Edward L. Schneider has resigned. We are currently recruiting a replacement for Dr. Schneider.

The acquisition of the Varian XL-200 NMR Spectrometer makes possible sophisticated structural studies in solutions that were not previously feasible. Unfortunately, our instrument, the first of its kind delivered in the U.S., has been plagued with difficulties, and has been available to us only one-third of the time. In spite of these problems, many interesting studies have already been carried out. We have great hopes for future studies using nuclear magnetic resonance.

The hypothesis that there is a relationship between aluminum and Alzheimer's disease has been strengthened recently by the confirmation of Crapper's findings in another laboratory. We have reinforced our efforts to study the possible biochemical intermediates that may be responsible for this relationship, as well as other manifestations of aluminum toxicity. The availability of a broad band probe with our Varian XL-200 NMR makes possible studies of the ^{27}Al nucleus. Very few studies have been carried out with this nucleus, and some effort has been exerted toward studying the usefulness of this nucleus. We have found that the ^{27}Al nucleus is extremely sensitive to changes in its coordination sphere, so that chemical shift due to different environments are spread far apart. Aluminum binding to phosphate (ortho, pyro, triphosphate, ATP, etc.) is particularly readily identifiable, since the resonance is shifted upfield from hydrated Al ion, while all other known Al binding causes downfield shifts.

One of the major goals of our work with Al is to understand its interaction with DNA. DNA greatly broadens the ^{27}Al signal, and heating the Al-DNA solution caused even more extensive broadening and subsequent cooling led to the original band width. No other method has been able to detect the subtle changes in structure that occur on cooling the DNA strands back together by Al crosslinks. The most likely explanation for the phenomenon is that the Al crosslinks at elevated

temperature are much more mobile than at room temperature.

The reaction of Al with ATP is of importance since the formation of Al-ATP complexes is believed to be a major reason for the toxic effects of Al on patients under dialysis treatment. By ^{27}Al studies it could be shown that even at pH as low as 1.5, 90% of Al is bound to ATP, and at pH 2, 98% becomes bound. ^{31}P and ^{1}H NMR studies on the interaction showed that Al binds to γ, and to a lesser extent to β phosphate, and also to the adenine of ATP. The β-phosphate was affected only at intermediate pH, where the Al apparently forms a chelate ring with β and γ phosphate oxygens. At low and high pH the Al binds to γ-phosphate.

Al interactions with the alternating copolymer poly d(AT) · d(AT) were studied as a function of Al concentration and pH. Since this polymer contains a repeating sequence, in the absence of divalent metals, the heat-denatured polymer can be completely renatured by cooling. Al-crosslinking prevents this renaturation by cooling, and the extent of renaturation can then be used as a measure of the amount of polymer that is not crosslinked by Al. The results indicate, for example, that at pH 5 and 1.25 Al/dAT residue, about 2/3 the polymer was crosslinked, and at 1.25 Al/dAT 100% was crosslinked. When the pH is raised to 7, very little crosslinking occurs. These studies are important because crosslinking could be responsible for the deleterious effects of Al in Alzheimer's disease - this is of course a speculation.

The effects of metal ions on genetic information transfer depend to a great extent on the modes of interaction of metal ions with nucleic acids, and to better understand the latter we carry out studies with homopolynucleotides. Hg^{2+} and Ag^{+} have been selected as metal ions that bind very strongly to the nucleotide bases, but have different charges and geometrics; the toxic properties of Hg^{2+} have long been recognized. The homonucleotides selected were poly(U), poly(I), and poly(X). A rather complex array of reactions between poly(U), poly(I), and poly(X) and Ag^{+} and Hg^{2+} can be explained by rather simple chemical principles: (1) low pH and high pH favors binding to unprotonated and protonated groups, respectively, (2) divalent ions shun proximity to each other, and (3) ready release of proton from a protonated group favors binding to that group instead of to an unprotonated group.

NMR studies with ^{31}P on a soluble chromatin preparation from rat liver showed a single line of $\sim$ 100 MHz width; according to its chemical shift with respect to an external reference it was assigned to the resonance of DNA phosphate. The signal surprisingly has a striking similarity to the signal from nucleosomes. The finding could be important, since it seems to indicate that the freedom of motion of the phosphate groups is independent of the additional structural restraints that are imposed on DNA in chromatin as compared to the simpler nucleosomes.

Magnesium ions decrease the line width of the DNA phosphate resonance by 40%; this can be interpreted by the metal disrupting protein-DNA interactions by competing with the protein for the DNA phosphate binding sites. Aluminum ions have a very different effect: the ^{31}P line is split, one line corresponding to uncomplexed DNA and the other, which is considerably broadened, to Al-bound DNA. These results indicate that Al binding increases the

rigidity of the DNA in its environment and that the Al ions bind to DNA much longer than Mg, i.e., the rate at which Al comes off the DNA is greatly decreased. We are encouraged by the ability to use NMR techniques to follow metal-DNA binding in a biologically relevant environment.

Several of the observations from the NMR experiments are confirmed by biochemical studies. Among these is competition between magnesium and protein for DNA Phosphate. Ordinarily chromatin melting occurs in one phase; the nucleosomal and internucleosomal DNA melt simultaneously. In the presence of Mg the melting is biphasic, with the internucleosomal DNA melting first. Thus the Mg lessens the ability of H-1 histone to protect internucleosomal DNA. Copper, which greatly decreases the stability of the DNA double helix and therefore decreases its melting temperature, has no such effect on chromatin; evidently the histones, both nucleosomal and H-1, protect the DNA against the copper. When H-1 histone is removed from chromatin, the latter melts biphasically, with internucleosomal DNA denatured before the nucleosomal DNA. Copper affects the melting of H-1-stripped chromatin in a manner similar to its effect on free DNA; apparently the loss of H-1 removes the protection of DNA chromatin from attack by copper. We had hoped that the dramatic effect of Cu on DNA would help to detect age differences in chromatin that would otherwise go unnoticed. However, no age changes of significance could be detected in the melting behavior of old and young chromatin, either in the presence or absence of copper.

In a collaborative study, Dr. Moudrianakis at John Hopkins University, has found a marked decrease in the activity of an enzyme that cleaves 15 amino acids from histone H2A. This cleavage may be involved in genetic regulation; thus the change in activity could be important.

We have refined our studies on the detection of age changes in rat liver ribosomes by testing fidelity of protein synthesis when young and old ribosomes are challenged with Mg^{2+} or paromomycin. Both of these are known to produce errors in protein synthesis. With Mg^{2+}, there is no apparent age difference in the error induced in protein synthesis. With paromomycin, there is an increase in error frequency of 6-9% in old when compared to young (at $p < 0.02$). Error was measured as leucine (Leu) to phenylalanine (Phe) ratio when poly(U) was used as a message - Phe represents correct and Leu incorrect incorporation. We do not know whether a change in error rate of such low magnitude has any biological significance. It should be emphasized that this study does not constitute a test of the "error" hypothesis, since we did not measure age changes in indigenous error rates. Instead, we challenged the ribosomes with agents known to induce error, and then measured age changes in this induced error. The results reflect a slight difference in the capacity of the ribosomes to prevent error.

The aminoglycoside antibiotic paromomycin (PM) elicits large misreading effects by binding to a small number of sites on eukaryotic ribosomes; high Mg concentrations, which may affect fidelity directly by enhancing codon-anticodon association, produce smaller effects. Mg is a required cation in protein synthesis, and at low Mg concentrations the error frequency is normally low. We have investigated further the mutual dependence of Mg and PM concen-

tration on correct and error elongation, using the same poly(U) programmed ribosome system we used in our study of ribosomal fidelity. With increasing PM there was a general shift of both Phe (correct) and Leu (error) responses toward lower Mg, and there was a decrease in the separation of their maxima. The dependences turn out to be such that at low Mg, e.g., 5 mM, the Phe as well as the Leu incorporation is increased (interestingly enough) by the addition of PM, while at higher Mg the Phe incorporation may be decreased by addition of PM as that of Leu is increased. The results demonstrate the wide variety of effects on fidelity that can be produced by the combined action of PM and Mg^{2+}.

Direct exposure of ribosomes to aluminum was tested by addition of aluminum lactate to ribosome solutions at neutral pH. An Al concentration-dependent inactivation of the ribosomes for polypeptide synthesis was observed. The inactivation was not reversed by lowering the free Al concentration either by dilution or by sedimentation of the ribosomes. The error frequency as measured at high Mg concentration was not affected by Al. Thus, if aluminum is presented at high enough levels directly to ribosomes, the ribosomes apparently become irreversibly inactivated, probably by tight binding of Al.

We are investigating the reason for the importance of metal ions in determining whether or not RNA polymerase (E. coli) can distinguish between ribo- and deoxy- nucleotides for incorporation into RNA. Studies on conformational changes induced by the three metals that can activate the enzyme (Mg^{2+}, Mn^{2+}, and Co^{2+}) have been previously reported. To further probe the effects of these metals double-label kinetic studies using ^{3}H-ATP and $^{32}P_{\alpha}$-dATP have been employed. Incorporation of ATP into cold TCA insoluble RNA, inhibition of this process by dATP, and misincorporation of the latter have been followed. The results are obtained as V_{max}, K_M, K_I and Q (amount of misincorporated dATP). The V_{max} and K_M of the ATP incorporation are in the order $Co^{2+} < Mn^{2+} < Mg^{2+}$; and K_I for inhibition by dATP, and dATP misincorporation, Q, are in the order $Mg^{2+} < Mn^{2+} < Co^{2+}$. The V_{max} differences are consistent with the differential effect of the metal cations on the enzyme conformation monitored by the previously reported melting studies. The K_M differences, however, demonstrate differences in enzyme-substrate affinity. The trend in the K_I values is best interpreted in terms of metal induced discrimination between the competing ligands, ATP and dATP, at the substrate binding step. Thus Co^{2+}, which has the lowest K_M, also has the largest K_I. This binding step, however, does not seem to be significant for fidelity, as is shown by the trend for the amount of misincorporated dATP. We conclude that the incorporation preference takes place at a later step, after the formation of the enzyme-substrate complex. This preference is also metal dependent, but in a manner not necessarily related to the trend in the competitive binding step.

The effect of aging on cellular response to DNA damage was investigated by several approaches. BrdU-differential staining techniques were utilized in vivo and in vitro to conduct SCE and cell replication studies. Ectopic bone implantation was employed for bone metabolic studies.

Spontaneous SCE levels were estimated in vivo in young and old mouse and rat bone marrow cells. These studies indicated that there were no young and

old cell animal cell populations. To examine the effect of young and old environment of SCE induction, Ehrlich Ascites tumor cells were implanted into young and old C57Bl/6J mice which were treated with various concentrations of mitomycin C. While SCE induction declined with aging in bone marrow cells, SCE Induction in the tumor cells was unchanged. SCE induction by mitomycin C in Chinese hamster cells was found to decrease as a function of the age of the serum donor in cells cultured in media containing human serum.

In utero studies of SCE induction in C57Bl/6J mice were expanded to examine differential transplacental transport of several mutagens and carcinogens. These techniques were also employed to demonstrate changes in mutagen and carcinogen transport at different stages of pregnancy. SCE induction was examined in several hepatic cell lines which contain the enzymes necessary to activate a wide range of carcinogens and mutagens. This system should provide a sensitive approach to examining those agents which require metabolic activation. While hormones such as hydrocortisone, testosterone, progesterone and dihydroepiandosterone (DHEA) do not induce SCE, they augment the frequency of SCE induced by other agents such as mitomycin C or ultraviolet light.. The cloning of cells and cell growth kinetics in the presence of mitomycin C were unchanged as a function of the age of the cell culture. The antibiotic tetracycline was demonstrated to induce SCE in vivo. Most of the induction was probably due to breakdown products of this antibiotic. In utero studies in mice indicated that certain mutagens and carcinogens have differential transport across the placenta. These studies have also demonstrated that placental transfer of mutagens and carcinogens changes with gestation.

The results of the studies of SCE and aging indicate that in vivo spontaneous or background SCE are not changed with aging, that environment does not appear to play a major role in the decline in induced SCE that is observed with aging, and that human serum appears to have a factor which augments SCE induction. This factor appears to lose its potency with aging. These findings increase our knowledge of cellular response to DNA damage with aging. In utero SCE examination provides a new approach to the examination of transplacental transport of mutagens and carcinogens.

The formation of ectopic bone from implanted bone powder decreased as a function of the age of the animal receiving the implant. This decrease provides an animal model system for studying alterations in bone metabolism with aging.

Previous work had shown that Dehydroeprandrosterone (DHEA) inhibited cytotoxicity induced by polycyclic hydrocarbon carcinogens (PHCs) and mutagenicity in rodent cells. The present work focused on human cells which are much less susceptible to PHCs. Our results indicate that DHEA in concentrations as low as 3×10^{-6} M inhibits metabolic activation of benzo(a)pyrene (BP). We have observed inhibition of BP-DNA adduct formation in human cells by DHEA. This phenomenon has not been studied previously in any cell type, and represents a possible mechanism of DHEA protection against PHC mediated DNA-damage. We have observed a significant effect of factors in human plasma on BP-metabolism in cultured human cells. This evidence comes from studies on cells from a single donor cultured on several media, differing only in the source of plasma. The factor appears to be inhibitory since increased concen-

trations of plasma result in decreased metabolism. The nature of the inhibitor and its mechanism are as yet unknown. We have conducted studies on the effects of DHEA and on plasma from different donors on excision of DNA adducts of the PHC 7-Bromomethyl benz(a)anthracene (7BMPA). In contrast to our studies on metabolic activation of PHCs, we observed no effect of DHEA or plasma from different donors on 7BMBA excision. Our assay measures only the excision step in the process of excision repair of DNA. The possibility of plasma effects on subsequent steps of DNA repair is as yet unexplored. Our results indicate that DHEA and other plasma factors may play a physiologic or pharmacologic role in the modulation of DNA damage by certain carcinogens. This may have importance to incidence of cancer and other age-related degenerative diseases in man.

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 may be improved. In the group of adrenergic blockers it has been established that attachment of drugs to a polysaccharide carrier by a stable bond sustains their activity. Such hydrolysis-stable conjugates do not penetrate cell membranes, a process which conditions many side-effects. Furthermore the conjugates have increased specific recognition compared to the parent drug. In the group of lipid soluble vitamins, the formation of a physical complex between the carrier which has been synthesized and vitamin A leads to sustenance of biological activities with some decrease in its toxic effects.

Beta-adrenergic antagonists are extensively used in clinical practice, to treat hypertension. Numerous studies with these drugs on cellular systems show that the drugs' effects are triggered by binding the drugs to the membrane located receptors. In vivo effects may also be independent of the ability of these drugs to penetrate the cellular membranes or the blood/brain barrier This makes the beta-adrenergic antagonists possible candidates for conversion into macromolecular form which would sustain pharmacological activity. The beta-adrenergic antagonist, alprenolol, was attached in an irreversible manner to macromolecular dextran via side arms that differed in length. The ability of these macromolecules to bind to the beta-adrenergic receptor of frog erythrocytes and to catecholamine-binding antibodies raised against partially purified receptors was studied. Compared to the parent drug the potency of binding of macromolecular alprenolol to the receptor decreased to about 1/10, 1/600, and 1/8000 when the length of the arm separating alprenolol from the dextran moiety was 13, 8, and 4 atoms, respectively. In contrast, the binding potencies of the parent drug and of all its macromolecular derivatives for the antibody were within the same order of magnitude. Thus, conversion of a drug to a macromolecular form may not only sustain its binding activity but may also lead to a higher selectivity. The macromolecular derivatives described here may be suitable probes for investigation of location and molecular properties of the binding sites for beta-adrenergic drugs.

Lipid soluble vitamins, in addition to their obvious nutritional values, have additional beneficial effects. Unfortunately, to obtain a significant measure of these additional effects, doses which already elicit toxicity are required. Thus retinoids, which are derivatives of vitamin A, have been shown

to reduce the incidence of lesions in epithelial sites of animals treated with chemical carcinogens. Work done in this program focuses on lessening the toxic effects of retinoids through transforming them into a water-soluble macromolecular form. A new, water-soluble, polymer-linked form of retinal was synthesized and tested for its ability to support the growth of vitamin A-deficient noninbred rats and to inhibit the proliferation of melanoma cells in culture. Retinal was conjugated to hydrazide of carboxymethyldextran in the presence of alpha-and beta-cyclodextrins. The aqueous solutions of the product contained between 200 and 1,000 μg retinal/mℓ as opposed to the low water solubility (<0.01 μg/mℓ) of retinal itself. The retinal-dextran complex, although barely resorbed from the gastrointestinal tract, supported the growth of rats fed a vitamin A-deficient diet when administered intraperitoneally at 2.3 μmol of retinal equivalent/kg body weight. Retinal and the retinal-dextran complex exhibited differential cytotoxicity toward S91 melanoma cells and caused cell lysis at 10 μM and 500 μM (retinal residue), respectively. At noncytotoxic doses both free retinal and its dextran-linked derivative reduced the cell proliferation rate in a time- and dose-dependent fashion with a median inhibitory dose of 1 μM and 4 μM (retinal residue), respectively. These data demonstrated that the water-soluble retinal-dextran complex retained certain biologic activities of retinal and was less cytotoxic.

We have previously found that zinc can be transported through the red blood cell membrane to the inside of the erythrocyte. Oxygenation studies on intact cells containing elevated levels of zinc indicate that zinc produces an increase in the oxygen affinity as well as dramatic changes in the sharpness and asymmetry of the oxygen binding curve. Other studies indicate that zinc binds to high molecular weight proteins on the cytoplasmic side of the membrane with an affinity similar to that for the binding of zinc to hemoglobin. The highly cooperative asymmetric oxygenation curve obtained with intact erythrocytes containing zinc can thus be explained by a shuttling of zinc between hemoglobin and the erythrocyte membrane. This mechanism for regulating oxygenation is particularly attractive because it is potentially sensitive to reagents in the plasma which can interact with the membrane, altering the membrane affinity for zinc and thereby the oxygenation of hemoglobin. Such a mechanism involving zinc and/or perhaps other intracellular substrates which bind to both the membrane and hemoglobin suggests the possibility of a new mechanism for fine tuning oxygen transport.

We have previously found that Cu(II) binds to the β-subunits of hemoglobin resulting in the oxidation of Fe(II) to Fe(III). The mechanism for this reaction depends on the relative location of the Cu(II) and the Fe(II). By utilizing magnetic resonance techniques it has now been possible to pinpoint the region of the molecule where the Cu(II) is located. The distance between paramagnetic Cu(II) and a nitroso spin label attached to hemoglobin can be determined by the decrease in the intensities of the spin label ESR spectra. By comparing the effect of Cu(II) on various labels attached to the reactive β-93 sulfhydryl group, as well as labels attached to the heme and the terminal α-amino groups, it has been possible to show that Cu(II) is located near the surface of the β-chain between the F and H helices. This places the Cu(II) some distance from both the heme and the iron atoms. Mossbauer studies indicate that, despite the distance between the Cu(II) and

Fe(II), under certain conditions Cu(II) binding does perturb the electronic environment of the iron. These effects may be related to the conformational change which we have previously found to take place subsequent to the binding of Cu(II). These results demonstrate that the transfer of electrons between Cu(II) and Fe(II) probably proceeds via the protein moiety.

We have previously shown that the rate of hemolysis which decreases with age is determined by membrane fluidity. Since the fluidity of the lipid bilayer and the mobility of the membrane proteins are thought to regulate many biological functions, we have begun a detailed investigation of the mobility of various components of the membrane in order to be able to detect the effect of age on membrane structure and function. Our studies thus far indicate that the mobility of the protein spin labels is sensitive to structural and functional alterations of the membrane, which can be triggered by altering the temperature, ionic strength, or divalent metal ion concentration. One of the most interesting effects we have found thus far is that the reversible phosphorylation of spectrin, which is thought to regulate the deformability of the erythrocyte in vivo, produces a rather dramatic decrease in the mobility of the spin labels.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00044-07 LCMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Effects of Metals and Proteins on Nucleic Acids, Information Transfer, and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. L. Eichhorn	Chief, LCMB	LCMB NIA
	J. J. Butzow	Commissioned Officer	LCMB NIA
	P. Clark	Research Chemist	LCMB NIA
	Y. A. Shin	Research Chemist	LCMB NIA
	G. A. Elgavish	Visiting Fellow	LCMB NIA
	E. Tarien	Chemist	LCMB NIA
	S. J. Karlik	Visiting Fellow	LCMB NIA (EOD 9/24/79)

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:
7.6	5.6	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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) age-related changes in chromatin structure; (3) aging of ribosomes; (4) the mechanism of involvement of aluminum in Alzheimer's disease; (5) crosslinking of nucleic acid strands by metal ions; (6) the effects of metal ions on RNA polymerase; (7) metal ions and cellular aging.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: (1) To study effects of metal 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 toxicity and aging, (5) to understand the participation of metal ions in the biological activities of nucleic acids and nucleoproteins, (6) to elucidate structural and functional changes in genetic information transfer that accompany aging.

Methods Employed: (1) The interaction of metal ions, proteins, and nucleic acids are studied by optical rotatory dispersion, circular dichroism, and uv spectrophotometry to determine conformational changes, and by infrared, nuclear magentic resonance, and electron spin resonance techniques to determine interaction sites. (2) Chromatin structure is analyzed by means of model systems to determine which structural features are responsible for its function. (3) Physical chemical techniques are employed to study age changes in chromatin. (4) 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. (5) The effect of metal ions on the enzymes responsible for genetic information transfer are studied. (6) The mechanisms by which enzymes and metal ions synthesize and degrade nucleic acids are elucidated. (7) Age changes in ribosomal fidelity are investigated by stressing the ribosomes with high concentrations of metal ions and with antibiotics.

Major Findings:

A. Chemistry and Biochemistry of Aluminum. The hypothesis that there is a relationship between aluminum and Alzheimer's disease has been strengthened recently by the confirmation of Crapper's findings in another laboratory. We have reinforced our efforts to study the possible biochemical intermediates that may be responsible for this relationship, as well as other manifestations of aluminum toxicity.

1. The Application of ^{27}Al NMR. The availability of a broad band probe with our Varian XL-200 NMR makes possible studies of the ^{27}Al nucleus. Very few studies have been carried out with this nucleus, and some effort has been exerted toward studying the usefulness of this nucleus. We have found that the ^{27}Al nucleus is extremely sensitive to changes in its coordination sphere, so that chemical shift due to different environments are spread far apart. Aluminum binding to phosphate (ortho, pyro, triphosphate, ATP, etc.) is particularly readily identifiable, since the resonance is shifted upfield from hydrated Al ion, while all other known Al binding causes downfield shifts.

2. Aluminum Equilibrium in Aqueous Solution. Equilibrium between Al^{3+}, water, OH^-, and lactate ion have been studied, leading to the identification

of 1:1 and 1:3 Al-lactate complexes, as well as their hydroxylated derivatives, $Al(OH)_4^-$ and $[Al_{13}O_4(OH)_{24}(H_2O)_{12}]^{7+}$. Substitution in Al complexes appears to be extremely slow. For example, when 1 mol lactate is added to 1 mol Al, the 1:1 complex is produced, but when 1 mol Al is added to 1 mol lactate, so that lactate is in excess under the reaction conditions, the 1:3 complex is produced, and apparently does not decompose into the 1:1 complex for weeks.

3. Al-DNA Interaction. One of the major goals of our work with Al is to understand its interaction with DNA. ^{27}Al NMR so far has not been very helpful in elucidating the Al-DNA binding, because DNA greatly broadens the ^{27}Al signal, so that the changes in chemical shift are observed. We are planning studies with smaller lengths of DNA, which may lessen the broadening. One very interesting discovery was made with the broadened line. Heating the Al-DNA solution caused even more extensive broadening and subsequent cooling led to the original band width. No other method has been able to detect the subtle changes in structure that occur on cooling the DNA strands back together by Al crosslinks. The most likely explanation for the phenomenon is that the Al crosslinks at elevated temperature are much more mobile than at room temperature.

4. Reaction of Al with ATP. This reaction is of importance since the formation of Al-ATP complexes is believed to be a major reason for the toxic effects of Al on patients under dialysis treatment. By ^{27}Al studies it could be shown that even at pH as low as 1.5, 90% of Al is bound to ATP, and at pH 2, 98% becomes bound. ^{31}P and ^{1}H NMR studies on the interaction showed that Al binds to γ, and to a lesser extent to β phosphate, and also to the adenine of ATP. The β-phosphate was affected only at intermediate pH, where the Al apparently forms a chelate ring with β and γ phosphate oxygens. At low and high pH the Al binds to γ-phosphate.

5. Reaction of Al with poly d(AT). Al interactions with the alternating copolymer poly d(AT) · d(AT) were studied as a function of Al concentration and pH. Since this polymer contains a repeating sequence, in the absence of divalent metals, the heat-denatured polymer can be completely renatured by cooling. Al-crosslinking prevents this renaturation by cooling, and the extent of renaturation can then be used as a measure of the amount of polymer that is not crosslinked by Al. The results indicate, for example, that at pH 5 and 1.25 Al/dAT residue, about 2/3 the polymer was crosslinked, and at 1.25 Al/dAT 100% was crosslinked. When the pH is raised to 7, very little crosslinking occurs. These studies are important because crosslinking could be responsible for the deleterious effects of Al in Alzheimer's disease - this is of course a speculation.

B. The Effects of Metal Ions on Polynucleotide Structure. The effects of metal ions on genetic information transfer depend to a great extent on the modes of interaction of metal ions with nucleic acids, and to better understand the latter we carry out studies with homopolynucleotides. Hg^{2+} and Ag^+ have been selected as metal ions that bind very strongly to the nucleotide bases, but have different charges and geometrics; the toxic properties

of Hg^{2+} have long been recognized. The homonucleotides selected were poly(U), poly(I), and poly(X).

The apparent paradox that low pH favored a 1:1 complex of Ag^{+} with poly(U) but a 2:1 complex with poly(I) and high pH had the reverse effect is readily explained by binding at low pH to groups that do not bind protons and at high pH to groups that bind protons that must therefore be displaced. The 2:1 low pH Ag-poly(I) complex involves binding to unprotonated N-7; the 1:1 high pH Ag-poly(I) complex involves binding to protonated N-3. The 1:1 low pH Ag-poly(U) complex involves binding of only one protonated N-3 to each Ag, and the 2:1 high pH complex involves 2 protonated N-3. The Hg^{2+} complexes sometimes resemble the Ag^{+} complexes; thus low pH favors 2:1 Hg-poly(I), with Hg bound to unprotonated N-7, and high pH favors a 1:1 complex that involves binding to both N-1 and N-7. A very important difference, however, occurs on binding to poly(U); Hg^{2+} forms only a 2:1 complex and no 1:1 complex. The reason for this difference becomes apparent when one considers that a complex like the 1:1 Ag-Poly(U) complex (which is dimeric and is really 2:2) brings the metals very close together - appropriate for monovalent Ag^{+} but not for divalent Hg^{2+}.

Although xanthine (X) resembles inosine (I) - they are both purines, with X containing an oxygen on C-2 - much more than the pyrimidine (U), reaction of Ag^{+} and Hg^{2+} with poly (X) resembles reaction with poly(U) in that N-1, (which is analogous to N-3 on the pyrimidine ring) is bound, rather than N-7. Probably the O on C-2 causes the much more ready release of proton from N-3, and consequent preference of binding to N-3 rather than N-7. In fact, neither Ag^{+} and Hg^{2+} seem to bind strongly at low pH, where N-7 binding would be favored.

Thus a rather complex array of reactions between poly(U), poly(I), and poly(X) and Ag^{+} and Hg^{2+} can be explained by rather simple chemical principles: (1) low pH and high pH favor binding to unprotonated and protonated groups, respectively, (2) divalent ions shun proximity to each other, and (3) ready release of proton from a protonated group favors binding to that group instead of to an unprotonated group.

C. Metal Ions and Chromatin Structure.

1. NMR Studies. A soluble chromatin preparation from rat liver was monitored by ^{31}P NMR spectroscopy. A single line of $\sim$ 100 MHz width was observed; according to its chemical shift with respect to an external reference it was assigned to the resonance of DNA phosphate. The signal surprisingly has a striking similarity to the signal from nucleosomes. The finding could be important, since it seems to indicate that the freedom of motion of the phosphate groups is independent of the additional structural restraints that are imposed on DNA in chromatin as compared to the simpler nucleosomes.

Magnesium ions decrease the line width of the DNA phosphate resonance by 40%; this can be interpreted by the metal disrupting protein-DNA interactions by

competing with the protein for the DNA phosphate binding sites. Aluminum ions have a very different effect: the ^{31}P line is split, one line corresponding to uncomplexed DNA and the other, which is considerably broadened, to Al-bound DNA. These results indicate that Al binding increases the rigidity of the DNA in its environment and that the Al ions bind to DNA much longer than Mg, i.e., the rate at which Al comes off the DNA is greatly decreased. We are encouraged by the ability to use NMR techniques to follow metal-DNA binding in a biologically relevant environment.

2. Biochemical Studies. Several of the observations from the NMR experiments are confirmed by biochemical studies. Thus, increases in the ionic strength of the chromatin solution, which lead to contraction of the chromatin as observed by EM, do not affect the melting (absorbance vs. temperature profile) of the DNA. Thus the nucleosome clustering in high salt does not affect the degree to which protein protects the DNA helix from unwinding. This is reminiscent of the constancy of the phosphate resonance in chromatin and isolated nucleosomes.

The competition between magnesium and protein for DNA Phosphate is also confirmed. Ordinarily chromatin melting occurs in one phase; the nucleosomal and internucleosomal DNA melt simultaneously. In the presence of Mg the melting is biphasic, with the internucleosomal DNA melting first. Thus the Mg lessens the ability of H-1 histone to protect internucleosomal DNA.

Copper, which greatly decreases the stability of the DNA double helix and therefore decreases its melting temperature, has no such effect on chromatin; evidently the histones, both nucleosomal and H-1, protect the DNA against the copper. When H-1 histone is removed from chromatin, the latter melts biphasically, with internucleosomal DNA denatured before the nucleosomal DNA. Copper affects the melting of H-1-stripped chromatin in a manner similar to its effect on free DNA; apparently the loss of H-1 removes the protection of DNA chromatin from attack by copper.

We had hoped that the dramatic effect of Cu on DNA would help to detect age differences in chromatin that would otherwise go unnoticed. However, no age changes of significance could be detected in the melting behavior of old and young chromatin, either in the presence or absence of copper.

In a collaborative study, Dr. Moudrianakis at John Hopkins University, has found a marked decrease in the activity of an enzyme that cleaves 15 amino acids from histone H2A. This cleavage may be involved in genetic regulation; thus the change in activity could be important.

D. Ribosomal Regulation of Protein Synthesis.

1. Age Changes in Fidelity. We have refined our studies on the detection of age changes in rat liver ribosomes by testing fidelity of protein synthesis when young and old ribosomes are challenged with Mg^{2+} or paromomycin. Both of these are known to produce errors in protein synthesis. With Mg^{2+}, there

is no apparent age difference in the error induced in protein synthesis. With paromomycin, there is an increase in error frequency of 6-9% in old when compared to young (at $p < 0.02$). Error was measured as leucine (Leu) to phenylalanine (Phe) ratio when poly(U) was used as a message - Phe represents correct and Leu incorrect incorporation. We do not know whether a change in error rate of such low magnitude has any biological significance. It should be emphasized that this study does not constitute a test of the "error" hypothesis, since we did not measure age changes in indigenous error rates. Instead, we challenged the ribosomes with agents known to induce error, and then measured age changes in this induced error. The results reflect a slight difference in the capacity of the ribosomes to prevent error.

2. Mutual Dependence of Magnesium and Paromomycin in their Effects on the Elongation Fidelity of Ribosomes. The aminoglycoside antibiotic paromomycin (PM) elicits large misreading effects by binding to a small number of sites on eukaryotic ribosomes; high Mg concentrations, which may affect fidelity directly by enhancing codon-anticodon association, produce smaller effects. Mg is a required cation in protein synthesis, and at low Mg concentrations the error frequency is normally low. We have investigated further the mutual dependence of Mg and PM concentration on correct and error elongation, using the same poly(U) programmed ribosome system we used in our study of ribosomal fidelity. With increasing PM there was a general shift of both Phe (correct) and Leu (error) responses toward lower Mg, and there was a decrease in the separation of their maxima. The dependences turn out to be such that at low Mg, e.g., 5 mM, the Phe as well as the Leu incorporation is increased (interestingly enough) by the addition of PM, while at higher Mg the Phe incorporation may be decreased by addition of PM as that of Leu is increased. The results demonstrate the wide variety of effects on fidelity that can be produced by the combined action of PM and Mg^{2+}.

3. Effects of Mg and Al Ions on Ribosomal Control of Polypeptide Elongation. Manganese can substitute for magnesium in many enzyme systems, and in the case of polymerases this replacement alters the fidelity of replication of the template. Because the ribosomal control of elongation fidelity is poorer at high Mg, we investigated the possible effects of Mn on a poly(U) programmed ribosome system. We also investigated the possible effects of Al, since the ribosome, like chromatin, is a nucleoprotein complex subject to the influence of metal binding to its components -- although there is no particular evidence for an involvement of Al with ribosomes in Alzheimer's disease.

In our initial study we find that Mn does not support polypeptide synthesis well. Mn inhibited synthesis when introduced in addition to Mg at usual concentrations; at very low Mg, Mn weakly supported synthesis so that a maximum of about 1/4 the usual Phe incorporation rate was reached by addition of about 8 mM Mn, somewhat higher than the optimum Mg concentration under

similar conditions. In either case, Mn did not affect the error frequency. The inhibition and poor response were not due to an effect of Mn on tRNA acylation.

Direct exposure of ribosomes to aluminum was tested by addition of aluminum lactate to ribosome solutions at neutral pH. An Al concentration-dependent inactivation of the ribosomes for polypeptide synthesis was observed. The inactivation was not reversed by lowering the free Al concentration either by dilution or by sedimentation of the ribosomes. The error frequency as measured at high Mg concentration was not affected by Al. Thus, if aluminum is presented at high enough levels directly to ribosomes, the ribosomes apparently become irreversibly inactivated, probably by tight binding of Al.

E. The Effect of Metal Ions on RNA Polymerase. We are investigating the reason for the importance of metal ions in determining whether or not RNA polymerase (E. coli) can distinguish between ribo- and deoxy- nucleotides for incorporation into RNA. Studies on conformational changes induced by the three metals that can activate the enzyme (Mg^{2+}, Mn^{2+}, and Co^{2+}) have been previously reported.

To further probe the effects of these metals double-label kinetic studies using ^{3}H-ATP and $^{32}P_{\alpha}$-dATP have been employed. Incorporation of ATP into cold TCA insoluble RNA, inhibition of this process by dATP, and misincorporation of the latter have been followed. The results are obtained as V_{max}, K_M, K_I and Q (amount of misincorporated dATP). The V_{max} and K_M of the ATP incorporation are in the order $Co^{2+} < Mn^{2+} < Mg^{2+}$; and K_I for inhibition by dATP, and dATP misincorporation, Q, are in the order $Mg^{2+} < Mn^{2+} < Co^{2+}$. The V_{max} differences are consistent with the differential effect of the metal cations on the enzyme conformation monitored by the previously reported melting studies. The K_M differences, however, demonstrate differences in enzyme-substrate affinity. The trend in the K_I values is best interpreted in terms of metal induced discrimination between the competing ligands, ATP and dATP, at the substrate binding step. Thus Co^{2+}, which has the lowest K_M, also has the largest K_I. This binding step, however, does not seem to be significant for fidelity, as is shown by the trend for the amount of misincorporated dATP. We conclude that the incorporation preference takes place at a later step, after the formation of the enzyme-substrate complex. This preference is also metal dependent, but in a manner not necessarily related to the trend in the competitive binding step.

Significance to Biomedical Research and to the 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: We shall continue our studies on the effects of metal ions on nucleic acid structure and function with emphasis on aluminum, because of its apparent relationship to Alzheimer's disease. The effects on the structure of chromatin and chromatin components will be probed. The NMR studies reported here look promising and will be continued. We hope to be able to study chromatin from aluminum-injected animals and from autopsies of dialysis dementia patients. We also intend to continue the investigations on RNA polymerase, using a variety of techniques, but mainly NMR. We hope to be able to understand the differences in the mechanism of RNA synthesis activated by different metal ions. We also hope to determine how the presence of the DNA template affects the enzyme-substrate interaction.

We intend to carry out studies on the effect of metal ions on the viability of cells and on the changes in the permeability of the cells to metals as a function of age. We then will look into the effects of the metals on DNA synthesis and DNA damage, and study any age changes of such effects.

We intend to continue work to try to understand the mechanism of the decrease in the fidelity of protein synthesis induced by magnesium and antibiotics. We hope to test the fidelity of ribosomes isolated from aluminum-injected animals and autopsies of dialysis dementia patients.

Publications:

Eichhorn, G., Shin, Y.A., Clark, P., Rifkind, J., Pitha, J., Tarien, E., and Rao, G.: Essential and Deleterious Effects in the Interaction of Metal Ions with Nucleic Acids. In Kharasch, N. (Ed.): Trace Elements in Health and Disease. New York, Raven Press, 1979, pp. 123-133.

Eichhorn, G.L.: The Function of Metal Ions in Genetic Regulation. In Sigel, H. (Ed.): Metal Ions in Biological Systems. New York, Marcel Dekker, Inc., 1980, Vol. 10, Chap. 1, pp. 1-21.

Marzilli, L.G., Kistenmacher, T.J., and Eichhorn, G.L.: Structural Principles of Metal Ion-nucleotide and Metal Ion-nucleic Acid Interactions. In Spiro, T.G. (Ed.): Nucleic Acid-Metal Interactions. New York, John Wiley & Sons, 1980, Chap. 5, pp. 181-250.

Shin, Y.A. and Eichhorn, G.L.: Induction of Helicity in Polyuridylic Acid and Polyinosinic Acid by Silver Ions. Biopolymers 19: 539-556, 1980.

Eichhorn, G.L., Butzow, J.J., Clark, P., von Hahn, H.P., Rao, G., Heim, J.M., Tarien, E., Crapper, D.R., and Karlik, S.J.: Metal Ion-Nucleic Interactions, Aging and Alzheimer's Disease. In Martell, A. (Ed.): Inorganic Chemistry in Biology and Medicine, in press.

Eichhorn, G.L.: Bioinorganic Chemistry. In: Encyclopedia of Science and Technology. New York, McGraw Hill, in press.

Karlik, S.J., Eichhorn, G.L., and Crapper, D.R.: Molecular Interactions of Aluminum with DNA. Neurotoxicology, in press.

Karlik, S.J., Eichhorn, G.L., Lewis, P.N., and Crapper, D.R.: Interaction of Aluminum Species with DNA. Biochemistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00046-10 LCMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Polymers as Biological Reagents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Pitha	Research Chemist	LCMB NIA
Other:	J. Kusiak	Special Expert	LCMB NIA (EOD 6/16/80)
	S. Zawadzki	Visiting Fellow	LCMB NIA
	J. Zjawiony	Visiting Fellow	LCMB NIA (DOD 4/07/80)

COOPERATING UNITS (if any)
Duke University Medical Center, Durham, NC; The Weizmann Institute of Science, Department of Biophysics, Rehovot, Israel; University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee.

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Macromolecules

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.8	2.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 may be improved. In the group of adrenergic blockers it has been established that attachment of drugs to a polysaccharide carrier by a stable bond sustains their activity. Such hydrolysis-stable conjugates do not penetrate cell membranes, a process which conditions many side-effects. Furthermore the conjugates have increased specific recognition compared to the parent drug. In the group of lipid soluble vitamins, the formation of a physical complex between the carrier which has been synthesized and vitamin A leads to sustenance of biological activities with some decrease in its toxic effects.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objective of the project is the innovative design of drugs used in age-related problems and diseases. Two ideas form the basis for such innovations. Activity of some drugs is contingent on the binding of the drug to the receptor located on the cell surface. Such a drug does not have to penetrate and saturate the whole cellular space to be active, a process which in this case leads only to side effects. Macromolecules, after being introduced into circulation, come into contact with the cell surface of many cells, but do not penetrate into them. Thus attachment of drugs acting on cell surface receptors to macromolecules by a stable chemical bond should sustain their pharmacological activity and lower their toxicity. In another part of the project water solubility of lipid soluble vitamins, hormones, and drugs are increased by physical complexation with specially designed carriers; this process may be expected to modify some of the toxic effects triggered by these compounds. Use of macromolecular or medium molecular weight carriers again represents an advantage since such compounds circulate longer in an organism.

Methods Employed: The study requires both chemical and biological methods. Chemical methods for attachment of small molecular weight compounds to polysaccharides were investigated and the stability of the attachment was measured. All synthetic work was done in the laboratories of the section; the prevalent part of biological and pharmacological testing was performed by outside collaborators. The intramurally performed biological tests were: (a) toxicity of synthesized compounds to cells grown in vitro (cells involved were Friend erythroleukemic cells and human fibroblast cells), (b) binding of synthesized compounds to beta-adrenergic receptors (membranes prepared from rat lungs were used for these purposes).

Major Findings:

A. Beta-Adrenergic Antagonists. Drugs of this group are extensively used in clinical practice, i.e. to treat hypertension. Numerous studies with these drugs on cellular systems show that the drugs' effects are triggered by binding the drugs to the membrane located receptors. In vivo effects may also be independent of the ability of these drugs to penetrate the cellular membranes or the blood/brain barrier (J.W. Hollifield et al., N. Eng. J. Med. 295, 68-73, 1976). This makes the beta-adrenergic antagonists possible candidates for conversion into macromolecular form which would sustain pharmacological activity. The beta-adrenergic antagonist, alprenolol, was attached in an irreversible manner to macromolecular dextran via side arms that differed in length. The ability of these macromolecules to bind to the beta-adrenergic receptor of frog erythrocytes and to catecholamine-binding antibodies raised against partially purified receptors was studied. Compared to the parent drug the potency of binding of macromolecular alprenolol to the receptor decreased to about 1/10, 1/600, and 1/8000 when the length of the arm separating alprenolol from the dextran moiety was 13, 8, and 4 atoms, respectively. In contrast, the binding potencies of the parent drug and of all its macromolecular derivatives for the antibody were within the same order of magnitude. Thus, conversion of a drug to a macromolecular form may not only sustain its

binding activity but may also lead to a higher selectivity. The macromolecular derivatives described here may be suitable probes for investigation of location and molecular properties of the binding sites for beta-adrenergic drugs.

B. Lipid Soluble Vitamins. These compounds, in addition to their obvious nutritional values, have additional beneficial effects. Unfortunately, to obtain a significant measure of these additional effects, doses which already elicit toxicity are required. Thus retinoids, which are derivatives of vitamin A, have been shown to reduce the incidence of lesions in epithelial sites of animals treated with chemical carcinogens. Work done in this program focuses on lessening the toxic effects of retinoids through transforming them into a water-soluble macromolecular form. A new, water-soluble, polymer-linked form of retinal was synthesized and tested for its ability to support the growth of vitamin A-deficient noninbred rats and to inhibit the proliferation of melanoma cells in culture. Retinal was conjugated to hydrazide of carboxymethyldextran in the presence of alpha-and beta-cyclodextrins. The aqueous solutions of the product contained between 200 and 1,000 μg retinal/mℓ as opposed to the low water solubility (<0.01 μg/mℓ) of retinal itself. The retinal-dextran complex, although barely resorbed from the gastrointestinal tract, supported the growth of rats fed a vitamin A-deficient diet when administered intraperitoneally at 2.3 μmol of retinal equivalent/kg body weight. Retinal and the retinal-dextran complex exhibited differential cytotoxicity toward S91 melanoma cells and caused cell lysis at 10 μM and 500 μM (retinal residue), respectively. At noncytotoxic doses both free retinal and its dextran-linked derivative reduced the cell proliferation rate in a time- and dose-dependent fashion with a median inhibitory dose of 1 μM and 4 μM (retinal residue), respectively. These data demonstrated that the water-soluble retinal-dextran complex retained certain biologic activities of retinal and was less cytotoxic.

Significance to Biomedical Research and Program of the Institute. The focus of the project was substantially re-oriented. Previously nucleic acids and their analogs were in focus. This group of compounds, in spite of their dominating role in living systems, have found only limited application in therapy and then again only in diseases which are not directly age-related (viral infections, neoplasias). The present work focuses on compounds which are heavily used in treatments for the elderly. Treatment by antihypertensive agents, a group of drugs to which the above used 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 effects of adrenergic blockers differ significantly in adult and elderly subjects (G.R. Wilkinson, Drug Metab. Rev. 10, 107-123, 1979 and T. Ishizaki et al., J. Pharmacol. Exp. Ther. 212, 173-181, 1980). Furthermore the elderly suffer more severely from these side effects, such as nervous system disturbances (J.J. Hammond & W.M. Kirkendall, Geriatrics, 27-36, 1979), which may be connected with the penetration of drugs through the blood/brain barrier. Equally another subject of the program, lipid soluble vitamins, are heavily used in alleviating age-related problems.

Proposed Course of the Project. By directed synthesis and study of the basic biological effects of polymeric drugs and macromolecular carriers for drugs, it is hoped to gain the knowledge necessary for the design of practical, useful compounds.

Publications:

Noronha-Blob, L. and Pitha, J.: Mechanism of enhancement of polynucleotide binding to cells by mutagens. Biochemistry 18: 3206-3209, 1979.

Pitha, J., Zjawiony, J., Lefkowitz, R.J. and Caron, M.G.: Macromolecular β-adrenergic antagonists discriminating between receptor and antibody. Proc. Natl. Acad. Sci. USA 77: 2219-2223, 1980.

Hughes, B.A., Roth, G.S. and Pitha, J.: Age-related decrease in repair of oxidative damage to surface sulfhydryl groups on rat adipocytes. J. Cell. Physiol., in press.

Pitha, J.: Nucleic Acids and Sulfate and Phosphate Polyanions. In Donaruma, L.G., Ottenbrite, R.M., and Vogl, O. (Eds.): Polymers in Biology and Medicine. New York, John Wiley & Sons, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00047-10 LCMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Structure-Function Relationships in Hemoglobin and Erythrocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. M. Rifkind	Research Chemist	LCMB NIA
OTHER:	J. T. Wang	Research Chemist	LCMB NIA (EOD 5/1/79)
	J. G. Mohanty	Visiting Fellow	LCMB NIA (EOD 4/1/80)

COOPERATING UNITS (if any)

Center for Disease Control, University of Pennsylvania, John 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:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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.

PHS-6040
(Rev. 10-76)

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. The interaction of zinc with intact erythrocytes. We have previously found that zinc can be transported through the membrane to the inside of the erythrocyte. Oxygenation studies on intact cells containing elevated levels of zinc indicate that zinc produces an increase in the oxygen affinity as well as dramatic changes in the sharpness and asymmetry of the oxygen binding curve. The increased oxygen affinity has previously been found with purified hemoglobin. However, the changes in shape of the oxygenation curve are observed only with intact cells.

Separate studies indicate that zinc binds to high molecular weight proteins on the cytoplasmic side of the membrane with an affinity similar to that for the binding of zinc to hemoglobin. The highly cooperative asymmetric oxygenation curve obtained with intact erythrocytes containing zinc can thus be explained by a shuttling of zinc between hemoglobin and the erythrocyte membrane.

This mechanism for regulating oxygenation is particularly attractive because it is potentially sensitive to reagents in the plasma which can interact with the membrane, altering the membrane affinity for zinc and thereby the oxygenation of hemoglobin. Such a mechanism involving zinc and/or perhaps other intracellular substrates which bind to both the membrane and hemoglobin suggests the possibility of a new mechanism for fine tuning oxygen transport.

B. Mechanism of Cu(II) oxidation of hemoglobin and the location of the copper binding sites. We have previously found that Cu(II) binds to the β-subunits of hemoglobin resulting in the oxidation of Fe(II) to Fe(III). The mechanism for this reaction depends on the relative location of the Cu(II) and the Fe(II).

By utilizing magnetic resonance techniques it has now been possible to pinpoint the region of the molecule where the Cu(II) is located. The distance between paramagnetic Cu(II) and a nitroso spin label attached to hemoglobin can be determined by the decrease in the intensities of the spin label ESR spectra. By comparing the effect of Cu(II) on various labels attached to the reactive β-93 sulfhydryl group, as well as labels attached to the heme and the terminal α-amino groups, it has been possible to show that Cu(II) is located near the surface of the β-chain between the F and H helices. This places the Cu(II) some distance from both the heme and the iron atoms.

We have obtained additional support for this conclusion by the failure to detect an effect of changes in the spin state of the iron on the Cu(II)-ESR spectrum, and the failure to detect an effect of binding Cu(II) to this site on the NMR spectra of the heme methyl groups.

None of these results, however, permit us to determine the amino acid residues associated with the Cu(II).

From previous studies of abnormal hemoglobins, we have evidence for the involvement of histidine-143 which is located in this region of the molecule. We have now been able to demonstrate the involvement of one of the carboxyl groups located in this same region of the molecule by showing that the binding constant and the rate of oxidation are dramatically reduced as the pH is lowered from 5 to 4 where carboxyl groups are the only protein groups titrated.

We are, currently, in collaboration with Dr. Abraham Levy of John Hopkins University, also investigating the Mossbauer spectra of the iron. These studies indicate that, despite the distance between the Cu(II) and Fe(II), under certain conditions Cu(II) binding does perturb the electronic environment of the iron. These effects may be related to the conformational change which we have previously found to take place subsequent to the binding of Cu(II).

These results demonstrate that the transfer of electrons between Cu(II) and Fe(II) probably proceeds via the protein moiety. Such a mechanism is different from the previously reported mechanisms for the oxidation of hemoglobin by other small molecules, which are thought to involve a direct interaction with the iron or the heme. There is, however, evidence that the in vivo enzymatic reduction of hemoglobin may proceed by a mechanism similar to the Cu(II) oxidation. Furthermore, transport of electrons through proteins are very likely involved in the electron transport systems.

C. Spin-label studies of membrane mobility. We have previously shown that the rate of hemolysis which decreases with age is determined by membrane fluidity. Since the fluidity of the lipid bilayer and the mobility of the membrane proteins are thought to regulate many biological functions, we have begun a detailed investigation of the mobility of various components of the membrane in order to be able to detect the effect of age on membrane structure and function.

For this purpose we have used various nitroso spin label probes whose ESR spectra are sensitive to mobility. In addition to our studies on the erythrocyte membrane, we are extending these studies, with the collaboration of Dr. George Roth of the Clinical Physiology Branch, to include membranes of adipocytes. These post- mitotic cells have been shown to undergo alterations in their response to hormones as a function of age, and therefore might possibly show changes in membrane structure.

Our studies thus far indicate that the mobility of the protein spin labels are sensitive to structural and functional alterations of the membrane, which can be triggered by altering the temperature, ionic strength, or divalent metal ion concentration.

One of the most interesting effects we have found thus far is that the reversible phosphorylation of spectrin, which is thought to regulate the deformability of the erythrocyte in vivo, produces a rather dramatic decrease in the mobility of the spin labels.

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 the 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 study the oxygenation of whole blood from individuals of various ages and try to correlate any observed changes with alterations in the erythrocyte composition and/or the hemoglobin molecule. (2) We plan to investigate the possible physiological significance of the reported zinc-induced increase in the oxygenation of erythrocytes. (3) We plan to further investigate the binding site for metal ions on hemoglobin and the mechanism whereby the bound metal ions alter functional properties of hemoglobin. (4) We plan to further study the interaction of metal ions with erythrocyte membranes and how these interactions alter the functional properties of the membrane. (5) We plan to further study membrane fluidity and possible changes with age in the mobility of different membrane components of erythrocytes and other cells.

Publications:

Rifkind, J.M.: The Oxidation of (Horse) Hemoglobin by Copper: An Intermediate Detected by Electron Spin Resonance. Biochemistry 18: 3860-3865, 1979.

Araki, K. and Rifkind, J.M.: Erythrocytes Membrane Cholesterol: An Explanation of the Aging Effect on the Rate of Hemolysis. Life Sciences 26: 2223-2230, 1980.

Araki, K. and Rifkind, J.M.: Age Dependent Changes in Osmotic Hemolysis of Human Erythrocytes. J. Gerontol. 35: 499-505, 1980.

Rifkind, J.M.: Copper and the Oxidation of Hemoglobins. In Sigel, H. (Ed.): Metal Ions in Biological Systems, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00088-08 LCMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Mechanisms of Cellular Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Schneider	Medical Officer, PHS	LCMB NIA
OTHER:	D. Kram	Staff Fellow,	LCMB NIA (DOD 11/30/79)
	Y. Nakanishi	Visiting Fellow,	LCMB NIA (DOD 4/09/80)
	G. Bynum	Medical Officer, PHS	LCMB NIA (DOD 7/18/80)
	R. Dean	Staff Fellow,	LCMB NIA
	J. Irving	Professor Emeritos,	LCMB NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Cellular Aging and Genetics

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.3	4.2	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effect of aging on cellular response to DNA damage was investigated by several approaches. Continuing studies of SCE with aging revealed: 1) spontaneous SCE levels do not change with aging in vivo 2) that induced SCE levels were not changed in EAT tumor cells implanted in young and old animals 3) induction of SCE in Chinese hamster cells grown in human serum was influenced by the age of the serum donor. Cloning studies of young and old human cell populations indicated that response to MMC induced DNA damage did not change with aging. Other SCE detection and the use of hepatic cell lines for detecting SCE induction by agents which require metabolic activation.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To examine age related alterations in cell replication in vivo and to determine if these alterations lead to significant declines in cellular function. 2) To complete the studies of the effect of aging on SCE. These cytogenetic events have been shown to be sensitive indicators of cellular DNA damage. 3) To create an animal model for studying age-related alterations in bone metabolism. 4) In addition, several studies were conducted to demonstrate the usefulness of SCE analyses in other areas.

Methods Employed: The BrdU-differential staining techniques were utilized in vivo and in vitro to conduct SCE and cell replication studies. Ectopic bone implantation was employed for bone metabolic studies.

Major Findings:

1) Spontaneous SCE levels were estimated in vivo in young and old mouse and rat bone marrow cells. These studies indicated that there were no young and old animal cell populations.

2) To examine the effect of young and old environment of SCE induction, Ehrlich Ascites tumor cells were implanted into young and old C57B1/6J mice which were treated with various concentrations of mitomycin C. While SCE induction declined with aging in bone marrow cells, SCE Induction in the tumor cells was unchanged.

3) SCE induction by mitomycin C in Chinese hamster cells was found to decrease as a function of the age of the serum donor in cells cultured in media containing human serum.

4) The formation of ectopic bone from implanted bone powder decreased as a function of the age of the animal receiving the implant.

5) In utero studies of SCE induction in C57B1/6J mice were expanded to examine differential transplacental transport of several mutagens and carcinogens. These techniques were also employed to demonstrate changes in mutagen and carcinogen transport at different stages of pregnancy.

6) SCE induction was examined in several hepatic cell lines which contain the enzymes necessary to activate a wide range of carcinogens and mutagens. This system should provide a sensitive approach to examining those agents which require metabolic activation.

7) While hormones such as hydrocortisone, testosterone, progesterone and dihydroepiandosterone (DHEA) do not induce SCE, they augment the frequency of SCE induced by other agents such a mitomycin C or ultraviolet light.

8) The cloning of cells and cell growth kinetics in the presence of mitomycin C were unchanged as a function of the age of the cell culture.

9) The antibiotic, tetracycline, was demonstrated to induce SCE in vivo. Most of the induction was probably due to breakdown products of this antibiotic.

10) In utero studies in mice indicated that certain mutagens and carcinogens have differential transport across the placenta. These studies have also demonstrated that placental transfer of mutagens and carcinogens changes with gestation.

11) Cell replication kinetics were determined in enriched B and T cell populations.

Significance to Biomedical Research and the Program of the Institute:

The results of our studies of SCE and aging indicate: 1) in vivo spontaneous or background SCE are not changed with aging 2) environment does not appear to play a major role in the decline in induced SCE that is observed with aging 3) human serum appears to have a factor which augments SCE induction. This factor appears to lose its potency with aging. The above findings increase our knowledge of cellular response to DNA damage with aging.

Cell kinetic studies of B and T cell populations can now be performed on young and old animals to examine how aging affects these cell populations. Thus, the relationship between immune decline and cell replication can be approached in vivo.

The decline in ectopic bone formation with aging provides an animal model system for studying alterations in bone metabolism with aging.

In utero SCE examination provides a new approach to the examination of transplacental transport of mutagens and carcinogens.

The development of hepatic cell lines for SCE analyses provides an in vitro approach to the screening of carcinogens and mutagens which require metabolic activation.

The observation that certain hormones augment the levels of induced SCE provides an interesting clue to the interaction of hormones and carcinogens in the production of cellular DNA damage.

The finding that tetracycline induces SCE is of particular interest, since this agent would be difficult to examine in the usual microorganism assays.

Hollenberg, M.D. and Schneider, E.L.: Receptors for Insulin and Epidermal Growth Factor-Urogastrone in Adult Human Fibroblasts Do Not Change with Donor Age. Mech. Ageing Dev. 11: 37-43, 1979.

Schneider, E.L., Mitsui, Y., Sternberg, H., Kram, D., Senula, G., Smith, J.R., Tice, R.R., and Bynum, G.: The Effect of Aging on Cellular Replication In Vitro and In Vivo. Excerpta Medica 469: 106-107, 1979.

Schneider, E.L. and Bickings, C.K.: Aging and Sister Chromatid Exchange. VI. The Effect of In Vitro Passage on Spontaneous SCE Frequencies in Human Fetal Lung Fibroblast Cultures. Cytogenet. Cell Genet. 26: 61-64, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00087-07 LCMB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Mechanism of the Parental Age Effects

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. L. Schneider	Medical Officer, PHS	LCMB NIA
OTHER:	G. Bynum	Medical Officer, PHS	LCMB NIA (DOD

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Cellular and Genetics

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

With increasing parental age, there is an exponential increase in the frequency of children born with chromosomal disorders. Most studies indicate that this effect is chiefly due to maternal aging. Current research is directed at the potential role of paternal aging. The latter area is being approached by measuring the genetic complement of sperm samples obtained from members of the Baltimore Longitudinal Study.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To examine if there is a paternal age effect in the etiology of chromosomal disorders in man.

Methods Employed: Sperm samples from longitudinal subjects were stained with flourescent stains and the number of Y bodies, representing Y chromosomes was examined as a function of paternal age.

Major Findings: There appears to be an increase in the frequency of double Y bodies representing an extra Y chromosome as a function of the age of the sperm donor. However this increase was slight, correlating well with the epidemiologic observation that if a paternal age effect does exist in the production of chromosomally abnormal offspring, this effect is quite small.

Significance to Biomedical Research and the Program of the Institute: Chromosomal disorders are extraordinarily common in man with a frequency of approximately 1 in 100 live births. This frequency is considerably higher if one considers that over one-half the spontaneous abortions that occur during pregnancy are due to chromosomal abnormalities.

The increased frequency of double Y bodies suggests that with paternal aging there may be an increase in nondisjunction for the Y chromosome. If this nondisjunction occurs for other chromosomes, such as the 21st pair, it may indicate that the father's age may play a role in the etiology of the Down syndrome.

Proposed Course: Studies of the paternal age effect will continue with flow microfluorometric and cytologic analysis of sperm samples obtained from volunteer members of the Baltimore Longitudinal Study. In addition, an attempt will be made to find a mouse model for the paternal age effects seen in humans.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00111-01 LCMB

PERIOD COVERED
October 1. 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Factors Affecting Polycyclic Hydrocarbon Carcinogen-induced DNA Damage and Repair in Human Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Evan Hadley Medical Officer, PHS LCMB NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cellular and Molecular Biology

SECTION
Section on Cellular Aging and Genetics

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project focuses on two important processes affecting polycyclic hydrocarbon carcinogen induced DNA damage in human cells, carcinogen activation and excision repair. Studies on carcinogen activation have focused especially on factors in human plasma affecting this process, particularly dehydroepiandrosterone, (DHEA), which inhibits activation. Studies on excision to date have indicated a lack of influence by DHEA or other plasma factors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: 1) To investigate the effect of plasma factors on metabolic activation of polycyclic hydrocarbon carcinogens (PHCs), by human cells, and on the formation of covalent carcinogen-DNA adducts which are thought to be an initiatory lesion in carcinogenesis and perhaps in other, degenerative diseases as well. 2) To investigate excision of PHC-adducts from DNA by human cells.

Methods Employed: Human lymphocytes and fibroblasts are cultured in the presence of radioactively labelled benzo(a)pyrene (BP) a common PHC. Metabolism of BP is measured by scintillation of water-soluble metabolites after extraction with cyclohexane. PHC adducts are measured by scintillation counting of DNA purified from these cells by isopycnic centrifugation and enzymatic digestion.

Major Findings:

1) Inhibition of PHC Activation in Human Cells by Dehydroeprandrosterone (DHEA). Previous work had shown that DHEA inhibited PHC-induced cytotoxicity and mutagenicity in rodent cells. The present work focused on human cells which are much less susceptible to PHCs. Our results indicate that DHEA in concentrations as low as 3×10^{-6}M inhibits metabolic activation of BP. We have observed inhibition of BP-DNA adduct formation in human cells by DHEA. This phenomenon has not been studied previously in any cell type, and represents a possible mechanism of DHEA protection against PHC mediated DNA-damage.

2) Effects of Plasma from Different Human Donors on BP-Activation. We have observed a significant effect of factors in human plasma on BP-metabolism in cultured human cells. This evidence comes from studies on cells from a single donor cultured on several media, differing only in the souce of plasma. The factor appears to be inhibitory since increased concentrations of plasma result in decreased metabolism. The nature of the inhibitor and its mechanism are as yet unknown.

3) Effects of Plasma Factors on PHC Excision from DNA in Human. We have conducted studies on the effects of DHEA and on plasma from different donors on excision of DNA adducts of the PHC 7-Bromomethyl benz(a)anthracene (7BMBA). In contrast to our studies on metabolic activation of PHCs, we observed no effect of DHEA or plasma from different donors on 7BMBA excision. Our assay measures only the excision step in the process of excision repair of DNA. The possibility of plasma effects on subsequent steps of DNA repair is as yet unexplored.

4) Functional Assays of PHC Damage in Human Lymphocytes. We have employed the phytohaemagglutinin (PHA) response as a sensitive indicator of PHC damage in human lymphocytes. We have found that concentrations of 7BMBA as low as 1×10^{-7}M can produce a total abolition of PHA response. The PHA assay

appears to be a more sensitive indicator of functional impairment in lymphocytes than vital staining and should be of use in relating DNA damage to functional impairment.

Significance to Biomedical Research and the Program of the Institute:

Our results indicate that DHEA and other plasma factors may play a physiologic or pharmacologic role in the modulation of DNA damage by certain carcinogens. This may have importance to incidence of cancer and other age-related degenerative diseases in man.

Proposed Course:

1) Effects of DHEA on PHC Activation. We are currently investigating the effects on BP metabolism of several derivatives of DHEA (DHEA sulfate, DHEA sulfatide) for which a metabolic role has been suggested.

2) Plasma Effects on PHC Metabolism. We are planning studies on various isolated plasma fractions to characterize more fully the effect of plasma factors on BP metabolism.

3) Donor Age Effects on PHC Excision and Metabolism. We are currently conducting preliminary studies of interassay variation to determine the worth of measurements of age-related differences if PHC metabolism and repair in human lymphocytes.

4) Species Comparison of Factors Affecting PHC Activation. An extremely strong inverse correlation between PHC activation and lifespan has been noted in interspecies studies. This suggests that the metabolic processes responsible for PHC activation may play some role in other age-associated processes besides carcinogenesis. Studies to clarify the nature of these interspecies differences are contemplated. In particular, comparisons of aryl hydrocarbon hydroxylase activity and hexose monophospate shunt activity, both of which play key roles in PHC activation, are planned.

The Laboratory of Molecular Aging has initiated pathophysiological studies on phosphorus and calcium homeostasis as part of a new research program on senile osteoporosis and osteomalacia. It has been demonstrated that the regulation of the renal and intestinal absorption of phosphate and calcium by diet and hormones, *i.e.*, parathyroid hormone and 1,25-dihydroxy Vitamin D_3, reflects specific alterations in the activities of carriers (proteins) in the plasma membrane. Thus, it should be possible to relate these changes to clinically observable disturbances in calcium and phosphorus balance noted in man as a consequence of aging.

To provide experimental models for the studies of phosphorous and calcium homeostasis the Laboratory of Molecular Aging has established two new research facilities. One is the setting up of an animal facility to produce 50-100 neo-natal chicks, Vitamin D-deficient or repleted, per week. The other resource, new to the Laboratory of Molecular Aging, is the establishment of a tissue culture facility for the provision, from established cell lines and primary cultures, of renal cells that respond to specific hormones.

Renal phosphate transport has been investigated by the Laboratory of Molecular Aging using the isolated proximal tubule brush border membrane vesicle to describe the mechanism by which the anion is removed from the filtrate and is accumulated against its concentration gradient into the renal cell. This model system permits the membrane transport step to be dissociated from cellular metabolism and allows the examination of the ionic driving forces that energize the active transport. The contribution of the Na^+ gradient in effecting the concentrative uptake of phosphate by an electroneutral mechanism has been further defined. Questions related to the effect of acid-base balance on phosphate uptake and the nature of the ionic species of phosphate that is transported have been addressed.

The Laboratory of Molecular Aging has discovered that in addition to a Na^+ gradient (extravesicular[Na^+]>intravesicular[Na^+]), a proton gradient (intravasicular[H^+]>extravesicular[H^+] can drive the uphill transport of phosphate. The efficacy of the proton-dependent system in transporting phosphate has been correlated with the magnitude of the H^+ gradient. The H^+ gradient-dependent system requires the presence of Na^+, although it functions in the absence of a Na^+ gradient, *i.e.*, extravesicular[Na^+] = intravesicular[Na^+]. This H^+ gradient-dependent phosphate system may be of particular significance in understanding the mechanism of urine acidification.

It was reported last year that animals fed a low phosphorus diet adapt to conserve filtered phosphate and that this adaptation is reflected at the level of the brush border membrane, vesicles of which show a doubling in their rate of phosphate uptake. Continued studies on the mechanism of this regulation by the Laboratory of Molecular Aging provides additional evidence consistent with the hypothesis that the adaptation involves a change in the interaction of the Na^+ gradient with the phosphate carrier.

Parathyroid hormone increases phosphate excretion by inhibiting phosphate transport in the renal tubule. In a study of the mechanism of this hormonal regulation, the Laboratory of Molecular Aging has found that, when renal cortical slices were incubated with parathyroid hormone (30 U/ml) for 2 min, the hormone elicited an activation of adenylate cyclase, an increase in tissue level of cyclic AMP, an enhancement of cyclic AMP-dependent protein kinase, and concomitantly an inhibition of the Na^+ gradient-dependent uptake of phosphate by brush border membrane vesicles isolated from the treated slices. These findings demonstrate that this *in vitro* system represents a model to examine further the mechanism by which parathyroid hormone regulates phosphate transport in the kidney.

Cultured LLC-PK_1 cells, a continuous line derived from pig kidney, orient themselves in a manner that maintains their polarity, *i.e.*, the basolateral segment of the plasma membrane abuts the substratum whereas the apical surface faces the media. When confluent the epithelial monolayers form domes, indicative of the accumulation of fluid between the serosal surface and the substratum, thus, of transepithelial transport. Using such cells, the LMA has initiated a study of phosphate transport. A Na^+-dependent phosphate uptake system has been found. NH_4^+ can partially substitute for Na^+, but other monovalent cations are without effect. Ouabain, the glycoside inhibitor of Na^+K^+ ATPase (the Na^+ pump), localized in the basolateral membrane, blocks the uptake of phosphate. Phosphate transport by the cultured cells is regulated by cell growth and density.

LLC-PK_1 cell adenylate cyclase activity has been found by the Laboratory of Molecular Aging to be markedly stimulated by the antidiuretic hormone, vasopressin, moderately stimulated by parathyroid hormone, but not enhanced by calcitonin. Guanyl nucleotides, which augment hormonal stimulation decrease the concentration of vasopressin needed to stimulate the enzyme f nM to 1.5 nM. Cells, preincubated with ^{32}P so that the γ-P of ATP has achieved a steady-state of radioactivity, and then exposed to vasopressin, show an increased phosphorylation in three distinct proteins. The precise relationship between the specific phosphorylation of a protein and the physiological response to the hormone remains to be determined.

The Laboratory of Molecular Aging has identified and partially purified and characterized an endogenous factor in the renal cortex cytosol that potentiates hormonal (parathyroid hormone and prostaglandin E_1) activation of renal adenylate cyclase without affecting basal enzyme activity or activity stimulated by guanyl nucleotides and F^-. The factor, probably a protein, is heat stable, sensitive to chymotrypsin, and distinct from calmodulin. This information is important to the understanding of how hormones regulate physiological functions.

The action of hormones, mediated via cyclic AMP, are propagated by cAMP-dependent protein kinases that catalyze the phosphorylation of specific proteins. The renal cortex cytosolic and brush border membrane protein kinase regulatory subunits, that bind the cyclic AMP, have been identified by the Laboratory of Molecular Aging using a photoaffinity labelling technique and molecular weights have been estimated. The cytosol contains Type I and II protein kinase, whereas the membrane possesses only a Type II protein kinase. Specific membrane proteins are phosphorylated by the kinase. Ca^{2+} stimulates phosphorylation. Calmodulin

does not appear to be involved. These findings contribute to our knowledge of the mechanism of hormone action.

In continuing studies on the age-dependent changes in the molecular structure and function of the renal brush border membrane, the Laboratory of Molecular Aging has developed an isolation procedure, including a novel affinity column chromatography technique, for the purification to homogeneity of the brush border membrane enzyme maltase. The specific activities of the pure enzymes from young (4-6 mo) and aged (24 mo) rats were 46 and 32 μmole/min/mg protein, respectively, a decrease of about 30%. Circular dichroism measurements indicate that the old enzyme has an altered conformation, from 63% β-helix and 37% random coil in the young to 75% β-helix and 25% random coil in the old. Like other extrinsic membrane proteins, maltase has been found to be a glycoprotein. There is a significant decrease in total carbohydrate in the old enzyme. The young and old enzymes do not differ in molecular weight, charge, substrate affinity, pH optimum, and sensitivity to inhibitors.

The LMA has found that renal brush border membrane vesicles from old rats have significantly decreased Na^+ gradient-dependent uptakes of phosphate and D-glucose.

The Laboratory of Molecular Aging has continued studies of the mechanism of the Na^+—D-glucose cotransport system in the renal brush border membrane. The findings are consistent with the views that (a) D-glucose binds to the carrier before Na^+, or the order of addition is random; (b) the membrane potential effects the transition of unloaded carrier from the interior to the exterior surface of the membrane; and (c) the membrane potential increases the affinity of the carrier for the sugar.

Utilizing known membrane permeable and non-permeable sulfhydryl reagents, the Laboratory of Molecular Aging has found that the Na^+ gradient-dependent transport systems of D-glucose and phosphate in the renal brush border membrane possess critical SH-groups and that these are localized exclusively on the inner surface of the membrane. The reagents affect the carriers themselves rather than the dissipation of the Na^+ electrochemical gradient.

To understand better altered membrane biochemical pathways associated with aging and disease, the Laboratory of Molecular Aging has carried out studies to (a) identify the intermediate reactions of the enzyme systems involved in the active transport of metal ions and (b) elucidate the mechanism of conversion of chemical to osmotic energy that occurs during active transport. Examination of interaction of ADP and Mg^{2+} with the sarcoplasmic reticulum Ca^{2+}ATPase phosphoenzyme has revealed the order of release of products from the enzyme during ATP hydrolysis. A model has been developed for the sequence of intermediate steps in the overall reaction. This information is needed for the further examination of the previously reported age-dependent decrease in heart muscle Ca^{2+} transport activity.

Rapid mixing (msec) studies of the interaction of (Na^+K^+)ATPase with inhibitors, vanadate and oligomycin, have enabled the Laboratory of Molecular Aging to ascertain essential features of the enzymatic reaction mechanism. Vanadate produces complete inhibition of the enzyme by binding to the K^+-stabilized (E_2)

conformation and by activating the reversal of dephosphorylation. The latter effect may involve the interaction of vanadate with a neighboring subunit in an oligomeric enzyme complex. Investigations of the effects of monovalent cations on oligomycin inhibition showed that oligomycin interacts with (Na,K)ATPase in the absence of Na^+, but preincubation with Na^+ potentiates its effect, suggesting that Na^+ stabilizes the formation of an oligomycin binding conformation.

In further studies of the age-linked changes in the activity of enzymes involved in major catabolic pathways in heart, the Laboratory of Molecular Aging has found that mitochondrial pyruvate dehydrogenase activity plays a crucial role in determining whether heart muscle oxidizes lipid or carbohydrate. Moreover, changes in the Ca^{2+} concentration in the range 0.1 to 1.0 μM result in large and reversible changes in the amount of catalytically active pyruvate dehydrogenase present in respiring rat heart mitochondria, with a higher content of the active form the consequence of a higher Ca^{2+} concentration. The effects of Mg^{2+}, which inhibits the electrogenic transport process by which Ca^{2+} enters heart mitochondria, and Na^+, which acts as a co-substrate for the electrically neutral process by which Ca^{2+} leaves heart mitochondria, has been determined. It is concluded from these findings that the free Ca^{2+} concentration of the mitochondrial matrix is in a steady-state, affected by both rates of ingress and egress across the mitochondrial membrane, and with the pyruvate dehydrogenase interconversion system acting as a sensor of this matrix free Ca^{2+} concentration.

The Laboratory of Molecular Aging has found that the rate at which acylcarnitine translocates across the mitochondrial membrane in heart mitochondria diminishes in old age, and that this may limit the ability of these organelles to oxidize fatty acids in the older animal. The diminished rate of transport is associated with a diminished mitochondrial pool of carnitine and acylcarnitine, when the mitochondria are prepared from senescent animals (24 mo old rats). Further, the whole-heart content of free carnitine is decreased with old-age, showing a 39% decrement and a 22% decrement in the sum of free carnitine and short-chain acylcarnitine ($p = 0.01$ to 0.025). On the other hand, the carnitine concentration in whole blood or serum does not show a statistically significant decrease with age. Therefore, there is apparently an age-linked lesion in carnitine transport into the heart from the serum.

Various tissues and cells exposed to α-adrenergic and cholinergic agonists show an increased turnover of phosphtidylinositol. This enhancement has been attributed to a stimulation in phosphatidylinositol degradation, concomitant with a secondary increase in the resynthesis of the phospholipid. It has been postulated that the degradation of phosphatidylinositol is associated with the opening of a Ca^{2+} gate, resulting in an increase in intracellular [Ca^{2+}]. The Laboratory of Molecular Aging has described a Ca^{2+}-dependent degradation of phosphatidylinositol in smooth muscle (vas deferens and aorta). On the other hand, removal of intracellular Ca^{2+} with the ionophore A23187 and EGTA increases the amount of phosphatidylinositol. The inhibition by Ca^{2+} of phosphatidylinositol synthesis has been attributed to the action of the divalent cation on the activity of CDP-diacylglycerol-inositol-3-phosphatidyltransferase, the enzyme that catalyses the formation of phosphatidylinositol. These findings indicate that the intracellular Ca^{2+} concentration has an important role in regulating the phosphatidylinositol content of the tissue.

The Laboratory of Molecular Aging has found that acetylcholine stimulation of the vas deferens enhances the degradation of phosphatidylinositol and effects a decrease in the tissue content of the phospholipid. The acetylcholine-induced degradation is blocked by the muscarinic antagonist, atropine, but not by the nicotinic antagonist, (+)-tubocuramine. The acetylcholine-induced decrease in the phosphatidylinositol content leads to the compensatory synthesis of phosphatidylinositol. Synthesis is distinguished from degradation in the same tissue. The degradative process is not mediated by an increase in cyclic GMP.

Dispersed cells of the rat parotid gland respond in a dose-dependent manner to α- and β-adrenergic and cholinergic agonists. The Laboratory of Molecular Aging has found no differences with age in β-adrenergic receptors (number and ligand affinity), β-adrenergic stimulation of amylase release (time-course, extent, inhibition by antagonists). Efflux of K^+, stimulated by α-adrenergic agonists, shows a significant difference between young and aged rats in initial rate and dose-response curves, with a decrease with age. An additional deficit in α-adrenergic stimulation of glucose oxidation is observed. No age-related differences are found in cholinergic- or β-adrenergic-stimulated glucose oxidation. Thus, a selective change in neurotransmitter responsiveness occurs in parotid glands during aging.

Cyclic AMP-dependent protein kinase from rat parotid gland has been characterized by the Laboratory of Molecular Aging. Isoproterenol activation of protein kinase *in situ* occurs within 30s and displays a temporal pattern with respect to amylase release consistent with a suggested role for a cyclic AMP-dependent phosphorylation step necessary in exocrine secretion. Three specific changes in the phosphorylation status of cell proteins are found; two proteins showed enhanced phosphorylation, the other, a diminished phosphorylation. The three alterations are time- and dose-dependent, inhibited by β-adrenergic blocking agents, and mimicked by dibutyryl cyclic AMP.

The Laboratory of Molecular Aging has found that the submandibular glands of young adult (5-8 mo) and aged (23-24 mo) male, but not female, rats differ in several descriptive biochemical parameters. These include protein, neutral sugar, and sialic acid contents, all are decreased in the aged. No differences in DNA content are found.

Continued studies by the Laboratory of Molecular Aging of the oral physiological status of participants in the Baltimore Longitudinal Study have focused initially on parotid saliva flow rate and gustatory function. Cross-sectional analysis of data obtained from 146 healthy subjects (98 male, 48 female) show unequivocally that production of stimulated parotid saliva does not change with increased age. Direct measurements of gustatory function (analysis of taste thresholds) show no apparent age-related alterations for sweet and sour tastes. Thresholds for bitter and salty tend to increase with age ($r = 0.272$ and 0.191, respectively). Self-perceived deficits in gustatory function and food enjoyment are modest during aging but occur more frequently in individuals who are medically compromised.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00041-07 LMA

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging I
Membrane Transport Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Sacktor	Chief, Lab. Molec. Aging	LMA, NIA
OTHER:	L. Cheng	Senior Staff Fellow	LMA, NIA
	S. Hilden	Senior Staff Fellow	LMA, NIA
	L. Noronha-Blob	Staff Fellow	LMA, NIA
	U. Reiss	Visiting Associate	LMA, NIA
	A. Elgavish	Visiting Fellow	LMA, 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:
7.8	5.4	2.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study defines the biochemical mechanisms whereby age-dependent changes in transport processes perturb physiological control systems and, thus, contribute to the failure to maintain homeostasis in the aged. Topics investigated include: (1) phosphate transport mechanisms; (2) sugar transport mechanisms; (3) molecular organization and orientation of membrane components in the brush border membrane; and (4) changes in membrane function with age.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: These studies are targeted to define the membrane transport systems in epithelial tissues, *i.e.* kidney and intestine, and to describe the changes in these processes with respect to age and disease. These investigations impact on the understanding of senile osteoporosis and osteomalacia, salt-water homeostasis, acid-base balance, absorption and retention of vital metabolites, and membrane alterations induced by ischemia. Experimental questions are focused on membrane biology, including (1) molecular organization; (2) selective vectorial transport systems; (3) dietary and hormonal regulation of the transport processes; (4) catalytic function; (5) turnover; and (6) failure to maintain structure and function, leading to cell death.

Methods Employed: Model systems include 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, are also used.

Major Findings:

Phosphate Transport Mechanisms. Renal phosphate transport was investigated using the isolated proximal tubule brush border membrane vesicle to describe the mechanism by which the anion is removed from the filtrate and is accumulated against its concentration gradient into the renal cell. This model system permitted the membrane transport step to be dissociated from cellular metabolism and allowed the examination of the ionic driving forces that energized the active transport. The contribution of the Na^+ gradient in effecting the concentrative uptake of phosphate by an electroneutral mechanism was further defined. Measurements of phosphate uptake at different pH values suggests that the preferred species of phosphate transported was the HPO_4^{2-} form, but some transport of the $H_2PO_4^-$ form was not ruled out. The Na^+-phosphate cotransport system was characterized by thermal energy of activation and heat-denaturation.

It was discovered that in addition to a Na^+ gradient (extravesicular[Na^+]> intravesicular[Na^+]), a proton gradient (intravesicular[H^+]>extravesicular[H^+]) could drive the uphill transport of phosphate. The efficacy of the proton-dependent system in transporting phosphate was correlated with the magnitude of the H^+ gradient. Thus, when the pH of the extravesicular medium was kept constant at 7.3, phosphate uptake was increased when the pH of the intravesicular medium was lowered from 7.3 to 5.5. Keeping the intravesicular medium at 5.5, the uptake was increased by increasing the extravesicular pH from 6.5 to 8.0. The H^+ gradient-dependent system required the presence of Na^+, although it functioned in the absence of a Na^+ gradient, *i.e.* extravesicular[Na^+] = intravesicular[Na^+]. The findings that the generation of a K^+ diffusion potential (inside negative), induced by the K^+ ionophore valinomycin, or a H^+ diffusion potential (inside negative), induced by the uncoupler FCCP, had no effect on the uptake of phosphate indicates that the H^+-energized as well as the Na^+-energized phosphate transport systems are electroneutral processes. This conclusion has implications on the effect of the cotransport systems on the membrane potential and on the stoichiometry of the cotransport. The H^+

gradient-dependent phosphate system may be of particular significance in understanding the mechanism of urine acidification.

It was reported last year that animals fed a low phosphorus diet adapt to conserve filtered phosphate and that this adaptation is reflected at the level of the brush border membrane, vesicles of which show a doubling in their rate of phosphate uptake. Studies on the mechanism of this dietary regulation of the membrane transport was continued. Membrane vesicles from animals on a low phosphorous diet differed strikingly from those on a high phosphorous diet in their response to pH. With the low phosphorous diet, the rate of the Na^+ gradient-dependent phosphate uptake increased with higher alkalinity. In contrast, the rate of uptake was unchanged as the pH was increased from 6.5 to 8.0, with membrane vesicles from an animal on a high phosphorous diet. Low phosphorous diet membrane vesicles had a higher H^+ gradient-dependent phosphate uptake then did those on a high phosphorous diet. The phosphate carrier in membranes from animals on the two diets did not differ with respect to thermal energies of activation nor in heat-denaturation.

Phosphate transport in the renal tubules is inhibited by parathyroid hormone. We have now found that brush border membrane vesicles isolated from renal slices preincubated with the hormone showed a specific decrease in the Na^+ gradient-dependent phosphate uptake. Phosphate uptake in the absence of Na^+ was not affected. In addition, parathyroid hormone treatment of the slices did not alter Na^+ gradient-dependent D-glucose uptake. This finding demonstrates that the hormone acted specifically on the Na^+-phosphate cotransport system rather than acting indirectly by dissipating the Na^+ electrochemical gradient.

A Na^+ gradient-dependent phosphate transport system was demonstrated in intestinal brush border membranes. The intestinal system differed from the renal system in its lower activity. The intestinal membrane vesicle system exhibited only a small Na^+ gradient-driven uphill uptake of phosphate. On the other hand, the Na^+ gradient-dependent uptakes of D-glucose in the two membrane vesicle preparations were similar.

Cultured LLC-PK_1 cells, a continuous line derived from pig kidney, orient themselves in a manner that maintains their polarity, *i.e.* the basolateral segment of the plasma membrane abuts the substratum whereas the apical surface faces the media. When confluent the epithelial monolayers form domes, indicative of the accumulation of fluid between the serosal surface and the substratum, thus, of transepithelial transport. Using such cells, a study of phosphate transport was initiated. A Na^+-dependent phosphate uptake system was found. NH_4^+ could partially substitute for Na^+, but other monovalent cations were without effect. Ouabain, the glycoside inhibitor of Na^+K^+ATPase (the Na^+ pump), localized in the basolateral membrane, blocked the uptake of phosphate. Phosphate transport by the cultured cells was regulated by cell growth and density. Although the total uptake of phosphate increased as cells approached confluency the amount of phosphate taken up per cell decreased strikingly as the density of the cells increased. For instance, at a density of 2×10^4 cells per coverglass 14 fmoles of phosphate were taken up per cell, whereas at a density of 2.5×10^5 cells/coverglass only 1.0 fmole of phosphate per cell was taken up.

Sugar Transport Mechanisms. Studies of the mechanism of Na^+-D-glucose cotransport in renal brush border membranes continued. Last year, initial studies were reported on a low affinity D-glucose uptake found in the presence of a membrane potential but in the absence of Na^+. Four characteristics suggest that this uptake is mediated by the Na^+-D-glucose transporter: stereospecificity, substrate specificity, phlorizin inhibition and potential dependence. In addition, the potential dependent, Na^+-independent glucose uptake shows the more general properties characteristic of all transport systems: saturability, accelerative exchange diffusion, osmotic sensitivity and temperature dependence. This study contributes to two questions currently under discussion. (1) What is the order of substrate addition? This study suggests that glucose binds before Na^+ or that the order of addition is random. (2) What is the effect of membrane potential on the Na^+-D-glucose cotransport mechanism? This study is consistent with two proposed suggestions: (a) membrane potential promotes the transition of unloaded carrier from the interior to the exterior surface of the membrane and (b) membrane potential decreases the K_m of the carrier for D-glucose or its competitive inhibitor phlorizin.

Molecular Organization and Orientation of Membrane Components in the Brush Border Membrane. Studies of the structural and functional organization of the renal brush border were continued with an examination of the loci of sulfhydryl groups critical to the different transport systems in the membrane. The question was approached by using sulfhydryl reacting reagents that exhibited marked differences in their solubility properties and therefore penetrated the membrane to different degrees. Two permeable reagents (NEM and CH_3HgCl) and one non-permeable reagent (DTNB) were used. Brush border vesicles treated with CH_3HgCl (1 mM for 10 min at 20°) showed a 63% inhibition at the peak of the overshoot in Na^+ gradient-dependent D-glucose uptake and 71% inhibition in Na^+ gradient-dependent phosphate uptake. After NEM exposure (1 mM for 1 hr at 20°), Na^+ gradient-dependent D-glucose transport was reduced by 58% and phosphate uptake by 48%. Thus, both D-glucose and phosphate transports were sensitive to sulfhydryl reagents that permeate the membrane, suggesting that either external or internal SH groups are involved in their transports.

However, when brush border vesicles were treated with the non-permeant reagent DTNB (1 mM at 20° for 1 hour) the Na^+ gradient-dependent uptake of neither D-glucose nor phosphate was affected, suggesting that the SH groups that were critical for transport were located on the inner surface of the membrane. Even concentrations of DTNB at 5 mM did not reduce uptake of either substrate whereas 2.5 mM CH_3HgCl inhibited transport of glucose and phosphate by 90% and 86%, respectively.

The inhibitory effect of pretreatment for 10 min with CH_3HgCl could be reversed by mercaptoethanol (10 mM), but after pretreatment for 60 min the inhibition was irreversible, probably due to nonspecific damage to the vesicles. The effects of NEM, however, were irreversible even at 100 mM mercaptoethanol.

In the absence of a Na^+ gradient but in the presence of equal Na^+ on both sides of the membrane, CH_3HgCl still inhibited both D-glucose and phosphate uptake, suggesting that the critical SH groups on the inner face of the membrane

must involve the two carrier systems and not the proteins involved in maintaining the Na^+ gradient.

Preliminary studies were carried out to reconstitute the D-glucose and phosphate carriers into synthetic phospholipid membranes. Brush border membrane proteins were dispersed by non-ionic (Triton X-100) and ionic (cholate) detergents and the carrier proteins reincorporated into asolectin phospholipid vesicles by sonication or by a dialysis method. Excess detergents were removed by dialysis (cholate) or by a Bio-bead chromatographic column (Triton X-100).

Changes in Membrane Function with Age. Renal brush border membrane vesicles from 24 mo old rats had decreased Na^+ gradient-dependent uptakes of phosphate and D-glucose when compared to the uptake of vesicles from 6 mo old animals. The initial rates for phosphate uptake for the young and the old were 605 $\pm$ 26 and 501 $\pm$ 17 pmoles/15 sec/mg of membrane protein. For D-glucose, the respective uptakes were 830 $\pm$ 33 and 580 $\pm$ 27.

Rat kidney maltase from young (4-6 mo) and old (24 mo) animals was purified to homogeneity (about 1000-fold purification with a 30-40% recovery of total activity). The purification procedure involved the release of the enzyme from the brush border membrane by papain hydrolysis, selective precipitation by ammonium sulfate, gel filtration and affinity chromatography, using a novel affinity procedure. Antibody was raised in rabbits against the pure enzyme, and immunodiffusion tests showed a single precipitin line of identity when "young" and "old" enzymes were cross-reacted with whole extracts.

The specific activity of pure enzymes obtained from young and old rats were 46 and 32.5 µmole/min/mg, respectively, a decrease of about 30%. No differences were found in molecular weight (1,300,000 daltons, with 4 subunits of 335,000 each), charge, K_m (1.6 mM), K_i for Tris (3.4 mM) and pH optimum (6.7) between "young" and "old" pure enzyme preparations. Renal maltase specificity hydrolyzes maltose, and no other disaccharide nor starch.

Circular dichroism measurements indicated that the old enzyme had an altered conformation, from 63% β-helix and 37% random coil in the young to 75% β-helix and 25% random coil in the old. Like other extrinsic membrane proteins, maltase was found to be a glycoprotein. There was a significant decrease in total carbohydrate in the old enzyme. Hexose constituted about 17.5% of the young enzyme molecule but only 7.5% of the old enzyme.

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 the Project: Studies on the mechanisms of membrane transport systems and their regulation, as related to the aging process, will focus on: (1) phosphate and calcium transport in membrane vesicles derived from renal

and intestinal cells and in intact cells, isolated or cultured; (2) sugar and amino acid transport mechanism; (3) carrier isolation and reconstitution of function in synthetic membranes; (4) changes in membrane structure and function induced by ischemia; and (5) changes in the molecular organization of the membrane during the aging process.

Publications:

Hilden, S. A. and Sacktor, B.: D-Glucose-dependent sodium transport in renal brush border membrane vesicles. J. Biol. Chem. 254: 7090-7096, 1979.

Sacktor, B.: Electrogenic and electroneutral Na^+ gradient-dependent transport systems in the renal brush border membrane vesicle. Curr. Top. Membr. Transport 13: 291-299, 1980.

Hammerman, M. R., Sacktor, B. and Doughaday, W. H.: The Na^+ electrochemical gradient-dependent transport of *myo*-inositol in rabbit renal brush border membrane vesicles and its inhibition by D-glucose. Am. J. Physiol., in press.

Lipsky, J. J., Cheng, L., Sacktor, B. and Lietman, P. S.: Gentamicin uptake by renal tubule brush border membrane vesicles. J. Pharmacol. Exp. Ther., in press.

Sacktor, B. and Schneider, E. G.: The singular effect of an internal K^+ gradient ($[K^+]_i > [K^+]_o$) on the Na^+ gradient ($[Na^+]_o > [Na^+]_i$)-dependent transport of L-glutamate in renal brush border membrane vesicles. Int. J. Biochem., Special Issue "Biochemical Aspects of Renal Function," in press.

Schneider, E. G., Hammerman, M. R. and Sacktor, B.: Sodium gradient-dependent L-glutamate transport in renal brush border membrane vesicles: evidence for an electroneutral mechanism. J. Biol. Chem., in press.

Schneider, E. G. and Sacktor, B.: Sodium gradient-dependent L-glutamate transport in renal brush border membrane vesicles: effect of an intravesicular> extravesicular potassium gradient. J. Biol. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00042-07 LMA

PERIOD COVERED
October 31, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging II
Mechanisms of Hormonal Regulation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Sacktor	Chief, Lab. Molec. Aging	LMA, NIA
	C. Filburn	Research Chemist	LMA, NIA
	T. Liang	Research Chemist	LMA, NIA
OTHER:	M. Freiberg	Medical Officer	LMA, NIA
	R. Balakir	Chemist	LMA, NIA
	J. Joseph	Senior Staff Fellow	LBS, NIA
	G. Roth	Research Chemist	CPB, NIA

COOPERATING UNITS (if any)
Laboratory of Behavioral Sciences, NIA and Clinical Physiology Branch, NIA.

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:
5.7	4.2	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study defines the biochemical mechanisms whereby age-dependent changes in hormonal regulation of transport processes perturb physiological control systems and, thus, contribute to the failure to maintain homeostasis in the aged. Topics investigated include: (1) hormonal regulation of phosphate, calcium, salt, and water transports in the kidney; (2) mechanism of action of cholinergic and α-adrenergic agonists: phosphoinositol turnover in membranes and calcium flux; and (3) relationships of dopamine receptor levels and dopamine stimulation of adenylate cyclase to dopaminergically mediated rotational behavior.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: These studies are targeted to define the biochemical mechanisms whereby age-dependent changes in hormonal regulation of transport processes perturb physiological control systems and, thus, contribute to the failure to maintain homeostasis 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. Investigations are focused on the actions of the hormones, parathyroid hormone, calcitonin, and vasopressin; and cholinergic, α- and β-adrenergic, and dopaminergic agonists. The research has direct impact on the understanding of the mechanisms underlying age-related changes in renal, smooth muscle, and neural function.

Methods Employed: Appropriate biochemical and endocrinological techniques for specific experimental questions are developed and/or adapted, as reported in published papers.

Major Findings:

Hormonal Regulation of Phosphate, Calcium, Salt, and Water Transports in the Kidney. Phosphate and calcium metabolism, salt-water balance, and acid-base balance are controlled, in part, by hormones whose mode of action are presumably mediated via adenyl and guanyl nucleotides and/or calcium. Thus, studies of plasma membrane-bound hormone receptors; enzymes that determine tissue levels of cyclic nucleotides, *e.g.* adenylate and guanylate cyclases and phosphodiesterases; enzymes whose activities may be regulated by cyclic AMP and calcium, *e.g.* protein kinases and phosphoprotein phosphatases; and the nature and function of the endogenous proteins, whose state of phosphorylation is affected by these kinase and phosphatases, are of great significance.

Parathyroid hormone increases phosphate excretion by inhibiting phosphate transport in the renal tubule. In a study of the mechanism of this hormonal regulation, it was found that, when renal cortical slices were incubated with parathyroid hormone (30 U/ml) for 2 min, the hormone elicited an activation of adenylate cyclase, an increase in tissue level of cyclic AMP, an enhancement of cyclic AMP-dependent protein kinase, and concomitantly an inhibition of the Na^+ gradient-dependent uptake of phosphate by brush border membrane vesicles isolated from the treated slices. These findings demonstrate that this *in vitro* system represents a model to examine further the mechanism by which parathyroid hormone regulates phosphate transport in the kidney.

LLC-PK_1 cell adenylate cyclase activity was found to be markedly stimulated by the antidiuretic hormone, vasopressin, moderately stimulated by parathyroid hormone, but not enhanced by calcitonin. Guanyl nucleotides, which augment hormonal stimulation, decreased the concentration of vasopressin needed to stimulate the enzyme from 5 nM to 1.5 nM.

An endogenous factor in the renal cortex cytosol that potentiates parathyroid hormone and prostaglandin E_1 activation of renal adenylate cyclase

without affecting basal enzyme activity or activity stimulated by guanyl nucleotides and F^- was discovered and partially purified and characterized. Partial purification of the factor was accomplished by $(NH_4)_2SO_4$ fractionation followed by chromatography on DEAE-cellulose and Sephadex G-75 columns. After the Sephadex column, the recovery of protein, units of activity (measured as the 50% potentiation of PTH activation of adenylate cyclase), and specific activity were, respectively, 2.7%, 37%, and 13-fold, relative to the crude $(NH_4)_2SO_4$ fraction. The molecular weight of the factor was about 15,000 daltons. The factor was heat stable (5 min at 100°), but relatively labile when frozen-thawed. Relative to basal activity, PTH and PTH + activator stimulated activity 138% and 384%, respectively. PGE_1 and PGE_1 + activator enhanced activity 48% and 228%, respectively. The factor was distinguished from endogenous GTP, since (1) activity remained after treatment with charcoal, which removed added GTP; (2) activity was lost after incubation with specific proteolytic enzymes; and (3) activation of adenylate cyclase by GMP-PNP was not affected by the factor. The factor was also distinguished from calmodulin, as factor activity was not decreased by removal of Ca^{2+}. That the factor is a protein was indicated by its sensitivity to partial digestion with chymotrypsin, although it was resistant to the action of trypsin.

The action of hormones, mediated via cyclic AMP, are propagated by cAMP-dependent protein kinases that catalyze the phosphorylation of specific proteins. The regulatory subunits of the cAMP-dependent protein kinases in cytosol and brush border membranes were isolated by DEAE-cellulose column chromatography and identified by photoaffinity labeling with [^{32}P]-8-N_3-cAMP, SDS-slab gel electrophoresis, and autoradiography. Molecular weights are: cytosol type I, 49,000; cytosol type II, 56,000; and membrane type II, 56,000.

Specific membrane proteins are phosphorylated by the protein kinase. To identify specific protein phosphorylations that are hormone responsive we obtained a line of cells (A_6), derived from toad kidney, from Dr. Joseph Handler (LKEM, NHLBI). As he has shown, these cells, when grown in monolayer, respond to the addition of cyclic AMP analogs with a decrease in transcellular electrical resistance and with a rise in short-circuit sodium flux, while potential difference is maintained. These effects are enhanced when the cells are pretreated with mineral-corticoids (aldosterone, triamcinolone). These electrophysiologic effects in response to cyclic AMP analogs are analogous to those one would predict from antidiuretic hormone, although the receptor to vasopressin has been lost in these cultured cells.

In preliminary experiments, it was necessary to develop conditions for the labelling of intracellular ATP and to establish that the radioactive specific activity of the γ-P of ATP had reached a steady-state. This was accomplished by incubating the cells with inorganic ^{32}P, labelling of the ATP, and determining the specific activity of the γ-P by exchange with GTP using the enzyme nucleotide diphosphate kinase. It was determined that isotopic equilibrium was achieved in these cultured cells in 3 hrs.

Phosphorylation of endogenous proteins in A_6 cell homogenates incubated with 8-bromo cyclic AMP showed multiple cAMP-dependent phosphoproteins. Gel

phoresis and radioautography of the separated proteins revealed a phosphoprotein of molecular weight 27,000 in the cytosol. Two membrane proteins responded to cyclic AMP, by showing an enhanced dephosphorylation. One had a molecular weight of 27,000 daltons, the other of 56,000 daltons.

Continuation of studies on the phosphorylation of proteins in the renal brush border revealed that the Ca^{2+}-stimulated phosphorylation of a 16,000 dalton brush border protein occurred only at relatively high Ca^{2+} (>100 μM) and appeared to be unaffected by trifluoperazine. Thus far, calmodulin does not appear to play a major role in phosphorylation of brush border membrane proteins.

Mechanism of Action of Cholinergic and α-Adrenergic Agonists: Phosphoinositol Turnover in Membranes and Calcium Flux. Various tissues and cells exposed to α-adrenergic and cholinergic agonists show an increased turnover of phosphatidylinositol. This enhancement has been attributed to a stimulation in phosphatidylinositol degradation, concomitant with a secondary increase in the resynthesis of the phospholipid. It has been postulated that the degradation of phosphatidylinositol is associated with the opening of a Ca^{2+} gate, resulting in an increase in intracellular [Ca^{2+}]. A Ca^{2+}-dependent degradation of phosphatidylinositol in smooth muscle (vas deferens and aorta) was found. On the other hand, removal of intracellular Ca^{2+} with the ionophore A23187 and EGTA increased the amount of phosphatidylinositol. The inhibition by Ca^{2+} of phosphatidylinositol synthesis is attributed to the action of the divalent cation on the activity of CDP-diacylglycerol-inositol-3-phosphatidyltransferase, the enzyme that catalyzes the formation of phosphatidylinositol. These findings indicate that the intracellular Ca^{2+} concentration has an important role in regulating the phosphatidylinositol content of the tissue.

An acetylcholine stimulation of the vas deferens enhanced the degradation of phosphatidylinositol and effected a decrease in the tissue content of the phospholipid. The acetylcholine-induced degradation was blocked by the muscarinic antagonist, atropine, but not by the nicotinic antagonist, (+)-tubocuramine. The acetylcholine-induced decrease in the phosphatidylinositol content led to the compensatory synthesis of phosphatidylinositol. Synthesis was distinguished from degradation in the same tissue. The degradative process was not mediated by an increase in cyclic GMP.

Relationships of Dopamine Receptor Levels and Dopamine Stimulation of Adenylate Cyclase to Dopaminergically Mediated Rotational Behavior. The interrelationships of dopamine receptors, dopamine-stimulated adenylate cyclase, and lugotril (a dopaminergic agonist)-stimulated rotational behavior in young and old male and female rats having 6-hydroxydopamine lesions of their left substantial nigra were examined in collaboration with Drs. James Joseph (LBS) and George Roth (CPB). Both ^{3}H-spiroperidol specific binding and dopamine stimulation of cyclase increased in the left side striata, indicating development of supersensitivity. The increase in cyclase response was most marked at a low dose (1 μM, 100% increase), and was less at a high dose (100 μM, 15-20% increase). Spiroperidol binding increased 30-40% compared to the right striata. Left/right ratios of these changes were compared to rotational response to lugotril in the same animals. While receptor number, *i.e.*, spiroperidol binding, showed a high

consistent correlation across age and sex, cyclase stimulation showed variable, usually negative correlation. No differences in changes in supersensitivity of receptors or cyclase were observed by comparisons of either sex or age. Thus, the age-deficit in rotational behavior seems to be associated with reduced numbers of dopamine receptors measured by spiroperidol binding, but not to receptors associated with dopamine stimulated adenylate cyclase.

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 on the mechanisms of hormone regulation as related to the aging process will focus on: (1) the regulation of phosphate and calcium metabolism by hormones and vitamin D_3 in cells and membrane preparations; and (2) the regulation of salt-water balance.

Publications:

Egawa, K., Sacktor, B. and Takenawa, T.: Ca^{2+}-dependent and Ca^{2+}-independent degradation of phosphatidylinositol in rabbit vas deferens. Biochem. J., in press.

Guarnieri, T., Filburn, C. R., Beard, E. S. and Lakatta, E. G.: Enhanced contractile response and protein kinase activation to threshold levels of β-adrenergic stimulation in hyperthyroid rat heart. J. Clin. Invest. 65: 861-868, 1980.

Guarnieri, T., Filburn, C. R., Zitnik, G., Roth, G. S. and Lakatta, E. G.: β-Adrenergic stimulation of senescent myocardium: biochemical correlates of diminished contractability. Am. J. Physiol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00043-07 LMA

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Physiological Control Systems and Aging III
Regulation of Intermediary Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Sacktor	Chief, Lab. Molec. Aging	LMA, NIA
	R. Hansford	Research Chemist	LMA, NIA
	B. Bulos	Research Chemist	LMA, 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:
3.2	2.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

These studies define the biochemical mechanisms whereby age-dependent changes in regulation of intermediary metabolism perturb physiological control systems and, thus, lead to a failure to maintain homeostasis in the aged. Investigations include: 1) regulation of pyruvate dehydrogenase activity by Ca^{2+} ions; 2) alterations in the carnitine content of the heart with age; 3) preparation of isolated heart cells, and the study of carnitine transport by these cells; and 4) regulation of the metabolism of 25-hydroxyvitamin D_3 in kidney mitochondria.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: These studies are designed to define the biochemical mechanisms whereby age-dependent changes in regulation of intermediary metabolism perturb physiological control systems, and, thus, lead to a failure to maintain homeostasis in the aged. The thrust of the work is focused on mitochondrial metabolism in the normal, diseased, and aged states. This research has impact on the mechanisms underlying age-related changes in cardiac function and in understanding the contributions of the control of renal and intestinal transports in effecting Ca^{2+} and phosphate homeostasis.

Methods Employed: Experimental preparations include the isolated rat heart cell, cardiac mitochondria from the rat, and renal mitochondria from the vitamin D-deficient and -repleted chick. Appropriate biochemical and enzymatic assays and procedures are employed.

Major Findings:

Regulation of Pyruvate Dehydrogenase Activity by Ca^{2+}-ions. The crucial role of pyruvate dehydrogenase activity in determining whether heart muscle oxidizes lipid or carbohydrate was discussed last year. We found (Annual Report 1979) that changes in the Ca^{2+}-ion concentration of the mitochondrial incubation medium in the range 0.1 to 1 μM (pCa = 7 to 6) gave large and reversible changes in the amount of catalytically active pyruvate dehydrogenase (PDH_A) present in respiring rat heart mitochondria, with a higher PDH_A content the consequence of a higher Ca^{2+}-ion concentration. This effect is of considerable physiological interest, as this range of Ca^{2+}-ion concentration is thought to be plausible for the cytosol of the living cardiac muscle, and was further investigated this year. We focussed mainly on the effect of Mg^{2+}-ions, which inhibit the electrogenic transport process by which Ca^{2+}-ions enter heart mitochondria, and Na^{+}-ions, which act as a co-substrate for the electrically neutral process by which Ca^{2+}-ions leave heart mitochondria. Elevated Mg^{2+}-ion concentrations were shown to diminish the pCa value at which 50% of the heart pyruvate dehydrogenase was in the form PDH_A, in experiments in which extramitochondrial Ca^{2+}-ion concentrations were stabilized in the range pCa 6 to 7 using $CaCl_2$/EGTA buffers. Elevated concentrations of NaCl (in the range 0 to 20 mM) were also shown to diminish the pCa value required for PDH_A to be 50% of the total. These effects are consistent with the free Ca^{2+}-ion concentration of the mitochondrial matrix being a steady-state value, affected by both rates of ingress and egress across the mitochondrial membrane, and with the pyruvate dehydrogenase interconversion system acting as a sensor of this matrix free Ca^{2+}-ion concentration. This concentration is not currently accessible by direct measurement techniques and so inferences based on the behavior of pyruvate dehydrogenase are of interest to those concerned with mitochondrial Ca^{2+}-ion transport, as well as those concerned with the control of dehydrogenase activity. Other experimental parameters which were studied as being possible effectors of mitochondrial Ca^{2+}-content were inorganic phosphate, acetate and bicarbonate-ion concentrations; in each case, an increased concentration was reflected in an increased PDH_A content.

Finally, controls were performed to establish that the effects of Mg^{2+}-, Na^{+}- and phosphate-ions were not a consequence of changes in mitochondrial energy-status, as pyruvate dehydrogenase interconversion is known to be sensitive to mitochondrial ATP/ADP and NADH/NAD^{+} ratios. This was studied by monitoring the mitochondrial content of NADH by fluorescence, and by recording the resting rate of O_2-uptake. PDH_A content was found to give large responses to changes in medium pCa without changes in mitochondrial NADH/NAD^{+} ratio, provided that pCa values of less than 6.05 are avoided. The concentrations of Na^{+}- and Mg^{2+}-ions had no influence on the NADH/NAD^{+} ratio.

This project is almost complete. It remains to establish whether Na^{+}-ions have a direct effect on one of the enzymes which catalyze the activation-inactivation mode of pyruvate dehydrogenase (the pyruvate dehydrogenase phosphatase) or act solely by diminishing the Ca^{2+}-ion concentration of the mitochondrial matrix, with the latter being the effector of the phosphatase. We have data supporting a role of Na^{+}-ions in the absence of added Ca^{2+}-ions, but the interpretation of this hinges upon knowing whether mitochondrial Ca^{2+}-ions had been withdrawn by the chelating agent EGTA. If not, Na^{+}-ions may still act solely on Ca^{2+}-transport. The (total) mitochondrial content of Ca^{2+} will be measured by atomic absorption spectroscopy, to resolve this matter.

It is suggested that the modulation of pyruvate dehydrogenase activity in heart by Ca^{2+}-ions may butress the regulation by ATP/ADP and NADH/NAD^{+} ratios in the response of PDH_A content to changes in work-load on the heart. However, modulation by the mitochondrial free Ca^{2+}-ion concentration may also mediate the response to hormones, notably insulin and α-agonists, as there is growing evidence in other tissues that a "second-messenger" leads to the mobilization of mitochondrial Ca^{2+}-ions.

The Heart Carnitine Content with Respect to Age. We established earlier (Annual Report 1978) that the rate of translocation of acylcarnitine into heart mitochondria diminishes in old age, and that this may limit the ability of these organelles to oxidize fatty acids in the older animal. The diminished rate of transport was associated with a diminished mitochondrial pool of carnitine and acylcarnitine, when the mitochondria were prepared from senescent animals (24 mo rats). Further, there was information from another strain of rat that the whole-heart content of carnitine diminished with old-age. We asked the questions: (1) Is it also true that the whole-heart content of the Wistar rat decreases in senescence? (2) If so, does the serum concentration also fall? (3) If the serum concentration does not fall, what has changed at the level of the plasma membrane transport of carnitine so that it is no longer accumulated so effectively from the serum in old-age? In answer to (1), we used a sensitive assay technique based on the carnitine acetyltransferase reaction and ^{14}C-acetyl-coenzyme A to measure carnitine in extracts of rat hearts, both before and after the hydrolysis of short-chain acylcarnitine species. We found a 39% decrement in carnitine in old-age (486 ± 65 and 297 ± 15 nmol/g wet wt in 12 mo and 24 mo animals, respectively, $p = 0.01$ to 0.025) and a 22% decrement in the sum of carnitine and short-chain acyl-carnitine

(1131 ± 55 and 887 ± 15, respectively, p = 0.01 to 0.025). The short-chain acylcarnitine content was itself unchanged with age. In answer to question (2), we measured carnitine in extracts of whole blood and found no measurable statistically significant decrease with age. Free carnitine was 32.5 ± 1.4 μM in blood from 12 mo animals and 29.8 ± 2.0 in 24 mo animals (p>0.2). After alkaline hydrolysis of acylcarnitines, the numbers were 53.2 ± 2.9 μM and 55.2 ± 2.4 μM for 12 and 24 mo animals, respectively (p>0.2). Similar experiments with serum instead of whole blood gave the same picture, but with more variation and somewhat higher values. All of these data were collected from male animals.

Thus, there is apparently an age-linked lesion in carnitine accumulation into the heart, from serum. What is the nature of this defect (question 3)? To attempt to answer this, we made preparations of isolated heart cells.

The Preparation of Isolated Heart Cells, and the Study of Carnitine Transport by These Cells. A great deal of time was expended in establishing a procedure that would give viable, isolated heart cells. Most of the preparations were based on the method of Clark and Berry, and used collagenase and hyaluronidase, in a "low-Ca^{2+}" medium, to effect disaggregation of the tissue. The Ca^{2+}-concentration is crucial, as less than 100 μM is needed to separate the cells, but the enzyme collagenase requires Ca^{2+}-ions for activity. In addition, once exposed to "low" Ca^{2+}-ion concentrations, the cells become hypersensitive to Ca^{2+}-ions and are readily killed by normally physiological concentrations. Systematic determinations were made of the optimal Ca^{2+}-ion concentration for each of three media with which the heart is perfused during the cell preparation. At the end of this work, cell preparations with 50% or more viability could routinely be made from the hearts of 2 to 6 month old rats. This assessment is based on microscopic examination and the exclusion of the dye, Trypan Blue. We also developed physiological criteria of integrity of these preparations, including the effects of oligomycin and uncoupling agents on the rate of O_2-uptake with pyruvate as substrate and the differential in the rate of O_2-uptake at high and low concentrations of succinate. Morphologically intact cells show a high rate of pyruvate respiration, a marked response to uncoupling agents, and a much lower rate of O_2-uptake at 1 mM than at 10 mM succinate.

These cells were used to study the transport of carnitine, experiments being terminated by filtration and washing. At 20° the cells showed a progressive uptake of carnitine over 30 min, this being slightly inhibited by the use of low-Na^+ (15 mM) media in the place of high-Na^+ (150 mM) media. The uptake was inhibited about 50% by the SH reagent mersalyl, but was essentially uninhibited by uncoupling agents (which will diminish the cellular ATP content) or by gramicidin (which will equilibrate the electrochemical potential of Na^+-ions across the plasma membrane). We assume that the concentrative uptake of carnitine into heart muscle is driven by the electrochemical potential gradient of Na^+-ions across the cell membrane. Whether the isolated cells are capable of generating a Na^+-ion gradient by substrate oxidation is not yet clear. We intend to

clarify the situation by (1) determining the intracellular volume of these cells, to know whether cellular carnitine is simply equilibrating with the concentration in the medium, and (2) determining the cellular Na^+- and K^+-ion content, by atomic absorption spectroscopy, to ascertain the size of the ion gradients discussed above. With this background, we can study carnitine transport more effectively. If it transpires that the cells cannot maintain an adequate Na^+-ion gradient, we may have to attempt to "re-seal" them by incubation with respiratory substrate in a low-Na^+ medium, before carrying out transport studies.

In Vivo and *In Vitro* Regulation of the Metabolism of 25-Hydroxyvitamin D_3 in Chick Kidney Mitochondria. It is known that 25-hydroxyvitamin D_3 is metabolized by chick kidney mitochondria to either 1,25-dihydroxyvitamin D_3 or to 24,25-dihydroxyvitamin D_3 depending upon the dietary and serum concentrations of calcium presumably through parathyroid hormone intervention and upon the Vitamin D and phosphate concentrations. Thus, in the presence of high concentrations of calcium, phosphate and 1,25-dihyroxyvitamin D_3, 24,25-dihydroxyvitamin D_3 is the major metabolite produced in the kidney; when dietary and, therefore, serum calcium, phosphate and Vitamin D levels are low, 1,25-dihydroxyvitamin D_3 is the major metabolite synthesized. That PTH is involved in the regulation of $1,25(OH)_2D_3$ synthesis has been suggested by the observation that rats maintained on a low calcium diet show a rapid decline in the previously elevated concentration of $1,25\text{-}(OH)_2D_3$ in plasma after parathyroidectomy and that this change was prevented by administration of PTH. Several laboratories have attempted to demonstrate that addition of PTH to surviving primary cultures of kidney cells would stimulate production of $1,25\text{-}(OH)_2D_3$. The results to date have been equivocal. What is clear is that primary kidney cell cultures from D-deficient chicks or rats synthesize $1,25\text{-}(OH)_2D_3$ from $25\text{-}(OH)D_3$ while cells from D-depleted animals synthesize $24,25\text{-}(OH)_2D_3$.

While apparent that the regulation of $1,25\text{-}(OH)_2D_3$ synthesis is extremely complex, the fact that the 1-hydroxylase is localized within mitochondria and that it is a cytochrome P_{450}-dependent monoxygenase requiring molecular oxygen and NADPH for activity has led us to investigate the ways of effecting enzyme activity in isolated kidney mitochondria from Vitamin D-deficient chicks. We have begun to examine if any variation in the mitochondrial concentration of NADPH could influence the activity of the 1-hydroxylase since mitochondria are impermeable to pyridine nucleotides and the needed NADPH must be generated internally. We are in the early stages of examining the total intramitochondrial concentrations of NAD, NADP, NADH and NADPH when mitochondria from kidneys of Vitamin D-deficient and -repleted chicks are allowed to oxidize glutamate + malate in State 4. The intramitochondrial NADPH is maintained in the reduced form by the action of an energy-linked (either ATP or substrate-drive) transhydrogenase. We have shown that kidney mitochondria from Vitamin D-deficient, rachitic chicks do indeed contain an active energy-linked transhydrogenase activity (a minimal specific activity of 2-3 nmoles NADPH formed/min/mg protein) but have not as yet compared this level of activity with that seen in mitochondria from Vitamin D-repleted chicks.

Mitochondria from Vitamin D-deficient rachitic chicks show coupled respiration using either glutamate + malate (respiratory control ratios of 4-5) or glutamine (RCR of from 4-6).

Differences in one or more components of the hydroxylase system, namely flavoprotein reductase, non-heme-iron protein (ferredoxin) and cytochrome P_{450} could account for the activation of the 1-hydroxylase in Vitamin D-deficient chicks and for the activation of the 24-hydroxylase in D-repleted chicks. Specificity of the hydroxylase activity is thought to reside in the P_{450} component. However, work from other laboratories has shown that the total cytochrome P_{450} concentration of chick kidney mitochondria remains unchanged regardless of whether 1- or 24-hydroxylation is the predominant activity. Furthermore, enzymatically active renal ferredoxin from mitochondrial extracts of chicks raised on D-deficient and D-repleted diets showed no significant difference in total units recovered and that the renal ferredoxin from D-repleted animals was fully functional in the reconstituted 1-hydroxylase system. These results strongly suggest that the activation of the 1-hydroxylase is not achieved by alteration of existing enzyme molecules but to the *de novo* synthesis of new enzyme or of some other protein whose presence is necessary to enhance the presentation of 25-$(OH)D_3$ to the intramitochondrial 1-hydroxylase. We plan to investigate this point together with the possible direct involvment of PTH on the regulation of the 1-hydroxylase by incubating primary cell cultures of chick kidney with PTH in the presence and absence of inhibitors of protein synthesis, *i.e.* cycloheximide and examine the activation of the enzyme.

While it is widely accepted that elevated calcium levels play a role in blocking the synthesis of the 1,25-$(OH)_2D_3$ and activating the synthesis of 24,25-$(OH)_2D_3$ *in vivo*, it is not clear whether calcium directly inhibits the 1-hydroxylase and/or activates the 24-hydroxylase. We plan to investigate this point by examining the effect of micromolar concentrations of calcium on the two enzyme activities from D-deficient and -repleted chicks using intact and partially disrupted mitochondria, and isolated and partially purified hydroxylases.

Before we can initiate these studies, we first must develop the methodology needed to assay the 1- and 24-hydroxylases in isolated mitochondria, in cultures of kidney cells, and isolated and partially purified enzymes. High performance liquid chromatography (HPLC) will be used to verify the presence of the various metabolites of 25-$(OH)D_3$.

Significance to Biomedical Research and to the Program of the Institute:
These studies define the mechanisms whereby perturbation in physiological control systems lead to the inability to maintain homeostasis. This fundamental information is needed to understand how cardiac function declines with age, and the biology of senile osteoporosis and osteomalacia. Such knowledge may eventually lead to the development of appropriate techniques and procedures that will prevent and/or enable the aged to cope effectively with their debilities.

Proposed Course of the Project: In addition to the transport experiments with heart cells which were outlined above, it is proposed to investigate lipid oxidation and acylcarnitine metabolism in mitochondria from skeletal muscle, and the effect of aging on these processes. This latter study probably depends upon the availability of a Visiting Fellow.

Investigations will continue on the regulation by Ca^{2+} and parathyroid hormone by the 1- and 24-hydroxylation of 25-hydroxyvitamin D_3 in renal mitochondria.

Publications:

Hansford, R. G.: Metabolism and Energy Production. In The Aging Heart: Its Function and Response to Stress. M. L. Weisfeldt, ed., Raven Press, New York, 1980, pp. 25-76.

Hansford, R. G.: Control of Mitochondrial Substrate Oxidation. In Current Topics in Bioenergetics, in press.

Hansford, R. G.: Review on Bioenergetics and Aging. In Handbook on the Biology of Aging, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00048-06 LMA

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

ATP-Linked Ion Transport Mechanisms

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Froehlich	Medical Officer	LMA, NIA
OTHER:	M. Kadoma	Visiting Fellow	LMA, NIA
	C. Filburn	Research Chemist	LMA, NIA
	R. W. Albers	Chief, Neurochemistry Section	LNC, NINDCDS
	A. S. Hobbs	Staff Fellow	LNC, NINDCDS
	E. N. Moudrianakis	Professor of Biology	Johns Hopkins U.
	R. Horner	Post-doctoral Fellow	Johns Hopkins U.
	P. Bungay	Chemical Engineer	R, BEI
	P. Krindle	Post-doctoral Fellow	R, BEI

COOPERATING UNITS (if any) Laboratory of Neurochemistry, National Institute of Neurological and Communicative Disorders and Stroke, NIH; Dept. of Biology, Johns Hopkins University; Biomedical Engineering and Instrumentation Branch, Division of Research Services, NIH.

LAB/BRANCH
Gerontology Research Center, Laboratory of Molecular Aging

SI
Biophysics and Intermediary Metabolism Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.1	2.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) The effects of the inhibitors vanadate and oligomycin on the (Na,K)ATPase partial reactions were examined using the rapid mixing acid-quench technique. Vanadate produces complete inhibition of the enzyme by binding to the K^+-stabilized (E_2) conformation and by activating the reversal of dephosphorylation. The latter effect may involve the interaction of vanadate with a neighboring subunit in an oligomeric enzyme complex. Investigations of the effects of monovalent cations on oligomycin inhibition showed that oligomycin interacts with (Na,K)ATPase in the absence of Na^+, but preincubation with Na^+ potentiates its effect, suggesting that Na^+ stabilizes the formation of an oligomycin binding conformation. 2) Studies of the interaction of ADP and Mg^{2+} with the sarcoplasmic reticulum ATPase phosphoenzyme reveals the order of release of products from the enzyme during ATP hydrolysis. 3) Rapid ultrafiltration is being evaluated to achieve rapid separation of bound and free ligands in enzyme-metal ion complexation reactions.

PHS-6040
(Rev. 10-76)

Project Description:

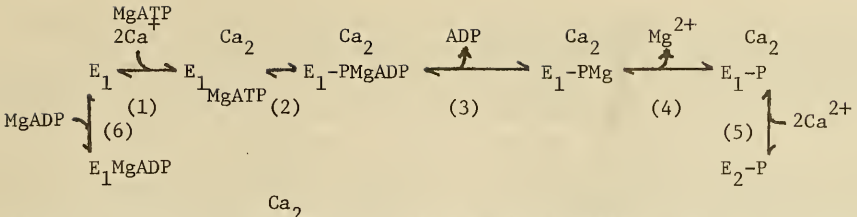
Objectives: 1) To identify the intermediate reactions of the enzymatic pathways involved in the active transport of metal ions. 2) To understand the mechanism of conversion of chemical to osmotic energy that occurs during active transport. 3) To elucidate the mechanism of the diminished transport rates in cardiac membranes prepared from old animals.

Methods Employed: The formation and decomposition of phosphoenzyme intermediates present during ATP hydrolysis were measured using the rapid-mixing acid-quench technique. A time accumulation of ^{45}Ca by sarcoplasmic reticulum vesicles was measured by Millipore filtration. Computer simulation of kinetic data was used to evaluate rate constants for the formation and breakdown of the various intermediates and products of the metal-ion dependent ATPase reactions.

Major Findings:

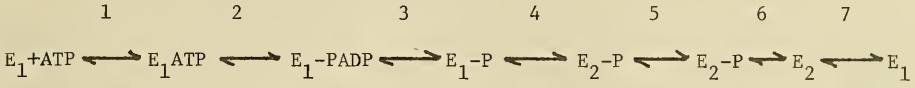
Interaction of the Ca^{2+}ATPase (SR) Phosphoenzyme with Mg^{2+} and ADP. The time course of phosphoenzyme decomposition following the addition of ADP was resolved into rapid ($>200s^{-1}$) and slow ($35s^{-1}$) phases. No Pi release accompanied the rapid phase (45% of the total E-P) while the slow phase (50% of total E-P) was accompanied by a less-than-stoichiometric amount of Pi release. The level of E-P remaining after dephosphorylation showed a two-step dependence on ADP concentration with the initial reduction in E-P level (10% of total E-P) occurring at concentrations below 4 μM. The second step of the curve which began at concentrations above 20 μM had a Hill coefficient of 1.4 indicating the involvement of more than one ADP binding site in dephosphorylation of the enzyme. Raising the Mg^{2+} ATP concentration shifted the curve to the right while lowering the Mg^{2+} concentration had the opposite effect. Decreasing the time of incubation of the enzyme with ATP prior to adding ADP reduced the amount of rapidly decaying E-P compared to that obtained at longer incubation times. If the enzyme was preincubated with EGTA to remove bound Ca^{2+}, the fraction of rapidly decaying E-P was reduced compared to that of enzyme initially exposed to Ca^{2+}. No rapid component was observed upon addition of ADP to enzyme that had been preincubated with EGTA and high Mg^{2+} (20 mM) and phosphorylated for a very short period of time. However, extending the time of incubation with ATP to much longer time intervals produced a rapid component in response to the addition of ADP. The effect of high Mg^{2+} in delaying the appearance of the rapid component was partially reversed either by raising the ADP concentration so there was an excess of ADP over Mg^{2+}, or by exposing the enzyme to Ca^{2+} prior to initiating phosphorylation. Determination of the fraction of rapidly and slowing decaying E-P formed after brief exposure to ATP in the presence of different Mg^{2+} concentrations showed that inhibition of the rapid component coincided with the formation of the MgADP complex.

The reactions of the CaATPase with Mg^{2+} and ADP are summarized in the following scheme which depicts a partial cycle of the pump:



Reaction of ADP with E_1-PMg produces the rapid phase of dephosphorylation while the slow phase represents the sum of two reactions, *i.e.* hydrolysis of E_2-P to E_2+Pi and binding of ADP to E_1 which reduces the level of E_1-PMgADP by shifting the equilibria in steps 1 and 2 to the left. Binding of ADP to E_1 is antagonized by high ATP concentrations and is potentiated by raising the Mg^{2+} concentration which increases the concentration of the metal-nucleotide complex. Mg^{2+} that is bound to the enzyme with ATP is released after the dissociation of ADP and before the conversion of E_1-P to E_2-P. Under conditions where essentially all of the nucleotide exists as the Mg^{2+} complex, the rate of release of Mg^{2+} follows the rate of appearances of the rapid decay phase which results from the combination of MgADP with E_1-P. In the case where there is free nucleotide present, the rapid decay phase is due to the combination of ADP with E_1-PMg.

Effects of Inhibitors on the (Na,K)ATPase Intermediate Reactions. Rapid mixing studies of the interaction of (Na,K)ATPase with inhibitors has enabled certain features of the enzymatic reaction mechanism to be elucidated. The mechanism of ATP hydrolysis catalyzed by (Na,K)ATPase consists of at least the following steps:



where E_1-P is the acid-stable phosphoenzyme that reacts with ADP to form E_1ATP, but is insensitive to K^+, and where E_2-P is insensitive to ADP but is hydrolyzed in the presence of K^+ to E_2+Pi. The conformation change in the final step is accelerated by the interaction of ATP with a low affinity binding site.

Vanadate, a potent-inhibitor of (Na,K)ATPase present in mammalian muscle, has been shown to prevent ATP hydrolysis at low concentrations by interacting with the low affinity ATP binding site (E_2 conformation) and at much higher concentrations by complexing with the high affinity ATP binding site or catalytic site (E_1 conformation). K^+ potentiates binding of vanadate to E_2 and dissociation of vanadate from this complex is slow in comparison to the reactions normally catalyzed by the Na^+ pump. The rapid acid quench technique was used to examine the effects of vanadate on the (Na,K)ATPase partial reactions under conditions which produced incomplete inhibition of the overall reaction. Results showed that incubation with vanadate in the presence of Na^+ and K^+ strongly inhibits phosphorylation. Enzyme remaining

under those conditions showed no change in the rate constants corresponding to phosphorylation and dephosphorylation although the height of the E-P overshoot was diminished suggesting that vanadate increases the reverse rate of dephosphorylation (k_{-5}). Because conditions of the experiment favor binding of vanadate to E_2 preventing its conversion to E_1 and all subsequent reactions, the effect of vanadate on the overshoot implies that either 1) there is an additional vanadate binding site on the enzyme that alters the kinetics of the reversal of dephosphorylation, or that 2) vanadate bound to one enzyme subunit alters the dephosphorylation kinetics of a neighboring uninhibited subunit. The latter explanation invokes the presence of an interaction between enzyme subunits as might normally occur in an enzyme consisting of multiple functionally-linked protein subunits.

In dephosphorylation experiments, phosphoenzyme formed in the presence of Na^+ and K^+ was found to consist of rapidly and slowly turning over components which differed in sensitivity to vanadate. At low concentrations (2 μM) vanadate inhibited formation of the rapidly decaying E-P while at higher concentrations it inhibited both the rate and level of formation of slowly decaying E-P. The latter was dependent on the presence of Na^+ suggesting that it might represent an isoenzyme or altered conformational state of the (Na,K)ATPase.

Oligomycin, a potent inhibitor of the mitochondrial ATPase, has been shown to produce inhibition of the (Na,K)ATPase. Inhibition of (Na,K)ATPase activity by oligomycin is accompanied by an increase in the steady state level of E-P and an increased rate of ADP-ATP exchange, suggesting that the inhibitor blocks the conversion of E_1-P to E_2-P.

The effect of varying the preincubation conditions on the degree of inhibition produced by oligomycin was studied. In the absence of preincubation with Na^+, oligomycin (100 μg/ml) increased the steady state level of E-P from 0.15 to 0.3 μmole/mg. If Na^+ (20 mM) was present during the preincubation interval, E-P rose to a maximum level of 0.6 nmole/mg. The Na^+ concentration dependence of this additional increase in E-P produced by oligomycin was sigmoidal with a $K_{0.5}$ of 3.5 mM. These results indicate there are two classes of oligomycin binding sites, one that requires Na^+ and the other not requiring Na^+. Alternatively, the enzyme may exist in conformational states that interact preferentially with oligomycin and which are stabilized by the presence of Na^+ (E_1 state). In the presence of Na^+ the dependence of the E-P level on oligomycin concentration had a Hill coefficient of 0.92.

Compared to results obtained in the absence of oligomycin, Pi release in the presence of oligomycin and following preincubation with Na^+ showed no early burst. A small burst was obtained if Na^+ was not present during the preincubation. The K^+-dependent conversion of E_1 to E_2 was slowed by oligomycin ($4s^{-1}$ as compared to $55s^{-1}$ in a control experiment), whereas oligomycin had no effect on the Na^+-dependent rate of conversion of E_2 to E_1. ATP-dependent acceleration of the rate of conversion of E_2 to E_1 was, however, completely prevented by oligomycin. Partial inhibition of these reactions was observed if Na^+ was absent during preincubation with the inhibitor. The reaction of ADP

with the phosphoenzyme formed in the presence of oligomycin consisted of rapid (kapp > $200s^{-1}$) and slow (kapp = $6s^{-1}$) phases accounting for 70% and 30% of the total E-P, respectively. No difference in the proportion of rapid and slow components was detected when the time of addition of ADP from the start of phosphorylation was decreased from 116 to 6 msec, indicating that ADP is rapidly dissociated from the enzyme.

The initial disappearance of E-P, resulting from the combination of ADP with E-P to form EATP, had a rate similar to the forward rate of phosphorylation indicating that the equilibrium for step 2 in the above reaction mechanism must be close to 1. In the absence of further effect of ADP this would prevent the decay of E-P to the low levels observed experimentally. The fact that E-P decays to zero can be explained by assuming that ADP binds to E_1 shifting the equilibria in steps 1 and 2 to the left. Consistent with this explanation, raising the concentration of ATP, which competes with ADP for the binding site on E_1, shifted the ADP concentration dependence of the E-P level following dephosphorylation to the right ($K_{I_{0.5}}$ = 110 µM at 10 µM ATP *vs.* $K_{I_{0.5}}$ = 195 µM at 100 µM ATP).

In the absence of oligomycin about 50% of the rapidly turning over phosphoenzyme was ADP-sensitive while virtually 100% of the stable E-P was ADP-sensitive.

Rapid Separation of Free and Bound Ligands by Ultrafiltration. The reactions of the Ca^{2+} and Na^{+} pumps involve steps in which the substrate and metal ions bind to and dissociate rapidly from the carrier protein. In the case where no suitable spectroscopic probe of the binding reaction exists, the isotope distribution method employing Millipore filtration can be used to determine the amounts of free and bound ligand. Time resolution of this method, however, is about 5 seconds limiting its application to very slow binding (conformationally-linked) reactions. In order to have a means of rapidly separating filterable (free) from non-filterable (bound) ligands two filtration chambers were constructed which could be used in combination with the quench-flow apparatus. In the hollow fiber unit, material flowing into the filtration chamber is divided into 15 separate flow paths constructed of tubular ultrafiltration membrane with an exclusion limit of 10,000 daltons. In the radial or sheet membrane design, material is spread out over the surface of an ultrafiltration membrane held in a cylindrical filtration chamber. In both units filtration is assisted by a positive back pressure of 40 psi. Experiments designed to test the effect of turbulent flow on vesicle integrity showed that damage due to rupture at flow rates well above the turbulence limit was only 5% greater than a hand mix control. The amount of protein present in the filtrate compared to protein in the retentate was significantly reduced by doubling the number of high salt extractions during purification of the enzyme and by reducing the pore exclusion limit from 30,000 to 10,000 daltons.

ATP Synthesis by Subchloroplast (CF_1) Particles. Rapid mixing acid-quench studies of subchloroplast particles prepared from spinach leaves were carried out in order to determine whether ADP formation is an obligatory step in the

reactions leading to ATP synthesis. Results of these studies showed that ADP formation following the addition of AMP and Pi to subchloroplast particles was too slow to account for the rate of appearance of ATP measured under turnover conditions. These results together with the absence of detectable ADP formation during steady state ATP synthesis strongly suggest that the mechanism for ATP synthesis does not include ADP formation as an intermediate step.

Pre-Steady State Ca^{2+} Accumulation by Young and Old Sarcoplasmic Reticulum. Studies of the effect of aging on active Ca^{2+} transport in SR vesicles isolated from rat myocardium are being continued using stopped-flow mixing and dual wavelength spectrophotometry to monitor the early time course of Ca^{2+} accumulation. Results of studies indicate the presence of differences in the initial rates of Ca^{2+} accumulation in young and old SR that are consistent with previous observations in the steady state.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the enzymatic pathways coupled to active ion transport will provide additional insight into the transport mechanism and enable comparison of the normal pattern of enzyme behavior derived from these with the altered biochemical pathways associated with aging and disease.

Proposed Course of the Project: Rapid kinetic methods will be used to monitor the progress of enzyme and metal ion transport reactions in isolated preparations of membranes prepared from muscle and nervous tissue. In order to examine further the effects of age on hormonally mediated Ca^{2+} transport in cardiac sarcoplasmic reticulum, stopped-flow mixing will be used to measure the initial baseline and cAMP-dependent Ca^{2+} transport rates in preparations isolated from young and old rat myocardium. Properties of the Ca^{2+} ATPase in cardiac SR will be examined in order to obtain further information on the mechanism of the age-dependent decrease in Ca^{2+} transport activity.

Publications:

Froehlich, J. P., Albers, R. W. and Hobbs, A. S.: Kinetics of the K^+-induced transition between the rapidly and slowly reacting conformations of Na,K-ATPase. In Skou, J. C. and Nørby, J. G. (Eds.): Na,K-ATPase: Structure and Kinetics. London, Academic Press, 1979, pp. 129-141.

Guarnieri, T., Spurgeon, H., Froehlich, J. P., Weisfeldt, M. L., and Lakatta, E. G.: Diminished ionotropic response but unaltered toxicity to acetyl-strophanthidin in the senescent beagle. Circulation 60: 1548-1554, 1979.

Sumida, M., Wang, T., Schwartz, A., Younkin, C. and Froehlich, J. P.: The Ca^{2+}-ATPase partial reactions in cardiac and skeletal sarcoplasmic reticulum. J. Biol. Chem. 255: 1497-1503, 1980.

Hobbs, A. S., Albers, R. W. and Froehlich, J. P.: Potassium-induced changes in phosphorylation and dephosphorylation of (Na^+,K^+)ATPase observed in the transient state. J. Biol. Chem. 255: 3395-3402, 1980.

Hobbs, A. S., Froehlich, J. P. and Albers, R. W.: Inhibition by vanadate of the reactions catalyzed by the (Na^+, K^+)-stimulated ATPase. J. Biol. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00049-02 LMA

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Studies on the Oral Physiological Status of Man During Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. J. Baum	Dental Officer	LMA, NIA
OTHER:	J. A. Bosma	Medical Officer	CID, NIDR
	R. O. Wolf	Dental Officer	CID, NIDR
	J. M. Weiffenbach	Research Psychologist	CID, NIDR
	W. H. Bowen	Dentist	NCP, NIDR
	M. F. Cole	Visiting Scientist	NCP, NIDR
	B. C. Sonies	Speech Pathologist	REHAB, CC
	T. H. Shawker	Medical Officer	DR, CC

COOPERATING UNITS (if any) Oral and Pharyngeal Development, and National Caries Program, NIDR; Rehabilitation Medicine and Diagnostic Radiology, Clinical Center; M. J. Levine, Dept. Oral Biology, SUNY, Buffalo; G. Bowden, Dept. Oral Biology, Univ. Manitoba, Canada; F. G. Oppenheim, Dept. Oral Biology, Boston University.

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:
1.1	0.5	0.6

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There has been little systematic investigation of tissues within the aging oral cavity. The purpose of this project is to assess the oral physiological status of participants in the Baltimore Longitudinal Study and thus provide baseline information which is lacking. The objectives of the study remain the same: (1) define subjects' dental and oral health; (2) examine biological factors likely to influence oral health. After 2 years, 220 subjects have been seen and approximately 150 pieces of information accumulated/subject. Major effort has been directed at methods of data handling and analysis. Data has been stored on computer discs and organized such that analysis of variables can be routinely performed. Initially comprehensive analyses have focused on stimulated parotid saliva flow rate and measurements of gustatory function.

PHS-6040
(Rev. 10-76)

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 3-4 years in "two alternating protocols." 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: All methods described following the first year report on this project are still utilized. These include the history, clinical oral and dental examinations, radiographic examination and evaluations of whole and parotid saliva and dental plaque. Two new procedures have been added. The first is an evaluation of taste acuity (thresholds) and perception of taste intensity. For both parameters four taste qualities are studied: sour (citric acid), sweet (sucrose), salty (sodium chloride) and bitter (quinine sulfate). Taste thresholds are determined using an up-down or staircase procedure, incorporating distilled water rinses between each tasting event. Perception of intensity is studied using a direct scaling of superthreshold concentrations of tastants with no fixed scale assigned to subjects. The second new procedure is an examination of tongue function during normal oral activites (speech, swallowing) using ultrasound methodology. Ultrasonic images of the tongue will be stored on videotape (using a mechanical seetor scan-β scan) for later analysis. Recordings will be obtained with the tongue at rest, during the act of swallowing and during a speech pathology examination (articulation of single phonemes, syllables and contextual speech).

In addition to these diagnostic methods, all data obtained in the oral physiology study have now been transferred to computer discs for storage and retrieval. Currently, analyses of data is being performed utilizing SPSS-pre-packaged programs. Also, the history and examination forms have been redesigned such that they are in a format suitable to direct placement of data on disc storage files (80 column format). Thus, continual update and analysis are now feasible.

Major Findings: Major efforts directed at problems in data handling and analysis have been successful and routine comprehensive analysis of results is now a practical exercise. Initial efforts in data review have focused in two areas, stimulated parotid salivary flow rate and gustatory function.

It is generally considered that salivary flow decreases in old age, particularly among post-menopausal women. Because of the physiological

roles of saliva, such a change would be important and likely compromise oral health. Cross-sectional analysis of data obtained from 146 healthy subjects (98 male, 48 female; defined as not taking prescription medication or currently under medical care) show unequivocally that production of stimulated parotid saliva does not change with increased age. The efficiency of fluid output (the simplest measure of function by this end-organ) thus remains intact throughout life. When data obtained from individuals of questionable medical status (taking prescription medication, under medical care) were compared to like aged healthy subjects, no differences were seen among males (n = 25). However, among post-menopausal women of questionable medical status (n = 32) a significant decrease in stimulated parotid flow rate (26% ↓, $p<0.025$) was found. This suggests that previously reported changes in salivary flow rates in post-menopausal women may reflect pathology or pharmacologic influences, rather than age-related alterations. In this regard, it is interesting that subjective complaints of xerostomia are relatively frequent in post-menopausal women, regardless of health status (∿25% for health subjects; ∿34% for subjects of questionable medical status). Since there appears to be no consistent relationship, in our population, between dry mouth and parotid saliva flow rate, it is probable that simple diminution of fluid flow does not trigger the sensations of xerostomia. Rather it may be that these symptoms reflect, among other things, changes in the concentration of specific salivary macromolecules, a possibility we are now exploring.

Decremental alterations in gustatory functions are also generally considered a concomitant of old age. Subjective data of gustatory function (self-assessments by participants) and objective measures of taste acuity and perception of intensity have been obtained and results analyzed. Subjective data has been compared in young (20-39 yrs), middle (40-59 yrs) and older (60-89 yrs) participants in both healthy and questionable status groups. Reports of diminished taste acuity increased with age, in both health groups. This response was 5%, 11.6% and 12.5% for healthy young, middle and older individuals. In subjects of questionable status (middle and older), this value approached 30%. Similarly when queried about food enjoyment almost all healthy subjects continued to enjoy food, while approximately 25% of questionable medical status persons noted diminished food satisfaction. These results suggested that self-perceived deficits in gustatory function and food enjoyment are modest during aging but occur more frequently in individuals who are medically compromised. Direct measurement of gustatory function has been performed on about 1/3 of our subjects. Analysis of taste thresholds (the minimal concentration of a tastant required to recognize as being different from water), showed no apparent age-related alterations in the thresholds for sweet (sucrose) and sour (citrate) tastes. However, thresholds for both quinine sulfate (bitter) and sodium chloride (salty) tend to increase with age ($r = 0.272$ and 0.191, respectively). Furthermore, heterogeneity of data for these two modalities are much more apparent in subjects 55 yrs and older. Scaling measurements of perception of intensity have also been performed. Preliminary analyses suggest that, for all four taste qualities, no differences occur in the ability to discriminate different levels of tastant concentration, with increased age.

Significance to Biomedical Research and to the Program of the Institute: This study has begun to define the physiologic status of the oral cavity with increased age. As witnessed by our initial results, our approach apparently allows for the distinction between age-associated oral alterations and those related to disease or therapeutic treatment. Thus, a re-examination of many "traditional" generalizations, associated with old age and oral health, is possible. Additionally, new insights to the understanding of certain oral conditions (*e.g.* complaints by post-menopausal women of xerostomia) may be obtained. Changes in the dental apparatus can have markedly adverse effects on facial appearance, speech and dietary patterns. Changes in oral motor functions can affect the quality of one's life as well as potentially being life threatening. 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.

Proposed Course of the Project: Examinations of oral tissues, as described above, will continue. Now that tools for data analysis are available, considerable effort will be spent on collating already obtained results, analyzing their meaning, and drawing appropriate conclusions.

Publications:

Baum, B. J.: Aging and oral motor function. In Oral Motor Behaviors: Impact on Oral Conditions and Dental Treatment, (NIA) DHEW Publ. No. (NIH) 79-1845, 1979, pp. 244-252.

Baum, B. J.: Current research on aging and oral health. J. Amer. Soc. Geriatric Dentistry, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00050-02 LMA

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Regulation of Salivary Gland Function During Increased Age.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. J. Baum	Dental Officer	LMA, NIA
OTHER:	C. Filburn	Research Chemist	LMA, NIA
	J. M. Freiberg	Medical Officer	LMA, NIA
	H. Ito	Visiting Fellow	CPB, NIA
	G. S. Roth	Research Chemist	CPB, NIA

COOPERATING UNITS (if any)

Endocrinology Section, Clinical Physiology Branch, NIA.

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:
1.6	0.9	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous clinical observations have suggested that with increased age, decremental changes in salivary gland function occur. To study the mechanism of this alteration in animal models, salivary gland functions from young adult and aged male and female rats have been characterized biochemically and regulation by neurotransmitters has been investigated. Included are studies of: (1) protein secretion from the parotid gland, and its control by the β-adrenergic system (β-receptor) characteristics, hormone responsiveness, cAMP-dependent protein kinase, endogenous phosphorylation); and (2) fluid and electrolyte secretion (K^+ efflux, α-adrenergic and cholinergic responsiveness) from the parotid gland. Additionally, examination of intermediary metabolic function (glucose oxidation) of the parotid gland has been initiated (basal; stimulated by adrenergic and cholinergic agonists). In the submandibular gland, basal protein and mucin biosynthesis and release, as well as stimulated exocrine release (β-adrenergic) have been investigated. Biochemical characterization of submandibular gland exocrine products has begun.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: These studies have as their overall objective to understand the biochemical mechanisms of saliva formation (*i.e.* regulation of the synthesis of secretory macromolecules and control of the release of salivary proteins and electrolytes into saliva) and the alterations occurring in these processes during aging. Constituents of saliva (organic, inorganic) have direct maintenance and protective functions in the oral cavity. Age-related perturbations in the formation and secretion of salivary gland products would likely be manifested as compromises to oral health.

Methods Employed: Rat salivary glands are used as model tissues. Descriptive biochemical characterizations of glands include information of DNA, protein, neutral sugar and sialic acid contents. *In vitro* studies are performed with enzymatically dispersed cells from parotid and submandibular glands. β-adrenergic receptors are measured using ^{3}H-dihydroalprenolol. Adrenergic and cholinergic receptor mediated events are studied using appropriate agonists and antagonists. Protein secretion is monitored by release of amylase or previously radiolabelled exocrine proteins. Fluid and electrolyte release is studied as K^{+} efflux. Oxidation of radiolabelled glucose is utilized as an index of intermediary metabolic function. Protein and mucin biosyntheses are studied using appropriate radiolabelled amino acid and carbohydrate precursors. Newly synthesized macromolecules are characterized by electrophoretic and chromatographic procedures. Cyclic AMP stimulated events, subsequent to β-Adrenergic agonist binding, are followed by protein kinase activity ratios and endogenous phosphorylation of salivary gland proteins. Newly phosphorylated proteins are identified by SDS gel electrophoresis and densitometry.

Major Findings: Submandibular glands of young adult (5-8 mo) and aged (23, 24 mo) male, but not female, rats displayed several significant differences in the descriptive biochemical parameters studied. These included protein content (μg/mg wet wt, 103.3 ± 4.6 young vs. 56.0 ± 3.5 aged, $p<0.01$), neutral sugar content (μg/mg wet wt, 2.26 ± 0.12 young vs. 1.4 ± 0.08 aged, $p<0.01$) and sialic acid content (μg/mg protein, 4.19 ± 0.25 young vs. 3.42 ± 0.13 aged, $p<0.05$). No differences in gland DNA content were found. An *in vitro* dispersed cell model for studying submandibular gland function has been established with young adult rat tissue. Cells (>90%) exclude trypan blue, synthesize protein linearly throughout the 2 hr period of experimentation and are hormone responsive (β-adrenergic, dose dependent).

Functions of parotid glands of young (3 mo), young adult, middle-aged (12 mo) and aged male rats have been studied *in vitro* using a similar dispersed cell model. These cells show hormone responsiveness (α- and β-adrenergic, cholinergic; dose dependent). No differences were observed in β-adrenergic receptors (number and ligand affinity), β-adrenergic stimulation of amylase release (time course, extent, inhibition by β-adrenergic antagonists), and dibutyryl cyclic AMP stimulation by amylase release. Young adult and aged rat parotid cells both responded to β-adrenergic stimulation with rapid elevations of protein kinase activity ratios.

Efflux of K^+, stimulated by α-adrenergic agonist was examined. Significant differences have been found between young and aged rats in both the time course (initial rates of release greater in young 25.6 ± 3.9%/min vs. aged 16.4 ± 2.3%/min, $p<0.05$) and dose response curves of K^+ release. Additionally, a deficit in α-adrenergic stimulated glucose oxidation was observed. Basal levels of glucose oxidation were identical in all age groups. Following α-adrenergic stimulation, however, responsiveness of aged rat cells had decreased by more than 50% that of young and middle aged rat parotid cells. No age-related differences in β-adrenergic and cholinergic stimulated glucose oxidation or in cholinergic stimulated amylase release and K^+ efflux were found. Thus, a selective change in neurotransmitter responsiveness occurs in parotid glands during aging.

Cyclic AMP-dependent protein kinase from the rat parotid gland has been characterized. It is a type II enzyme (anion exchange chromatography, photoaffinity (labelling). Activity is ATP (K_m = 32 μM) and substrate (histone VII, K_m = 200 μg/ml) dependent. These criteria, other kinetic parameters (K_a cAMP = 0.5 μM; K_a cGMP = 50 μM) and its subcellular distribution suggest its similarity to the parotid cyclic AMP-dependent protein kinase from other species (mouse, pig, cow) and suggest that our rat model system is a satisfactory and representative choice.

β-Adrenergic agonist activation of protein kinase *in situ* occurs within 30 sec and displays a temporal pattern, with respect to amylase release, consistent with a suggested role for a cyclic AMP-dependent phosphorylation step necessary in exocrine secretion. Examination of dispersed rat parotid cells for such a β-adrenergic agonist induced phosphorylation event has demonstrated three specific changes in cell phosphoproteins; two proteins are phosphorylated and one protein dephosphorylated in response to β-agonist treatment. All three events are time-dependent, dose-dependent (50% effect between 5×10^{-8} to 5×10^{-7} M isoproterenol), completely abolished by a β-adrenergic antagonist and mimicked by dibutyryl cyclic AMP.

Significance to Bio-medical Research and the Program of the Institute: These studies continue the examination of salivary gland function throughout the adult life span of the rat. Based on morphologic and biochemical data, the rat salivary glands are suitable models for the human condition. Many clinical studies have suggested a variety of age-related changes in the human oral cavity likely, at least in part, to be related to decrements in salivary gland function. These include complaints of xerostomia, increased prevalence of cervical caries, alterations in taste acuity and perception, "atrophy" of the oral mucosa. Salivary constituents include a number of factors which function in the maintenance of oral health. By studying the mechanisms which regulate salivary gland function, one can begin to understand physiological malfunctions and thus be better equipped at designing therapeutic regimens.

Proposed Course of the Project: Further studies with dispersed submandibular gland cells will focus on the characterization of newly synthesized exocrine products, proteins and mucins (chromatographic, electrophoretic and structural

parameters). The *in vitro* model will be utilized with aged rat tissue. Hormone responsiveness and secretory product synthesis will be compared to young adult rats. In addition to basal biosynthetic rates, synthesis of exocrine products following prior stimulation (*i.e.* re-synthesis) will be examined.

The observed age-related decrements in α-adrenergic mediated parotid gland functions will be further studied. This includes examination of α-adrenergic receptor characteristics, the role of Ca^{2+} in these α-adrenergic responses, and pharmacologic manipulations of the α-adrenergic response in various aged animals. Production of protein will also be studied in dispersed parotid cells. Preliminary studies suggest that in older animals the protein content of the parotid gland is diminished (similar to the submandibular gland). Methods developed with the submandibular gland will be used to examine rates of synthesis, turnover and macromolecule characteristics, with young adult and aged rat parotid cells.

Endogenous phosphorylation studies will examine the subcellular locales of the three proteins whose phosphate content is specifically modulated in response to β-adrenergic agonists. We will attempt to identify secreted phosphoproteins, which likely are important to Ca^{2+} handling in the oral cavity (remineralization of teeth, dental calculus formation) and study factors important to their phorphorylation. The pattern of phosphoproteins made basally, and in response to β-adrenergic stimulation, will also be examined in aged rats.

Publications:

None

Annual Report of the Laboratory of Neurosciences
National Institute on Aging

The Laboratory of Neurosciences 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, disease and aging. This report summarizes the following basic sciences projects: (A) Blood-brain barrier and central nervous system function, (B) Cerebral metabolism, relation to brain function and aging, (C) Function of peripheral nerve and muscle, (D) Pharmacology of central and peripheral catecholaminergic nervous systems, (E) Synapse development, specificity and mechanism in culture and (F) Central nervous system binding sites for the neurotoxin kainic acid. In addition, the report discusses the following clinical sciences project: (G) Brain function in aging and disease in man. I am primarily responsible for Project A, Drs. E.D. London and N.L. Shinowara for Project B, Drs. H. Levitan and N.L. Shinowara for Project C, Dr. C.C. Chiueh for Project D, Dr. J.M. Thompson for Project E, Dr. E.D. London for Project F and Drs. R. Margolin, E. A. Robertson-Tchabo and myself for Project G.

A. Blood-Brain Barrier and Central Nervous System Function.

Studies of blood-brain interaction in health, disease and aging require quantitative measurements of cerebrovascular permeability and transport, and adequate pharmacokinetic theories to interpret drug distribution within the various brain compartments in relation to plasma concentrations and 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, experimentally and theoretically, a new quantitative procedure that can be used in conscious animals, and that is independent of cerebral blood flow and is at least 1000 times more sensitive than any other currently available technique.

A major problem in psychopharmacology is that a central drug response often cannot be related directly to drug dosage or plasma concentration. In the elderly, furthermore, the high incidence of neurotoxicity may be caused by a high brain concentration of a drug at a dosage that is not toxic in young individuals, or by altered receptor sensitivity to a drug. Using our method as discussed above, we established a general relation between cerebrovascular permeability and the octanol/water partition coefficient of a drug. We also formulated a multi-compartment (brain-plasma) kinetic model that incorporates the observed relation between permeability and partition coefficient, and that can be used to predict brain concentration of a psychoactive drug from the dosage and plasma concentration.

We used our pharmacokinetic procedure and analysis to demonstrate that synthetic opioid peptides are significantly permeant at the blood-brain barrier. Prior to our studies, other methods suggested that they were not. Our findings suggest that feedback may operate between circulating peptides that have potential central nervous system effects and brain sites that regulate their release into the systemic circulation.

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 the last years, we experimentally refined the osmotic method in the rat, and quantified changes of cerebrovascular permeability in relation to infusate concentration and infusion time, as well as in the recovery period. We showed that, when the barrier opens transiently, brain metabolism is stimulated, there is uncoupling of metabolism from cerebral blood flow, and a temporary brain edema is produced. 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. We do not know, however why metabolism is stimulated by barrier disruption. We have demonstrated that the effect is not mediated by central α- or β-adrenergic receptors, nor due to entry of potassium (which can depolarize and stimulate neurons) from plasma into brain.

We also employed the osmotic method as a pharmacological tool. We demonstrated in rats and dogs that osmotic barrier opening can be used to increase brain uptake of methotrexate, an anticancer agent, to a sufficient extent so as to treat possibly certain sensitive brain tumors. We used the osmotic method to allow exogenous enzymes (e.g., α-mannosidase) into the rat brain, and showed that they are taken up within neuronal lysosomes where they retain their activities for days. Finally, in collaboration with Dr. E.A. Neuwelt, we successfully applied the osmotic procedure to man, and showed with computerized assisted tomography that the blood-brain barrier can be opened up in man without producing apparent neurological changes. A clinical Phase I study has been initiated to evaluate the osmotic method for the treatment of unilateral brain tumor in man, with methotrexate and other appropriate anti-tumor agents.

Proteins that enter the central nervous system may be viruses, or antibodies to brain tissue. One hypothesis of aging is that brain senescence is mediated by anti-brain antibodies. However, the factors that govern protein entry into cerebrospinal fluid are not understood. We derived a model to analyze experimental spinal fluid/serum protein concentration ratios in normal man. With it we concluded that large proteins enter spinal fluid at the choroid plexus via vesicles, and that small proteins enter by vesicles as well as by intercellular pores. Diseases which modify cerebrovascular integrity alter the normal relation between protein concentration in spinal fluid and protein size.

The blood-ocular barrier regulates the environment and function of the eye. We demonstrated that retinal blood vessels are much less vulnerable to acute hypertension than are cerebral blood vessels in the rhesus monkey. Absence of vascular disease in the retina in hypertensive man may, therefore, not reflect absence of cerebrovascular disease. We also showed that

hypercapnia (increased partial pressure of carbon dioxide) in rats increases permeability of the retinal pigment epithelium to intravascular sodium fluorescein. The findings suggest that hypercapnia may be of use to allow organic acid drugs into the retina for chemotherapeutic purposes.

We showed, in collaboration with Dr. S. Gruneau that decompression of animals in a hyperbaric N_2-O_2 environment results in blood-brain barrier damage only if the partial pressure of nitrogen is reduced by at least two-fold. No damage is produced for an equivalent reduction in the partial pressure of oxygen. These findings may help to design diving schedules in man that will reduce the incidence of decompression sickness (the "bends") with accelerated decompression from hyperbaric conditions.

B. Cerebral Metabolism, Relation to Brain Function and Aging.

Regional cerebral glucose utilization and regional blood flow reflect the functional state of the brain. With aging in man, blood flow appears to decrease in the frontal lobe and increase in the parietal lobe in the absence of evidence of cerebrovascular disease, and mean brain metabolic rate may be unchanged. Dr. London employed the ^{14}C-2-deoxy-D-glucose method of Sokoloff et al. (1977) to examine regional rather than overall rates of cerebral glucose utilization in Fischer-344 rats, at 1,3,12,24 and 34 months of age. She wanted to evaluate age-related alterations in functional states in specific brain regions, because of published studies on age-related changes in neurotransmitter systems. Regional glucose utilization was found to increase between 1 and 3 months of age, when the rat brain continues to grow, but then, remarkably, to fall between 3 and 12 months. The cause of this fall is not known, but may be related to neuroendocrine changes. In addition, glucose utilization did not change further after 12 months of age in the resting rat. This indicates that resting glucose utilization is not a measure of the many functional changes which occur during senescence, and is of relevance to current studies on regional cerebral metabolism in man in relation to aging. Pharmacological or physiological stress may be necessary to demonstrate defective cerebral function with the 2-deoxy-D-glucose technique.

We also reported, in abstract form, changes in local cerebral blood flow in the brain of the Fischer-344 rat, at ages of 1,3,12,24 and 34 months. Blood flow, as proposed by Roy and Sherrington (1890), responds to functional demands and under normal conditions is a dynamic measure of cerebral activity. We used ^{14}C-iodoantipyrine to measure flow because we recently demonstrated that this radiotracer provides accurate values for blood flow. Blood flow, like glucose utilization (see above), increased in most brain regions between 1 and 3 months of age, but continued to increase from 3 to 12 months when glucose utilization simultaneously fell. Thus, there was a dissociation in the time courses of cerebral flow and metabolism in relation to aging. Changes in blood flow after 12 months of age probably reflect functional deafferentation of specific brain regions.

We also employed the ^{14}C-2-deoxy-D-glucose method to examine the regional metabolism of the retina in the Fischer-344 rat. Dr. N. Shinowara showed that glucose utilization increases between 1 and 3 months in the rat retina, but then declines in relation to the gradual loss of photoreceptors due to both aging and light damage. Glucose metabolism decreases only slightly, however, at the superior colliculus (part of the central visual pathway) despite retinal degeneration with age.

The 2-deoxy-D-glucose method has been used to map central pathways of altered functional activity, but has rarely been used as a pharmacological tool to examine functional responses of the brain to specific drugs, in relation to receptor density for those drugs. Dr. E. London has initiated two projects, one with a muscarinic cholinergic agonist (oxotremorine), the other with a series of GABAergic drugs, to examine their specific dose-related effects on the central nervous system of Fischer-344 rats. Drs. London and Dow-Edwards showed in the conscious rat that oxotremorine stimulated glucose utilization in the sensory-motor and parietal cortices, the hippocampus, globus pallidus, caudate nucleus, several diencephalic nuclei and the red nucleus and superior colliculus of the mesencephalon. Some but not all of these regions (e.g., the red nucleus) have high levels of receptors for acetylcholine. The effect of the drug was specific and could be blocked by prior administration of scopolamine, which crosses the blood-brain barrier, but not of atropine methyl bromide, which does not. Dr. London and colleagues also mapped out specific brain regions that have a markedly increased metabolic rate following administration of the GABAergic drugs, muscimol and bicuculline. Because the increase was not clearly related to receptor density, it now is established that specific pharmacological activation of the central nervous system depends on neuronal input as well as receptor density.

C. Function of Peripheral Nerve and Muscle.

In order to understand regulation of peripheral nerve function by the blood-nerve barrier, we perfused an excised cylindrical portion of the frog sciatic nerve perineurium (a sheath that surrounds nerve axons), and measured permeability and electrical properties of the perineurial wall. Low permeabilities to ^{14}C-sucrose and to Na^+, Cl^- and K^+ demonstrate that perineurium represents a major restriction to blood-nerve exchange. There is a selectivity to K^+ as compared to Na^+. Furthermore, the frog perineurium does not appear to actively transport either Na^+ or K^+. Perineurial permeability can be increased reversibly by 10% stretch or irreversibly by further stretch or hypertonicity.

We also examined the electrical properties of the isolated frog perineurium. An AC current was passed across the membrane and the voltage across the wall was measured at frequencies from 1 Hz to MHz. The DC resistance of the perineurium was high, 430 ohm cm^2, further indicating that it is a diffusion barrier to ions. Analysis of the impedance data in terms of a four-element model suggested that there are two capacitances at the perineurium, a small one whose value equals 0.1 $\mu F/cm^2$ and which represents the multiple cell layers of the perineurium, and a large one with a value of 20 $\mu F/cm^2$ and

which probably is due to polarization of the matrix of intercellular tight junctions that connect some perineurial cells. Experimental manipulation of this large capacitance may help us to understand the chemical nature of the junctions.

We also demonstrated that sectioning the sciatic nerve of the frog, so as to produce retrograde (Wallerian) degeneration in the distal section, increased the permeability of the perineurium as the compound action potential declined over the following 14 days. The results show that the peri-perineurial integrity depends on the integrity of nerve function, and establishes a feedback relation between adequacy of function of nerve and the blood-nerve barrier system.

We analyzed data and prepared them for publication on muscle metabolism in relation to muscle fatigue. Fatigue of single muscle fibers from the frog appeared as a decline in tension output during prolonged tetanic stimulation, and semitendinosus occurred even though significant quantities of ATP, the energy source for muscle contraction, remained in the fiber. By applying caffeine to fatigued fibers, we showed that they could produce contractures and at the same time deplete residual ATP concentrations. Thus, the ATP in fatigued fibers was not sequestered and was available for contraction. From our measurements of fiber lactate, we suggested that H^+ accumulates in fatigued fibers so as to uncouple excitation from contraction. Uncoupling may be a protective mechanism to prevent total depletion of energy stores, which would result in irreversible muscle contracture and cell death.

D. Pharmacology of Central and Peripheral Catecholaminergic Nervous Systems.

Dr. C.C. Chiueh's work is concerned with the effect of aging, stress, and antihypertensive drugs on the neuronal activities of both the central and peripheral catecholaminergic nervous systems, and on the function of the cardiovascular system in the rat. An age-dependent increase in blood pressure was found in Fisher-344 rats, Wistar rats and spontaneously hypertensive rats. Immobilization stress induced increases in plasma catecholamines, corticosteroid and blood glucose, but the increases were not related to age. However, the aged rats appeared to release these substances into the circulation more slowly than did the younger groups. In physiological measurements, immobilization stress produced an α-adrenergic vasopressor response in young rats but a β-adrenergic vasodepressor response in old rats, which suggests that there is an age-related change in α-adrenergic receptors in rats. Furthermore, older rats usually died following stress. Dr. Chiueh also found that the age-dependent increase in blood pressure could be blocked by administration of Uncarine A, the active ingredient of a Chinese herbal medicine. Uncarine A also reduced the blood pressure of spontaneously hypertensive but not of normotensive animals, for up to 48 hr. His findings suggest that it may eventually be of use in treating hypertension in man.

E. Synapse Development, Specificity and Mechanism in Culture.

Dr. 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 retina or chick spinal cord form long-lived synapses with muscle cells in culture. Each type of neuron, therefore, possesses a synapse-competent state during its development, when it can form abundant synapses with muscle cells in culture. Dr. Thompson's 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. Dr. Thompson's studies also demonstrate that synapse formation and retention can be studied in a controlled fashion in tissue culture. His findings may be critical for our interpretation of neuronal cell death in the aging nervous system.

Dr. Thompson also examined the role of methylation reactions in synapse formation and neurotransmission. Neurons from 8-day chick embryo retina, that were grown in culture with rat striated muscle cells, formed synapses within 24 hr. Synapses were detected by recording spontaneous depolarizing synaptic muscle responses with intracellular microelectrodes. The cultures were treated with inhibitors of methyltransferases, enzymes which catalyze methylation reactions in cell membranes. Dr. Thompson demonstrated that methyltransferase inhibition was associated with inhibition of synapse formation, and that the effect was reversible. His findings strongly suggest that there is a methyltransferase-mediated reaction during neurotransmission, and open the possibility of a new class of pharmacological agents which interfere with neurotransmission by acting at biochemical reactions rather than at receptors.

F. Central Nervous System Binding Sites for the Neurotoxin Kainic Acid.

In addition to examining regional cerebral metabolism in relation to aging (see above), Dr. E. London examined central nervous sytem binding sites for the neurotoxin, kainic acid, which appears to be specific for neurons that contain glutamic acid receptors. She found maximal binding in brains of the frog, dogfish, goldfish and chick, to both high and low-affinity receptors. In mammals, maximal binding occurred in the forebrain, moderate binding in the cerebellum, and little binding in the medulla. The widespread distribution of highly specific binding sites in neural tissue of different species, together with the observation that binding characteristics are similar, suggest that there is an endogenous neuronal system that has not changed appreciably through evolution.

Dr. London also noted cooperative effects between L-glutamate and kainate at the receptor level. When taken with the observation that kainate is not toxic when glutamatergic innervation is removed, the results suggest that, at the receptor level, kainate sensitizes neurons to the neurotoxicity potential of glutamate-induced depolarization. The demonstration of binding sites

in human brain, with kinetic characteristics similar to those in the rat model of Huntington's disease, indicates that kainate's neurotoxicity may be relevant to human disease. She showed that high affinity binding sites were severely reduced in the caudate nucleus and putamen of the human brain in Huntington's disease. The profound loss of these sites in these specific brain regions supports the concept that the pathogenesis of Huntington's disease involves an imbalance in neurotransmitter systems which interact with afferent nerves that release glutamate, a central neurotransmitter, within the basal ganglia.

G. Brain Function in Aging and Disease in Man.

A clinical protocol was written to study cerebral glucose utilization in man in relation to aging, when employing positron emission tomography and ^{18}F-2-deoxy-D-glucose as a tracer of cerebral glucose utilization. The protocol was approved. Subjects were screened for the absence of neurological disease and for the study, which will commence in the forthcoming year.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00120-03 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Blood-Brain Barrier and Central Nervous System Function

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.I. Rapoport	Chief	LNS	NIA
	N.L. Shinowara	Staff Fellow	LNS	NIA
	M. Ohata	Visiting Fellow	LNS	NIA
	E.D. London	Sr Staff Fellow	LNS	NIA
Others:	K. Ohno	Neurosurgeon	LNP	NIMH*
	W.R. Fredericks	Biologist	LNS	NIA
	U. Sundaram	Bio Lab Tech	LNS	NIA
	J.A. Barranger	Neurologist	DMN	NINCDS
	K.D. Pettigrew	Statistician	B	NIMH
	W. Klee	Neurochemist	LGCB	NIMH
	S. Gruneau	Psychologist	NAMRI	

COOPERATING UNITS (if any) *Tokyo Medical and Dental University
E. Neuwelt, Neurosurgeon, Southwestern Med Sch, Dallas, Texas
Lab of Cerebral Metabolism, NIMH; Developmental and Metabolic Neurol. Br, NINCDS, Div of Biometry and Epidem, NIMH

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.9	.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A new method was developed to measure cerebrovascular permeability to poorly permeable as well as rapidly permeable substances. Pharmacokinetic principles for the central nervous system were established that demonstrate in rats that cerebrovascular permeability of nonelectrolytes and organic electrolytes is related linearly to the octanol/water partition coefficient. Synthetic opioid peptides are significantly permeable at the blood-brain barrier. Entry of blood-proteins into spinal fluid is governed by pores and vesicles at the choroid plexus. Barrier permeability can be increased by 10 to 20 fold, by infusing a hypertonic solution of arabinose or mannitol into the carotid artery of animals or man, and has been used to increase brain uptake of methotrexate (an anti-tumor agent) in humans with brain tumors, and of exogenous enzymes (for use in enzyme replacement therapy). Barrier opening is accompanied by a transient elevation of brain metabolism and uncoupling of flow from metabolism. Hypertension increases the permeability of cerebral but not retinal blood vessels, whereas hypercapnia increases permeability of retinal pigmented epithelium to fluorescein.

PHS-6040
(Rev. 10-76)

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 prevent 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 normal and aging brain, (2) to determine the quantitative rules that govern drug entry into the brain from plasma, (3) to develop a method to reversibly modify barrier permeability, and to apply it to understanding the central actions of systemically-administered drugs and other agents, (4) to determine how the barrier regulates behavior and brain metabolism, and (5) to examine the function of the blood-ocular 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 pathology, and electronmicroscopy was employed to evaluate transfer mechanisms at the barrier and barrier ultrastructure. Radioisotope techniques including autoradiography were used to quantitate drug and metabolite transfer from blood to brain. Computers were used to analyze data and to generate relevant models.

Major Findings: 1. Blood-Brain Barrier.

a. Quantitative Aspects of Drug Entry into the Central Nervous System.

(1) Central nervous system pharmacokinetics. The blood-brain barrier limits exchange of drugs between plasma and brain, and makes it difficult to interpret dose-response relations of centrally-acting drugs. We therefore decided to establish empirical rules to quantitatively predict drug entry into the brain from the plasma concentration and the physical propeties of the drug (e.g., lipid solubility). Radiotracers of organic electrolytes and of nonelectrolytes, with differing octanol/water partition coefficients, were injected intravenously in conscious rats. Arterial plasma concentrations were followed, and regional brain concentrations of tracer were determined at various times after injection. A model for blood-brain exchange was formulated and applied to the data. Calculated cerebrovascular permeability was found to be related linearly to the octanol/water partition coefficient of a solute. For a drug, whose partition coefficient is known, the rate of brain accumulation can be predicted now from the linear relation and the history of the plasma concentration. Cerebral blood flow, which was measured in a separate series of experiments with ^{14}C-iodoantipyrine, must also be known for predicting brain accumulation of very permeable drugs (Ref. 1).

(2) Entry of opioid peptides into central nervous system. Our new method for measuring permeability and predicting drug entry into the brain is at least 1000 times more sensitive than any currently available (Indicator Dilution Technique or Brain Uptake Index technique) and should be applicable to pharmacokinetic studies in man. In conscious rats, it was used to show that synthetic opioid peptides -- [D-Ala^2]-methionine enkephalin amide, [D-Ala^2, ^{14}C-Homoarg]-β-endorphin, [D-Ala^{62}, ^{14}C-Homoarg^{69}]-β-lipotropin 61-69 and [D-Ala^2, ^{14}C-Homoarg^9]-α-endorphin - which have been reported by other less sensitive methods not to be permeable at the blood-brain barrier, have significant cerebrovascular permeability coefficients which should allow these peptides to fill the brain extracellular space with half-times of 4-12 min, following a step increase in plasma concentration. Our findings suggest that central effects to systemically administered peptides can be explained on the basis of their entry into the brain at the blood-brain barrier, and suggest, furthermore, that feedback may operate between circulating peptides that have potential central effects and brain sites that regulate their release into the systemic circulation (Ref. 2).

(3) Protein entry into the central nervous system. It is critical to understand the processes that govern entry of proteins into the central nervous system. Proteins that enter may be virus particles or antibodies to brain tissue. Alteration of the normal pattern of spinal fluid protein can reflect specific mechanisms of blood-brain barrier disruption by diseases of the central nervous system. A negative relation exists between the steady-state, cerebrospinal fluid/blood concentration ratio and the hydrodynamic radius of blood-derived proteins. A model for protein transfer at the choroid plexus from blood to brain was proposed and applied to this negative relation. The most likely interpretation of the data is that there are two pathways for protein transfer at the plexus epithelium, a set of 117-A radius pores which allow transfer of smaller proteins by diffusion or ultrafiltration, and a set of 250-A radius pinocytotic vesicles which account for exchange of larger proteins as well. Pores with a 117-A radius have not been described at the choroidal epithelium, but may represent a 0.08% defect in the normally continuous tight junctions that surround and closely connect choroidal epithelial cells (Ref. 3).

b. Reversible Modification of Blood-Brain Barrier Permeability.

(1) 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-solute (e.g. arabinose or mannitol) into the carotid artery of rats, rabbits or monkeys, dogs and 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.

(2) Transient functional and metabolic consequences.

Osmotic barrier opening can be accomplished in animals without producing long-term changes in behavior, brain water or electrolyte content or brain histology (Abstract: Smith, M., Girton, M., Rapoport, S.I., Brady, R.O., Barranger, J.A. Pathology of reversible blood-brain barrier opening. Abstr. J. Neuropathol Exper. Neurology, 1980). However, if the procedure is employed in conscious rats, then transient changes occur that are reversed within 2 to 24 hr. Brain water is elevated by about 1%, due to barrier opening to salts and loss of the protective barrier action against brain edema, and cerebral blood flow no longer is tightly coupled to cerebral metabolism. Finally, behavioral and cerebral metabolic changes reminiscent of convulsions (some changes are prevented by Valium) are produced by barrier opening. Regional uptake of ^{14}C-2-deoxy-D-glucose is increased. This tracer is a glucose analogue that is transported and phosphorylated like glucose within the brain but not metabolized further (Ref. 4,5).

The metabolic stimulation that is evidenced by increased brain uptake of ^{14}C-2-deoxy-D-glucose, following osmotic barrier opening in the rat, is not caused by stimulation of central α- or β-adrenergic receptors, as it is not prevented by prior administration of either phenoxybenzamine (an α-adrenergic blocker) or propranolol (a β-adrenergic blocker). Furthermore, it is not due to entry of potassium from plasma to brain, with consequent neuronal activation, because it occurs in potassium-deprived animals in which plasma potassium is reduced below potassium in the brain extracellular space. Metabolic stimulation does occur after hypertensive as well as osmotic barrier opening, however, and therefore is not specific to barrier insult. The cause at present is not known, but we suggest that such stimulation, which probably represents local convulsive activity and is accompanied by a reduced cerebral blood flow, may contribute to changes in the level of consciousness and to brain damage in maladies of the central nervous system in which the blood-brain barrier may be disrupted (e.g. Alzheimer's disease, multiple sclerosis, hypertensive encephalopathy). An abstract of this study has been published. (Rapoport, S.I., Fredericks, W.R., Dow-Edwards, D., and London, E.D. Increased local cerebral glucose utilization (LCGU) following opening of the blood-brain barrier by hypertonic arabinose infusion and by acute hypertension. Abstracts Tenth Annual Meeting Society of Neuroscience, 1980).

(3) Quantification and time course. In order to apply the osmotic method to studies of brain pharmacology in animals, and possibly to regulating drug entry into the brain in man, it was important to determine thresholds for infusate concentration and infusion time that produce maximum opening, as well as the time course of such opening. To do this, we employed the quantitative methodology discussed above, and used ^{14}C-sucrose as an intravascular tracer to measure and quantify osmotically-induced cerebrovascular permeability changes in the rat.

Retrograde external carotid infusion of a hypertonic arabinose solution in the conscious rat reversibly increased cerebrovascular permeability to ^{14}C-sucrose. PA (product of permeability and capillary surface area) rose from 11 x 10^{-6} sec^{-1} in control brains to above 200 x 10^{-6} sec^{-1} in experimental brains. The rise correlated with an increased brain staining by intravascular Evans blue-albumin, and was accompanied by a transient 1-1.5% increase in brain water content. At least 20 sec of carotid infusion was required for 1.6 m arabinose solution to effectively increase cerebrovascular permeability. Prolongation of infusion time or elevation of infusate concentration had no further effect. The increased permeability remained for about 1 hr, during which time it is suggested that drugs of pharmacological interest should be administered for maximum central effectiveness (Ref. 4-7).

In order to eventually apply the osmotic method to facilitate drug entry into the brain of man, we developed an *in vivo* method to monitor blood-brain barrier disruption, using computerized axial tomography. In the dog, a hypertonic mannitol solution was infused into the internal carotid artery, following the intravenous injection of Conray 60 as a radiocontrast agent. Immediately after osmotic treatment, methotrexate was infused through the carotid circulation. Computerized tomograms of the brain demonstrated reversible osmotic blood-brain barrier disruption. These *in vivo* measures of disruption correlated with brain uptake of methotrexate, an anti-tumor drug that normally enters the central nervous system very slowly (see below) (Ref. 8).

(4) Pharmacological uses of reversible osmotic barrier opening. Osmotic barrier opening has been employed by us to allow the following normally-excluded agents into the brain:

(a) Methotrexate, for therapy of brain tumors. One of the major problems in cancer chemotherapy is the inability of systemic drugs with proven effectiveness to act on metastatic brain tumors, because of the blood-brain barrier. It would be extremely important, especially in cases of single brain metastases in association with pulmonary or breast carcinoma, to be able to open the blood-brain barrier so as to allow proven effective chemotherapeutic agents into the brain. We performed animal experiments to quantify methodology and effectiveness of osmotic barrier opening as a possible adjunct to brain chemotherapy in man. In anesthetized rats, the blood-brain barrier to ^{3}H-methotrexate was opened by infusing 1.6 m arabinose solution into the external carotid artery, in a retrograde direction, for 30 sec. PA (product of cerebrovascular permeability and capillary surface area), as measured by methods that we developed (see above), increased from a mean of 3.3 x 10^{-5} sec^{-1} in brains of control rats to as high as 20 x 10^{-5} sec^{-1}.

At the cerebral hemisphere ipsilateral to the infused carotid artery, brain uptake was increased by a factor of 50 (a factor of 7 due to elevation of PA, multiplied by 7 due to infusion by the carotid route), as compared to uptake by normal untreated brain with infusion into a peripheral vein. The study shows that osmotic barrier opening followed by carotid drug administration should be of use in chemotherapy of the central nervous system (Ref. 9).

Carotid infusion of hypertonic mannitol solution also was used to open the blood-brain barrier to methotrexate in dogs. Ipsilateral methotrexate brain levels ranged from 90-216 ng/g tissue following intraarterial injection of drug in control animals, whereas a mean value of 1420 ng/g was measured with carotid infusion of mannitol. Brain levels remained high for 6 hrs. There were no neuropathological changes 24 or 72 hrs after infusion, or up to 2 weeks later. The methotrexate levels reached under these conditions would be sufficient to treat certain cerebral tumors (Ref. 8).

(b) Reversible blood-brain barrier disruption in man in chemotherapy of malignant brain tumors. Successful experimental reversible disruption of the blood-brain barrier in animals provided the basis for a clinical evaluation of osmotic disruption in five patients with primary and metastatic malignant brain tumors. Reversible, transient osmotic barrier disruption was achieved 15 times in the 5 patients without additional toxicity, using 25% mannitol which was infused into the internal carotid artery via a catheter placed in the femoral artery. Computed tomography and radionuclide brain imaging were shown to be useful noninvasive monitors of the adequacy and extent of barrier disruption. These studies also provided further evidence that the barrier is at least partially intact in human tumors, because one patient demonstrated a cerebral metastasis only after barrier disruption. These studies were performed by Dr. E. Neuwelt.

(c) Access of enzymes to brain following osmotic alteration of blood-brain barrier. Enzyme replacement therapy has as its goal the permanent restoration of a deficient enzymatic activity in an organ. The lysosomal enzyme deficiencies (e.g. Tay-Sachs disease, Fabry disease, Gaucher's disease) are unique in that the organelle in which the deficiency is found is the organelle into which incorporated macromolecules are delivered. Intravenously infused enzymes are incorporated into lysosomes of mammalian liver cells, and human lysosomal enzyme deficiencies can be corrected in tissue culture by enzyme administration. The application of enzyme replacement within the normal brain by peripheral administration is precluded by the impermeability of the blood-brain barrier to the enzymes. We demonstrated in the rat, however, that reversible disruption of the blood-brain barrier by carotid infusion of a hypertonic arabinose or mannitol solution resulted in significant brain uptake of peripherally administered enzymes -- α-mannosidase and horseradish peroxidase. These findings were demonstrated with direct enzyme assay. Furthermore, histological studies showed uptake within lysosomes of neurons but not glia. The increased brain enzyme activity indicates that enzyme replacement therapy may eventually be used to augment low endogenous levels encountered in genetic disorders involving the central nervous system of man (Refs. 10, 11).

(d) Blood-brain barrier opening after explosive decompression from hyperbaric N_2-O_2 mixtures. The basis of decompression sickness in man is not completely understood, but may be related to damage to the lung and brain following rapid decompression from hyperbaric exposure. In order to examine pulmonary and blood-brain barrier changes that may be related to decompression sickness, we exposed guinea pigs for 30 min to different hyperbaric N_2-O_2 mixtures, following which they were explosively decompressed. Evans blue, a marker of lung and blood-brain barrier integrity, was injected intravenously and at various times after decompression. Neither the lung nor brain showed staining in unexposed control animals, but lung staining occurred in experimental animals. Brain staining, caused by opening of the blood-brain barrier to dye, occurred only when the hyperbaric mixture contained at least 2 ATA (absolute atmospheric pressure) pN_2, but did not occur at less than 2 ATA pN_2, even with exposure to a N_2-O_2 mixture of 6.5 ATA. Blood-brain barrier opening was reversible. Blood-brain barrier damage may be caused by nitrogen-rich bubbles that remain in the brain for at least 6 min, the time required for barrier opening by intravascular emboli (Ref. 12).

5. Breakdown of the Blood-Eye Barrier.

(a) Hypertensive breakdown of cerebral but not of retinal blood vessels in the rhesus monkey. Acute hypertension, induced either by intravenous injection of Aramine (metaraminol) or by infusion of isotonic saline into the common carotid artery, or a combination of both procedures, led in the rhesus monkey to breakdown of the blood-retinal barrier to intravenous Na fluorescein. Whereas the cerebral vasculature was made permeable to blood-borne dye at carotid pressures above 160 mm Hg, the retinal blood vessels were intact even at pressures as high as 310 mm Hg. Hypertensive breakdown of the blood-brain barrier was associated with neurological deficits and brain edema. The results indicate that the retina is more resistant to acute hypertension than is the brain, (Ref. 13).

(b) Reversible opening of the retinal pigment epithelium to fluorescein by hypercapnia. In the rat, hypercapnia consistently and reversibly increased the permeability of the retinal pigment epithelium to intravascular fluorescein, but much less consistently modified blood vessels in the retina and brain. The epithelial change was not mediated by systemic hypertension, because it occurred when CO_2-induced hypertension was blocked by phenoxybenzamine. The change may be related to altered transport of Na fluorescein at the pigment epithelium, as one of several organic acids that are transported. Other dyes and tracers of different chemical structure do not show increased entry into the pigment epithelium followed hypercapnia (Ref. 14).

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 to interpreting changes of

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. An altered response in relation to age could be due to an altered rate of drug entry into the brain or to changes in cerebral receptor sensitivity.

3. Our demonstration that opioid peptides have a significant cerebrovascular permeability, supports experiments that show that they have central effects when injected systemically, provided their plasma half-life is longer than several minutes. Feedback may operate between circulating peptides with potential central effects and brain sites that regulate their release into the systemic circulation.

4. The analysis of factors that determine protein entry into the central nervous system is relevant to questions of entry of protein viruses and antibodies into the brain. Alteration of the normal concentration profile of spinal fluid protein reflects specific brain diseases that may involve blood-brain barrier disruption.

5. 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. Because of its potential use in man, our studies that quantify threshold of infusate concentration and infusion duration for osmotic opening are critical, and our description of the time course of increased permeability provides a basis for determination of therapeutic regimens.

6. Our demonstration that osmotic barrier opening can be employed in man provides a stimulus for examining its use in the chemotherapy of brain tumors and for enzyme replacement therapy of lysosomal storage diseases that involve the central nervous system.

7. Our finding that osmotic and hypertensive barrier opening increases local cerebral glucose utilization by the brain, reduces cerebral blood flow and produces brain edema demonstrates that an intact blood-brain barrier is necessary for the maintenance of normal cerebral metabolism and function, coupling between cerebral blood flow and metabolism, and prevention of brain edema. Changes in consciousness in central nervous system diseases that may involve blood-brain barrier damage (e.g. multiple sclerosis, hypertensive encephalopathy) may be related directly to altered cerebrovascular permeability.

8. Decompression sickness that involves the central nervous system may be related to disruption of the blood-brain barrier. Our demonstration that barrier damage occurs only when pN_2 in the inspired air is reduced two-fold, independently of the reduction of pO_2, is unexplained, but should help to design hyperbaric conditions during diving schedules in man that reduce the incidence of decompression sickness.

9. The blood-ocular barriers regulate the environment and the function of the eye. Our finding of the low vulnerability of the retinal as compared to cerebral vasculature to acute hypertension suggests that examination of the retinal vasculature in clinical conditions of hypertension will not reflect brain changes. Studies of the effect of hypercapnia on permeability of the pigment epithelium may help to enhance drug entry into the eye.

Proposed Course: (1) 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, (2) further application of the osmotic method will be made in human and animal studies, for its eventual use as a pharmacological tool, (3) we will try to determine why changes in blood flow and metabolism accompany opening of the blood-brain barrier, (4) the effect of hypercapnia will be studied on the blood-ocular barrier to tracers of differing properties, (5) further ultrastructural studies will be performed to define the basis of increased cerebrovascular permeability following osmotic treatment.

Publications:

1. Rapoport, S.I., Ohno, K. and Pettigrew, K.D.: Drug entry into the brain. Brain Research. 172: 354-359, 1979.

2. Rapoport, S.I., Klee, W.A., Pettigrew, K.D. and Ohno, K.: Entry of opioid peptides into the central nervous system. Science 207: 84-86, 1980.

3. Rapoport, S.I. and Pettigrew, K.D.: A heterogenous, pore-vesicle membrane model for protein transfer from blood to cerebrospinal fluid at the choroid plexus. Microvascular Research 18: 105-119, 1979.

4. Rapoport, S.I., Fredericks, W.R., Ohno, K. and Pettigrew, K.D.: Quantitative aspects of reversible osmotic opening of the blood-brain barrier. American Journal of Physiology 238: R421-R431, 1980.

5. Rapoport, S.I., Ohno, K., Fredericks, W.R. and Pettigrew, K.D.: A quantitative method for measuring altered cerebrovascular permeability. Radio Science 14: 345-348, 1979.

6. Rapoport, S.I.: Cerebrovascular permeability in normal brain and following osmotic opening of the blood-brain barrier. In: J.G. Cunha-Vaz (Ed.): The Blood-Retinal Barriers. NATO Advanced Study Institutes Series A. Life Sciences. New York, Plenum Press, 1980, vol. 32.

7. Rapoport, S.I.: Quantitative aspects of osmotic opening of the blood-brain barrier. In: Weiss, L., Gilbert, H.A. and Posner, J.B. (Eds.): Brain Metastases. Boston, Mass., G.K. Hall & Co., 1980, pp. 100-114.

8. Gruneau, S.P., Folker, M. and Rapoport, S.I.: Blood-brain barrier opening after explosive decompression from hyperbaric N_2-O_2 mixtures. Experimental Neurology 66: 238-247, 1979.

9. Neuwelt, E.A., Maravilla, K.R., Frankel, E.P., Rapoport, S.I., Hill, S.A. and Barnett, P.A.: Osmotic blood-brain barrier disruption: computerized tomographic monitoring of chemotherapeutic agent delivery. J. Clinical Investigation 64: 684-688, 1979.

10. Ohno, K., Fredericks, W.R. and Rapoport, S.I.: Osmotic opening of the blood-brain barrier to methotrexate in the rat. Surgical Neurology 12: 323-328, 1979.

11. Barranger, J.A., Rapoport, S.I. and Brady, R.O.: Access of enzymes to brain following osmotic alteration of the blood-brain barrier. In: Desnick, R.J. (Ed.): Enzyme Therapy in Genetic Diseases: 2. Birth Defects: Original Article Series. March of Dimes. New York, Alan R. Liss, Inc., 1980. vol. XVI, No. 1, pp. 195-205.

12. Pentchev, P.G., Kusiak, J.W., Barranger, J.A., Furbish, F.S., Rapoport, S.I., Massey, J.M. and Brady, R.O.: Factors that influence the uptake and turnover of glucocerebrosidase and α-galactosidase in mammalian tissues. In: Gatt, S., Freysz, L. and Mandel, P. (Eds.): Enzymes of Lipid Metabolism. New York, Plenum Press, 1978, pp. 745-752.

13. Laties, A.M., Rapoport, S.I. and McGlinn, A.: Hypertensive breakdown of cerebral but not of retinal blood vessels in the rhesus monkey. AMA Archives Ophthalmology 97: 1511-1514, 1979.

14. Rapoport, S.I., Fredericks, W.R. and Laties, A.M.: Reversible opening of the retinal pigment epithelium by hypercapnia. Experimental Eye Research 30: 129-141, 1980.

15. Rapoport, S.I.: Book Review: "The Concept of a Blood-Brain Barrier" by Michael Bradbury. Nature 283: 414, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00121-03 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Function of Peripheral Nerve and Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Levitan	Physiologist (IPA)	LNS	NIA
	N.L. Shinowara	Staff Fellow	LNS	NIA
	S.I. Rapoport	Chief	LNS	NIA
Other:	J.V. Passonneau	Chief	LNS	NINCDS
	R.E. Taylor	Research Physiologist	LN	NINCDS
	A. Weerasuriya	Visiting Fellow	LN	NINCDS

COOPERATING UNITS (if any)
Lab of Neurochemistry and Lab of Biophysics, NINCDS
V. Nassar-Gentina, Res. Scientist, Dept of Physiol, Univ of Santiago, Chile
R.A. Spangler, Biophysicist, Dept of Biophysics, Univ of Buffalo

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. Fatigue of single frog muscle fibers appeared as a decline in tension output during prolonged tetanic stimulation. The concentration of ATP (energy source for contraction) remained normal in fatigued fibers. Furthermore, ATP was not sequestered or unavailable for contraction. Fatigue therefore was due to an inability of the muscle to use energy in the form of ATP. We suggest that H^+, as lactic acid, accumulated in these fibers so as to interfere with excitation-contraction coupling. B. The perineurial sheath which surrounds peripheral axons may regulate nerve function and development. The permeability of the sheath of the frog sciatic nerve is very low to water-soluble nonelectrolytes and to ions, but can be increased by stretch or osmotic treatment. Ionic diffusion takes place through cells and intercellular tight junctions, but is not an active process. Following sectioning of the frog sciatic nerve, the perineurium undergoes a gradual increase in its permeability to ^{22}Na during Wallerian degeneration with a time course that corresponds to the decline in the amplitude of the compound action potential. This indicates that perineurial integrity depends on functional integrity of the nerve.

Project Description: Z01 AG 00121-03 LNS

The object of this project is to relate metabolism and morphology of peripheral nerve and muscle to the functioning of these tissues in health, disease and aging.

Objectives:

1. Peripheral nerve and perineurium. Peripheral nerve function may be governed in part by the local axonal milieu, which is separated from body fluids by a perineurial sheath and by endoneurial capillaries. We wanted to study the function and morphology of the perineurium, in order to understand the role of the perineurium in regulating and/or maintaining the endoneurial environment of axons, and in affecting peripheral nerve function. Towards this end, the ability of the perineurium to act as a permeability barrier to radiotracer nonelectrolytes and ions was examined in normal and degenerating nerves, and when the tonicity and ionic strength of the medium surrounding the perineurium was altered.

2. Muscle contraction and muscle fatigue. When a striated muscle is stimulated repetitively, its contractile force decreases and it is said to be fatigued. Fatigue characterizes fast twitch muscles whose metabolism is glycolytic, rather than slow twitch muscles whose metabolism is oxidative. Muscle fatigue generally is thought to be caused by depletion of available energy reserves. However, some studies in single fibers suggest that fatigue can be due to uncoupling of steps that connect excitation with contraction. We explored the relation between fatigue and metabolism in single muscle fibers rather than in whole muscles, because, in whole muscles, some fibers can fatigue faster than others and measured metabolite concentrations are averaged over a heterogeneous, often disparate fiber population. Furthermore, intercellular diffusion limits physiological studies of whole muscles but not of single fibers.

Methods Employed:

1. Perineurium. For electrical and flux studies, the perineurium of the frog sciatic nerve was isolated and mounted on cannulae within a bath of stirred Ringers solution and perfused by Ringers solution which could be collected. Radioisotopes were placed in the bath. Transport through the sheath was quantified by measuring isotope entry into perfusate.

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 transperineurial potential difference produced by it.

To examine the permeability of the perineurium during Wallerian degeneration, the right sciatic nerve was transected in ether-anesthetized frogs with the

left serving as control. The compound action potential and the sodium (^{22}Na) permeability of the distal segment of the nerve were examined 3,7,10,14,21,28 and 42 days after transection, and compared to observations in the control nerve.

For morphological studies, the frog sciatic nerve was fixed with glutaraldehyde in situ and/or in vitro by immersion. More rapid preservation was achieved by using an osmium tetroxide-glutaraldehyde fixative. For tracer studies, colloidal lanthanum or lanthanum chloride was presented for 3 to 4 hours externally to small segments of nerve, or internally by injection. Modifications in the standard lanthanum procedure were done as controls for the effectiveness of lanthanum as a tracer. Horseradish peroxidase was used as an in vitro tracer in the bath for one hour at 25° or 5°C and reacted with 3,3-diamino-benzidine and hydrogen peroxide. Tissues were prepared for electron microscopic examination.

2. Muscle. A single fiber was dissected from the frog semitendinosus muscle and mounted in a bath of flowing Ringers solution at 15°C. Tension was recorded by a RCA 5734 transducer tied to one end of the fiber; the output was displayed on an oscilloscope face. Sarcomere length was measured by a laser diffraction technique. The fiber was stimulated at different frequencies via external platinum electrodes, so as to produce twitches or tetanic contractions for up to 200 sec.

Major Findings:

1. Perineurium. a) Electrical and flux studies. The permeability of the normal isolated perineurium to ^{14}C-sucrose was found to equal 5.6×10^{-7} cm/sec. This value is very low and shows that the sheath is extremely impermeable to water-soluble solutes. Permeability to ^{14}C-sucrose was increased reversibly by stretching the perineurium by 10%, but was increased irreversibly by further stretch or by immersing the sheath in a Ringers solution made hypertonic with either NaCl or sucrose. We suggested that stretch or hypertonicity modifies the dimensions of tight junctions that connect perineurial cells, and thereby increases permeability (ref. 1).

In order to examine the electrical and ionic permeability properties of the perfused perineurium of the frog sciatic nerve, an AC current was passed across the perineurial cylinder wall and voltage across the wall was measured. Frequency of current varied from 1 Hz to 0.1 MHz. The DC resistance of the perineurium was 430 ohm cm^2, and impedance measurements demonstrated two dispersions with center frequencies of 5 KHz and 20 Hz. Exposure to high conductance Ringers decreased the DC resistance; a low conductance Ringer increased the resistance. Analysis of the data in terms of a four-element model (two resistors and two capacitors) for an equivalent circuit suggested that small (0.1 $\mu F/cm^2$) and large (20 $\mu F/cm^2$) capacitances exist at the perineurium. The small capacitance indicates the presence of six or more layers of cells and was unchanged by experimental manipulations. The large capacitance could be ascribed to polarization of charge, perhaps at intercellular tight junctions, and was affected by experimental manipulation.

The perineurial permeability for ^{22}Na equaled 1.68×10^{-6} cm/sec and was unaffected by inhibitors of active transport. The $^{42}K/^{22}Na$ permeability ratio exceeded the ratio of limiting conductances of these ions in free solution, but the $^{36}Cl/^{42}K$ permeability ratio did not differ from the respective limiting conductance ratio. Immersion of the perineurial cylinder in hypertonic Ringer solution increased the absolute permeability coefficients of the three ionic tracers but did not affect their permeability ratios. The flux ratio of $^{22}Na/^{14}C$-sucrose, however, was decreased by hypertonic treatment. It was concluded that there is no evidence of active Na or K transport across the perineurium, and that the extracellular path in the perineurium exhibits selective size-dependent permeability. Low rates of transperineurial permeation of ions and water-soluble nonelectrolytes are comparable to those in epithelia with tight junctions, and show that the perineurium acts as a tight diffusion barrier that protects the nerve environment (ref.2).

(b) Wallerian degeneration. Following transection of the frog sciatic nerve, an increase in perineurial permeability to ^{22}Na accompanied the decrement in the amplitude of the compound action potential during Wallerian degeneration of the nerve. Three days after transection there was no significant change in either perineurial permeability or compound action potential, but by 6 weeks the compound action potential was absent, and perineurial ^{22}Na permeability had increased more than eight-fold. From the third to the sixth day after nerve transection, the permeability increased gradually, with the greatest changes occurring between the 14th and 21st day. On the other hand, the sharpest decrement in compound action potential was between the 10th and 14th day. The results show that perineurial integrity depends on the integrity of the nerve itself.

An abstract of this work has been published (Weerasuriya, A., R.E. Taylor and S.I. Rapoport. Permeability increase in the frog perineurium during Wallerian degeneration. Fed. Proc. 39: 381, 1980).

2. Muscle. We first distinguished the time course of the fatigue process in single fibers from post-tetanic potentiation of the twitch, which is characterized by elevation and prolongation of the twitch and is due to intracellular calcium accumulation. Fatigue appeared after 20 sec of tetanic stimulation (at 20 Hz) as a decline in tetanic tension that took as long as 1 hr to be reversed. We then correlated the decline and recovery of tension with the instantaneous metabolic profile of the fiber. We found that prolonged tetanic stimulation reduced fiber phosphocreatine (PCr) in proportion to the internal work performed by the fiber, as measured by the isometric time-tension integral. However, despite the fact that ATP is the immediate energy source for muscle contraction, ATP concentration was normal or close to normal in fatigued fibers. ATP was not sequestered in internal compartments in these fibers, as residual ATP could be consumed when caffeine was applied to the fibers to produce maximal and irreversible contractures. Thus, fatigue was not due to depletion of available energy stores for muscle contraction, but to failure of coupling between excitation and contraction.

On the basis of measured lactate and ATP/PCr ratios in fatigue fibers, we suggested that H^+, as lactic acid, accumulates in fatigue repetitively stimulated muscle and interferes with the action of calcium in activating muscle contraction.

Significance to Biomedical Research and the Program at the Institute:

1. Little is known about how the perineurium helps to regulate and maintain the metabolic and ionic environment of peripheral nerve axons. Defects in the perineurium may contribute to peripheral neuropathy in disease and aging. Basic information on the perineurium is required to address these questions.

2. Our finding that muscle fatigue develops, despite high levels of ATP corresponds to observations of "power failure" in stimulated peripheral nerve and sympathetic ganglion. In these tissues, function also fails before energy supplies are exhausted. Protection of energy supplies from exhaustion, may preserve survival of neuronal and muscle cells. Muscle wasting and fatigue commonly accompany senescence in man, but may be partially reversed, as endurance training can reduce fatigue despite age (Suominen et al., J. Gerontol. 32: 33, 1977). Endurance training also increases muscle aerobic metabolism. If our hypothesis is correct, that lactate accumulation rather than energy exhaustion contributes to fatigue, then the results in man may reflect an enhanced ability of endurance-trained muscles to oxidize lactic acid.

Proposed Course:

1. We will continue to investigate the function and morphology of the perineurium in relation to regulation of the peripheral nerve environment.

2. The data on energy consumption during excitation-contraction uncoupling by stretch and hypertonic solutions, and in relation to caffeine contractures, will be analyzed and prepared for publication.

Publications:

1. Weerasuriya, A., Rapoport, S.I. and Taylor, R.E.: Modification of the permeability of the frog perineurium to ^{14}C-sucrose by stretch and hypertonicity. Brain Research 173: 503-512, 1979.

2. Weerasuriya, A., Rapoport, S.I. and Taylor, R.E.: Ionic permeabilities of the frog perineurium. Brain Research 191: 405-415, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00122-03 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Pharmacology of Central and Peripheral Catecholaminergic Nervous System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Chiueh	Senior Staff Fellow	LNS	NIA
Others:	S.I. Rapoport	Chief	LNS	NIA

COOPERATING UNITS (if any)

C.C. Chang, National Taiwan University
M. Kuehne, University of Vermont

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The main goal of this project is to study the effect of aging, stress and drugs on the blood pressure of spontaneously hypertensive rats. (1) Three oxindole derivatives of Uncarine A alkaloid have been synthesized and tested for an Uncarine-like antihypertensive and tranquilizing action. (2) A gas chromatographic-Mass Spectrometric assay procedure for simultaneous measurement of nanogram level of dopamine and its metabolite, 3-methoxytyramine in brain tissue has been developed. Results indicate that O-methylated rather than deaminated metabolite of dopamine is a better index for the measurement of neuronal activity in the central dopaminergic systems. The striatal level of 3-methoxytyramine and behavioral activity in the spontaneously hypertensive rats is higher than that in the normotensive rats after administration of amphetamine or stress. (3) A 30 min immobilization induced an irreversible cardiovascular failure in the aged Wistar and Fischer-344 rats while it produced little effect on 12 month old rats.

PHS-6040
(Rev. 10-76)

Objectives:

(1) The project investigates the etiology of an age-dependent increase in blood pressure of spontaneously hypertensive rats and uses these hypertensive rats as a rodent model for the screening of new therapeutic agents for the treatment of essential hypertension. (2) One of the objectives is to investigate the hypothesis of a defective central control of blood pressure in the aged or hypertensive animals. (3) The final goal of the project is to develop pharmacological or physiological treatments for essential hypertension or for stress-induced cardiovascular failure in the elderly.

Methods Employed: (1) Chronic indwelling tail arterial catheter. One or two days before an experiment, an indwelling catheter filled with heparin saline was inserted into the tail artery. The catheter was led subcutaneously up to the back and out at the back of the neck, where it was threaded through a spring wire which was anchored at the neck. The catheter was flushed twice daily with heparin saline. Rats were housed individually in 30 x 30 x 8 cm plastic cages.

(2) Measurement of blood pressure and heart rate in conscious and unrestrained rats. The systolic and diastolic blood pressures of rats were monitored by a pressure transducer which was connected to the indwelling arterial catheter and recorded polygraphically. The heart rates were triggered by the pulses of the blood pressures and recorded by a polygraph.

(3) Assay for plasma catecholamines, corticosterone and blood sugar. Plasma samples (50 µl) were assayed for catecholamines by a radioenzymatic procedure. Corticosterone in 50 µl of plasma was measured by a spectrofluorometric procedure. Sugar in 10 µl of plasma was determined by an enzymatic procedure using a Beckman glucose analyzer.

(4) Stress. Immobilization stress of rats were performed by tying down four limbs of the animals to a board.

(5) Gas Chromatographic - Mass Spectrometric Identification of Uncarine A and its synthetic oxindole derivatives. The purity of the synthetic 3-spiropyrrolidyl-2-oxindole derivatives of Uncarine A were analyzed by a Gas Chromatographic-Mass Spectrometric procedure.

Major Findings:

(1) Antihypertensive effect of Uncarine A. Uncarine A, an alkaloid obtained from a Chinese herbal medicine, decreased the blood pressure of conscious, hypertensive rats. During the hypotensive stage, the heart rate and plasma catecholamines were increased. There was no effect on the content of norepinephrine in the heart or on the pressor responses to the administration of norepinephrine, angiotensin II or to sympathetic stimulation. Uncarine A decreased the exploratory behavior of rats which indicates that it may act at the central nervous system.

(2) Screening of antihypertensive and sedative effect of the synthetic oxindole compounds. Less than 0.5 gm of three 3-spiropyrrolidyl-2-oxindole derivatives of Uncarine A have been synthesized. NMR and GCMS analyses of these compounds reveal that it consists of side chain of $N-CH_2-\emptyset$, $N-CH_3$ and N-H, respectively on the spiropyrrolidyl ring. Despite the minute quantity and supply of drugs, preliminary tests indicate that some of these oxindole compounds can produce both antihypertensive and tranquilizing effects. At a dose regima of 8 mg/kg (i.v.), $N-CH_2-\emptyset$ compound produces the greatest pharmacological effects while N-H compound produces little or no antihypertensive effect but does have a tranquilizing effect.

(3) Development of a simultaneous assay for dopamine and 3-methoxytyramine in brain samples by employing a Gas Chromatographic-Mass Spectrometric procedure. The gas chromatographic-mass spectrometric assay procedure of monoamines requires deuterium labeled internal standards. d_4-Dopamine was obtained from Merck Sharpe and Dohme Company. The d_4-dopamine was catalyzed by catechol-O-methyltransferase and O-methylated to form d_4-3-methoxytyramine in the presence of S-adenosyl-L-methionine. The brain extract was purified and derivatized with pentofluoro-propionic anhydride. The pentofluoro-propionic derivatives were assayed gas chromatographic-mass spectrometrically. Specific mass ion pairs of dopamine (428/431) and 3-methoxytyramine (296/299) were used for monitoring the effluent of gas chromatographic column. The sensitivity of this assay procedure was about 10 nanograms per sample. Results indicate that striatal 3-methoxytyramine increases after the administration of amphetamine or stress. The increment of 3-methoxytyramine is greater in the spontaneously hypertensive rats than in the normotensive rats.

(4) Stress induced irreversible cardiovascular failure in the aged Wistar and Fischer-344 rats. A 30 min immobilization stress produced an increase in blood pressure of 12 month old rats while it produced a decrease in blood pressure of 2 - 3 year old rats. The stress-induced cardiovascular collapse was irreversible in the aged.

Significance to Biomedical Research and to the Program of the Institute:

By studying the pharmacological mechanism of Uncarine A and its synthetic oxindole derivatives we may find a new class of drugs for the treatment of essential hypertension. The tranquilizing effect of oxindole compounds may be useful for the relief of stress-induced anxiety or fear. The measurement of dopamine and 3-methoxytyramine rather than acidic metabolites by the newly developed gas chromatographic-mass spectrometric procedure may provide a better index for brain dopaminergic activity or functional turnover rate in the aging brain since there is a positive correlation between striatal level of 3-methoxytyramine and the behavioral arousal after the activation of dopaminergic neurons by either administration of amphetamine or stress.

Proposed Course:

(1) Study of the therapeutic index of antihypertensive and tranquilizing effects of oxindole compounds. A large quantity of oxindole compounds will be synthetized in order to provide enough drug for the toxicological tests. The possibility of involvement of peptidergic systems in the regulation of blood pressure of hypertensive animals will be explored.

(2) Dopaminergic mechanism in aging, stress and hypertension. The hypothesis of involvement of brain dopaminergic system in the regulation of cardiovascular, autonomic and neurohumoral functions will be investigated in order to provide a neurochemical basis for understanding of type "A" personality or hyperadrenergic responsiveness in the hypertensive subjects.

(3) Pharmacological management of stress-induced cardiovascular collapse in the elderly. Experiments are planned to improve the survival rate and cardiovascular performance of the aged rats after stress by the pretreatment of the rats with drugs in order to increase the pulse pressure and cardiac output, to prevent the metabolic acidosis or to find a receptor blocking agent for the unknown myocardial depressant factor.

Publications:

Chang, C.C., Tung, L.H., Chen, R.R.L. and Chiueh, C.C.: A study on the antihypertensive action of Uncarine A, an alkaloid of Uncara Formosana used in Chinese herb medicine. J. Formosan Med. Assoc. 78: 61-69, 1979.

Chiueh, C.C. and Thoa, N.B.: Turnover of hypothalamic catecholamines in spontaneously hypertensive rats. In Usdin, E (Ed.): Catecholamines: Basic and Clinical Frontiers. New York, Pergamon press, 1979, pp. 1455-1457.

McCarty, R., Chiueh, C.C., and Kopin, I.J.: Differential behavioral responses of spontaneously hypertensive (SHR) and normotensive (WKY) rats to d-Amphetamine. Pharmacol. Biochem. and Behavior 12: 53-59, 1980.

Perlow, M.J., Chiueh, C.C., Lake, R., and Wyatt, R.J.: Increased dopamine and norepinephrine concentrations in primate CSF following amphetamine and phenylethylamine administration. Brain Res. 186: 469-493, 1980.

Lee, T.J.-F., Chiueh, C.C. and Adams, M.: Synaptic transmission of vasoconstrictor nerves in rabbit basilar artery. Europ. J. Pharmacol. 61: 55-70, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00123-02 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Synapse Development, Specificity and Mechanism in Culture

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.M. Thompson	Sr Staff Fellow	LNS	NIA
Others:	S.I. Rapoport	Chief	LNS	NIA
	N.E. Bennett	Biochem Lab Tech	LNS	NIA
	M. Nirenberg	Chief	LBG	NHLBI
	G.L. Cantoni	Chief	LGCB	NIMH
	P.R. Chiang	Sr Staff Fellow	LGCB	NIMH

COOPERATING UNITS (if any)
Laboratory of Biochemical Genetics, NHLBI
Laboratory of General and Comparative Biochemistry, NIMH

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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. These results suggest a mechanism of synapse specificity based on a synaptogenic period during development for each type of neuron. In addition, it appears likely that a methyltransferase reaction is involved in neurotransmission.

PHS-6040
(Rev. 10-76)

Objectives: Neurons in culture form synapses with other neurons and/or muscle as shown by electrophysiological recording and electron microscopy. 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 synapses. The objective of this study is to examine the specific properties that determine synapse formation during development in relation to neuronal type and target organ. In addition, the objective is to examine which parameters determine whether a synapse is appropriate and is retained or whether a synapse is inappropriate and is lost during further development. In examining these questions, neurons and their target cells are established in a culture environment which can be manipulated in order to study a) neurotransmission, b) synapse formation, c) synapse termination and d) 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 with microelectrodes in target muscle cells and electrophysiological recording of miniature endplate potentials. Biochemical measurements are made of neurotransmitter levels, release and synthetic enzymes. In addition, morphological measurements at the light and ultrastructural levels are made on the cultured cells.

Major Findings: 1) Synapse development. The ability of neurons at different development ages to form synapses in culture was studied by combining neurons from chick embryos with rat striated muscle cells as target organs. Synaptic connections were examined after 24 hr of co-culture by looking for miniature endplate potentials in muscle cells with microelectrodes. Three populations of cholinergic neurons from the chick embryo were examined. Neurons from the retina formed synapses with muscle cells between 6 and 16 days of embryonic age with a maximum rate of formation at 8 days. Rat retinal neurons formed synapses only between the 18th day of embryonic development and day 7 after birth, with a maximum number formed from pups 1 day old. Neurons from the chick embryo spinal cord formed synapses after 2 days, the maximum number with 4-day neurons. The capacity to form neurons decreased with age, and was observed after 51 hr, but not after 24 hr, of co-culture with neurons from 18-day embryo spinal cord. Thus, spinal cord neurons do not completely lose their ability to form synapses with muscle cells. All neurons show decreased ability to form synapses as neurons age during development. All neurons also lose their ability to form synapses when maturing *in vitro* in a manner and time course which parallels the loss of their ability to form synapses during *in ovo* maturation.

It has been proposed (Ruffolo, R., Eisenbarth, G., Thompson, J. and Nirenberg, M., Proc. Natl. Acad. Sci. USA. 75: 2281-2285, 1978) that 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 synaptic 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. An abstract of this work has been published (J. Thompson, Critical Periods for Synapse Formation: Implications for Plasticity. In Modern Aging Research, Vol. 1, (eds), Alan R. Liss, Inc., New York, 1980, in press).

2) Mechanism of neurotransmission. The role of methylation reactions in synapse formation on neurotransmission was examined with biochemical and electrophysiological techniques. Methylation reactions are catalyzed by methyltransferases, which can be inhibited by S-adenosyl homocysteine (AdoHcy) or by analogues of this compound. Analogues which either increase levels of AdoHcy or inhibit methyltransferases directly include 3-deazaadenosine (DZA), adenosine-2', 3'-diazido-5'-carboxamide (744-99), 5'-deoxy-5-isobutylthioadenosine (SIBA) and 5'-deoxy-5'-isobutylthio-3-deazaadenosine (DZ-SIBA). Neurons from 8-day chick embryo retina grown in culture with rat striated muscle cells for 24 hr formed synapses. Synapses were detected by recording spontaneous depolarizing synaptic muscle responses with intracellular microelectrodes. After treating the cultures for 2 hr with one of the AdoHcy analogues, the number of muscle responses/min in these synapses was inhibited up to 95% by DZ-SIBA (ED_{50}=1.5 x 10^{-6}M), SIBA (ED_{50}= 3 x 10^{-5}M), DZA (ED_{50} = 1.5 x 10^{-5}M) and 744-99 (ED_{50}=1 x 10^{-4}M). Homocysteine thiolactone, 5'-deoxyadenosine and tubercidin, which do not increase levels of AdoHcy or inhibit methyltransferases, had no effect. DZ-SIBA inhibited the muscle responses with a half-time of 3.5 min. No inhibition of the number of synapses formed was seen. Inhibition of the number of muscle responses/min by DZA was reversed within 2 hr after removal of the compound. Further, homocysteine thiolactone potentiated the inhibition by DZA by 6-fold when the two compounds were added together. These results strongly suggest that a methyltransferase-mediated reaction occurs during neurotransmission. An abstract of this work has been published: J.M. Thompson, P.R. Chiang, R.R. Ruffolo, Jr., G.L. Cantoni and M. Nirenberg. Methyltransferases are Involved in Neurotransmission Soc. Neurosci. Abstr. 5: 747, 1979.

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.

Inhibition of a methyltransferase which inhibits neurotransmission suggests that enzyme mediated reactions can influence neuronal communication. This finding opens the possibility of a new class of pharmacological agents which act not at receptors, but at biochemical reactions specific for transmission.

Proposed Course: Neuronal and neuronal-muscle co-cultures will be used to study: (a) pharmacology of synapses and synaptic circuits, (b) pharmacology of neurotransmitter release and changes during development and aging, (c) comparison of neuroblastoma cell lines as model systems for study of neuronal properties and (d) control of synapse-competent states of neurons.

Publications: none

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00124-02 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Central Nervous System Binding Sites for the Neurotoxin Kainic Acid

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: E.D. London Sr. Staff Fellow LNS NIA

Others: None

COOPERATING UNITS (if any)
J.T. Coyle, Assoc. Prof. Dept. Pharmacol., Johns Hopkins Sch. of Med., Balto, Md.
H.I. Yamamura, Prof, Dept Pharmacol, Univ. of Arizona Hlth Sci Ctr, Tucson, AZ

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.1	.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) Inhibition of the specific binding of [^{3}H] kainic acid was studied in membranes isolated from rat and human cerebellum. Whereas the Hill coefficient for unlabelled kainate is 1.0, the other compounds exhibit coefficents near 0.5, suggestive of cooperative interactions at the receptor. (2) In human postmortem brains, samples from patients who died with Huntington's disease, show decreased [^{3}H] kainic acid receptor binding in the caudate nucleus, putamen and frontal cortex, but not insular or temporal cortex, hippocampus or cerebellum. High affinity binding is profoundly reduced in the caudate nucleus and putamen. These regions of brain are most affected in Huntington's disease.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives:

Intrastriatal injection of the neurotoxin kainic acid (KA) to rats has provided an animal model for the human neurodegenerative disorder Huntington's disease (HD). Subtle modifications in the structure of KA eliminate its neurotoxic effects, indicating a high selectivity in the recognition sites mediating kainate's action. The marked potency of KA as a depolarizing agent indicates that these sites have a high affinity for the molecule. Accordingly, the specific binding of [^{3}H]KA to neural membranes has been studied. The aims of this project are as follows: (a) to characterize possible receptor recognition sites that mediate KA neurotoxicity, (b) to determine the phylogenetic distribution of these sites in neural tissue, (c) to explore possible cooperative effects between KA and glutamic acid at the receptor level, and (d) to determine if there is a selective loss in [^{3}H] KA binding sites in postmortem brains of HD patients.

Methods Employed:

1. Preparation of washed membranes. Frozen neural tissue is sonified in glass distilled water, and the suspensions centrifuged at 48,000 x g for 10 min. at 2°. The membrane pellets are resuspended, pelleted and washed sequentially in 100 vol. glass distilled water and 50mM Tris-citrate buffer, pH 7.1. Finally, the membranes are suspended in 100 vol of 100 mM Tris citrate buffer, pH 7.1. Protein is assayed by the method of Lowry et al. (1951).

2. Standard [^{3}H]Kainic Acid Binding Assay. 1000 µl of washed membrane suspension and various concentrations of radioligand displacers are placed in 15 ml Sorvall teflon centrifuge tubes. Ten to 300 pmoles of freshly diluted [^{3}H]KA are added in a volume of 100 µl to start the reaction. The incubation volume is adjusted to 2 ml with distilled water. Incubations are run for 60 min at 2° and are stopped by centrifugation. Bound radioactivity in pellets is estimated by liquid scintillation spectroscopy. Nonspecific binding is determined in the presence of 0.1 mM unlabeled KA.

To characterize separately binding to high and low affinity sites, advantage is taken of the fact that the radioligand dissociates slowly from high affinity sites but nearly instantaneously from low affinity sites in rat and human brain. After 60 min of incubation with [^{3}H] KA, 200 nmoles of unlabeled KA are added to some tubes, bringing the final concentration to 0.1 mM. After another 5 min of incubation, the samples are centrifuged and bound radioactivity measured. The addition of unlabeled ligand 5 min prior to centrifugation displaces radioligand from low affinity binding sites but does not affect nonspecific or high affinity binding.

Major Findings:

1. In inhibition studies using unlabelled kainic acid, L- and D-glutamic acids and dihydrokainic acid as antagonists of specific [^{3}H] kainic acid binding, the order of potencies for inhibition in human and rat cerebellum is: Kainic acid > L-glutamic acid > dihydrokainic acid > D-glutamic acid. Whereas the Hill coefficient for unlabelled kainate is 1.0, dihydrokainic acid and D- and L-glutamic acids exhibit negative cooperativity with Hill coefficients of near 0.5. This allosteric interaction of glutamic acid at the kainic acid recognition site suggests a biochemical correlate for the synergistic effects of these compounds in vivo.

2. When postmortem alterations in [^{3}H] kainic acid receptor binding are assessed in rat brain incubated for up to 14 hr at temperatures simulating those to which human postmortem brains are normally subjected, no receptor alterations are observed. In the caudate nucleus of human Huntington's disease (HD) brains, total specific binding is significantly reduced by 51%, and binding to high affinity sites is virtually abolished. HD putamen shows a 77% reduction in total specific binding with a 90% reduction in high affinity binding. While HD frontal cortex shows a 47% reduction in total specific binding, no changes are seen in insular cortex or temporal cortex. Similarly, no alterations are observed in HD hippocampus or cerebellum. Thus, losses of kainic acid binding sites are mainly localized to those regions of HD brain that are most severely affected by the disease, and the HA site is profoundly affected. These results have been published in abstract form (London, E.D., Schattschneider, G., Yamamura, H.I. and Coyle, J.T., 1980, Decrease of kainic acid binding sites in Huntington's diseased brain. Fed. Proc. 39: 388).

Significance to Biomedical Research and to the Program of the Institute:

The cooperative effects noted between L-glutamate and kainic acid(KA) at the receptor level, taken with the observation that KA is not toxic when glutamatergic innervation is removed, indicate that at the receptor level KA sensitizes neurons to the neurotoxic potential of glutamate-induced depolarization. Such an action of an endogenous brain substance in over-abundance might be responsible for the degeneration seen in HD and other human disorders. Alternatively, an abnormality in membrane structure may render neurons more sensitive to the neurotoxic action of an endogenous substance, as KA renders striatal neurons sensitive to their afferent input.

Recently, local injections of KA have been used to selectively reduce the cholinergic innervation of the cortex in rats, resembling the major reported deficit in neurochemical senile dementia of the Alzheimer's type. Thus, the information obtained in these studies seem relevant not only to HD, but to several human diseases where specific cell loss may be linked with a membrane receptor-mediated phenomenon.

The demonstration of [^{3}H] KA binding sites in human brain with kinetic characteristics similar to those in the rat model for Huntington's disease (HD) indicate that kainate's neurotoxicity may be relevant to human disease. Notably, the caudate nucleus and putamen, which are severely affected in HD, exhibit the highest densities of high affinity KA binding sites in human brain. The profound loss of [^{3}H] KA binding sites in these brain regions supports the concept that the pathogenesis of HD involves an inbalance in systems which interact with glutamatergic afferents to the basal ganglia.

Proposed Course: The results obtained with [^{3}H] KA binding studies in human postmortem brain will be prepared for publication.

Publications:

London, E.D. and Coyle, J.T.: Cooperative interactions at [^{3}H] kainic acid binding sites in rat and human cerebellum. Eur. J. Pharmacol. 56: 287-290, 1979.

London, E.D., Klemm, N. and Coyle, J.T.: Phylogenetic distribution of [^{3}H] kainic acid receptor binding sites in neuronal tissue. Brain Res. 192: 463-476, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00125-02 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cerebral Metabolism, Relation to Brain Function and Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E.D. London	Sr. Staff Fellow	LNS	NIA
	D. Dow-Edwards	Staff Fellow	LNS	NIA
	N. Shinowara	Staff Fellow	LNS	NIA
	S.I. Rapoport	Chief	LNS	NIA
Others:	H. Holloway	Biol. Lab Techn.	LNS	NIA
	S. Nespor	Biol. Lab Techn.	LNS	NIA
	W. Fredericks	Biologist	LNS	NIA
	M. Ohata	Visiting Fellow	LNS	NIA
	M. Dam	Visiting Fellow	LNS	NIA
	U. Sundaram	Student	LNS	NIA

COOPERATING UNITS (if any)

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

SECTION

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, MD 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.6	3.4	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

(1) Several parameters of cerebral function were studied as a function of age in male Fischer-344 rats: (a) Local cerebral glucose utilization (LCGU) measured by the ^{14}C-2-deoxy-D-glucose technique (Sokoloff et al., 1977) increased in all brain regions between 1 and 3 mo; decreased in 11 of 19 regions between 3 and 12 mo; but remained unchanged between 12 and 34 mo. (b) Regional cerebral blood flow (rCBF) measured with ^{14}C-iodoantipyrine (Sakurada et al., 1978; Ohno et al, 1979) increased between 1 and 12 mo in anterior but not posterior brain regions, and tended to decline between 12 and 24 mo. (c) In the retina, there were comparable age-associated decreases in outer nuclear layer thickness, number of photoreceptor nuclei and glucose utilization. These decrements were correlated with decreased LCGU in the superior colliculus between 3 and 12 mo. (2) In adult rats, central muscarinic stimulation with oxotremorine caused regional increases in LCGU. (3) GABAergic agonist drugs produced variable decreases in LCGU, but increases in the red nucleus of the adult rat.

PHS-6040
(Rev. 10-76)

Objectives: The aim of this project is to examine the morphological, biochemical and functional changes in the brain that occur in relation to development, maturation and aging, to study coupling between regional cerebral blood flow and metabolism in relation to brain aging, and in response to pharmacologic agents. Specifically, the project is designed to 1) describe the time course of changes in local cerebral glucose utilization (LCGU) in the rat during development and aging of the Fischer rat, 2) to measure regional cerebral blood flow (rCBF) during development and aging of the rat brain, 3) to relate retinal morphology to local retinal glucose utilization during aging of the rat, 4) to study the effects of central muscarinic, and 5) GABAergic drugs on LCGU.

Methods Employed: 1. For studies of LCGU, experiments are performed on conscious, partially restrained rats with indwelling femoral artery and venous catheters. ^{14}C-2-deoxy-D-glucose (^{14}C-2-DG) (125 μCi/kg) is injected i.v., and timed arterial blood samples are collected over the next 45-50 min until the rat is killed. Regional brain radioactivity is determined by dissection and liquid scintillation spectroscopy, or by quantitative autoradiographic procedures on 20 μ frozen sections. LCGU is calculated from the tissue radioactivity at kill time, the plasma histories of glucose and ^{14}C-2-DG, and the kinetic constants for ^{14}C-2-DG uptake and metabolism by brain (Sokoloff et al., 1977, J. Neurochem. 28: 897).

2. For studies of rCBF, conscious, restrained rats, prepared as described above, are infused i.v. with ^{14}C-iodoantipyrine and decapitated 45-55 sec later. Regional brain radioactivity and whole blood radioactivity are determined by liquid scintillation spectroscopy. rCBF is calculated by applying the Kety-Schmidt analysis to the data (Sakurada et al., 1979, Am. J. Physiol. 234: H59; Ohno et al., 1979, Stroke 10: 62). Arterial blood pH, P_{CO_2} and P_{O_2} are measured with specific electrodes.

3. Local retinal glucose utilization (LRGU) is measured as is LCGU (see above) on light adapted retinas removed from rats administered ^{14}C-2-DG. Morphological observations are made by light microscopy on plastic-embedded 1 μ-sections of retina strips.

4. The effects of central muscarinic stimulation on LCGU are assessed in adult rats treated with oxotremorine (0.75 mg/kg, i.p.) 2 min before i.v. ^{14}C-2-DG. Atropine methylbromide (1 mg/kg, s.c. 20 min before oxotremorine) is given to block peripheral muscarinic effects. Scopolamine (2.5 mg/kg, i.p.) is given 10 min before oxotremorine to test the specificity of the effect of oxotremorine on central muscarinic receptors.

5. The effects of GABAergic drugs on LCGU are assessed in adult rats injected i.v. with one of several doses of GABAergic agonist (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) drugs prior to the administration of ^{14}C-2-DG.

Major Findings: 1. Age-related changes in local cerebral glucose utilization (LCGU) in the Fischer-344 rat. Between the ages of 1 and 3 mo, LCGU increases in all brain regions (by an average of 20%), but between 3 and 12 mo, LCGU decreases significantly in 11 of 19 regions, with most striking reductions evident in the inferior colliculus, medulla and pons. The average decrease equals 24%, but the nucleus accumbens and septum show no changes in LCGU. LCGU remains unchanged between 12 and 24 mo and between 24 and 34 mo of age. The results demonstrate a rise in LCGU with development, a decline during late maturation in the first year of life, and a constancy in the second and third years that is dissociated from reported senescence-associated alterations within the brain. The results have been presented in abstract form (London, E.D., Nespor, S., Moore, L., Mahone, P. and Rapoport, S.I., 1980, Age dependent changes in local cerebral glucose utilization. Age 2: 131, and London, E.D., Nespor, S. and Rapoport, S.I., Age-associated decreases in local cerebral glucose utilization, in Neural Regulatory Mechanisms During Aging, ed. by R.C. Adelman, G. Baker, V. Cristofalo and J. Roberts. Alan R. Liss, Inc. New York, 1980).

2. Age-related changes in regional cerebral blood flow (rCBF) between 1 and 34 months in the Fischer-344 rat. In 1 mo old rats, mean rCBF in 9 anterior brain regions is 92 ml.100 g^{-1}.min^{-1} and is 76 ml.100 g^{-1}.min^{-1} in 4 posterior brain regions. rCBF increases significantly ($p < 0.05$) in the anterior regions between 1 and 3 mo of age, and continues to increase after 3 mo to a mean of 132 ml.100 g^{-1}.min^{-1} at 12 mo, but does not change significantly in the posterior brain regions between 1 and 12 mo. rCBF tends to decline between 12 and 24 mo, more consistently in the posterior than anterior brain regions. White matter rCBF does not change with age. The findings indicate that rCBF increases during development of the anterior portion of the rat brain, between 1 and 12 mo, whereas the posterior part of the brain, which is more mature than the anterior part by 1 mo, shows no significant changes during this period. The decline in rCBF in some regions after 12 mo of age may reflect some of the degenerative changes that occur within the brain from 12 mo to death at 36 mo. rCBF does not follow the same temporal pattern during maturation and aging of the rat brain as does LCGU (see above). Separate time courses suggest that the sensitivity of the cerebrovascular bed, to metabolic factors which regulate rCBF, increases between 3 and 12 months. An abstract of this work has been published (Ohata, M., Sundaram, U., Fredericks, W.R., London, E.D. and Rapoport, S.I. Age-associated alterations in regional cerebral blood flow in the Fischer-344 rats. Abstr. Tenth Annual Meeting Soc. Neurosciences, 1980).

3. Retinal morphology and glucose utilization at different ages of the Fischer-344 rat. There is a progressive loss of photoreceptors in the albino Fischer-344 rats in relation to aging and light exposure. The changes correlate in part with changes in the light-adapted retina of local retinal glucose utilization (LRGU), which can be taken as a measure of functional activity of the retina. Between 1 and 3 mo, LRGU does not change when, at the same time, there is a 32% loss in photoreceptor nuclei number in the peripheral, equatorial and central portions of the retina and also in the

superior colliculus, which receives input from retinal ganglion cells. LRGU decreases between 3 and 12 months of age from 59 to 37 μmol.100 g^{-1}.min^{-1}, remains at 34 μmol.100 g^{-1} at 24 months and then declines to 15 μmol.100 g^{-1}.min^{-1} at 34 months of age. These decreases closely parallel the continuous decreases by up to 86%, in mean number of photoreceptor nuclei between 3 and 34 months of age. Thus, LRGU does not reflect photoreceptor loss between 1 and 3 mo but after time demonstrates that such loss correlates with a reduced retinal metabolism. Small decreases of LCGU in the superior colliculus after 3 mo of age indicate that this structure retains substantial ganglion cell input despite severe retinal degeneration. Abstracts of this work have been published (Shinowara, N.L., London, E.D. and Rapoport, S.I. Comparisons of retinal changes in morphology and glucose utilization in aging Fischer-344 rats. In Neural Regulatory Mechanisms During Aging, ed by R.C. Adelman, G. Baker, V. Cristofalo and J. Roberts. Alan R. Liss, Inc. New York, in press, 1980, and Shinowara, N.L., Rapoport, S.I., Hoover, M.J. and London, E.D. Changes in regional retinal morphology and local glucose utilization in 1 to 34 month Fischer-344 rats. Abstr. Tenth Annual Meeting Society Neurosciences., 1980).

4. Effect of a muscarinic cholinergic agonist on cerebral metabolism in the Fischer-344 rat. Although many cerebral regions show no change in LCGU following oxotremorine administration, LCGU falls significantly ($p < 0.05$) in the sensory-motor and parietal cortices, the hippocampus, globus pallidus, caudate nucleus, nucleus accumbens, medial and lateral septal nuclei, olfactory bulb, and cerebellar gray matter. Several diencephalic nuclei show increased LCGU; these include the ventral and anteroventral thalamic nuclei, the lateral geniculate nucleus and the lateral habenula. In the mesencephalon, the red nucleus and superior colliculus show 2-fold increases in LCGU, and a 50% increase in the substantia nigra and pars compacta. In every region where oxotremorine increases LCGU, this increase is blocked by the antimuscarinic drug scopolamine (2.5 mg/kg, i.p., 10 min before oxotremorine) but not by atropine methylbromide (1 mg/kg, s.c. 20 min before oxotremorine), which does not readily penetrate the blood-brain barrier. Scopolamine (2.5 mg/kg i.p.) without oxotremorine has no significant effect on LCGU. These findings indicate that increased LCGU in response to oxotremorine is caused by stimulation of central muscarinic receptors. However, there is no simple correlation between the magnitude of the increase in LCGU and reported densities of high-affinity muscarinic binding sites. Specific pharmacological stimulation of cerebral metabolism therefore depends on the presence of relevant receptors for the administered drug and on neuronal circuitry of a given brain region. An abstract of this work has been published (Dow-Edwards, D., Peterson, J.M., Mahone, P., Rapoport, S.I. and London, E.D.: Effect of oxotremorine, a muscarinic agonist, on local cerebral glucose utilization (LCGU) in the rat. Abstr. Tenth Annual Meeting Soc. Neurosciences, 1980).

5. Effect of GABAergic drugs on cerebral metabolism in the Fischer-344 rat. GABA agonists induce dose-dependent depressions in LCGU in most brain regions assayed. Decreases in LCGU are most dramatic in forebrain regions, especially cerebral cortex, caudate nucleus and thalamic nuclei. There are variable reductions in LCGU within the hippocampus. In contrast, LCGU in the red nucleus is increased by up to 100% with a high dose of muscimol (7 mg/kg). A subconvulsant dose of bicuculline (294 μg/kg) increases LCGU in several regions, notably the auditory cortex and inferior colliculus. As with the effect of oxotremorine on LCGU, there is no simple correlation between the magnitude of effect on LCGU and reported densities of GABA receptors. This is further evidence that specific pharmacologic activation or inhibition of LCGU is influenced by the presence of relevant receptors for the administered drug and by the neuronal input to a given brain region. An abstract of this work has been published (London, E.D., Palacios, J.M., Rapoport, S.I. and Kuhar, M.J. Local cerebral glucose utilization (LCGU) following systemic administration of GABAergic agonist and antagonist drugs. Abstr. Tenth Annual Meeting of the Society of Neurosciences, 1980.

Significance to Biomedical Research and the Program at the Institute:

1. A knowledge of the regional disturbance of metabolic deficits with age can direct future research to identify particular transmitter systems which may be defective in senescence in man. Our observation that LCGU in the resting state does not decline with aging of the rat may mean that functional defects in the brain in relation to aging must be identified by pharmacological or physiological challenges.

2. Cerebral function is thought to be reflected by rCBF as it is by LCGU, because of the coupling of both parameters to neuronal activity. The different time course of rCBF as compared to LCGU, during development and aging of the rat brain, suggests that rCBF and LCGU are not indices of the same functional parameters, and that both must be examined in man when interpreting aging changes of the brain.

3. Retinal degeneration is a process which may be influenced by age, light and/or disease. Our present study establishes a foundation for correlating changes in glucose utilization in the light-adapted albino rat retina with morphological changes involved in retinal degeneration.

4. Local cerebral metabolic responses to stimulation by the muscarinic agonist oxotremorine are relevant to the pharmacology of aging and senile dementia of the Alzheimer's type. In view of presynaptic muscarinic loss in aging and to a much greater extent in dementias, treatment has been designed to enhance central muscarinic (cholinergic) neurotransmission. Our findings indicate the potential for untoward effects of direct receptor agonist therapy, particularly due to activation of the extrapyramidal motor system.

5. The pattern of local cerebral metabolic responses to GABAergic drugs can help elucidate functional GABAergic pathways in the brain. In view of the known interaction between GABAergic systems and benzodiazepines, such information could help explain the untoward central effects of benzodiazepines in the elderly.

Proposed Course: The work on age-related changes in LCGU, rCBF, retinal morphology and retinal glucose utilization in the rat will be prepared for publication. The results with rCBF will be correlated with those for LCGU during aging. The effects of oxotremorine and GABAergic drugs on LCGU will be further investigated by increasing the sample size and testing different drug dosages.

Publications:

Rapoport, S.I., Ohata, M. and London, E.D. Regional cerebral blood flow and local cerebral glucose utilization following opening of the blood-brain barrier and during maturation of the rat brain. Fed. Proc., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00126-01 LNS

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Brain Function in Aging and Disease in Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.A. Margolin	Staff Fellow/Clinical Associate	LNS	NIA
	E.A. Robertson-Tchabo	Psychologist (IPA)	LNS	NIA
	S. I. Rapoport	Chief	LNS	NIA
Others:	E.D. London	Senior Staff Fellow	LNS	NIA
	L. Sokoloff	Chief	LCM	NIMH
	R.M. Kessler	Staff Physician	CC	NM

COOPERATING UNITS (if any)
D. Ingvar, Neurologist, University of Lund, Sweden

LAB/BRANCH
Gerontology Research Center, Laboratory of Neurosciences

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 (200 words or less - underline keywords)

A clinical protocol was written to study cerebral glucose utilization in man in relation to aging, which employs positron emission tomography and ^{18}F-2-deoxy-D-glucose as a tracer of cerebral glucose utilization. The protocol was approved and technical aspects of the emission tomography method were refined. Subjects were screened for the absence of neurological disease and for the study, which will commence in the forthcoming year.

PHS-6040
(Rev. 10-76)

Project Description

Objectives: The aim of this project is to relate behavioral and cognitive performance in normal man at different ages to local cerebral glucose utilization (LCGU).

Methods Employed: Male subjects in three age categories: 20-30 years, 40-50 years and 65+ years, are screened by medical diagnostic procedures for the absence of neurologic and cardiorespiratory disease, including arteriosclerosis. Personality and psychometric tests and a comprehensive neuropsychological battery are administered to evaluate cognitive performance. Electroencephalography and computerized axial tomography are employed to evaluate neurological status. Positron Emission Tomography (PET scanning) is employed to evaluate the brain uptake of intravenously injected ^{18}F-2-deoxy-D-glucose. Computerized techniques are used to calculate local cerebral glucose utilization (LCGU) from brain uptake and time courses of plasma radioactivity and glucose consumption.

Major Findings: A clinical protocol was written and was approved in April 1980. Fifty volunteers from the community were identified for the study and have been medically screened. Of these, twelve are free of evident primary or secondary neurological disease and are undergoing complete evaluation prior to PET scanning. Procedures were established for the measurement of LCGU in Bethesda. This year was used to initiate an operational clinical program at the Gerontology Research Center (GRC) and the Baltimore City Hospitals (BCH), and to establish liaison with Nuclear Medicine and collaborators at NIH in Bethesda.

Significance to Biomedical Research and the Program at the Institute: It is critical to establish the relation between cognitive function and regional cerebral metabolism in normal man as a function of age. Not only will these measures help us to understand age-related changes in the way man thinks, but they will establish reference base lines for the study of central nervous system diseases.

Proposed Course: Cerebral metabolism will be measured in screened subjects with positron emission tomography.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00010-07 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Echocardiographic Assessment of the Left Ventricle and Mitral Valve in Aging Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA

Other: None

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	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Earlier echocardiographic work from this laboratory has shown that in normal men the left ventricular thickness and aortic root diameter increase as a function of age whereas cavity size, ejection fraction and velocity of shortening are unchanged and mitral valve closure slope is reduced. Since this initial project was limited to men with a maximum blood pressure of 140/90, we have extended this study to women and extended the blood pressure range for entrance into the study of both sexes across a spectrum of ages (18-95).

A research contract involving the Cardiology Division of Johns Hopkins Hospital to evaluate cardiac anatomy and function at rest and during exercise began on 1 November 1977 and will extend for three years. Two-dimensional exercise echocardiography and thallium^{201} myocardial perfusion scanning are being employed. This is discussed in the appendix to this report.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: Echocardiography is an ideal non-invasive method for assessing age-related changes in cardiac anatomy and function. We have been studying the pattern of these changes in normal women, to compare with the findings in normal men. Computer-assisted analysis of the relationships among echocardiographic parameters is also being performed in both sexes. Similarly, the relationships between blood pressure and various echocardiographic indices are being investigated. Hypertension, for example, is known to induce hypertrophy (abnormal thickening) of the left ventricular walls. Echocardiography is being employed to quantify this hypertrophy and determine whether age-associated increases in blood pressure are correlated with ventriculary hypertrophy. Similarly, age-related changes in left ventricular filling rates, as measured by the mitral valve closing velocity, are being correlated with ventricular wall thickness, blood pressure and other parameters. These analyses will provide a framework for understanding the factors responsible for the age-related changes in cardiac structure and function.

Methods: Echocardiograph assessment of the aging heart includes two major projects. Normal men and women in the Baltimore Longitudinal Study and those with uncomplicated hypertension receive resting echocardiograms. Left ventricular wall thickness, systolic and diastolic dimensions and rate of endocardial shortening, mitral valve closure rate, plus aortic root and left atrial dimensions are measured. The data are entered into a computer and stepwise regression analysis is performed.

Major Findings: The results in normal men were presented by Gerstenblith et al., Circulation 56: 272-278, 1977. In normal women, the aortic root diameter, left ventricular dimensions and left ventricular ejection fraction and velocity of fiber shortening were not age-related whereas the following were:

	n	Age Regression	r	P
LA/m^2	98	0.05 age + 16.4	.297	0.001
E-F	97	-0.685 age + 113.9	.411	0.001
$LVPWT_D/m^2$	91	0.02 age + 3.35	.356	0.001
$LVPWT_S/m^2$	90	0.02 age + 6.70	.283	0.01
IVS_D/m^2	85	0.04 age + 3.10	.488	0.001
IVS_S/m^2	81	0.04 age + 5.61	.416	0.001

LA = left atrial size, E-F = mitral valve closure rate, LVPWT = left ventricular posterior wall thickness in diastole and systole, IVS_2 = interventricular septal thickness in diastole and systole, m^2 = per square meter of body surface area.

These findings indicate that aging in women as in men is

associated with mild but definite left atrial enlargement, concentric left ventricular hypertrophy, and diminished diastolic filling rate. The magnitude of these age-related changes appears to be smaller in women than in men.

When basal systolic blood pressure and $LVPWT_D/m^2$ in a given subject were compared across the age range, $LVPWT_D/m^2 = 0.02\ SBP_b + 23$, $P<0.002$. This suggests that the moderate progressive increase in SBP_b that occurs as man ages is a significant determinant of the mild left ventricular hypertrophy which occurs with aging. We suspect that the age-related diminution in left ventricular filling rate may be related at least in part to this increase in LVPWT.

Significance to Biomedical Research and the Program of the Institute: Information concerning the anatomic and functional aging changes of the normal human heart is critical to understanding the aging process of the cardiovascular system. Similar information is needed in aged "hypertensives" (over 10% of the population) to examine the effects of hypertension on the heart and their interrelationships with the aging process.

Proposed Course: The current project will be continued at least through the next fiscal year.

Publications: None.

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. of Medicine
M. L. Weisfeldt, Dir., Div. of Cardiology
J. Weiss, Asst. Prof. of Medicine
L. Beacker, Asst. Prof. of Medicine

Money Allocated: $614,168.00

Objectives: Two-dimensional echocardiography is a 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 goal is to examine subjects from the Baltimore Longitudinal Study over a three year period (ages 18-95) during rest and maximal semi-supine bicycle exercise to determine age-related differences in regional and global myocardial function.

Thallium^{201} myocardium imaging allows non-invasive assessment of regional left ventricular blood flow 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. About 150 new subjects will be studied for each of three years. In year 5 the subjects in year 1 will be retested.

Methods: Subjects perform maximal graded semi-supine bicycle ergometry and simultaneous ECG and blood pressure monitoring every minute. Two-dimensional echograms are made in longitudinal and cross-sectional views of the left ventricle at rest, maximal exercise and during recovery with regard to the following echocardiographic indices (1) myocardial mass, (2) left ventricular chamber size, (3) mean and maximal velocity of fiber shortening and lengthening, (4) percent change plus mean and maximal velocities of regional left ventricular systolic thickening and diastolic thinning, (5) percent, mean and maximal rates of change in regional left ventricular radius and (6) duration of left ventricular ejection and filling.

Thallium imaging is performed in four views following peak exercise of a multi-stage maximal treadmill test. Subjects found to have perfusion defects after stress are re-imaged in 2 hours (without further nuclide injection) to determine whether there is any "filling in" of the defect.

Major Findings: To date approximately 300 subjects have undergone two-dimensional echocardiography. Analysis of the resting echocardiograms is in progress. Exercise tracing have been difficult to obtain, especially in elderly subjects. We have compared the echocardiographic responses to supine bicycle exercise in young subjects (mean age 31±1 yr) versus old subjects (mean age 64±3 yr). 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 suggest age-related differences in the mechanisms of increasing stroke volume during exercise.

Nearly 300 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 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 suggests that stress thallium scintigraphy may provide a useful epidemiological tool to enhance the detection of CHD.

Significance to Biomedical Research and the Program of the Institute: Resting two-dimensional echocardiography allows detailed non-invasive analysis of cardiac structure and ventricular function and should expand our findings from M-mode echocardiography. Exercise two-dimensional echocardiography may help to elucidate the mechanisms for the diminution in maximal cardiac performance with age and may help 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 exercise electrocardiography and thallium scanning represents a new epidemiologic approach 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 of the disease process.

Proposed Course: Both two-dimensional echocardiography and thallium^{201} myocardial scanning should prove valuable tools for longitudinal assessment of cardiac wall motion and myocardial perfusion respectively and should allow the response to numerous medications and physiological interventions to be determined, both in normals of various ages and in patients with heart disease. A two year extension of this contract is being sought, hopefully allowing us to complete our cross-sectional study of the Baltimore Longitudinal Study population.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00024-04 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Response to Cardiac Glycosides in Senescent Dogs, Rats, and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
Other:	J. P. Froehlich	Medical Officer	LMA, NIA

COOPERATING UNITS (if any)
T. Guarnieri, Dept. of Medicine, Johns Hopkins Medical Institutions
G. Gerstenblith, Div. of Cardiology, Dept. 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:
0	0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Investigation of the role of aging on the: (1) effect of cardiac glycosides on electrophysiology and mechanical performance in intact dogs; and (2) effect of cardiac glycosides and Na^+-K^+ ATPase inhibition in cardiac muscle from dogs.

Combined into Project Z01 AG 00029-03 CPB.

GRC/CPB-247

Project Description:

Combined into Project Z01 AG 00029-03 CPB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00026-04 CPB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Age-Associated Alterations in Response to Catecholamines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	C. R. Filburn	Staff Fellow	LMA, NIA
	E. S. Beard	Chemist	CPB, NIA
	G. S. Roth	Research Chemist	CPB, NIA

Others: None

COOPERATING UNITS (if any)

T. Guarnieri, 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:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work from this laboratory has demonstrated that when compared to the young adult rat myocardium, that from aged rats exhibits a diminished inotropic response to catecholamines. In addition, the response to these agents in both the canine model and in man is diminished in senescence, compared to that observed in mature adults. The present work includes measurements of cyclic nucleotide levels, and protein kinase activation in perfused rat interventricular septa which have been stimulated with isoproterenol and dibutyryl cAMP, and in which the mechanical response to these agents has been quantitated. -receptor number and affinity as well as cAMP-protein kinase stimulation of Ca^{++} accumulation in isolated sarcoplasmic reticulum have also been measured.

Combined into Project Z01 AG 00034-01.

GRC/CPB-249

Project Description:

Combined into Project Z01 AG 00034-01.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00029-03 CPB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Efficacy of Chronic Digoxin Therapy in Stable Congestive Heart Failure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. L. Fleg	Staff Cardiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)

S. H. Gottlieb, Div. of Cardiology, Dept. of Medicine, Baltimore City Hospital
Div. of Chronic Medicine, Dept. of Medicine, Baltimore City Hosp.

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 (200 words or less - underline keywords)

Despite the nearly universal employment of digitalis in the treatment of chronic congestive heart failure (CHF), there is no objective documentation of its long-term efficacy. The current project is designed to evaluate, in a double blind crossover fashion, the effects of maintenance digoxin therapy in chronic stable CHF, utilizing clinical findings, chest radiography, echocardiography and systolic time intervals to assess baseline left ventricular function non-invasively and maximal exercise testing to determine aerobic work capacity.

GRC/CPB-251

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: For some 200 years, digitalis glycosides have been the cornerstone of therapy for CHF. The low toxic therapeutic ratio of digitalis results in the development of toxicity in 20-25% of patients and death in up to 20% of patients developing toxicity. Despite this universal employment of digitalis in the treatment of CHF, there is no objective documentation of its long-term efficacy. The current project utilizes several non-invasive parameters of cardiac function to assess the effects of maintenance digoxin therapy in stable CHF in a double blind crossover manner.

Methods: Patients in the Baltimore City Hospital Cardiology Clinic and the Chronic Hospital in normal sinus rhythm who had been on the same CHF medical regimen for at least 3 months were studied. After a baseline clinical history and physical exam, each patient received a resting echocardiogram, systolic time intervals (STI), chest x-ray, and when possible, a maximal stress test. He was then randomly assigned in a double blind fashion to either digoxin or placebo for 3 months and the studies repeated at the end of this period. Crossover then occurred and the testing was repeated again 3 months later.

Major Findings: None of the 30 patients studied required reinstitution of digoxin. No difference was found in symptoms or clinical signs, including resting heart rate (HR), blood pressure, and weight between digoxin and placebo regiments. Similarly the cardiothoracic ratio (CTR) on chest x-ray, the ratio of pre-ejection period to ejection time (PEP/LVET) as well as the duration of maximal treadmill exercise (TM_{max}) were not affected by withdrawal from digitalis. Echocardiographically determined left ventricular end-diastolic dimension (LVED) was slightly larger and velocity of circumferential fiber shortening (V_{cf}) slightly diminished on placebo. The duration of electromechanical systole ($QS2_i$) corrected for heart rate was prolonged on placebo. These results are summarized in the table below and have been presented in part at the American Heart Association's Annual Scientific Sessions, November 1979 (Fleg et al., Circulation 60: Suppl. 2, II-178, 1979).

	HR	Weight (lb)	CTR	TM_{max} (min)
Digoxin	68.0±2.5	155.6±5.7	0.54±0.01	7.64±0.75
Placebo	69.8±2.5	155.0±5.8	0.54±0.01	7.80±0.80
P	NS	NS	NS	NS
N	30	30	27	12

	LVED (mm)	V_{cf} (circ/sec)	PEP/LVET	$QS2_i$ (msec)
Digoxin	55.8±2.3	0.90±0.08	0.467±0.02	525.6±4.6
Placebo	57.8±2.2	0.82±0.06	0.467±0.02	548.5±4.5
P	0.001	0.05	NS	0.001

N	21	19	28	28

These results suggest that chronic digoxin administration has no apparent clinical effect, at rest or during exercise in ambulatory patients in normal sinus rhythm with stable CHF. Chronic digoxin therapy elicits small improvements in noninvasive resting indices of cardiac pump and muscle function. Though statistically significant, these changes have no apparent clinical impact. The population studied, representative of the majority of ambulatory patients who are treated with digoxin, can be successfully managed without this drug.

Significance to Biomedical Research and the Program of the Institute: If maintenance digitalis can be discontinued without ill effect in a sizable percentage of patients with stable chronic CHF, many episodes of toxicity (and death) could be avoided and medical expenses reduced. It should be noted that a sizable portion, if not the majority, of persons in whom digitalis preparations are prescribed are over 60 years of age.

Proposed Course: Long-term follow-up study of the patients reported on here is planned. In addition exercise radionuclide cineangiography and 24-hour ambulatory electrocardiography both on and off digitalis are planned, to search for changes in ventricular size and function during exercise as well as changes in cardiac rhythm elicited by digoxin. New patients in addition to those previously studied will be solicited for these studies.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00030-02 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Altered Relaxation and Stiffness in the Senescent Rat Heart: Relation to Hypertrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. A. Spurgeon	Physiologist	CPB, NIA
	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	E. S. Beard	Chemist	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)
F. C. P. Yin, Div. 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 (200 words or less - underline keywords)

With advancing age, several characteristic alterations occur in the cardiovascular system. One of the most commonly described changes in senescence is a moderate degree of myocardial hypertrophy in both animals and in man. Other alterations seen in the senescent hearts are a prolonged time course of relaxation (Weisfeldt et al., Am. J. Physiol. 220: 1921-1927, 1971; Lakatta et al., J. Clin. Invest. 55: 61-68, 1975), a diminished rate of sarcoplasmic reticulum calcium accumulation (Froehlich et al., J. Mol. Cell. Cardiol. 10: 427-438, 1978) and an increased active dynamic stiffness in senescent compared with mature heart muscle (Spurgeon et al., Am. J. Physiol. 232(4): H373-H380, 1977) and intact heart (Templeton et al., Circ. Res. 44: 189-194, 1979). Although the extent to which these functional alterations seen with senescence are related to the concurrent hypertrophy is unknown, a relationship is suggested since prolonged contraction duration, diminished sarcoplasmic reticulum function, and increased stiffness have been observed in experimental models of hypertrophy. GRC/CPB-254

Project Description:

Objectives: The goals of this study were (1) to determine the extent of hypertrophy and its relationship to alterations in relaxation and stiffness as a function of age in the rat model, (2) to create a mechanical overload hypertrophy of the same extent in adult rats to delineate the resulting functional alterations, (3) to ascertain whether or not the alterations due to experimental hypertrophy are qualitatively and quantitatively analogous to the changes seen in the normal hypertrophied senescent heart, and (4) to determine whether physical conditioning would prevent the age-related changes in contraction duration and stiffness.

Methods: (1) Heart weight and heart weight to tibial length ratios were measured in adult rats ages 6, 15, and 24 months of age to determine the extent of myocardial hypertrophy. (2) Mechanical parameters of the isometric twitch were studied in muscle isolated from hearts of rats of the ages noted above. (3) Aortic banding was implemented in middle-aged rats in order to induce hypertrophy of the same extent found in the senescent heart. (4) Male rats at 3 and 19 months were run in motorized wheels (.7KMH) for 30 minutes, 5 X week, for 5 months and sacrificed with matched controls, at age 8 (adult) and 24 (senescent) months.

Major Findings: Using tibial length as a reference, we found 14% LV hypertrophy in senescent compared to both young and middle-aged rats, indicating that the hypertrophy occurred during the last quarter of life. The senescent muscles demonstrated a 25% prolongation in contraction duration and a 30% increase in slope of the active stiffness-tension line (α_A) compared to both young adult and middle-aged muscles. Compared to middle-aged muscles the banded muscles demonstrated a similar spectrum of change in mechanical properties as the senescent muscles (8% increase in contraction duration and 15% increase in α_A), but the quantitative differences between the banded and senescent muscles were significant. Over the functional range of developed tensions, the banded muscles demonstrated the lowest and the senescent muscles the highest values of stiffness. The findings suggest that a portion of the mechanical property alterations seen in the senescent heart are due to the underlying hypertrophy. However, the hypertrophy produced by mechanical loading of the LV cannot explain all of the senescent changes. The moderate exercise protocol did not alter body or heart weight in either adult or senescent. Isometric left ventricular trabeculae studied at L_{max}, 24 min^{-1}, 29°C, in Krebs buffer (Ca^{++} = 2.5 mM) produced the following results:

	N	Developed Tension (g/mm^2)	Maximal Rate of Tension ($g/mm^2/sec$)	Contraction Duration (msec)
Adult-Control	11	2.38±0.34	39.3±4.6	188±7
Senescent-Control	9	2.65±0.22	39.7±4.9	232±12
Adult-Exercised	14	2.66±0.21	43.4±3.4	193±4
Senescent-Exercised	9	3.14±0.47	52.3±8.5	199±5

Developed tension and maximal rate of tension development were not age-related and were not altered by exercise. Contraction duration, while prolonged in senescent-control relative to adult -control, $P<.01$, was unaltered by exercise in the adult; exercise prevented the prolonged contraction duration in senescent -exercise, $P<.05$ versus senescent-control, and adult-exercise or adult-control versus senescent-control were not different. Exercise altered slope stiffness in the senescent group only and changes in stiffness paralled those in contraction duration. The effect of exercise to prevent prolongation of contraction duration in senescent may be mediated by a change in velocity of sarcoplasmic reticulum Ca^{++} sequestration which has been shown to be diminished in senescent and enhanced by physical conditioning.

Significance to Biomedical Research and the Program of the Institute: The results are significant to biomedical research because they encourage further studies to elucidate the mechanism for the age difference in relaxation and stiffness. If this can be determined it will extend our knowledge regarding the contraction and relaxation process in heart per se. The results are significant to the program of the Institute in that two of the most distinctive features of aged hearts of many species, including man, are hypertrophy and slowing of relaxation. This is of potential clinical significance during times of stress, at high heart rates, when diminished relaxation could interfere with ventricular filling and lead to enhanced dyspnea and functional impairment. It appears that the diminished filling and slowed relaxation may be in part due to the hypertrophy per se. Furthermore, the results of the physical conditioning study indicate that increased stiffness and prolonged contraction duration that occur in the senescent heart are not fixed but can be modified by physical conditioning.

Proposed Course: To expand the physical conditioning studies in view of the promising initial results.

Publications:

Yin, F. C. P., Spurgeon, H. A., Weisfeldt, M. L., and Lakatta, E. G.: Mechanical properties of myocardium from hypertrophied rat hearts: a comparison between hypertrophy induced by

senescence and by aortic banding. Circ. Res. 46: 292-300, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00031-02 AG

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Length-Dependence of Excitation-Contraction Coupling in Cardiac Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
Other:	None		

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	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Recent evidence suggests that the mechanism underlying Starling's law of the heart involves a length-dependence of excitation-contraction process, or in other words, at shorter muscle lengths, contractile activation is less than at longer lengths. Further evidence to support this concept is sought by examining the force staircase following a change in the frequency of stimulation or bathing fluid [Ca^{++}] of isolated cat papillary muscles.

Combined into Project Z01 AG 00035-01 CPB

GRC/CPB-258

PHS-6040
(Rev. 10-76)

Project Description:

Combined into Project Z01 AG 00035-01 CPB.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00032-02 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Contractile, Biochemical and Electrical Catecholamine Responses in Hyperthyroid Heart

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	C. R. Filburn	Staff Fellow	LMA, NIA
	E. S. Beard	Chemist	CPB, NIA
	J. Y. Wei	Staff Fellow DOD 7/4/80	CPB, NIA
Other:	None		

COOPERATING UNITS (if any)
T. Guarnieri, Department of Medicine, Johns Hopkins Medical Inst.

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 (200 words or less - underline keywords)

The apparent increase in adrenergic activity observed in the organism in the hyperthyroid state in the presence of a normal level of circulating catecholamines may result from enhanced responsiveness to basal or threshold concentrations of catecholamines. This may result from an increase in the number of β-receptors demonstrated recently. The purpose of the present study was to measure the cAMP-dependent protein kinase activation and contractile response subsequent to threshold levels of β-stimulation with isoproterenol in the hyperthyroid and euthyroid heart. In addition, since the response to catecholamines is mediated by an increase in transsarcolemmal Ca^{2+} influx this was monitored via transmembrane action potential measurements in the hyperthyroid and euthyroid hearts.

GRC/CPB-260

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The objectives of these studies are to characterize (1) the near threshold and maximal response to contractile performance and biochemical mediators of contractile performance in hyperthyroid and euthyroid hearts of β-adrenergic stimulation, and (2) the response of simultaneously measured transmembrane action potential and isometric twitch to threshold concentrations of isoproterenol in cardiac muscle isolated from hearts of euthyroid and hyperthyroid rats.

Methods: (1) Perfused interventricular septal preparations from hyperthyroid and euthyroid rats were employed in part one of this study. Contractile performance was measured under (a) basal conditions, and (b) during low levels of β-adrenergic stimulation (10^{-9} isoproterenol). Under each condition the septum was quick frozen and analyzed for the level of cAMP-dependent protein kinase activation. (2) Stimultaneous transmembrane action potential and isometric contraction were measured in right ventricular papillary muscles from hyperthyroid (6.4 mg/kg/day i.m. thyroxine) rats. Measurements were made at control and after low concentrations of isoproterenol.

Major Findings: (1) Perfused Septa. The contractile response measured as maximal rate of force development (dF/dt) to a near threshold concentration of isoproterenol (10^{-9}M) was enhanced in perfused interventricular septa from hyperthyroid (128±4% control) compared to euthyroid rats (105±2%, $P<.01$). This enhanced contractile response was accompanied by a significant activation of cAMP-dependent protein kinase (protein kinase activation ratio increased from .159±.008 to .218±,019, $P<.005$, while no significant changes from baseline occurred in euthyroid septa .152±.007 to .179±.012, NS). No difference between hyperthyroid and euthyroid hearts was observed in the contractile response to $1X10^{-4}$ dibutyryl cAMP (126.5±2.5% and 122.0±9.2% in hyperthyroid and euthyroid, respectively). The magnitude of the response to dibutyryl cAMP was comparable to that observed in the hyperthyroid group with 10^{-9} isoproterenol. These results suggest that the mechanism for enhanced protein kinase activation and contractile response to low concentrations of isoproterenol in the hyperthyroid heart is at or proximal to cAMP generation. (2) Isolated Papillary Muscles. The enhanced contractile response to isoproterenol noted in the septal preparation of hyperthyroid rats was reproducible in isolated papillary muscles. Moreover, the increase in Ca^{2+} influx, as evidenced by the transmembrane action potential parameters (area above -40mV and time to 75% repolarization) was also enhanced in hyperthyroid hearts. This data was presented at the 30th Fall Meeting of the American Physiological Society in New Orleans, October 1979 and is published in The Physiologist 22: 130, 1979.

	N	*Δ RMP (mv)	Δ A-40 (mv·msec)	Δ T75 (msec)	Δ dT/dt ($mN/mm^2/sec$)
Hyperthyroid	7	1.9±1.1	29.8±7.2	21.1±7.4	46.7±18.4
Euthyroid	8	-0.1±0.4	10.8±2.1	4.2±2.2	3.2±3.8
		NS	<.05	<.05	<.05

*Changes from control in response to 10^{-9} isoproterenol.

Significance to Biomedical Research and the Program of the Institute: The enhanced threshold contractile response, which we have demonstrated for the first time, provides a mechanism to explain in part the apparent hyperadrenergic state in hyperthyroidism. Furthermore, the results strongly suggest that the supersensitivity to catecholamines in the hyperthyroid heart is mediated via a greater Ca^{2+} influx across the sarcolemma in response to β-adrenergic stimulation. The results also provide a logical explanation why treatment of hyperthyroidism with β-adrenergic blocking agents has been successful.

Proposed Course: To determine whether the enhanced threshold contractile response to β-adrenergic stimulation in the hyperthyroid heart is in part related to a concomitant alteration in cholinergic modulation.

Publications:

Guarnieri, T., Filburn, C. R., Beard, E. S., and Lakatta, E. G.: Enhanced contractile response and protein kinase activation to threshold levels of β-adrenergic stimulation in hyperthyroid rat heart. J. Clin. Invest. 65: 861-868, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00033-02 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Ambulatory Electrocardiography in a Healthy Elderly Population

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. L. Fleg Staff Cardiologist

Other: E. G. Lakatta Chief, Cardiovascular Section CPB, NIA

COOPERATING UNITS (if any)
H. L. Kennedy, Div. of Cardiology, USPHS Hospital, 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:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although anti-arrhythmic medications and cardiac pacemakers are used most frequently in the elderly, relatively little attention has been focused on characterizing the patterns of the heart beat in normal elderly subjects over an extended period. In the current project, 104 healthy men and women from the Baltimore Longitudinal Study (BLS) over the age of 60 have undergone ambulatory electrocardiography for a 24-hour period, during part of their regular 2 1/2 day visit to the Gerontology Research Center. A small 2-channel Holter recorder has been employed.

GRC/CPB-263

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The prevalence of supraventricular and ventricular ectopy of all types, conduction disturbances, disorders of impulse formation, repolarization abnormalities and characterization of diurnal variation has been obtained over a 24-hour period via a 2-channel Holter monitor.

Methods: One hundred and four men and women from the Baltimore Longitudinal Study (BLS) over the age of 60 have been studied. Subjects with a BP greater than 160/95, diabetes requiring drug or dietary therapy, overt or suspected heart disease, or chronic obstructive lung disease were excluded. Heart disease was ruled out by history and physical exam, ECG, maximal treadmill exercise test and chest x-ray. Patients with an FEV_1 less than 60 percent of their predicted value were no studied.

A small 2-channel Holter recorder weighing about 1 lb. was attached to the patient after informed consent had been obtained. The subject wore the device for a 24-hour period, while he underwent the standard battery of tests for participants in the BLS. He kept a diary of activities and symptoms (particularly chest pain, dizziness, palpitations or shortness of breath) on a standard form which is routinely used for that purpose. At the end of 24 hours, the recorder was detached and the tape send to Drs. Harold Kennedy and Dennis Caralis at the USPHS Hospital for analysis.

The tapes were analyzed by a high speed scanning system with manual validation. Hourly quantitation of ventricular and supraventricular premature beats (VPB's and SVPB's) was made as well as changes in heart rate and ST segments. This data will be correlated with the extensive information available on these subjects (activity, smoking pattern, weight, nutritional history, etc.). The prognostic significance of the Holter monitor findings should be readily assessed by clinical followups of these BLS participants over a 5-year period.

Major Findings: Supraventricular and ventricular ectopy occur in the majority of a healthy active geriatric population. High degree AV conduction disturbances, profound sinus bradycardia and ischemic repolarization patterns are rare 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.

Significance to Biomedical Research and the Program of the Institute: Because such a large percentage of healthy elderly subjects demonstrates ectopic beats, the significance of such findings in patients with cardiac symptoms is diminished unless the

symptoms and dysrhythmias occur concomitantly. Profound sinus bradycardia and high degree atrioventricular block, however, should be considered distinctly abnormal.

Proposed Course: Ambulatory electrocardiographic studies should be extended to include younger and middle age participants in the BLS. Holter monitoring should be combined with ambulatory blood pressure recording in normals, hypertensives, and patients with cardiac and cerebrovascular disease. A protocol employing Holter monitoring to study the effects of digoxin on cardiac rhythm in patients with stable congestive heart failure has recently been approved.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00034-01 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Electro-Mechanical Coupling in Cardiac Muscle from Adult and Senescent Rats

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
	H. A. Spurgeon	Physiologist	CPB, NIA
	J. Y. Wei	Staff Fellow DOD 7/4/80	CPB, NIA
Other:	None		

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:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Although it is well established that certain changes occur in the cardiac contraction in muscle from senescent rats, simultaneous measurements of ventricular transmembrane action potential and muscle contraction have not been investigated. Such measurements are important since contraction is modulated by events that occur during the excitation of the muscle.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To measure the cardiac contraction and transmembrane action potential that initiates that contraction in cardiac muscle from adult and senescent rats.

Methods: Right ventricular papillary muscles are mounted in a chamber that permits measurement of isometric force and transmembrane action potential. The latter is measured via glass tipped microelectrodes with which the cell is impaled. The microelectrode is connected to an amplifier via AgCl wire, with reference provided by a similar electrode in the bathing fluid. The electrical and contractile events are recorded in analog form on tape which is then edited to eliminate contractions in which mechanical artifact distorts the action potential. The edited tape is played through a program that measures the conventional contractile and action potential indices.

Major Findings: The preliminary results indicate that the prolonged cardiac contraction is preceded by a prolonged action potential. Similar age differences were found at both high and low levels of contractility, which was altered by changing the bathing fluid Ca^{2+}. Resting and developed force was not age-related. Contraction duration (stimulus artifact to half twitch relaxation) was prolonged in senescent (322.7±18.2 ms) versus adult (269.1±12.6 ms, $P<.02$). Resting membrane potential was not age-related, (-73.2±1.1 mv in senescent and -74.2±1.1 mv in adult). However, the following were greater in senescent than in adult: AP amplitude in mv, 97.1±1.4 versus 92.1±1.9, $P<.05$; time above zero mv in ms, 17.6±2.1 versus 6.7±0.7, $P<.001$; time to repolarization at -40 mv, 44.0±6.7 versus 20.1±1.3, $P<.005$; time to 75% repolarization, 64.9±6.1 versus 33.4±2.3, $P<.001$; time to 90% repolarization, 102.6±8.6 versus 65.9±7.2, $P<.002$. Thus, the extent of depolarization and time of repolarization are greater in senescent compared to adult. These alterations in transsarcolemmal ionic flux may in part explain the prolonged contractile activation that has been repeatedly observed in the senescent heart.

Significance to Biomedical Research and the Program of the Institute: Prolonged contractile activation in cardiac muscle from aged organisms has been observed in species ranging from rats to man. The present results suggest a possible mechanism for the prolonged contraction.

Proposed Course: To undertake studies to determine whether a cause-effect relationship between the age changes in action potential and contraction can be demonstrated.

Publications: None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00035-01 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Fluctuations in the Intensity of Light Scattered through Diastolic Cardiac Muscle

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. G. Lakatta	Chief, Cardiovascular Section	CPB, NIA
Other:	K. P. Brin	Staff Fellow	CPB, NIA

COOPERATING UNITS (if any)
D. L. Lappé, Div. of Cardiology, Johns Hopkins Medical Inst.

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	1.1	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Intensity fluctuations in a laser beam scattered by nonbeating isolated rat cardiac muscle varied directly with the calcium concentration in the bathing fluid. The steady-state level of these fluctuations varied directly with calcium-dependent force suggesting that the intensity fluctuations reflect an interaction of calcium ions with the myofilaments. The demonstration that both a portion of resting force and the frequency of intensity fluctuations vary directly with calcium even in quiescent conditions indicates that some contractile activation is present in the resting muscle.

GRC/CPB-268

Project Description:

Objectives: To determine whether measurements of fluctuations in the intensity of light scattered through cardiac muscle provide useful information regarding the contractile process. To determine whether cardiac muscle in the diastolic period is totally quiescent or whether contractile activation can be detected via fluctuations in intensity of a laser beam measured at a point after having been scattered through the muscle.

Methods: A 5 mm Helium-Neon laser is passed through right ventricular papillary muscles isolated from the rat. The muscle is isometric and the bathing fluid is maintained at 29°C. Experimental conditions are varied, i.e., the rate and pattern of stimulation are changed, $[Ca^{2+}]$ in the bathing fluid is altered, and/or pharmacologic agents known to alter the contractile state are added to the bathing fluid. 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 amplified and a-c coupled to an analog 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. With the current methodology these measurements are made while the muscle is in the diastolic period (i.e., unstimulated state or during the period between beats at a low frequency).

Major Findings: The major findings are (1) that fluctuations in scattered light are present, (2) that the frequency of these fluctuations is not fixed but rather varies with alterations in experimental conditions, and (3) changes in fluctuations in rat cardiac muscle are accompanied by small but detectable changes in resting force.

Significance to Biomedical Research and the Program of the Institute: The results for the first time demonstrate that during diastole isolated cardiac muscle is not quiescent. This in itself is of fundamental importance in modeling the muscle contraction and stiffness. Furthermore, since the fluctuations in scattered light increase under conditions in which the capacity to develop force on excitation is also increased, the two may be related, i.e., the fluctuations may be a clue to the mechanisms that couple excitation to contraction in cardiac muscle. Currently no technique is available to probe these mechanisms in living muscle with intact sarcolemmae. 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.

Proposed Course: To determine the precise relationship between the level of diastolic fluctuations measured before an excitation and force development subsequent to that excitation over a range of contractile states. It is expected that these measurements will be made in the rat as well as other species. In addition, equipment will be purchased to permit time gated measurements of the fluctuations in regularly beating muscle both in systole and diastole.

Publications:

Lappé, D. L., and Lakatta, E. G.: Intensity fluctuation spectroscopy monitors contractile activation in "resting" cardiac muscle. Science 207: 1369-1371, 1980.

Lakatta, E. G., and Spurgeon, H. A.: Force staircase kinetics in mammalian cardiac muscle: modulation by muscle length. J. Physiol. (Lond.) 299: 337-352, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00011-08 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging I. Aging and hormone-sensitive adenylate cyclase.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. M. Dax	Visiting Associate, CPB, NIA
	T. M. Kelly	Clinical Associate, CPB, NIA (resigned 6-31-80)
	R. I. Gregerman	Chief, Endocrinology Section, CPB, NIA
OTHER:	None	

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 (200 words or less - underline keywords)

These studies deal primarily with influences of age on the biochemistry of hormone-sensitive adenylate cyclase in a variety of tissues. The purpose of these studies is to explore the mechanisms of age-related alterations of hormone responsiveness and biological membranes, with special emphasis on the relationship between adenylate cyclase and hormone receptors. Other aspects include ongoing literature review of the field of aging and hormones in general and of certain aspects of clinical medicine as they relate to aging and endocrinology.

GRC/CPB-271

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The experimental portion of this project explores age-related effects on the hormone-sensitive adenylate cyclase system. A number of hormones act at the level of the cell surface by initiating a series of biochemical events which lead to the characteristic expression of the hormone's action. Such hormones include a number of proteins (thyrotropin, luteinizing hormone), small polypeptides (glucagon, vasoactive intestinal polypeptide, etc.), the catecholamines (norepinephrine, epinephrine, and dopamine), and other biologically active amines (histamine, serotonin). In each instance the hormone interacts with a specific chemical entity termed a "receptor". This interaction leads to the activation of an enzyme, adenylate cyclase (AC), which promotes the conversion of adenosine triphosphate (ATP) to adenosine-3'-5' monophosphate (cAMP). cAMP in turn activates a number of protein kinases, a process which leads to initiation or acceleration of many biochemical events.

The biochemistry of adenylate cyclase, a multi-component, membrane-bound enzyme, is rapidly being elucidated. Receptors are being quantitated and isolated, as are other components of the system. As a result we have in recent years undertaken a series of investigations that aim to a) identify those AC mediated hormonal responses that are age-dependent b) elucidate the biochemical mechanisms by which the changes occur. In this latter aspect, we are specifically attempting to quantitate the components of the activating system, i.e., receptors, AC, and the control of their interrelationships or "coupling". Since the hormone receptors and AC are integral parts of the cell's outer membrane, our work is also a probe of the effects of aging on cell membranes. A number of age-related changes in this system have been described earlier from our laboratory.

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: 1) Biochemistry of the Adenylate Cyclase System.

a) Cytosolic activators of adenylate cyclase activity. During our earlier work, loss of epinephrine and glucagon-sensitive adenylate cyclase activity was noted during preparation of membranes from crude homogenates. The mechanism of loss was shown to be due to removal of one or more cytosolic proteins which interact with the membranes to stimulate hormone-sensitive cyclase. These proteins were then partially characterized and a technique for their quantitation developed. These results have been previously published.

In the past year we have continued our efforts to purify these substances. Although they seemed at first to be quite stable, purification schemes utilizing our quantitative assay have now shown consistent loss of activities with even the mildest spearation procedures. Since a number of "unstable" proteins are in fact destroyed by tissue proteinases and since our materials are known to be readily destroyed by enzymes such as trypsin and pepsin, extensive efforts were directed toward control of tissue proteinases with inhibitors of various types (pepstatin, diisopropyfluorophosphate, trasylol, etc.). Various protein stabilizers (SH compounds, glycerol, etc.) have also been tried; none of these maneuvers has been successful to date. Recently other workers have devised schemes for isolation of similar, perhaps identical, activators from tissues other than liver. Although these methods do not appear very different from our own, our further attempts will be patterned after those reports.

The origin of the cytosolic activators has been further explored. Activators similar or identical to those in cytosol can be isolated from washed membranes after their sonication and extraction with non-detergent buffers. The amount of such material is about 15% of the total in the tissue. We have now raised the possibility that there exist two pools of activator, one loosely bound to the membranes that is released into cytosol during homogenization and another that is tightly associated with the membranes. In either case, the close association of activator with the membranes further suggests that they are important components of the adenylate cyclase system. Our cytosolic/membrane-bound activator appears to be clearly distinct from the "G" protein, a membrane-bound GTP-GDP binding protein which is closely linked to the β-receptors and modulates adenylate cyclase activity. This work has been submitted for publication.

b) Human fat cell adenylate cyclase. Several aspects of this system have been clarified. Although GTP appears to be an essential modulator (activator or inhibitor, depending on conditions) of adenylate cyclase systems generally, past work has failed to demonstrate that this nucleotide was active in human fat cells. Nonetheless, a role for guanine nucleotide was suspected since our earlier publication that the GTP analog 5'-guanylyl-imidodiphosphate (GMP-P(NH)P) allowed expression of epinephrine stimlation. We have now clarified this issue by showing that temperature is the variable which determines the GTP response. At 37°, as opposed to 30°, GTP becomes a stimulator. Only in the presence of both GTP and epinephrine is true stimulation observed. Addition of GTP alone is inhibitory and epinephrine alone merely prevents a decrease of the initial rate of basal activity. Temperature apparently produces a crucial alteration in the membranes, possibly fluidity, which controls the interactions of the membrane-bound cyclase components. This work has been submitted for publication.

c) Anions and cations as stimulators of liver adenylate cyclase. We earlier published the first demonstration of anion stimulation of adenylate cyclase. This study extended those observations to include the effect of cations which which are now shown to interact with anions and AC in a complex fashion. Moreover, we have now demonstrated a specific role for Na^+ as a requirement for stimulation of fat cell adenylate cyclase by GTP. Although Na^+ has been recently recognized as being involved in the inhibition of cyclase by GTP, the present work demonstrates a stimulatory role for the first time. Some of this work is in press and other aspects have been submitted for publication.

2) Concentrations of β-receptors in Rat Liver and Fat Cells.

a) Liver. We were previously able to achieve β-receptor quantitation in rat liver membranes only when the membranes were obtained from weanling animals. In older animals--those in which we wished to relate observed increases in adenylate cyclase to β-receptor number--excessive non-specific binding of the labeled antagonists ^{3}H-dihydroalprenolol (DHA) and ^{125}I-hydroxybenzylpindolol (I-HYP) was seen in renewed efforts to delineate this problem. Other laboratories have now also observed similar problems in heart and skeletal muscle with these ligands and have suggested that impurity of the ligand materials may contribute. Still others have recently readvocated ^{3}H-epinephrine as a suitable ligand. Although use of labeled epinephrine for this purpose was abandoned several years ago, new data suggest that the problem is worth further examination, and experiments along these lines are now underway.

b) Fat cells. Further studies on adrenergic receptors in fat cells using the α antagonist, [^{3}H] dihydroergocryptine (^{3}H-DHE) reveal that α-receptors are not measurable in fat cells of the rat but preliminary results indicate that they are demonstrable in human fat. In this regard the human resembles the rabbit and hamster. Measurements of α-receptors in the human correlate with biochemical demonstrations of α-inhibition of lipolysis in man. Further experiments are in progress.

3) Recharacterization of the Fat Cell β-adrenergic Receptor. Scatchard analyses of [^{3}H]DHA binding in adipocyte membranes showed curvilinear plots. This indicated that multiple orders of receptors were present or that binding was negatively cooperative. In kinetic binding experiments no negative cooperativity was demonstrated between the sites. At least two orders of binding sites were thus shown to exist in fat cell membrane. This is a new finding and demands reinterpretation of a number of published studies. Presently, our own studies are aimed at determining the physiologic significance of the different sites. This material is being prepared for publication.

4) Other Receptor-Binding Studies. Previously it had been reported from this laboratory that lipolytic activity of epinephrine was increased during chronic dietary restriction. This was tested in the present experiments by acute starvation. β-adrenergic binding, adenylate cyclase activation, and cAMP accumulation were measured in conjunction with lipolysis. Dose-response relationships were again examined. In acutely starved rats it was found that adipocytes were more sensitive to epinephrine while binding parameters, adenylate cyclase, and cAMP accumulation were unchanged between the starved and control groups. It is planned to extend these studies into the aging rat to investigate whether the membranes of older rats respond to the physiological stress of starvation in a similar manner as do those of young animals. In addition, the mechanism by which sensitization occurs will be further explored, since it is not presently apparent in any of the measurements made to date.

Studies on adipocytes have classically been carried out on cells isolated from epididymal fat pads. This approach limits one to use of only male animals and to relatively small amounts of fat. Furthermore, in the human regional differences of lipolytic responsiveness have been reported. It was therefore thought to be useful to investigate whether all fat depots of the rat behave in the same way. Fat cells were isolated from four depots--epididymal, perirenal, mesenteric, and subcutaneous. Binding parameters for β-receptors, epinephrine stimulated lipolysis, and adenylate cyclase activity were similar

in each of the adipocyte depots. This study was carried out only in mature rats, but we are interested in testing whether the uniformity is present at all ages. This information should be of practical use in the design of experiments in this area and is being prepared for publication.

α-adrenergic receptors in rat liver were characterized using the [^{3}H]DHE ligand. No changes in the binding parameters were seen during aging.

5) Age-Related Alterations of Lipolysis in Rat Fat Cells. A number of studies, the best of which has only recently been published, have indicated that β-receptor mediated lipolysis decreases with aging. In the meantime, we had initiated a comprehensive study of our own in order a) to quantitate age-related changes of lipolysis b) to identify the molecular mechanisms of such changes. The variables examined were a) lipolysis induced by epinephrine including dose-response curves b) β-receptor number and affinity for epinephrine c) adenylate cyclase activity d) phosphodiesterase activity and e) net cAMP production. A decrease of lipolysis has been established in senescent (24 mo.) rats. The mean at 24 mo. was about 50 per cent than at 12 mo. The epinephrine dose-responses suggest that the sensitivity to epinephrine increases at advanced age, sensitivity being conventionally defined as the K_a or concentration for half-maximal activation. These results are statistically significant; however, they may be artifactual. If maximal response decreases, the K_a will appear to decrease also. However, in our studies no shift (parallelism) of the dose-response occurs. The same finding applies to the concentration of epinephrine producing half-maximal production of cAMP. Maximal cAMP production is not significantly decreased in the cells from the old animals. Phosphodiesterase does not appear to change with age. Adenylate cyclase and β-receptor number were also unchanged. These results in comparision to those available from other studies suggest that strain and/or dietary influences determine the magnitude of the age-related alteration of lipolysis. Decreased lipolysis may yet in fact result from decreased intracellular cAMP, which in these experiments was not measured directly, or from decreased phosphorylation in the kinase reaction. A new possibility is altered membrane content of the "G"-protein, the receptor-cyclase mediator which determines the interaction of receptor with the catalytic component of adenylate cyclase. Evidence is already at hand in other systems that this protein may modulate cAMP formation in certain patho-physiologic states.

Significance to Biological Research and the Program of the Institute. Our ongoing studies are producing basic information upon which depends an understanding of age-related changes of hormone sensitivity. In addition, new insights are being obtained concerning the biochemistry of receptors and adenylate cyclase.

Proposed Course of the Project: Further attempts to isolate the cytosolic adenylate cyclase activator will related to efforts to stabilize the protein. In the case of lipolysis and aging, additional data will be obtained to define the mechanism of decreased lipolytic responsiveness. In order to explore the basis of the age-related increase of sensitivity to epinephrine, experiments will be undertaken to examine the variables which determine such sensitivity by a comparison of all components of the adenylate cyclase system in two strains of rats whose responsiveness differs by 100-fold. The mechanism by which dietary composition affects lipolysis will also be examined.

Publications:

Katz, M. S., Partilla, J. S., Piñeyro, M. A., and Gregerman, R. I.: Determinants of the Stimulation of Fat Cell Adenylate Cyclase by High Concentrations of Sodium and Magnesium Salts. Implications for the Role of Magnesium in Regulation of Enzyme Activity. Biochim. Biophys. Acta 613: 229-237, 1980.

Katz, M. S., Kelly, T. M., Piñeyro, M. A., and Gregerman, R. I.: Anions and Cations as Stimulators of Liver Adenylate Cyclase. Biochim. Biophys. Acta, in press.

Gregerman, R. I.: The Thyroid Gland, Chapter 76. In Harvey, et al. (Eds): The Principles and Practice of Medicine--ed. 20. Appleton-Century-Crofts, 1980.

Gregerman, R. I. and Bierman, E. L.: Aging and Hormones, Chapter 29. In Williams, R. H. (Ed.): Textbook of Endocrinology--ed. 6. Saunders, 1980, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00012-08 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors and aging. II. Aging and hormone responsiveness.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. S. Roth	Research Chemist, CPB, NIA
OTHER:	H. Ito	Visiting Fellow, (EOD 5/1/79), 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 (200 words or less - underline keywords)

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. 10-76)

Project Description:

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 population 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) Mechanisms of Steroid Action. We have continued our examination of age associated changes in the mechanisms of steroid hormone action. Although we have been unable to generate specific antisera against purified glucocorticoid receptors, antiserum against uterine estrogen receptors was obtained from Drs. G. Greene and E. V. Jensen. As with glucocorticoid receptors in various tissues, we observed that rat uterine estrogen receptors appear to decrease in concentration by about half between 6 and 22 months of age. Thus, the specific antiserum was used to determine whether some estrogen receptors are actually lost or merely become non-functional as is the case with certain enzymes during aging. Our experiments suggest that equal amounts of antisera are required to shift the sedimentation coefficients (from 4 to 8S) of equal amounts of receptors from mature and senescent rats. Thus, the apparent loss of receptors during aging does not appear to be due to some type of inactivation, blockage or malformation within the limits of immunochemical detection. Moreover various physiochemical properties of these same receptors are also unchanged during aging. These include thermostability, degree of stabilization by binding of estrogens, and binding specificty for various steroids. Thus, apparent age related loss of some types of steroid hormone receptors is probably more the result of altered control of biosynthesis and/or degradation than inactivation as was reported for rat adipocyte glucocorticoid receptors last year.

Attempts to determine whether reduced sensitivity to steroid modulation may be responsible for loss of responsiveness to certain other hormones during aging have also been continued. Collaborative experiments have confirmed last year's preliminary findings that glucocorticoids are necessary to maintain maximal sensitivity to calcium for catecholamine stimulation of cardiac muscle contraction. A similar glucocorticoid requirement appears to exist for gonadotrophin stimulation of testosterone production in Leydig cells. Both of these systems show reduced hormonal responsiveness during aging.

2) Adipocyte Aging. Studies using adipocytes as models for post-mitotic cellular aging have continued. As reported in previous years rat epididymal fat pad adipocytes exhibit reduced glucocorticoid sensitivity during aging, apparently as the result of receptor loss and changes in the cell membrane glucose transport system. Preliminary experiments with agents potentially capable of increasing glucocorticoid sensitivity such as thyroxine and mercaptoethanol have not been able to restore the responsiveness of aged cells.

Other experiments suggest that the ability of prostaglandin E1 to stimulate rat adipocyte glucose oxidation is reduced by 50% between 12 and 24 months of age. Since some reports have suggested a relationship between prostaglandins and glucocorticoid receptors, various problems at the level of regulation of glucocorticoid sensitivity may exist in aged adipocytes.

Since the adipocyte membrane glucose transport system changes with age and surface sulfhydryl groups become progressively more oxidized, attention has been directed toward other possible age changes in this cellular component. Collaborative studies with Drs. Rifkind and Wang have employed electron paramagnetic resonance probes to assess membrane fluidity during aging. Preliminary experiments at 25°C have not revealed age changes in the mobility of several labels associating with proteins, although the increase in fluidity at higher temperatures may be age related.

Studies employing brown adipocytes from rat interscapular fat have been initiated to determine whether altered hormonal regulation of energy metabolism in this depot plays a role in the reduced ability of aged individuals to adapt to cold stress. Glucose oxidation in these cells is stimulated markedly by catecholamines. Very preliminary results suggest that maximal stimulation occurs in 3-6 month old animals.

3) Other In Vitro Hormone Action Systems. In an effort to extend our previous findings of altered hormonal responsiveness in aged myocardium we are developing a system of isolated myocardial cells which are responsive to catecholamines and steroids *in vitro*. Regulation of glucose oxidation has been used as an index of hormonal responsiveness. The major problem with this system at present appears to be maintenance of viability. If this obstacle can be overcome, it will be possible to eliminate many problems inherent in using isolated cardiac muscle and also provide a useful post-mitotic cell complement to the adipocyte system.

A more successful system for studying hormone action *in vitro* has been the isolated rat parotid cell aggregate. The ability of catecholamines to stimulate DNA synthesis and cell division in salivary glands *in vivo* is progressively impaired with increasing age. Thus, it is of interest to examine hormonal control of other metabolic function in this tissue. In collaboration with Dr. Baum, we have observed no age changes in β-adrenergic receptors, β-adrenergic stimulation of amylase release or dibutyryl cAMP stimulation of amylase release. No age differences were observed in cholinergic stimulation of amylase release or glucose oxidation. In contrast, α adrenergic stimulation of potassium release was reduced between 3 and 12 months of age and remained constant thereafter. α-adrenergic stimulation of glucose oxidation was progressively reduced from 3 to 24 months of age. No age differences in basal levels of glucose oxidation were observed. It appears, therefore, that selective changes in adrenergic responsiveness occur during aging in parotid glands.

Proposed Course of the Project: 1) The mechanisms responsible for changes in steroid responsiveness during aging will be further examined. Further immunochemical titration of receptor levels will be attempted in systems where receptor levels change with age, if specific anti-sera can be produced or otherwise obtained. Where possible, age changes in control of receptor biosynthesis and degradation will be examined as is currently being performed for epididymal fat pad adipocytes.

The effect of age on the permissive action of glucocorticoids in modulating other hormone actions will also be further examined. In particular, the effects of adrenalectomy and glucocorticoid restoration will be compared in mature and senescent rats on catecholamine stimulation of cardiac contraction and gonadotrophin stimulation of Leydig cell testosterone production. If steroid removal and reconstitution produce less marked effects in the older aminals, receptor metabolism and other steps involved in steroid action will be examined.

2) Attempts to restore maximal glucocorticoid responsiveness in aged adipocytes will be continued. Consideration will be given to agents and manipulations potentially capable of increasing receptors and sensitivity or reversing oxidative damage. These include thyroid hormones, prostaglandins and regulators of their metabolism, mercapthoethanol and dehydroepiandrosterone.

Studies of possible age changes in adipocyte membrane fluidity will be continued using probes for both protein and lipid mobility. Various conditions will be employed including different temperatures and ionic concentrations.

Brown adipocytes from control and cold-stressed rats of various ages will be employed to determine the sensitivity to regulation of energy metabolism by various hormones (catecholamines, steroids, thyroxine). If age differences are detected, receptors and other components of hormone action will be examined.

3) Attempts to improve the viability of isolated myocardial cells will be continued. Techniques to separate viable from non-viable cells such as sephacel beads and albumin gradients will be employed. If adequate yields of viable cells can be obtained from rats of various ages, the effects of catecholamines, steroids, insulin and thyroid hormones on carbohydrate, lipid and protein metabolism will be examined. This system may thus provide a useful extension of hormonal studies performed on isolated cardiac muscles.

Parotid cell aggregates will continue to be employed for hormone action studies during aging. Since β-adrenergic amylase release has been found not to change with age, the resynthesis of this enzyme will next be examined. Further studies on the mechanisms by which α adrenergic responsiveness is reduced during aging will now be carried out. These will include analysis of α adrenergic receptors. The effects of steroid hormones on parotid metabolism will also be examined during aging.

Our general focus will continue to be on elucidation of age changes in the mechanisms of hormone action. Although receptor changes appear to be important in many cases, other cellular components may also be responsible for altered hormone responsiveness during aging. These changes will be examined in as much detail as possible, and attempts will be made to restore altered control mechanisms to the functionality characteristic of healthy mature animals.

Publications:

Chang, W-C., and Roth, G. S.: Changes in steroid action during aging. J. Steroid Biochem. 11: 889-892, 1979.

Chang, W-C., and Roth, G. S.: Biosynthesis of putative glucocorticoid receptors in adipocytes in vitro. Biochim. Biophys. Acta. (in press).

Hughes, B., Roth, G. S., and Pitha, J.: Changes in surface sulfhydryl groups during aging in rat adipocytes. J. Cellular Physiol. (in press).

Roth, G. S.: Hormone action and receptors during aging. In Korenman, S. (Ed.): Endocrine Aspects of Aging. Maryland, National Institutes of Health, 1979, p. 7.

Roth, G. S.: Receptor changes and the control of hormone action during aging. In Borek C., Fenoglio, C. M., and King, D. W. (Eds.): Aging, Cancer and Cell Membranes. New York, Thieme-Stratton, Inc., 1980, p. 228.

Roth, G. S.: Age related changes in hormone action: the role of receptors. In Schimke, R. (Ed.): Biological Mechanisms of Aging, Maryland, National Institutes of Health, (in press).

Roth, G. S.: Age-related changes in hormone action: the role of hormone receptors. In Adelman, R. C., and Roth, G. S. (Eds.): Hormonal Regulatory Mechanisms. Florida, Chemical Rubber Co., (in press).

Roth, G. S.: Changes in hormone action during aging: glucocorticoid regulation of adipocyte glucose metabolism and catecholamine regulation of myocardial contractility. Proc. Soc. Exp. Biol. and Med. (in press).

Schocken, D. D., and Roth, G. S.: Age-associated loss of beta adrenergic receptors. In Adelman, R. C., Roberts, J. and Cristofalo, V. J. (Eds.): Pharmacological Intervention of the Aging Process. New York, Plenum Press, 1978, p. 273.

Filburn, C. E., Guarnieri, T., Zitnik, G., Roth, G. S. and Lakatta, E. G.: Mechanisms of Altered Cardiac Inotropic Responsiveness During Aging in the Rat. American Journal of Physiol. (in press).

Roth, G. S.: Altered Biochemical Responsiveness and Hormone Receptor Changes during Aging. In Behnke, J. A., Finch, C. E., and Momemt, G. B. (Eds.): The Biology of Aging. New York, Plenum Press, 1978, p. 291.

Zitnik, G. and Roth, G. S.: Effects of Thyroid Hormones on Cardiac Hypertrophy and β-adrenergic Receptors during Aging. Mech. Aging and Devel. (in press).

Roth, G. S.: Interaction of Hormones with Receptors and Alterations of these Processes with Age. In Oota, K., Makinodan, T., Iriki, M., and Baker, L. S. (Eds.): Aging Phenomena, Relationships Among Different Levels of Organization. New York, Plenum Press, 1980, p. 157.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00013-06 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Hormones, hormone receptors, and aging. III. Aging and human endocrine regulation.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. M. Harman, Senior Investigator, CPB, NIA

OTHER: P. D. Tsitouras, Visiting Associate, CPB, NIA
C. E. Martin, Senior Investigator, CPB, NIA
R. E. Wehmann, Staff Fellow, CPB, NIA
M. R. Blackman, Visiting Scientist, CPB, NIA

COOPERATING UNITS (if any)
NICHD, RRB and Dept. of Medicine, Baltimore City Hospitals; and Dept. of Pediatric Endocrinology, Johns Hopkins University School of Medicine

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Endocrinology Section and Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.95	1.75	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A series of tests of reproductive endocrine function have been carried out on male volunteers in the Baltimore Longitudinal Study on Aging. Cross sectional data have been obtained on androgen, estrogen, and gonadotropin levels in serum. Level of sexual activity is being related to hormone levels. Results show no change with age in serum sex steroid levels in men, but significant elevations of serum gonadotropins. There is a modest decrease in pituitary gonadotropic response to LRH and a small reduction in testosterone secretion in response to chorionic gonadotropin. Sexual activity appears significantly correlated with serum testosterone in men over 60. New work is underway to assess effects of age in metabolic clearance of glycoprotein hormones.

PHS-6040
(Rev. 10-76)

Project Description:

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.

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 currently available 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. None of the available studies have attempted to correlate changes in sex hormone regulation with such variables as sexual behavior, body composition, or cardiovascular 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.

C. Present study - 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. has been defined. In addition, we have attempted to correlate altered function of the above variables with changes in libido, coronary disease, and body composition.

Methods employed: (1) Plasma gonadotrophins are assayed using a double antibody radioimmunoassay. (2) Plasma testosterone and dihydrotestosterone are measured with a very precise recently developed radioimmunoassay. (3) Plasma estrone and estradiol are assayed using a charcoal radioimmunoassay method. (4) Semen analysis has been performed by standard techniques of counting and staining. (5) The fraction of free testosterone in plasma is estimated by an ion-exchange column method developed in our laboratory. (6) Blood samples are obtained from a healthy, non-obese subgroup of the BLS population before and after intravenous injection of 100 μg of LHRH to test for pituitary gonadotrophin reserve and then after intramuscular injection of human chorionic gonadotrophin to test for testis secretory reserve. (7) Freeze-dried plasma samples from the BLS subjects, taken in previous years, have been used to survey relationships between testosterone level and sexual function in collaboration with Dr. C. Martin. (8) Computerized techniques for regression and covariant analysis have been developed to evaluate data. (9) Subjects will be restudied at 5 year intervals to provide longitudinal data.

Major Findings: 69 subjects over the age range 25 to 89 have participated to date. There are a minimum of 10 subjects in each decade. No new data have been produced during the preceeding year, which has been devoted to devising and executing computerized analysis of information gained from the first cycle of the study and to preparing and revising manuscripts describing our findings. A brief summary of findings is as follows: (1) No changes (contrary to previous reports) in serum androgens, estrogens, or free testosterone, despite a small increase in testosterone binding protein; (2) Significant increases in serum LH and FSH, despite the fact that steroid levels did not fall; (3) A modest reduction in Leydig cell response to stimulation with chorionic gonadotropin; and (4) a small reduction in pituitary responsiveness to LRH. The latter information could only be obtained after a new method for comparing response criteria of groups whose basal hormone levels differ was devised in collaboration with Drs. Sherins and Loriaux of NICHD. A manuscript describing this method, and two manuscripts describing respectively steroid data and gonadotropin data have been prepared and submitted for publication. A paper which describes our novel semiautomated assay for testosterone and compares it with prior methodology was prepared in collaboration with Dr. A. A. Kowarski of the Johns Hopkins Dept. of Pediatric Endocrinology and is scheduled for publication in August, 1980. Another manuscript in preparation reports the facts that sperm counts do not decrease with age in BLS men, but that there is some failure

of sperm maturation after age 50 and also the finding that sexual activity decreased greatly with age despite maintenance of normal testosterone levels. Nonetheless the oldest group (70-89) showed a weak but significant correlation between testosterone and frequency of intercourse.

Sexual activity levels, serum testosterone, body composition (muscle mass and percent fat), diagnosis of coronary artery disease, and alcohol intake have been examined retrospectively in a group of 180 men in the BLS aged 60-80 years old. We found no fall in plasma testosterone over this age range. Increased frequency of intercourse was associated with higher levels of testosterone and low testosterone levels with lower sexual activity. High alcohol consumption reduced sex but not testosterone and increased body fat correlated with lower testosterone but not reduced sex. Therefore the association of testosterone and sexual activity was not mediated by obesity or alcohol intake as a common cause. Other variables were not related to testosterone or sexual activity. These findings are described in a manuscript submitted for publication.

A study of hormone subunits secreted during LRH stimulation has revealed a decrease in ratio of α to β subunits as intact hormone secretion increases. The ratios are similar in both old and young subjects, suggesting that no loss of synthetic efficiency occurs with advancing age. A manuscript describing these findings will be completed before October 1, 1980, and submitted for publication.

Significance to Biomedical Research and the Program of the Institute: The above studies have contributed to the fund of normative aging data and suggested that the decreased endocrine function previously reported in aged men may not be an effect of aging per se, but of other variables such as illness, obesity, etc. Decreased libido and sexual activity occurs despite the continued normal testosterone levels so that the former cannot be considered a product of hypogonadism. Nonetheless the correlation of sexual performance with testosterone suggests that controlled trials of testosterone therapy might have value in selected elderly men complaining of impotence. The fact that basal steroid levels are maintained by increased gonadotropin secretion in the face of reduced Leydig cell reserve highlights the working of compensatory mechanisms to maintain homeostasis in the aging organism and may serve as a paradigm for future studies of the physiology of aging.

Proposed Course: The second cycle of the male longitudinal study will begin in Fall 1980. A TRH infusion test will be substituted for the LRH bolus stimulation and data on pituitary secretion of prolactin and TSH will be collected. Measurements of basal TSH will be done with a new highly sensitive assay which can distinguish normal from diminished levels. Measurements of prolactin will be done also. Other parameters added to the new cycle will include examination of serum obtained before coming to the GRC and 24 hour diurnal sampling on a subset of subjects to determine the effect of time of day and stress of being admitted to the GRC on the serum testosterone and gonadotropin levels.

We have initiated investigations of the rate of metabolic clearance of glycoprotein hormones with age. A pilot study of clearance of LH in women in different phases of their menstrual cycles and on steroid suppression is under way. These data will add to our ability to interpret the meaning of elevated gonadotropins and altered gonadotropin responses in older persons, since our previous techniques have not differentiated whether effects of secretion or altered metabolism were being observed.

A new study proposed but not yet initiated is based on reports that post-menopausal gonadotropin elevations in women become less prominent with advancing age. We plan to assess the pituitary reserve for FSH, LH, TSH, and prolactin in post-menopausal women of various ages using TRH, LRH, and metclopramide (Reglan) (a drug which stimulate prolactin release) to stimulate pituitary response.

Another study proposed and approved by the Institutional review board, but not yet initiated will investigate the effects of three physiologic doses of long acting parenteral estradiol (the physiologic estrogen) on various metabolic and endocrine responses to estrogen in young (castrate) and old (post-menopausal) hypogonadal women. This study may help to elucidate the minimum effective dose for physiologic estrogen replacement and tell us whether this dose differs for young vs. old women.

Publications:

Harman, S. M. and Tsitouras, P. D.: Reproductive hormones in aging men I. Measurement of sex steroids, basal luteinizing hormone, and Leydig cell response to human chorionic gonadotropin. J. Clin. Endocrinol. Metab. 51: 35, 1980.

Harman, S. M., Tsitouras, P. D., Kowatch, M. A., and Kowarski, A. A.: Advantage of Florisil over charcoal separation in a mechanized testosterone radioimmunoassay. Clin. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00014-10 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Biochemistry of renin and renin substrate.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G. Pourmotabbed, Visiting Associate (resigned, 9-30-79), CPB, NIA
R. I. Gregerman, Chief, 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 (200 words or less - underline keywords)

This project explores the biochemistry of renin and renin-substrate, proteins involved in hypertension. Other studies include development of renin inhibitors.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The enzyme renin, produced and secreted from specialized cells of the renal glomerulus, appears to be involved in several varieties of hypertension. Secretion of the enzyme is under control of a variety of physiologic and pathophysiologic factors, e.g., salt restriction, volume depletion, sympathetic nervous system control. In the circulation the enzyme acts on a plasma glycoprotein to release the decapeptide, angiotensin I, which is then activated by C-terminal cleavage to a smaller octapeptide, angiotensin II, in the lung and other tissues. Angiotensin II in turn influences the secretion of aldosterone, principal mineralocorticoid hormone of the adrenal, and has other direct effects on the cardiovascular and central nervous systems. In certain pathologic states renin has a direct role in the pathogenesis of hypertension, as it does in renovascular stenosis. Recent work suggests that renin may be more directly involved in common or "essential" hypertension. Preliminary reports indicate that an inhibitor of angiotensin II formation results in amelioration of hypertension in many patients. Finally, the secretion of renin (and aldosterone) is markedly influenced by aging in man, while hypertension is an age-dependent disease.

Progress in these areas has depended on advances in our knowledge of the basic biochemistry of renin and its substrate. Our own laboratory has been involved in the development of new techniques for measurement of the enzyme and its peptide products, in the biochemistry of the enzyme and its substrate, and the relevance of the renin-angiotensin-aldosterone system to normal and pathologic aging.

Methods Employed: Renin has been assayed by our previously published polymeric substrate assay and by immunoassays of angiotensin I. Renin substrate has been purified from porcine and human plasma by column chromatography on DEAE cellulose, Con-A Sepharose and Sephadex G-100. Other techniques are standard biochemical methods (amino acid analysis, high voltage electrophoresis, etc.).

Major Findings: 1) Kinetics of Renin Inhibition: Previous kinetic analyses of inhibition used renin preparations contaminated with non-specific proteases and pseudorenin. Since we have described a method for preparation of protease and pseudorenin-free renin, we have now used this material in a reexploration of this problem in order to resolve a number of discrepancies and criticisms which could be directed toward the earlier work.

Pseudorenin-free enzyme exhibits kinetics of inhibition which differ markedly from those of crude material. The purified enzyme is inhibited by pepstatin in a pseudo-irreversible ("tight-binding") manner. The contaminating proteases exhibit reversible kinetics, showing them not to be cathepsin-like, as earlier believed, but belonging to some other category of enzyme. These studies clarify earlier kinetic analyses by ourselves and others and have been submitted for publication.

2) Inhibition of Pseudorenin-free Renin by a Pepstatin-Dextran Conjugate. We have earlier described this high molecular weight inhibitor of renin. The present study examined its interaction with purified renin. Inhibition kinetics were similar to those for non-conjugated pepstatin. This material should have characteristics suitable for use in vivo. The results have been submitted for publication.

3) Protease Activity of International Reference Standard Renin. This preparation has been available from the Medical Research Council in Great Britain for about 10 years. From our own work we know that crude renin preparations are contaminated by proteases. These materials may give rise to angiotensin and to a host of spurious peptides which could be mistakedly measured in immunoassays for antiotensin I. Accordingly, we examined the International Standard for probable contamination by proteases. The preparation was indeed shown to be heavily contaminated. This finding emphasizes the need for a new International Reference Standard.

Significance to Bio-medical Research and the Program of the Institute. This work should clarify certain aspects of the inhibition of renin by inhibitors. These agents in turn may help clarify the nature of the hypertensive states and their biochemical bases.

Proposed Course of the Project: Active work in this area has been terminated Studies will be resumed when new personnel can be recruited. Existing material has been submitted for publication.

Publications: Chou, H. J., Pourmotabbed, G., Workman, R. J., and Gregerman, R. I.: Proteinase Activity of Human Renin Preparations: The International Reference Preparation (Renin Standard 68/356). Clinical Science, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00023-05 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Hormones, hormone receptors, and Aging, IV. Testis, pituitary, and hypothalamic function in aging and uremia.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. M. Harman, Senior Investigator, CPB, NIA

OTHER: P. D. Tsitouras, Visiting Associate, CPB, NIA
M. R. Blackman, Visiting Scientist, CPB, NIA
R. E. Wehmann, Staff Fellow, CPB, NIA

COOPERATING UNITS (if any)

G. Briefel, Dept. of Medicine, Nephrology Section, 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:
2.7	2.0	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Leydig cells isolated from rat testis and thyrotropic and gonadotropic cells from rat pituitaries are studied in vitro to compare their physiology in old and young rats and in normal vs. uremic rats. Testosterone production, cell number of gonadotrophin receptors, and cAMP production are assayed in order to investigate the alterations of Leydig cell function in aging and uremia. Production of TSH in response to TRH, production of LH and FSH and their subunits in response to LRH and receptors for TSH and LRH will be measured in an attempt to elucidate altered function of pituitary secretory cells with age and in uremia. Hypothalamic content of LRH, dopamine, catecholamines and α and β adrenergic and dopaminergic receptors will also be determined. Work to date indicates that the Leydig cell defect is reversible by in vivo treatment of aged rats with gonadotropin and that it is not mediated by loss of receptors, cyclase, or any single enzyme in the steroidogenic cascade. Deficient function of aged pitutiary cells in vitro has been confirmed and a workable assay for LRH has been initiated in our laboratory.

GRC/CPB-290

Project Description:

Objectives: The Leydig cells of the testis secrete the major male hormone, testosterone, which is essential to normal male development and function, and 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 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 hypothalamic LRH and which interacts with membrane receptors to activate 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 the present study is to define and investigate the nature of the defect appearing in an animal Leydig cell system with advancing age. Previous findings have shown that a significant minority of older rats studied were uremic and uremia is known to inhibit gonadal function. Furthermore, 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. Therefore we have expanded our studies to investigate the effects of uremia on Leydig cell function and of aging and uremia on pituitary cell physiology and hypothalamic biochemistry.

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. In uremia studies young animals are made uremic by partial nephrectomy and studied with paired sham-operated rats after 6 weeks of uremia. Tubular and interstitial elements are separated by filtration, and, for cAMP experiments, Leydig cells are further purified by density gradient centrifugation. 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, an LH-like hormone) to determine cell production of testosterone or cAMP. Cell membrane binding capacity and affinity for radioactively labelled hCG is determined to estimate receptor number and quality. Testosterone is analyzed by radioimmunoassay using Florisil for separation of bound and free hormone. cAMP present in washed cells (total) and bound to intracellular protein (presumably protein phosphokinase) are estimated by standard techniques of radioimmunoassay. Pituitaries 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. Hypothalami are removed, homogenized, and aliquots set aside for determination of LRH by radioimmunoassay and neurotransmitted by radioenzymatic assay. Hypothalamic membranes will also be incubated with radiolabelled receptor agonists and antagonists to establish content and characteristics of catecholamine and dopamine receptors.

Major Findings: The previous year's experiments, in which old and young rats were hemicastrated, Leydig cell function determined, and then the second testis studied after 3 days in vivo treatment with hCG, have shown that the "aging" defect is reversible with hCG treatment (i.e., post-treatment aged Leydig cells are indistinguishable in their ability to secrete testosterone, from similarly treated young cells). An improvement in performance occurs 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. Our study of uremic rats has demonstrated reduction in testosterone secretion similar to that seen in aged cells, but uremic cells differ in that they improve their response to hCG with continued exposure in vitro, while aged cells do not, and uremic cells appear to have fewer receptors than aged cells.

Incubation of Leydig cells with hCG and steroid precursors has not restored old cells to levels of testosterone production similar to young cells even when androstenedione, the immediate precursor for testosterone, is available. This suggests that one or more of the enzymes in the steroid synthetic pathway proximal to the final step may be deficient in activity and that the final enzyme in the pathway (17α keto reductase) is deficient. Pinpointing of other deficient enzymes (if any) awaits measurement of other steroid intermediates formed during incubation with various precursors.

Initial experiments with pituitary cell suspensions have demonstrated deficient performance of cells isolated from old rats when stimulated with LRH. This appears to remain constant even 3 weeks post-castration which suggests that performance of old pituitaries is not restored by increasing endogenous LRH secretion in vivo.

An assay for hypothalamic LRH has been set up in our laboratory. Preliminary results indicate that total hypothalamic content of LRH determined by our assay is similar to values reported by previous investigators and that uremia may be associated with a reduction in hypothalamic LRH content.

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: Work will continue in an effort to elucidate the nature of the "repair" process occurring in vivo, and to demonstrate the site or sites of the aging defect demonstrated in vitro in isolated Leydig cells. Young and old cells' performance will continue to be compared in the presence and absence of various steroid precursors, cofactors, and energy sources. Rate limiting

enzyme activities will be assayed. Comparison of uremia and aging will continue. More emphasis will be placed on working out the physiology and biochemistry of aging pituitaries and hypothalami with determinations of receptors, hormones, and neurotransmitters.

Publications:

Tsitouras, P. D., Kowatch, M. A. and Harman, S. M.: Age related alterations of isolated rat Leydig cell function: Gonadotropin receptors, cAMP response, and testosterone secretion. Endocrinology 105: 1400-1405, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00015-22 CPB

PERIOD COVERED
October 1, 1989 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Baltimore Longitudinal Study of Human Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	A. H. Norris	Chief, Human Performance Section	CPB NIA
	N. W. Shock	Scientist Emeritus	NIA
OTHER:	S. K. Sharma	Sociologist	CPB NIA

Other workers who are associated with the Longitudinal Study describe their involvement in their individual reports.

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.08	2.03	8.05

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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. 10-76)

Project Description: Z01 AG 00015-22 CPB

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 by other participants in the program. Recruits agree to return to GRC in Baltimore for 2-1/2 days of testing every 12 months (age 70 and over), 18 months (age 60-69) or 24 months (under age 60) for an indeterminate period. At entry into the program, 86% of subjects reported at least some college, 87% were identified with professional, technical or managerial occupations, 90% were presently married, 83% described 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 June 30, 1980 a total of 1125 men and 237 women have participated in the testing program on one or more visits to GRC. Since the inception of the study 224 men have died and another 252 have withdrawn from the program, leaving an active sample of 649 men. As of this date, 853 men have completed three or more visits for testing, 657 visited five times or more, 279 ten times or more, 133 twelve times or more, and 37 fifteen times or more. In all, these subjects account for a grand total of 7047 participant visits.

During the year 75 women were newly admitted to the program as compared to 17 men. Of the 237 women who have joined the BLSA since January 1978, three have died and one withdrew leaving an active sample of 233. Seventy-seven women have been tested two times; three have been tested three times.

Determination of the characteristics of participants who have withdrawn from the BLSA as compared to those who have remained active is the primary focus of the Illness and Disability (I & D) Study, an integral part of the BLSA.

The primary objectives of the I & D Study are:

1. To locate, contact and gather information pertaining to the subsequent health and illness experiences of those subjects who dropped out from the BLSA between February 6, 1958 to July 1, 1977.

2. To ascertain the illness and disability experiences of drop-outs subsequent to their last visit to the BLSA until present and to compare it with the illness and disability experiences of those who remained active in the study.

3. To determine whether subjects who dropped out from the BLSA differ significantly from subjects who remained active in the study with regard to a) demographic variables, b) psychological variables, and c) physiological variables, as measured at the time of first visit to the BLSA.

4. To ascertain the risk factors for dropping out from the BLSA and to recommend some control measures aimed at reducing the dropout rate.

The I & D Study is now in its identification and location phase. First, an attempt is made to locate all the drop-outs (those who have failed to return for tests and those who have formally withdrawn). After the subjects are located, a letter is mailed to the subjects explaining this study and requesting their cooperation. This letter is followed by a telephone call approximately 10 days after the letter is mailed. Subjects are encouraged to return to the BLSA, for one or more visits. If the subject can not come to the BLSA for any reason, an attempt is made to get their consent for a home visit by the investigator. If a home visit is not possible (due to distance), an attempt is made to get consent for an interval medical history by mailed questionnaire. In addition, these subjects are asked to provide consent for the investigator to obtain information from any physician/s and hospitals that they may have visited during this interval period. If the subjects refused the questionnaire, an attempt is made to get consent for a telephone interview.

As of July 1, 1977, there were 1088 subjects who had made one or more visits to the BLSA since the inception of the study in February 1958. Of those 1088, six hundred and fifty-eight (60.5%) were still active in the study. One hundred and fifty seven (14.4%) failed to return to the BLSA. Ninety-six (8.8%) formally withdrew from the BLSA. The remaining one hundred and seventy-seven (16.3%) were know to be dead as of July 1, 1977. (Table 1)

Table 1. Frequency Distribution of BLSA Subjects by Status
N = 1088 (July 1, 1977)

Status	No	Percent
Active	658	60.48
Failure	157	14.43
Withdrawn	96	8.82
Deceased	177	16.27
	1088	100.00

Total attrition in 20 years by reasons other than death	23.25%
Annual attrition rate	1.16%

The first phase of the study was to locate and contact those subjects who dropped out of the BLSA and obtain their consent to cooperate in this study. It was decided to begin first with subjects who had failed to return to the BLSA (N=157).

The location rate was 100% in this study. The response rate was 98%.

Table 2. Current Status (Alive/Dead) of Subjects who Failed to Return to the BLSA and Response Rate in I & D Study.

Total Subjects who failed to return.
(N=157) 100%

Step I Locating	Step II Contact Response	Step III Outcome
Dead (n=13) 8.3%		
Alive (n=144)	Will return (n=67)	Did return (n=59) 88.1%
91.7%	46.5%	
	Home Visit (n=17) 10.8%	Definite Appointment (n=5) 7.5%
	Mailed Questionnaire (n=56) 38.9%	Questionnaire Received (n=44) 78.6%
	Dropped (n=1) .7%	Questionnaire Not Received (n=12) 21.4%
	Others (n=3) 2.1%	Refused Questionnaire Agreed for Telephone Interview Only (n=2)

Of these 157 subjects, 13 subjects (8.3% were found dead at the time of first contact. Information with regard to data of death and cause of death has been obtained on these deceased subjects.

Suceeding phases of the I & D Study will include:

1. Location and contact of subjects who have formally withdrawn from the BLSA as of July 1, 1977, (N=96). Our procedures will remain the same as in the case of subjects who failed to return as described above.

2. Data with regard to health, illness and disability experiences of subjects who failed to return will be analyzed.

Findings of this study will update the BLSA records. The validity of the data already obtained and the generating of the research findings would be enhanced. The identification of risk factors for dropping out of the study may be used to facilitate the development of control measures aimed at reducing the dropout rate in the BLSA.

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 interrelationships. The relation of functional changes in an individual to age at death, age of 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 major summary of all aspects of this program is in progress.

Publications:

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00016-25 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Age Changes in Human Performance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. H. Norris	Chief, Human Performance Section	CPB NIA
	S. P. Tzankoff	Sr. Staff Fellow	CPB NIA
OTHER:	N. W. Shock	Scientist Emeritus	NIA

COOPERATING UNITS (if any) A.T. Welford, Collaborator
S.M. Garn, Ctr. for Human Growth & Development, U. of Michigan, Ann Arbor,
G.A. Borkan, S.S. Bachman, Boston Normative Aging Study, V.A. Outpatient Clinic, Boston

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.99	0.89	3.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the mechanisms and the limitations of a variety of physical activities in old and young individuals. Muscular activity ranges from brisk walking on an inclined treadmill to tapping between targets of various widths drawn on paper and separated by various distances. Exercise responses are measured for blood pressure, heart rate, pulmonary ventilation, carbon dioxide elimination, and oxygen uptake. Responses of metabolites, such as lactic acid and hormones, such as catecholamines and growth hormone are measured. The oxygen cost of exercise is measured and compared to the total amount of physical work performed to estimate the mechanical efficiency of the subjects' neuromuscular and psychomotor control systems. Responses of the pulmonary system are interpreted in terms of standard spirometry and dead space (residual volume) measurements as well as studies of respiratory control. Limitation on performance imposed by cerebrovascular, cardiovascular, and pulmonary disease is assessed. Reflex time, reaction time and speed and accuracy of movement are measured and compared with exercise responses.

GRC/CPB-299

Project Description: Z01 AG 00016-25 CPB

Objectives: This project is designed to study the effects of aging on the physiological responses to and recovery from exercise--to describe age changes and to elucidate the mechanisms of these effects of aging. It is designed to identify underlying factors in the limitation of work performance and reduced mechanical efficiency in older people. For this purpose, detailed evaluation of pulmonary function and pulmonary response to stressful agents are carried out. Other factors such as the metabolic cost of limb movement and psychomotor control of limb movement are being studied.

An additional goal is to identify and explain the role of disease-altered physiological function in age-related limitation of work performance. Cerebrovascular, cardiovascular and pulmonary disease and functional measures such as blood pressure, reflex time, and reaction time will be considered.

Methods Employed: Measured amounts of physical work are administered to subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill is used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and electrocardiogram are made before, during and after standardized amounts of exercise. The functional capacities of the pulmonary system are evaluated. Alterations in respiratory function as a result of the stimulation of low oxygen and high oxygen and carbon dioxide in the inspired air are evaluated by pressure changes induced by occlusion of airflow ($P_{0.1}$).

Subjects of the BLS continue to be evaluated by participating in the multi-purpose maximal treadmill exercise tests. Subjects are instructed to walk on the motor-driven treadmill at a constant speed of 5.6 km/hr. They start on the level and the grade is elevated by 3% increments every two minutes until either exhaustion or, at the discretion of the attending physician, the test is terminated. Before the walk, as well as during the exercise and recovery from it, electrocardiographic tracings are displayed and monitored on a CRT, recorded on magnetic tape, and periodic samples reproduced on strip-chart paper. In addition while walking, subjects breathe through a mouthpiece-valve arrangement which allows for the inspiration of room air and expiration into spirometers for measurement of pulmonary ventilation, and after gas analyses, for the calculation of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio for each level of exercise. Blood pressure determinations are made at rest, in the last twenty seconds of each grade, and during recovery from exercise.

A few male subjects representing the adult age-range, highly selected in terms of absence of cardiovascular, renal, metabolic disorders, and, in addition, with demonstrated excellent endurance on the treadmill test on previous visits, were asked to undergo the treadmill exercise test while blood sampling was performed through an indwelling venous catheter at rest,

at each stage of exercise, and during recovery. Blood samples were assayed for catecholamines concentrations using the sensitive radioenzymatic method which allows for individual quantification of nonrepinephrine and epinephrine following thin-layer chromatography.

In the recovery phase venous blood samples are obtained at 3, 5, and 7 min. for the determination of lactic acid concentration, a by-product of anaerobic metabolism. Each subject who progresses through the test until exhaustion is asked to identify his limiting symptom, e.g., muscle pain, shortness of breath, general fatigue, and this response recorded. In addition, the investigator makes a subjective evaluation regarding whether or not the performance represented a maximal effort. Exercise and recovery electrocardiographic tracings are evaluated for signs of ischemic coronary heart disease (ICHD) according to the World Health Organization standards.

Major Findings: Details of a study of metabolic responses of primates to exercise and behavioral heart rate conditioning which involves collaboration with LBS is reported in Z01 AG 00063-13 LBS.

Details of a study of metabolic and adrenergic response to exercise with and without behavioral heart rate conditioning which inolves collaboration with LBS is reported in Z01 AG 00067-13 LBS.

Both catecholamine responses and myocardial oxygen consumption were found to be adequate in older men during maximal exercise:

CATECHOLAMINE RESPONSES. Data was analyzed for 17 subjects who have undergone the treadmill test with blood catecholamine determinations before, during and after the exercise. Resting levels for nonrepinephrine (NE) and epinephrine (E) did not differ significantly with age and were well within ranges cited in the literature. Both NE and E increased progressively with work load, linearly up to about 55-65% of maximal work load and exponentially thereafter. Over the entire range of work loads, plasma concentrations of NE and E were higher for the older men. At maximum work, values for NE were significantly and positively related with age: $NE(pg/ml) = 17 + (40 \cdot Age)$, $r = 0.50$, $p < .05$. Heart rate at this work load showed the expected age-related decrease: $HR(B/min = 200 - (.63 \cdot Age)$, $r = .72$, $p = <.001$ as did maximal aerobic capacity: $\dot{V}O_2(ml/kg \cdot min) = 45 - (.17 \cdot Age)$, $r = .50$, $p < .05$. Changes in E with age were similar but more variable. These findings indicate that age-related diminution in cardiovascular performance during exercise cannot be attributed to decreases in either peripheral or adrenomedullary catecholamine secretion; this suggests an age-related decline in target organ responsivity in the elderly during maximum muscular exercise.

The completed study will include some 30 men. Follow-up studies are under consideration and will include the use of blocking agents for adrenergic (propranolol) and cholinergic (atropine) receptors, used separately and in combination.

MYOCARDIAL OXYGEN CONSUMPTION. Cardiovascular function data during treadmill exercise have been analyzed on some 200 male BLS participants representing the adult age-range who were clinically clean for cardiovascular abnormalities, i.e., normotensive without medication, euthyroid, exhibited no ECG abnormalities at rest or with maximal exercise, and when available, had no adverse findings on stress thallium scanning or echocardiography. Subjects were selected into age-decade groups (25-34, 35-44, 45-54 yrs., etc.) and values for systolic blood pressure (SBP), heart rate (HR), and rate-pressure product (RPP) (an index of myocardial oxygen consumption) were analyzed for age-related differences in terms of a wide range of submaximal work loads and also with maximal work load.

Both the absolute values and the increments of HR with submaximal work load were similar across the age range studied. Values for SBP at any submaximal work load were higher with age. However, increments of SBP with work load were similar across the age groups. RPP differences with age, therefore, indicate a higher myocardial oxygen requirement with age for the performance of any one submaximal task.

Maximal values of HR assocated with the highest work rate individual subjects could perform decreased in the expected manner (9.5% decade). Maximal values of SBP increased slightly and linearly with age. Maximal RPP exhibited only a slight linear decrement with age which averaged 2.3% per decade, indicating that age-related decrements in cardiovascular function during maximal exercise cannot be attributed to deterioration in myocardial metabolism.

Significance to Bio-Medical Research and the Program of the Institute: The decline of the ability of some older people to perform their day-to-day activities and to engage in pursuits which contribute to the economic and social strength of our society represents a national loss. Identification of the physiological, medical and social correlates of high levels of physical strength and psycho-motor performance in middle and old age, as well as declines in these abilities, should lead to techniques designed to reduce the rate of decline in performance capacities with age.

Proposed Course: Measurements of muscle strength and maximum power generating ability during arm exercise will be continued. Cardiovascular, ventilatory and metabolic responses to standardized arm ergometer exercise and monitored treadmill exercise will be used to classify participants into fitness categories and to explore the age relationships of biochemical and metabolic responses to exercise. Measurements of lung volumes and uniformity of pulmonary ventilation will be made to characterize the respiratory competence of the longitudinal studies participants.

Publications:

Borkan, G.A., Bachman, S.S. and Norris, A.H.: Comparison of visually estimated age with physiologically predicted age as indicators of rates of aging. Social Science & Medicine. In press.

Borkan, G.A. and Norris, A.H.: Assessment of biological age using a profile of physical parameters. J. Gerontol. 35(2):177-184, 1980.

Tzankoff, S.P. and Norris, A.H.: Age-related differences in lactate distribution kinetics following maximal exercise. Eur. J. Appl. Physiol. 42:35-40, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00017-22 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Age Relationships of Body Composition, Nutrition and Physical Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A.H. Norris	Chief, Human Performance Section	CPB NIA
	R. Andres	Chief, Clinical Physiology Branch	CPB NIA
	S.P. Tzankoff	Sr. Staff Fellow	CPB NIA
OTHER:	N.W. Shock	Scientist Emeritus	NIA
	J.D. Tobin	Medical Officer	CPB NIA
	D. Elahi	Staff Fellow	CPB NIA
	R. Aamodt	Chief, Whole Body Counter Section	NM CC

COOPERATING UNITS (if any)
P.T. Davis, Dept. of Medicine, University of Buffalo, Buffalo, N.Y.
S.M. Garn, Ctr. for Human Growth & Development, University of Michigan, Ann Arbor
G.A. Borkan, Boston Normative Aging Study, V.A. Outpatient Clinic, Boston

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.89	1.04	0.85

CHECK APPROPRIATE BOX(ES)
[X] (a) HUMAN SUBJECTS [ ] (b) HUMAN TISSUES [ ] (c) NEITHER
[ ] (a1) MINORS [ ] (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of the interrelationships of body composition, nutrition and physical activity is a longitudinal study of aging. It provides a description of these characteristics for participants in the Baltimore Longitudinal Study. It provides opportunity to relate changes in these basic characteristics of the individual participants to changes in other biochemical, physiological and psychological measurements. A variety of non-invasive techniques are employed. They include the Behnke Anthropometric Index, skinfold thickness measurements, height, weight, twenty-four hour creatinine excretion, total body potassium determination, basal metabolism determinations, Garn X-ray fat thickness measurements, a diet diary, and an activity questionnaire. Previously, measures of total body density and total body water have been made in longitudinal studies participants. Body density corrected for differences in body water content have been compared with the Behnke Index and other conventional anthropometric indices (such as ponderal index).

GRC/CPB-304

Objectives: This project is designed to describe age differences and age changes in body composition, nutrition, and physical activity. Mechanisms of interaction of these functions and behaviors will be sought. The relationship of these measurements to other physiological, psychological and biochemical variables will be examined.

Methods Employed: Height, weight, and body circumferences of longitudinal study participants are obtained by standard anthropometric methods. Roentgenographic and anthropometric estimates of skeletal mass are combined with height, weight, and body circumferences to provide an estimate of body fat. Other estimates of fat include skinfold thickness measurements and fat thickness measurements from X-rays. Indices of lean body mass include: (1) basal metabolic rate determinations, (2) twenty-four hour urinary excretion of creatinine, (3) total body potassium, and (4) total body water and extracellular water determinations by indicator dilution. Nutrient intakes and activity calories are estimated from a diary and physical activity from a self-administered questionnaire. All such measurements are repeated in the course of each subject's participation in the longitudinal program.

Analysis of the nutrition data followed the Schaie-Baltes model in order to separate the effect of aging from secular and cohort effects. The analytic objective was to determine how diet varies with age and how diet may have changed since the early 1960's. To answer these questions it is necessary to follow a group of men as they age to distinguish aging, secular and cohort effects on the nutritional variables of interest.

Physical activity has been estimated in a variety of ways. The results of the activity questionnaire developed for the Baltimore Longitudinal Study of Human Aging (BLSA) have provided two measures, 1) the number of calories expended per day in activity and 2) a Sportsman-non-sportsman classification. In addition, two questionnaires have been used to infer physical activity status for participants in the BLSA. They are: 1) A questionnaire attributable to Burgess, Cavan, and Havighurst, Your Activities and Attitudes and 2) The Cornell Medical Index Health Questionnaire.

Major Findings: Details of studies of bone loss in participants in the Baltimore Longitudinal Study of Aging are reported in project no. Z01 AG 00022-04 CPB.

BIOLOGICAL AGE AND PHYSICAL ACTIVITY. With the goal of examining the association of lifestyle with aging variability, we have developed a new approach to assessment of biological age in adulthood (Borkan and Norris, 1979, reference in report no Z01 AG 00016-25). This method uses a profile of 24 biological age scores reflecting various aspects of physical function. The 24 scores included measurements of psychomotor speed, pulmonary function, blood biochemistry, body composition, sensory perception and muscle strength. The profile approach allows the possibility that different body systems may age at different rates within the same individual.

The purpose of the present analysis was to utilize the profile approach to biological age to determine, in the Baltimore Longitudinal Study population, whether physically active men were biologically more youthful than their inactive counterparts. Because the profile technique allows analysis of 24 aging parameters, it was also possible to test whether activity influenced certain aspects of aging more than others.

Among the measures of physical activity available in the Baltimore Longitudinal Study data set, three were selected for analysis. These were used to divide the sample into subpopulations representing high and low amounts of activity.

1) Frequency of Illness and Fatigability--This variable was based on the I and J sections of the Cornell Medical Index Health Questionnaire, a self reported history of physical and mental health. This particular score indicates whether an individual views himself as frequently ill and/or easily fatigued. We viewed that men who scored poorly on this parameter were less energetic and active, as well as in poorer health, than those with favorable scores. Two subpopulations were compared based on their responses: those with a perfect score (zero yes answers) versus those with one or more yes answers.

2) Sportsman Classification--Data collected on exercise patterns of participants were used to classify men as sportsmen or non-sportsmen. Sportsmen were categorized by participation in any particular form of regularly scheduled and frequent physical activity. This classification involves components of exercise, sociability, and self-discipline.

3) Calories Expended in Activity per Kilogram--Each study participant recorded the time spent in various activities per day on a questionnaire. These activities were converted to calorie expenditure based on body weight, yielding the total calories expended in activity per day. For the present analysis these data were reconverted to activity calories per kilogram of body weight to obtain a measure of activity independent of body size. The subpopulations compared men below the 33rd percentile (less than 41.28 calories/kg) and men above the 66th percentile (more than 44.20 calories/kg).

The results of this study support the view that physically active individuals are biologically more youthful than their inactive peers. However, present findings should not be viewed as comparing athletes and non-athletes but rather the more active with less active segments of a middle class sample of adult males. Differences between the subpopulations were not significant for all profile variables, nor was this expected since activity may influence certain aspects of aging and not others. The main association of activity status was with lung function and psychomotor speed. Less divergence was found for blood biochemistry, body composition, or sensory perception variables in the profile.

Although this analysis indicates greater biological youthfulness for active men, extrapolation to the conclusion that activity itself slows aging is perilous. This issue has been discussed in depth with reference to the effect of activity on coronary heart disease by Keys (1970). He observed that active and inactive individuals differ in many more ways than simply their activity. People who are inclined towards active lifestyles might come from different socioeconomic classes and differ in health, smoking, alcohol consumption, diet, and perhaps other areas. People who select active occupations may initially differ in physical health and other traits from those who choose sedentary work. Even an intervention study in which one group begins to exercise and a similar control group remains sedentary would not be conclusive if the active group began to change smoking, drinking or diet habits as they became more health conscious.

Such problems are compounded in studying the effects of activity on aging because the dependent variable is more difficult to measure than heart disease. Activity may be hypothesized to influence aging directly by its physiological effects, or through intermediary steps such as improving health, promoting weight loss, or leading to quitting cigarettes. On the other hand, the relationships seen could simply reflect self-selection into activity patterns based on previous health, body weight, or smoking history. Finally, to add further complexity to the problem, it is quite reasonable to assume that biological age itself is a determinant of activity level, and men who age faster are less likely to be active because they are physically debilitated.

To begin to investigate this issue, the analysis in Table 1 can indicate the effect of activity (sportsman status and activity calories) in men of constant health, weight, and education.

TABLE 1

Multiple Regression Analysis: Mean Biological Age Predicted by Lifestyle Parameters (R^2 = .09)

Independent Variable	B	Beta	St. error of B	F
Weight (kg)	-.004	-.126	.001	15.57[1]
Health rating (Cornell Index)[2]	.009	.206	.001	40.37[1]
Education[3]	-.018	-.191	.006	9.35[1]
Sportsman classification[4]	-.042	-.103	.013	9.93[1]
Activity (cal/kg)	-.004	-.044	.003	1.77

1 - P < .05
2 - Number of "yes" answers (lower score is more favorable)
3 - Scale = 0 to 7 (7 is highest)
4 - 1 = non-sportsman, 2 = changed status, 3 = sportsman

Results demonstrate that sportsman status was still a significant predictor of biological age after correcting for differences in these three covariates. However, this approach statistically removes the possible influence of activity on aging mediated through its relationship with health or weight. By removing the role of these potential "indirect" pathways we may be underestimating the total effect of activity on aging. As such, this analysis may come closer to measuring the interrelationship of activity and aging directly, but does not satisfactorily differentiate the other indirect pathways involved.

Analyses presented here demonstrate that more active men in the Baltimore Longitudinal Study were biologically younger than their inactive counterparts, particularly in lung function, work capacity, and psychomotor speed. To extrapolate that these findings indicate that activity retards aging is hazardous because of self-selection into activity habits on the basis of other parameters. These results do demonstrate that the widely held view, that active people are physically youthful, is valid.

Significance to Bio-Medical Research and the Program of the Institute: Nutritional deficiencies in the aged are known to be common and are generally attributed more to the socio-economic deprivation of this group than to biological or physiological aging effects. The volunteers in the Longitudinal Study Group are not a deprived group--it may be characterized as upper-middle class and has a very high educational level. It, therefore, offers a unique opportunity to study nutritional status under very favorable conditions. The nutritional effects of biological age per se may, therefore, be separated from what might be called "social aging."

Certain age changes in organ systems and various diseases are thought to be affected by diet, level of physical activity, and body composition. From the repeated assessment of these factors over time, it may be possible to determine their relative contributions to longevity and the maintenance of health and vigor in later life. Difficulties associated with obtaining retrospective estimates of eating habits, activity and body composition in the past make a prospective approach necessary for the collection of reliable information.

Proposed Course: Studies of diet, physical activity and body composition will continue. Data already collected will be further analyzed. Interactions of changes in body composition, food intake, food composition, kind and amount of physical activity, disease, and age will be examined. Specifically, body fat and lean body mass estimates, nutrient intakes and physical activity will be used in an analysis of risk of cardiovascular disease and of rate of aging of several organ systems.

Publications:

Borkan, G.A. and Norris, A.H.: Biological age in adulthood: comparison of active and inactive U.S. males. Human Biology. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00018-14 CPB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Marital, Sexual and Social Factors in Aging

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: C. Martin Sociologist CPB NIA

COOPERATING UNITS (if any)

Baltimore City Hospitals

LAB/BRANCH

Gerontology Research Center, Clinical Physiology Branch

SECTION

Human Performance Section

INSTITUTE AND LOCATION

NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.80	.70	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Systematic data concerning sexual, marital and social experience were obtained by structured interviews with males taking part in the Baltimore Longitudinal Study of Aging. In the course of the year, subjects aged 79 and younger at interview with a positive diagnosis of coronary heart disease (CHD) prior to July 1, 1979, were closely matched on age at interview to subjects negative for CHD. In over one hundred comparisons between CHD subjects and their controls, a remarkable correspondence between them was found in regard to a wide range of personal and experiential attributes pertaining to marriage and sexual activity. In sum, present findings clearly suggest that these phenomena play no role in the etiology of CHD.

GRC/CPB-309

Project Description: Z01 AG 00018-14 CPB

Objectives: Present objectives are to: 1) continue to pursue the question as to why some older men are more active sexually than others of similar age, 2) determine whether various diseases of unknown etiology may be related to one or another kind of marital or sexual history, and 3) conduct interviews with male study members who have not been interviewed.

Methods Employed: In the course of his participation in the longitudinal program, each male is asked to contribute an interview concerning his history of marriage and sexual activity. In making this request the investigator delineates study objectives, provides assurance of confidence, and emphasizes the voluntary nature of such a contribution. Over the years the refusal rate has varied from 2 to 3 percent.

To facilitate communication and help insure systematic data collection, the questions asked were committed to memory by the investigator along with whatever categories are required for classifying responses. To aid recall, help establish rapport and generate data not otherwised obtained, various aspects of occupational, educational, religious, military and parental-home experience are reviewed before introducing questions pertaining to marital adjustment and sexual conduct. Because of the excellent mental status of respondents and their evident interest in this part of the program, the data obtained are thought to be of exceptional quality.

Major Findings: The principal analytic endeavor this past year concerned the question of whether any variables derived from the above-described interviews may be related to the occurrence of coronary heart disease (CHD). Cases were defined as those respondents, aged 79 or younger at interview who acquired a positive diagnosis for CHD before July 1, 1979. Controls were selected from among those who remained free of CHD during that time and who were of the same ages at interview. A group of subjects classified as borderline for CHD were included for comparison, although they averaged four years younger at interview. Since mean year at interview for all three groups was 1969, there was a ten-year lapse for most subjects between time of interview and most recent diagnostic information. As the following data indicate, 70 percent of subjects were negative for CHD and only 16 percent had definite CHD at time of report.

	Diagnosis at Interview		Diagnosis at Last Interview		
	N	%	Negative	Borderline	Positive
Negative	256	70.5	132	68	56
Borderline	48	13.2		30	18
Positive	59	16.2			59
	363	99.9	132	98	133
Mean age at interview			62.6	58.4	62.3
Age range at interview			38-81	38-77	38-79

Case and control comparisons with reference to more than 100 experiential and personal attributes revealed only two minor factors (subjects religiosity and whether either parent had been previously married) emerged as significant correlates of CHD at the .05 level of confidence. No important differences were found in the areas of: Demographic Attributes; i.e. birth year, early farm residence, occupational status or occupational sector, highest academic degree... Parental-Home Experience; i.e. religiosity of parents, broken home, harmony within the parental home, age left parental home... Marital History; i.e., duration of engagement, age at marriage, number of years married, number of changes of marital status, number of children, marital success or failure... Sexual Anatomy and Function; i.e., erectile potency, history of premature ejaculation, circumcision status... Quantity of Sexual Activity; i.e., quantity of sexual activity during the year prior to interview, maximum number of coital events ever in any single week of marriage, customary frequency of coitus in the first year or two of marriage, and quantity of coital and total activity recalled for the years between 20 and 39 and between 40 and 59 years of age... Behavioral Attributes; i.e., prepubescent sex play, dating frequency in high school, ages at onset of petting and coitus, number of coital partners... Subjective Reactions; i.e., time comfortable without sex, attitudes toward a restoration of sexual vigor, erotic arousal from certain visual stimuli... Stress Indicators; i.e., history of intoxication, previous episodes of acute emotional stress, professional consultation for personal problems...

Significance to Bio-Medical Research and the Program of the Institute: Pages would be required to review the hypotheses that have been proposed to account for the distributive aspects of CHD by sex, social class, marital status, geographic area of residence and the like. Perhaps the major importance of these results is to narrow the range of likely explanations by an examination of marital and sexual factors that have long been ignored.

Proposed Course: In addition to preparing the above data for publication, it is planned to consider whether other entities of disease may be related to the kinds of experiential attributes that were reviewed in the current analysis.

Publications:

Martin, C.E.: Factors affecting sexual functioning in 60-79 year-old married males. Archives of Sexual Behavior. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00021-17 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Dermatoglyphics in: 1. Populations 4. Families
2. Medicine 5. Twins
3. Aging 6. Methodology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous System Studies	CNS NINCDS
	R. Garruto	Sr. Staff Associate	CNS NINCDS
	B.D. Bricker	Computer Specialist	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:
0.64	0.54	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project represents an ongoing joint collaborative effort, involving the WHO and other national and international biological laboratories to coordinate the collection, evaluation and interpretation of dermatoglyphic data. Specifically the objectives of this project are: 1) to establish dermatoglyphic markers in various diseases (clinical dermatoglyphics); 2) to establish the dermatoglyphic frequencies in normal control samples (control dermatoglyphics); 3) to study the distribution of dermatoglyphics among the various human populations (population dermatoglyphics); 4) to study the dermatoglyphics of the aged: 5) to study the genetics of dermatoglyphics; and, 6) to utilize dermatoglyphics as an added tool in twin diagnoses (twin dermatoglyphics).

PHS-6040
(Rev. 10-76)

Cooperating Units: Z01 AG 00021-17 CPB

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, Md.

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. M. T. Newman
   Department of Anthropology
   University of Washington
   Seattle, Washington

8. R. W. Hornabrook
   New Guinea Institute of Medical Research
   Wadestown, Wellington, New Zealand

9. Y. Ahuja
   Department of Genetics
   Osmania University
   Hyderabad, India

10. G. M. Flickinger
    Department of Biology
    Xavier University of Louisiana
    New Orleans, Louisiana

11. Paul T. Baker
Chairman, Department of Anthropology
Pennsylvania State University
College Station, Pennsylvania

12. W. Pollitzer
Department of Anatomy
The University of North Carolina at Chapel Hill
Chapel Hill, North Carolina

13. J. Larrick
Duke Medical School
Duke University
Durham, North Carolina

14. B. Schaumann
Neurology Section
The Veterans Administration Hospital and
The University of Minnesota
Minneapolis, Minnesota

15. R. G. Schamschula
The Institute of Dental Research
The United Dental Hospital of Sydney
Sydney, Australia

16. M. H. Seltzer
St. Barnabas Medical Center
Livingston, New Jersey

17. Paul Tchen
Groupe De Recherches De Genetique Epidemiologique
Paris, France

Project Description:

Objectives: This ongoing project represents an extensive collaborative effort, in conjunction with WHO and other national and international institutions, to study all aspects of dermatoglyphics. The specific objectives of this study are: 1) To utilize dermatoglyphic markers in clinical investigations, with special emphasis on diseases of the middle and older age groups, i.e. cancer, diabetes, coronary heart disease, etc. 2) To establish dermatoglyphic frequencies in non-affected, control samples from different human populations, which will be used in comparisons with patient groups. 3) To utilize dermatoglyphic markers in studies of population genetics, twin diagnosis and other genetic investigations.

Methods: 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.

Major Findings: (A) Caucasian American women with breast cancer showed significantly higher frequencies of digital whorls and pattern intensity index, than a sample of non-affected controls. A third sample "High Risk" composed of non-affected women, 1) with family history of cancer, 2) without children, 3) having had cysts or any other type of tumor, had digital dermatoglyphic frequencies between those of the patients and controls. (B) A second study involving diabetic patients and non-affected controls from Hyderabad, India also showed higher frequency of digital whorls and pattern intensity index among patients. Larger studies are now underway to confirm these results and ascertain the biological significance of these patient control differences. (C) In other investigations we found no significant differences between patients with Amyotrophic Lateral Sclerosis/Parkinsonism Demential Complex of Guam and Guamanian controls, or between patients with endemic cretinism and controls from Papua, New Guinea. (D) We prepared a standardized atlas of the principal dermatoglyphic frequencies among the major human groups as well as a detailed atlas of dermatoglyphic frequencies among all Amerindian populations.

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 intrauterine 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 intrauterine 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.

Proposed Course: To continue the analysis of the collected data and publish the results. To expand the study of possible associations between dermatoglyphics and breast cancer and diabetes in order to confirm the preliminary findings. To ascertain the dermatoglyphic frequencies of coronary heart disease and cancer of all types among BLSA participants as well as among participants of collaborative units. To continue the population and genetic dermatoglyphic analysis for characterization of appropriate control samples.

Publications:

Pollitzer, W. and Plato, C.C.: Anthropology and Dermatoglyphics. In Wertelecki, W. and Plato, C.C. (Eds.): Dermatoglyphics--50 Years Later. New York, Alan Liss Publishers, 1979, pp. 211-224.

Garruto, R.M., Plato, C.C., Hoff, C.J., Newman, M.T., Gajdusek, D.C. and Baker, P.T.: Characterization and Distribution of Dermatoglyphic Features in Eskimo and North, Central and South American Indian Populations. In Wertelecki, W. and Plato, C.C. (Eds.): Dermatoglyphics--50 Years Later. New York, Alan Liss Publishers, 1979, pp. 277-334.

Editor: Wertelecki, W. and Plato, C.C. (Eds.): Dermatoglyphics--50 Years later. The National Foundation March of Dimes. Birth Defects: Original Articles Series, Volume XI, Number 6. New York, Alan R. Liss, Inc., 1979, 822 pp.

Plato, C.C.: The worldwide distribution of dermatoglyphics. Proceedings of the International Symposium on Dermatoglyphics, Patiala, India, Feb. 1980. In press.

Ahuja, Y., Plato, C.C., Igbad, M.A. and Sahay, B.K.: Dermatoglyphics of diabetes mellitus: revisited. Proceedings of the All Indian Seminar on Human Variation, Patiala, India. In press.

Plato, C.C., Garruto, R.M. and Newman, M.T.: Total and lateral and a-b palmar interdigital ridge counts among Northern and Southern Peruvian Quechua. Human Biology. In press.

Wertelecki, W., Plato, C., and Plato, C.C.: Absent d triradius and dotting of the ridges in siblings. Human Heredity. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00022-04 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Investigations of Osteoarthritis and Bone Loss

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
	A.H. Norris	Chief, Human Performance Section	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies	CNS NINCDS
	R.M. Garruto	Sr. Staff Associate	CNS NINCDS
	W.W. Greulich	Dept. of Anatomy, Stanford U.	
	R.T. Yanagihara	NINCDS Research Center, Guam	NINCDS
	K.M. Chen	NINCDS Research Center, Guam	NINCDS

COOPERATING UNITS (if any)
Tecumseh Michigan Community Health Study
The University of Michigan, Ann Arbor, Michigan

LAB/BRANCH
Gerontology Research Center, Clinical Physiology Branch

SECTION
Human Performance Section

INSTITUTE AND LOCATION
NIA, NIH, Baltimore, Maryland 21224

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.50	0.80

CHECK APPROPRIATE BOX(ES)

XX (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 restriction 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.

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), and (3) among patients afflicted with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex of Guam. (4) Guamanian children of ages 7-17 who lived on Guam during periods of severe nutritional deprivation.

GRC/CPB-317

Objectives: The objectives of this study are: 1) To study the cross-sectional and longitudinal changes in bone measurements, bone mineral content and bone loss at different parts of the skeletal system of male and female participants of the BLSA and to identify factors which may affect them. 2) To evaluate the cross-sectional longitudinal and bilateral aspects of osteoarthritis in the digits and the vertebral column. 3) To compare age of onset and rate of bone loss between BLSA male and female participants and other populations. 4) To investigate the longitudinal, familial and secular changes in bone measurements and bone density by examining Guamanian x-rays of the same individuals collected 33 years apart. 5) To study a) the possible differences in bone density between patients with Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PD) of Guam and a non-affected Guamanian sample and b) the effect of long term immobilization, due to paralysis, on bone mineral content. (See project Z01 AG 00028-04.) 6) To investigate the possible relationship between lateral functional dominance and osteoarthritis and bone measurements.

Methods Employed: Radiographs were graded for osteoarthritis for each of the proximal and distal interphalangeal joints utilizing the internationally accepted grading system of J. H. Kellgren. Grades 0 and 1 were considered negative (normal), and grades 2, 3 and 4 were considered as having osteoarthritis (affected). Bone measurements were made for the total width, medullary width and the length of the second metacarpal bone. From these measurements we calculated the combined cortical thickness, the cortical area, the cortical area index and the cortical volume. The cross-sectional study was based on both the left and the right hands. The longitudinal study included participants with at least three x-rays; only left hands analyzed. Lateral functional dominance was established through a series of tests involving gross as well as fine manipulations of the hands. Foot and eye preferences were also ascertained as well as grip strength.

Major Findings: (A) Our longitudinal studies on osteoarthritis suggested that 1) joint degeneration of the hand is a relatively slow process. 2) The rate of joint degeneration at the proximal interphalangeal joints is much lower than that of the distal. 3) The progress of degeneration in the distal interphalangeal joints is directly related to the age of the participant and the time interval between visits. 4) These results reenforce our previous suggestions that osteoarthritis of the distal and proximal interphalangeal joints may have different etiology. It is possible that osteoarthritis in the distal interphalangeal joints represents a progressively deteriorating condition due to biological factors associated with aging, while osteoarthritis of the proximal joints may be caused primarily by occupational and other environmental factors whose effects are increasing in terms of years of exposure rather than biological age. (B) Evaluations of bilateral measurements of the second metacarpal bone resulted in the following significant conclusions. 1) After adulthood is reached, there is a progressive loss of bone with increasing age due to bone resorption from the endosteal surface. Specifically, the medullary diameter increases with increasing age and cortical thickness decreases. Total

diameter and length of the second metacarpal do not change with age. 2) Comparisons of bone measurements between left and right handed participants are suggestive of an inherent tendency for right second metacarpals to have more bone than the left, regardless of hand dominance. Differential physical stress applied to the bone, due to hand dominance, will increase the bilateral difference in the right handed and reduce it in the left handed. The apparent association between physical strength and bone size has its origin prior to reaching adulthood. 3) Total bone diameter, combined cortical thickness and percent cortical area are positively correlated with physical stress, while bone loss as indicated by increasing medullary diameter is not. C) Comparative measurements of the second metacarpals of individually matched Chamorros of Guam and Caucasian GRC participants demonstrated that: 1) Guamanians as well as GRC participants have more cortical bone on the right than the left metacarpals. 2) Guamanian males have significantly smaller bones than Caucasians although there is no significant difference in amount of cortical bone between the two groups. 3) Caucasian women start loosing bone at an earlier age and do so at a higher rate than Guamanian women. Biological as well as sociocultural differences, such as activity, nutrition and artificial menopause, suspected to be contributing to these differences, are now being investigated. 4) Guamanian men born during the last 35-40 years have less cortical bone than those born prior to the 1940's. This dichotomy corresponds to the beginning of radical changes in the socio-cultural life of the Chamorros, which include, nutrition, occupation, area of residence, medical care, family structure and exercise and activities. The possible contributions of these secular changes to bone mineral content are presently under further investigation.

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 things may be compared.

Proposed Course: To finish the evaluation of the collected data and publish the results. To continue the collection of bilateral x-rays from the male as well as from the female participants of the Baltimore Longitudinal Study. Now that the prevalence and rates of change in bone loss and joint degeneration have been described, comparisons of these processes with other characteristics and processes in these participants have been undertaken. Such things as nutrient intakes, medications, socio-economic status, physical activity and muscle strength will be considered. To expand the data on the ALS/PD patients, suspected patients and non-affected Guamanian controls. To study the cross-sectional and longitudinal aspects of bone density and osteoporosis in the thoracic spine and the ulna and radius of the arm, and to compare the relative severity and rate of bone loss at different skeletal sites. To ascertain possible associations between the two types of bone changes (osteoarthritis and bone loss) and certain diseases as well as physiologic and anthropometric

variables. To investigate the relationship of bone loss to blood levels of calcitonin, parathyroid hormone and its subchains and other hormones, as well as vitamin D, calcium, phosphorus, flouride and magnesium. To compare bone density values obtained through x-rays with those of other methods.

Publications:

Plato, C.C. and Norris, A.H.: Osteoarthritis of the hand: Longitudinal studies. Am. J. Epidemiol. 110:740-746, 1979.

Plato, C.C., Wood, J., and Norris, A.H.: Bilateral asymmetry in bone measurements of the hand and lateral hand dominance. Am. J. Phys. Anthrop. 52:27-31, 1980.

Plato, C.C. and Norris, A.H.: Bone measurements of the second metacarpal and grip strength. Human Biology. 52:131-149, 1980.

Plato, C.C. and Norris, A.H.: Bone measurement of the second metacarpal and lateral hand dominance. Proceedings of International Symposium on Human Growth, Patiala, India (February 1980). In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AG 00028-04 CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Epidemiologycal and Genetic Studies of Amyotrophic Lateral Sclerosis/ Parkinsonism Dementia (ALS/PD) Complex of Guam

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.C. Plato	Geneticist	CPB NIA
OTHER:	D.C. Gajdusek	Chief, Lab. of Central Nervous Systems Studies	CNS NINCDS
	R.M. Garruto	Sr. Staff Associate	CNS NINCDS
	R.T. Yanagihara	Research Associate	CNS NINCDS

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	0.30	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 established genetic and anthropological markers among the normal Guamanian population and compare them with those of the ALS/PD patients, and 3) to ascertain the effects of immobilization due to paralysis on bone density.

PHS-6040
(Rev. 10-76)

Project Description: Z01 AG 00028-04 CPB

Objectives: Our involvement in this multidisciplinary project has the following objectives: 1) to identify the epidemiological variables which contribute to the very high incidence of Amyotrophic Lateral Sclerosis/Parkinsonism Dementia (ALS/PD) Complex of Guam, 2) to ascertain the possible changes in the incidence of ALS/PD, 3) to determine the extent of genetic involvement in the etiology of the disease, 4) to study the distribution of several established genetic markers such as blood groups, serum proteins, red cell enzymes and dermatoglyphics in the normal Guamanian (Chamorro) population to be used as controls in comparisons with patients, 5) to determine the prevalence of osteoarthritis, osteoporosis and other bone diseases in Chamorro ALS/PD patients and non-affected controls, and 6) to study bone growth and development of normal Guamanian children and adolescents in a 33 year follow-up study (See project #Z01 AG 00022-04 CPB).

Methods: Twenty years ago we established two approaches towards the study of familial and genetic aspects of ALS/PD. First, the patient control registry panels, which consist of 136 patients, 136 individually matched controls and their respective sibs, parents, offspring and spouses. Second, collection of complete pedigree data for each patient as well as complete genealogy of all individuals born in Umatac, the village with the highest prevalence of ALS/PD. Both of these studies are presently being updated and the information is being evaluated. X-rays from patients and controls are collected and evaluated using the methods described in project #Z01 AG 00022-04 CPB.

Major Findings: Continuing epidemiologic investigations on ALS/PD of Guam showed that the disease is not limited to native Chamorros. It was also found among long term Filipino migrants on Guam.

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 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 a twenty year follow-up analyses of the ALS/PD Patient Control Registry data and Umatac Pedigrees. To complete the evaluation of bone loss data among patients with ALS/PD and non-affect Chamorros. And, to prepare reports for publication.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00093-08-CPB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Cellular Basis of Regulation of the Humoral Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. 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:
1.7	0.8	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The regulatory mechanisms of lymphoid cells are being investigated in vitro using a T-cell dependent as well as a T-cell independent antigen. The cellular requirements and functions involved in the in vitro immune response are being established for normal adult mice. These are then being compared to those in aged mice of the same strains. The possibility that regulatory mechanisms observed in young adult mice are amplified in old mice resulting in immunosenescence is being investigated.

PHS-6040
(Rev. 10-76)

Objectives: The goal of this project is to characterize the cells regulating the immune response by cellular elements in both young and aged mice. Efforts to determine the origin and mechanism of action of these cells are of prime interest.

Methods Employed:
(1) The in vitro culture techniques and the assay for plaque-forming cells are routine methods.

(2) The carbonyl-iron treatment of spleen cells is accomplished by adding 25 mg of sterile carbonyl iron to 100 x 10^6 normal spleen cells. After a 30 minute incubation at 37°C in a 5% CO_2 environment, the iron and cells with ingested iron are removed by magnetic attraction. This process is repeated and the spleen cells free of iron-ingesting cells are used as a source of T and B lymphocytes.

(3) Peritoneal exudate cells are collected from unstimulated mice and used as a source of accessory cells. These cells are either used directly or an adherent layer prepared from them before other cell types are added. Supernatants of peritoneal cells collected 24 hours after culture initiation are used in some instances instead of the peritoneal cells.

(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 haptenic group. The preparation used here contains 48 moles of hapten per mole of Ficoll.

Major Findings: The requirement for macrophages in the in vitro immune response to the T-independent antigen, DAGG-Ficoll, can be replaced by culture fluids collected from peritoneal cell cultures. Investigations to characterize the factor(s) showed that:

1. The factor(s) can be separated from the macrophage culture fluids by affinity chromotography on lectin bound Sepharose.

2. The factor(s) does not pass through an exclusion membrane which retains molecules larger than 25,000 daltons.

3. The factor(s) is heat resistant (56° C for 30 min) and is denatured by 4 M urea.

Attempts were made to find a more abundant source of macrophages that would produce sufficient quantities of culture medium needed to expedite the characterization of the factor. These studies showed:

1. Murine macrophage cell lines, WEHI and Raw failed to yield active culture fluids.

2. Thioglycolate stimulated peritoneal cells were no more active in producing the factor(s) than normal peritoneal cells.

3. Normal rat peritoneal cells produced slightly more factor(s) than murine cells. The factor(s) derived from rat macrophages does not show the high affinity for the lectin-Sephrose that was used to purify the factor(s) from murine peritoneal cells.

The cellular mechanism(s) through which the peritoneal cell factor(s) function was investigated to determine which cell type(s) may be responding or affected by the factor(s). Cell separation and purification techniques were used in conjunction with in vitro cell culturing. These studies showed:

1. Spleen cells depleted of macrophages and then reacted with monoclonal anti-Thy 1.2 and rabbit complement responded to DAGG-Ficoll only if the peritoneal cell factor(s) was present. The magnitude of the response was however only 50% of that observed in the appropriate control cultures.

2. The response of the monoclonal anti Thy 1.2 treated cell suspensions used above is restored to control levels when normal splenic T-cells are added to the cultures. The addition of splenic T cells without the presence of the peritoneal cell factors(s) was not able to restore the response.

3. The peritoneal cell factor(s) must be present at the initiation of the cultures or the response is severely reduced.

Significance to Biomedical Research and the Program of the Institute: The goal of this research program is to examine the cellular populations that are regulating the humoral immune response. The mechanisms by which the regulation takes place would be of significance not only to the field of immunology but would 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. The role of regulatory mechanisms in explaining the phenomena of immunosenescence may be of considerable significance.

Proposed Course: Purification of the factor(s) derived from peritoneal cells will be further pursued. Both mouse and rat peritoneal cells will be used as a source for the factor(s) while we continue to examine other cell lines that may produce the factor(s). Chemical characterization of the factor will be undertaken when sufficient material of exceptible purity is available.

The mechanism by which the factor(s) function will be continued and expanded. The influence of T-cells on the in vitro response will be approached by using fluorescent probes and cell-sorting to obtain selected T-cell subpopulations. Such purified cell populations will be studied to identify more precisely the cellular basis of the immune response. In

addition, the response of the purified T cell populations to selected mitogenic agents will be undertaken.

Publications:

Nordin, A. A. and Buchholz, M.: The Effect of Age on the In Vitro Immune Response of C57BL/6 Mice to a T-independent Antigen. In Segre, D. and Smith. L (Eds.), Immunological Aspects of Aging, Marcel Dekker, Inc., New York . (In press).

Saxena, R. K., Adler, W. H. and Nordin, A. A.: Monoclonal Anti Thy 1.2 Antibodies from Hybridoma H013-4 Do Not React with Mouse Natural Killer Cells. Immunol. Communications, (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00094-07-CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Characterization of Immune System of Aging Mice with Immunodeficiency

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. A. Nordin	Research Chemist	CPB NIA
Other:	A. J. Russo	Staff Fellow	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:
1.75	0.85	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to characterize the cellular basis for the immunodeficiencies observed in aging mice. Both in vivo and in vitro studies are undertaken in an attempt to evaluate the extent and consequences of immunodeficiency in aging mice. Cell-mediated and humoral immune responses to various antigens are investigated to determine the effect of age on the immune system.

GRC/CPB-327

Objectives: The goal of this project is to characterize the immune system in young and aging mice. The relationship between the immunological disorders and the cellular changes in the various type of lymphocytes that may be responsible for the immunodeficiencies is investigated.

Methods Employed: (1) A modification of the spleen cell culture system of Mishell and Dutton is used. Spleen cells from individual mice are cultured with mitomycin-C treated or irradiated allogeneic spleen cells, F_1 spleen cells or heterologous erythrocytes at 37°C for various days. The double chamber culture system is also used.

(2) Cytotoxicity assay - ^{51}Cr labelled EL-4 or P-815 cells are mixed with cultured spleen cells and incubated for various times. After incubation, cold PBS is added, the tubes centrifuged and the radioactivity of the supernatant counted.

(3) Plaque-forming cell assay-routine technique used to detect IgM and IgG antibody-producing cells.

(4) A radioactive (^{51}Cr) micro tube leukocyte adherence inhibition assay was used. Mononuclear cells from murine spleen were separated using Ficoll-Hypaque, labelled with ^{51}Cr, and 5 x 10^4 spleen cells are incubated in microculture plates, with flat bottom wells (37°, 5% CO_2), for 1 hr with specific or control tumor membrane extracts. The C.P.M. of labelled non-adherent cells were used as a parameter of adherence.

Major Findings: Nonphagocytic, nonadherent spleen cell populations are enriched in natural killer activity which is not due to the removal of a suppressor cell population. Using these enriched spleen cell preparations, it was shown that natural killer activity decreased progressively with age when both K562 and YAC were used as target cells. Natural killer activity of enriched spleen cell populations from different age group mice with a progressively growning syngeneic MCA-38 (colon adenocarcinoma) tumor was shown to be significantly less than that of spleen cells from normal mice. Cells expressing the receptor for the Fc portion of IgG also decreased with age and tumor growth.

The *in vitro* humoral immune response to a T-independent antigen, DAGG-Ficoll, in aging (24 mo) mice is more severely depressed than the *in vivo* response. Cell survival *in vitro* of spleen cells from old mice is equivalent to that observed using spleen cells from young (2-4 mo) mice.

Significance to Biomedical Research and the Program of the Institute: The significance of these studies concerns the understanding of the cellular mechanisms responsible for the decline in the immune systems as a result of the aging process. Attempts to re-establish the immune function in aging individuals demands that cellular deficiencies in the immune network be fully described.

Proposed Course: The reduced humoral immune response of aging mice to the T independent antigen, DAGG-Ficoll, will be investigated both in vivo and in vitro. A variety of techniques will be used to purify lymphocyte types involved in the response in an attempt to characterize the cellular basis for the immunodeficiency. Purified lymphocyte types derived from young mouse spleen cells will be co-cultivated with lymphocytes from old mice in an attempt to re-establish the immune system in vitro. The size of the responsive clone of cells able to respond to the antigen and the burst size of the committed cells will be determined in old mice and compared to that in young mice.

Publications:

Russo, A. J., Nordin, A. A. and Goldrosen, M. H., A Radio (^{51}Cr) Micro-Tube leukocyte Adherence Inhibition Assay: Specific Tumor-Associated Immunity in 3 Murine Tumor Systems, J. Immunol. Meth. 31, 259-269, 1979.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00095-07-CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

The Role of Cell Membrane Structures on Cellular Recognition

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	CPB NIA
Other:	J. E. Nagel	Clinical Associate, PHS	CPB NIA
	R. K. Saxena	Visiting Fellow	CPB NIA
	Q. B. Saxena	Visiting Fellow	CPB NIA
	R. Yanagihara	Clinical Associate, PHS	CPB NIA
	M. A. Brock	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:
4.2	3.3	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project will define the functional capability of lymphocyte subpopulations in aging mice. Assays for functional ability and techniques for cellular manipulation will allow for correlation of type with particular function. The use of a cell sorting machine 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.

GRC/CPB-330

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: To correlate certain immunologic functions with morphologically identifiable populations 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. The tests of immune functions will include responses to mitogen and antigen in in vitro culture conditions, responses to antigens and oncogenic stimuli in vivo, and the development of immunogically 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. There will be both short term for the investigation of mitogen and antigen responsiveness and for the generation of antibody-forming cells, and longer time for the generation of cytotoxic lymphocytes and antibody-forming cell colonies. In vivo methods will primarily be cell transfer studies and transplantation studies with syngeneic tumor cells. The cells will be from various lymphoid organs and from varying aged donors. The cells will be treated by physical separation methods and with specific antisera to eliminate certain populations, or to quantitate various cellular population representation. The use of a cell sorter with appropriate antisera will allow separation of distinct subpopulations. An Ortho-Cytofluorograph Cell Sorter will be used to define cell types in lymphoid tissue, changes in the types with age and stages of differentiation and selective isolation of specified subpopulations.

Major Findings: Some cells in human peripheral blood and mouse spleen have the ability to kill tumor cells in vitro without the necessity to have a preceeding immunization procedure. These so called natural killer (NK) cells have been studied to determine the factors or agents which can modulate their level of activity. Previously it was found that alloantisera directed against the NK cell could promote NK cytotoxic activity. This augmentation proceeded without the elaboration of interferon which also can augment NK activity. The mechanism of alloantisera effects appears to be an augmentation of lymphocyte attachment to the tumor cell. Other augmenting agents such as mitogens have variable times of action and variable levels of interferon induction. The cells responsible for NK activity may be an immature thymic derived cell, or a cell carrying little or no thymic associated antigen. Various anti T-cell antisera have variable effects on NK cells.

The natural killer cell activity in both mice and humans was shown to be dependent on outside influences. Both diet and ethanol use could be shown to modulate NK activity. Malnutrition induced by dietary restriction or in vivo tumor growth caused a marked decrease in NK activity. On the other hand chronic ethanol ingestion raised NK activity levels. It is important to determine the effects of environmental factors on cellular function to be able to distinguish their effects from the effects of aging.

Many in vitro assays, depend on the presence of a responsive lymphocyte population and also a non-responsive (in terms of cell division) but necessary macrophage population. The mechanism of the interaction between lymphocytes and macrophages is not known. In time lapse cinematography studies the mitogenic actions of plant lectins were investigated. What appears to be a standard feature of these stimulated cell cultures is a clumping of lymphoid cells around a macrophage with cell division preceeding in the clumped cells. Unclumped, single lymphocytes do not appear to divide in vitro. The implications of this interaction are many, especially with the appreciation of the known changes in the composition of the lymphoid tissue of aging humans or animals.

Cytofluorograph analysis of human and mouse lymphoid cells was facilitated by the use of dyes other than acridine orange. It was possible to determine differential counts, immunoglobulin positive cells and rosette forming cells easily and rapidly. Sorting of cell types with subsequent functional assays is proceeding to determine the types of cells responsible for a given function and the interactions necessary between cells in order to obtain a function.

Studies with auto-immune prone NZB mice have demonstrated the presence of an immuno-stimulatory substance in the serum from old mice. This substance is apparently produced or released by an adherent, phagocytic cell population in the spleen. The factor is only produced by cells from mice with auto-immune illness. Further studies will determine the mechanism of activity of the factor and its usefulness as a potential immuno-stimulator in mice.

Significance to Biomedical Research and the Program of the Institute: We are gaining a better definition and appreciation of the term immunodeficiency. Since a relative immunodeficiency is seen in aging, it is important to develop better diagnostic criteria, so that possible remedial measures can be undertaken. Studies on the resistance of old animals to infection will help to outline theraputic regimens for dealing with a major problem in elderly humans.

Proposed Course: To continue to outline the connection between form and function and to expand out technical ability to measure function. To develop better diagnostic criteria and tests to describe immune capacity.

Publications:

Saxena, R. K., Saxena, Q. B. and Adler, W. H.: Augmentation of NK Activity by Alloantisera. Cellular Immunol., in press.

Collins, G. D., Chrest, F. J. and Adler, W. H.: Maternal Cell Traffic in Allogeneic Embryos. J. Reproductive Immunol., in press.

Saxena, R. K., Saxena, Q. B. and Adler, W. H.: Regulation of Natural Killer Activity In Vivo. I. Loss of Natural Killer Activity During Starvation, Indian J. Exp. Biol., 1980, in press.

Saxena, R. K., and Adler, W. H.: Monoclonal Anti-Thy 1.2 Antibodies from Hybridoma H013-4 Do Not React with Mouse Natural Killer Cells. Immunological Communications, V. 9, July 1980, in press.

Adler, W. H.: The Use of the Mitogen Assay in Research on Aging and Immune Function. CRC Series on Techniques In Aging Research, W. H. Adler, and A. A. Nordin Eds. in press.

Yanagihara, R. H., Nagel, J. E. and Adler, W. H.: Cells of the Immune Response. CRC Series on Techniques In Aging Research, V. Cristafalo Ed., in press.

Chrest, F. J. and Adler, W. H.: The Use of Fluorescent Microscopy to Identify Cellular Subpopulations. CRC Series on Techniques In Biological Research J. Johnson., Ed., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00096-07-CPB

PERIOD COVERED

October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)

Low Temperature Effects on Cells of Aging Individuals

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. A. Brock	Research Biologist	CPB NIA
Other:	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:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to characterize age-related differences in the in vitro responses of murine lymphohemopoietic cells to mitogens and their differential susceptibility to freezing damage. 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. 10-76)

Project Description: Z01-AG-00096-07-CP

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 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 non-viable 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, phytohemagglutinin (PHA) and Concanavalin A (Con A), and B-lymphocytes by lipopolysaccharide (LPS).

Major Findings: The viability and functional recovery of cryopreserved lymphocytes were compared to their unfrozen controls in splenic cell suspensions from young (4-6 mo old) and older mice. Previous results have shown that cooling splenic lymphocyte suspensions using the microprocessor-controlled cooling system at rates ranging from -0.5° to -5.0° C/min resulted in 60-100% recovery of viable and functional cells of young mice. The ratios of T-cell to B-cell function, that is, the ratios of the incorporation of ^{3}H-thymidine in response to stimulation by T-cell and B-cell mitogens, were unchanged from the values for unfrozen, control cells cyropreserved at all of the cooling rates tested. This indicates that there was no selective injury to either lymphocyte subpopulation and is in contrast to results on the cryopreservation of cells from older mice. When splenic lymphocytes from 15 mo old mice were cooled at -1.0°, -2.5° and -5.0° C/min, the recovery of viable cells, those which were stained by fluorescein, ranged from 47 to 69%. However, T-lymphocyte function was markedly impaired with ^{3}H-thymidine incorporation close to the levels for unstimulated cells in 8 of 10 frozen-thawed suspensions. Studies on this impairment of T-cell function are continuing, with current experiments to explore whether sub-lethal injury has occurred and can be reversed and whether cryopreserved lymphocytes from 24 mo old mice are similarly impaired in function.

Because the development of the microprocessor-controlled cooling system and the cryopreservation experiments have been in progress for some time, it has been possible to assess the in vitro functional capacity of mouse splenic lymphocytes as a function of time. Continuous observations for 18 months have confirmed the results reported previously that there is an

endogenous circannual rhythmicity in the splenic lymphocytic response to mitogens in vitro. The seasonal rhythms were evident despite the absence of known exogenous environmental signals which could synchronize the responses. Young mice (4-6 mo old) were housed at constant environmental temperature and with a photoperiod of 12 hours of light followed by 12 hours of dark. They were sacrificed at the same time of day, and splenic cell suspensions were cultured with the T- and B-lymphocyte mitogens. The minimum responses of both lymphocyte subpopulations were observed in December 1977 through February 1978 and in December 1978 and were followed by increases to maximal levels. Peak responses to LPS by B-cells and to Con A and PHA-P by T-cells were 2, 3, and 5, times, respectively, those observed at the troughs. The free-running period for these rhythms was slightly less than 365 days.

Significance of 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 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: Differential sensitivity to temperature steps in the circannual rhythm of hydranth longevity in the marine cnidarian, Campanularia flexuosa. Comp. Biochem. Physiol. 64A: 381, 1979.

Baartz, Guenter and Mary Anne Brock: A microprocessor - controlled rate controller for use in cryopreservation. Cryobiology 16: 497, 1979.

Brock, M. A.: Cryopreservation of Murine Lymphocytes. In: Methods in Immunology and Aging Research. Eds.: W. H. Adler and A. A. Nordin, CRC Press Inc., In Press.

Brock, Mary Anne: Comparison of circasemilunar rhythmicity in east and west coast cnidarians. Proceedings of the 4th International Coelenterate Conference, Elsevier/North Holland, The Netherlands. In press.

Brock, Mary Anne and Guenter Baartz: Cryoprotection of murine lymphocyte subpopulations using a microprocessor-controlled cooling system. Cryobiology, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-AG-00104-04-CPB

PERIOD COVERED
October 1, 1979 to September 30, 1980

TITLE OF PROJECT (80 characters or less)
Clinical Immune Survey of the Longitudinal Project Participants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. H. Adler	Medical Officer, PHS	CPB NIA
Other:	A. A. Nordin	Research Chemist	CPB NIA FW/SP 8/1/80
	J. E. Nagel	Medical Officer, PHS	CPB NIA
	Q. B. Saxena	Visiting Fellow	CPB NIA
	R. Yanagihara	Clinical Associate, 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:
4.1	1.8	2.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The immune function of an aging human population will be evaluated to determine the age associated changes and the possible effects on the incidence and types of disease. The individuals demonstrating decreased immune function will be evaluated closely in this regard. The eventual goals will be to determine which age related diseases are associated with a decrease in immune function and therefore can be modulated by augmenting the function. The accurate assessment of function of the human population will be a major goal with the development of newer assays and the refinement of existing assays.

PHS-6040
(Rev. 10-76)

Project Description:

Objectives: The purpose of this project is to assess various human immune functions and to correlate these results into an overall profile of the individuals' immunologic abilities. Since many different assays are being performed, each is presented individually with the understanding that though correlational analysis of the data it may be possible to arrive at the most useful assays to employ in measuring human immune function.

The three broad areas of the program are data collection, research and service. The data collection and research aspects are dealt with specifically in terms of investigation of serum antibody and lymphocyte responses. The service aspect is a consultative function for other investigators wishing to utilize assays that use immunological methods, such as radioimmunoassays for hormones, or fluorescent antibody staining in histopathology.

The specific assays of human immune function which are used in this study are as follows.

1. The measurement of serum immunoglobulin levels by radial immunodiffusion and by the paper radioimmunosorbant test.

2. The measurement of specific antibody to immunogens, such as diphtheria or tetanus toxoids, and pathogens, such as pneumococci, influenza, E. coli.

3. The measurement of peripheral blood granulocyte function.

4. The identification and quantitation of lymphoid cell subpopulations in the peripheral blood.

5. Quantitation of the response of lymphocytes to cellular activators (PHA, Con A, PWM, SPA, tetanus toxoid, candida, phorbal myristate acetate).

6. Determination of the number of lymphocytes in peripheral blood which can differentiate to antibody-forming cells. There is evidence to indicate that immunoglobulin synthesis in aging mice and humans becomes restricted and often converts to a monoclonal nature. However, it is not yet appreciated if such a restriction in any way affects the integrity of the immune system.

7. The measurement of Epstein-Barr virus (EBV) infection will be determined in cells which formed lymphoblast cell lines in tissue culture. We will also determine the presence of anti-EBV antibody in the serum as evidence of exposure.

8. Measurement of serum autoantibodies. Distinct profiles of antinuclear antibodies are associated with certain systemic rheumatic diseases.

Methods Employed:

1. Lymphoid cell preparations:
   Human peripheral blood lymphocytes (PBL) will be isolated from freshly drawn herparinized blood of the participants in the Baltimore Longitudinal study of Aging (BLSA). The blood is diluted with an equal volume of tissue culture medium, carefully layered over Ficoll-Hypaque and centrifuged. Lymphocytes are retained at the interface and are easily collected. The cells are thoroughly washed to remove Ficoll-Hypaque and resuspended in a tissue culture medium. These cells will be identified and cultured in standard systems. Granulocyte enriched cell populations are obtained by dextran sedimentation of heparinized peripheral blood with subsequent collection of granulocytes from the dextran mixture.

2. Tissue culture assay:

   a. The detection of cytotoxic T lymphocyte activity will be by chromium isotope labelling of K-%62 tumor target cells followed by incubation with the lymphocytes. Measurement of the isotope released by the target cells will be the measure of the cytotoxicity indiced by the lymphocytes.

   b. Assays of immunoglobulin synthesizing cells will be accomplished by plating pokeweed-stimulated lymphocyte cultures with sheep erythrocytes that have been coated with staphylococcal protein A. The development of plaques will be accomplished using specific antisera and a complement source.

   c. EBV titers will be accomplished by standard methods using lymphoblast cell lines, serum titration and a fluorescent anti-immunoglobulin antisera.

   d. A flow cytometry system with attached cell sorter will be utilized to study specific subsets of lymphocytes, as well as other cell populations.

Major Findings: A total of 560 individuals have been studied using the various assays. To date 162 of 235 (69%) of the participants in the women's program have been studied. Twenty-seven women have now been examined two times. A total of 298 men have been studied, which represents approximately 60-65% of the active male BLSA participants. One hundred and four men have been studied two times and 19 studied three times.

Data analysis is cross-sectional except for peripheral blood leukocyte studies which are longitudinal. The findings are:

1. No decrease with age of the ability of pokeweed mitogen activated peripheral blood lymphocytes to synthesize immunogloublin using a plaque assay.

2. An age related decrease in T lymphocyte proliferative responses to mitogens; however no decrease in B lymphocyte proliferation.

3. No age related changes in serum immunoglobulin levels.

4. An age-associated decrease in natural killer (NK) activity among individuals less than age 70 years; however, responses of those over age 70 were variable and did not continue the pattern of change seen among younger subjects.

5. No changes in numbers of peripheral blood white cells, lymphocytes, granulocytes, monocytes, or in numbers of lymphocytes forming rosettes (T cells) or bearing surface membrane immunoglobulin (B cells).

6. Levels of anti-pneumococcal antibody increase with age, however levels of anti-tetanus antibody decrease with age. Much lower titers of anti-tetanus antibody were found among women participants compared to the men. Elevated rheumatoid factor was found in only 8 of 560 subjects, however only 2 of these individuals manifest clinical rheumatoid disease.

7. A new assay of peripheral blood granulocyte function is being performed which assesses both phagocytic and metabolic activities. Prior metabolic studies indicated that there was no age related decrease in granulocyte metabolic capacities; however it was also found that an increasing percentage of granulocytic cells were obtained from the peripheral blood of older individuals by dextran sedimentation.

8. Lymphoblast lines were established on longitudinal study participants with approximately a 10-15% success rate. All cell lines were found to be EB virus positive, and serum anti-EBV titers of individuals forming lines were higher than those of non-transformers. Possible significance of this finding is being evaluated.

Significance to Biomedical Research and the Program of the Institute: There is no doubt that these studies along with the other information available on BLSA participants will yield valuable data to show the presence or absence of a correlation of age-associated diseases and disorders with a deficiency of immune function. At present, there is no information on this subject. If we find that an age-associated immunodeficiency does lead to specific diseases, we could, hopefully, avoid the disorders by augmenting immune function. It may also be possible to alter immunogens to make them more effective in the aging host.

Proposed Course: The number of subjects studied has increased and within the next two years immune function data will be available for all BLSA participants. It is planned over the next few years that this baseline data from the immune function assays will be compared to other information about these individuals. The types of questions to be asked will be: What are the effects of drugs, diseases, gender, and diet on immune function; How do alterations in immune function relate to disease incidence and severity; What alterations in immune function may be predictive of subsequent disease? In all our population studies there are always

individuals who demonstrate levels of immune performance outside the normal range. These individuals will be chosen for more careful scrutiny in terms of the above questions. Lastly, we will continue to restudy BLSA participants to determine the stability of immune function over a period of one to two years, so that an appropriate interval for reassessment of host defense mechanisms can be determined.

Publications:

Adler, W. H. and Nagel, J. E.: Studies of Immune Function in a Human Population. in Aging and The Immune Response, D. Segre, ed. Plenum Press, New York, 1980.

Nagel, J. E. and Chrest, F. J.: Enumeration of Individual Antibody synthesizing Cells In Vitro. in Techniques in Immunological Aging Research, W. H. Adler and A. A. Nordin, eds. CRC Press, Boca Raton, FL, 1980.

Saxena, Q. B., Mezey, E., and Adler, W. H.: Regulation of Natural Killer Activity In Vivo II The Effects of Alcohol Consumption on Human Peripheral Blood Natural Killer Activity. International Journal of Cancer, 1980, in press.

Office of Planning and Extramural Affairs

The Office of Planning and Extramural Affairs (OPEA) has responsibilities in the following areas: planning and evaluation, grant referral and review, legislative analysis, program analysis and policy development, international affairs, training policy and analysis, and program coordination. These responsibilities are shared by the three components of OPEA: the Planning and Evaluation Office, the Scientific Review Office and the Program Analysis Office.

Planning, Evaluation and Legislation

During FY 1980, OPEA staff developed and initiated a new planning process and format, "The NIA Planning Sourcebook." The Sourcebook will serve as the repository for information pertaining to the history, organizational structure, goals and plans of the Institute and each of its organizational components. It will form the basis for the annual NIA Director's review of programs, for the NIA Annual Report, Research and Evaluation Plans, Scientific Directory and Bibliography and Budget reports. In addition, it will be used to prepare responses to inquiries from Congress, the scientific community, other agencies and the public. The Sourcebook was utilized for the first time as the format for the FY 1980 program reviews and will be refined and integrated into a revised annual planning process.

In early FY 1980, OPEA staff developed the NIA Research Plan for FY 1982-84, based on the NIA Director's program reviews held in late FY 1979. The Research Plan was finalized after a collegial review with the Director, NIH. OPEA staff also prepared the FY 1981 NIA Evaluation Plan and initiated two evaluation projects funded by the HHS "evaluation set-aside program," evaluations of the NIA Genetic and Cellular Resource Program and Animal Resource Program. Plans were initiated as well for the evaluation of the Geriatric Medicine Academic Award, with the subject of evaluation being discussed at the First Annual Meeting of Geriatric Medicine Academic Awardees in 1980.

Also in the area of evaluation, OPEA staff evaluated the Special Initiative Grant mechanism (R21). Approval and funding patterns for this grant mechanism were analyzed to determine whether it had achieved its goal of stimulating new, high-quality research in the field of aging.

OPEA staff is responsible for a number of legislative activities, including: the preparation and transcript editing of Congressional testimony for the Director, NIA; legislative liaison with the Division of Legislative Analysis, NIH; monitoring of legislative activities relevant to the NIA mission; responding to Congressional inquiries on NIA programs; and providing legislative information and analyses to NIA staff. During FY 1980, an OPEA staff member prepared the following testimony for the Director, NIA:

- "Energy and the Elderly," presented before the Select Committee on Aging, U.S. House of Representatives, October 5, 1979;

- Statement on "The Veterans Senior Citizen Health Care Act," presented before the Veterans Affairs Committee, U.S. Senate, October 29, 1979;

- "How Old is Old? The Effects of Aging on Learning and Working," presented before the Special Committee on Aging, U.S. Senate, April 30, 1980;

- "The Impact of Alzheimer's Disease on the Nation's Elderly," presented before the Subcommittee on Aging and the Subcommittee on Labor-HEW Appropriations, U.S. Senate, July 15, 1980 (prepared in conjunction with the Information Office, NIA);

- "Research Frontiers in Aging and Cancer," presented before the Select Committee on Aging, U.S. House of Representatives, September 25, 1980.

The preparation of a "Legislative and Administrative History of the National Institute on Aging," prepared under contract, was coordinated by an OPEA staff member. This comprehensive document traces the history of aging research at NIH and the various forces responsible for the eventual creation of the NIA in 1974.

In June 1980, NIA awarded a logistical and technical support services contract to Prospect Associates (Contract No. NO1-AG-0-2104) with Dr. Don C. Gibson serving as Project Officer and Ms. Judith L. Howe acting as Assistant Project Officer. This contract administered by OPEA staff, will provide services in support of a HHS inventory of research on aging, a comprehensive evaluation of NIA research and research resource programs, the updating of the HHS Research Plan on Aging, "Our Future Selves," and NIA activities associated with the 1981 White House Conference on Aging. During the latter half of FY 1980, all OPEA staff members have been working with the contractor in developing methodologies for the various components of this multitask contract.

NIA has been designated as the lead Institute in the implementation of the "airline pilots study" mandated by P.L. 96-171. This law, enacted on December 29, 1979, requires the Director of NIH, in consultation with the Secretary of Transportation, to conduct a study to determine whether any mandatory age limitation for pilots, and specifically a mandatory age limitation of sixty years, is medically warranted; whether the rules governing eligibility and frequency of first- and second-class medical certification and examinations are adequate; and the effects of aging on the ability of individuals to perform the duties of pilots with the highest level of safety. An OPEA staff member has worked with the Office of the Deputy Director, NIA, in resonding to numerous Congressional, Departmental, and public inquiries about the study, and through its representative on the Inter-Institute Committee, was involved in initial study implementation plans.

In support of the Medical/Scientific Work Group of the Under Secretary's Task Force on Long Term Care, OPEA contributed to the development of the

initial submissions of four primary long term care initiatives and four potential initiatives. The former includes: "Epidemiology of Long Term Care Utilization," "The Relationship of Medical and Behavioral Interventions to the Need for Long Term Care," Epidemiological Study of Fall Injuries Among the Aged," and "Senile Dementia." Potential initiatives identified include "Sleep Disorders and Aging," "Comprehensive Diagnosis in Long Term Care," "Protheses and Rehabilitation," and The Teaching Research Nursing Home." Editing, budget preparation and justification were completed by OPEA staff for submission through Dr. Butler, Task Force Chairman, to the Under Secretary, HHS.

A workshop on Dietary Restriction, DHEA and Aging was organized by OPEA to review current knowledge of the effects on longevity and physiological correlates of aging of dietary restriction and administration of dehydroepiandrosterone, and to make appropriate scientific and programmatic recommendations to enhance progress of the field.

A member of the OPEA staff is coordinating preparation of a Data Book on Social Indicators which will be utilized for planning purposes by the staff of the White House Conference on Aging as well as planning operations of the NIA. The topics to be addressed are:

- Population and Retirement Social Indicators for the Elderly U.S. Population
- Housing and Living Arrangements of the Elderly U.S. Population (1946-1979)
- Continuing Care and Social Services Delivery for the Elderly U.S. Population (1946-1979)
- The Design of Time-Series Indicators of Health Status, Health Care Delivery and Health Care Utilization

An OPEA staff member is also responsible for coordinating the selection of authors for preparation of a series of scientific papers on topics of interest to delegates to the 1981 White House Conference on Aging. These papers will include such topics as:

- Osteoporosis
- Digestion and Aging
- Geriatric Dentistry
- Exercise and the Elderly

OPEA staff coordinated the addition of seven members to the NIA Aging Review Committee. This expansion was approved by the Secretary, DHHS and a

slate has been submitted to the Director, NIA. This expansion will allow the Committee to review a broader range of projects, particularly in the area of social and behavioral research.

A report entitled "Mammalian Models for Research on Aging" prepared by experts selected by the Institute of Laboratory Animal Resources of the National Academy of Sciences, was released in 1980. This project was supported by Contract No. N01-AG-7-2118 from the National Institute on Aging, Dr. Don C. Gibson, Project Officer.

During FY 1980, an OPEA staff member developed an NIA Contract Policy Manual. This task was recently accomplished and the manual should be available for review and comment in the immediate future. The manual will provide important information to all staff regarding the process, method and terminology involved in contract activities.

OPEA staff also coordinated development of a Sources Sought Announcement on Alternatives to Long Term Care. Approximately 90 responses were received. These were reviewed by staff and an outside panel of experts. Approximately 20 responders were considered to have innovative demonstrations for research projects which could form the basis for a state-of-the-art conference on Alternatives to Long Term Care.

An OPEA staff member was the NIA liaison to the Interdepartmental Working Group on National Manpower Policy for the Field of Aging. This group was established to carry out the legislative mandate of the Older Americans Act Amendments of 1978 "to develop and implement a national manpower policy and to do this in collaboration with such other Federal departments and agencies as may be appropriate..." The plan calls for an inventory of present and potential involvement of Federal departments and agencies in supporting programs for trained manpower. In carrying out this charge, OPEA canvassed all the NIH Institutes and served in an advisory capacity to the AoA staff.

OPEA staff also participates in the following committees:

- Committee for Program Policy and Management
- Extramural Program Management Committees
- Review Policy Committee
- Arthritis Interagency Coordinating Committee
- NIA Biological Resources Committee

SCIENTIFIC REVIEW

OPEA staff members in the Office of Scientific Review are responsible for providing initial merit review on a variety of grants and projects. The staff handles a diversified group of applications in the basic and clinical aspects of the biological, behavioral, and social sciences.

Applicants for program project grants or institutional training grants are usually site-visited by a team (assembled by OPEA staff) consisting of members of the Aging Review Committee (ARC), the ARC Executive Secretary from this office, the extramural scientists, and a grants management representative. Site visit reports are prepared by this office and are used by ARC as an important adjunct in their review and rating of applications for scientific merit. OPEA staff made a total of 26 site visits in FY 1980 (as of August 1980).

Another series of award reviews prepared by the office through ad hoc extramural review committees are the Geriatric Medicine and Dentistry Academic Awards. These awards support and encourage faculty and curriculum development in the areas of geriatrics and gerontology, and represent an important mechanism for the establishment of permanent didactic programs and staff in these areas in medical and dental schools throughout the country.

A third function of this office is the technical merit review of all contract proposals initiated by NIA. Again, OPEA staff is responsible for the selection of expert reviewers, and the production of summary statements used in evaluation and subsequent negotiations with top ranked contractors, contributing to obtaining maximum performance for each dollar of expenditure.

The centralization of contract review for the first time allowed all NIA contracts to be brought to best and final offer status during the same fiscal year in which they were initiated.

Other accomplishments of this office during the past year include revision of the NIA Referral Guideline document, which is the central guide used by DRG referral officers and others to assign grants to NIA. The new referral guidelines are designed for quick reference, yet they fully and accurately reflect the broad range of interests of all NIA programs. The new guidelines will result in increased accuracy and more unequivocal assignments of meritorious proposals to our Institute. Finally, this office also has initiated coordination of NIA policies with respect to training, assignment, and referral by program within NIA.

The activities of the Scientific Review Office are directed toward ensuring that all functions of the Institute receive the highest possible degree of review and coordination, so that material presented to the Director (NIA), to the National Advisory Council on Aging, and to other units of NIH accurately reflect merit, ability, and sound judgment as determined by expert objective review.

The increasing volume and diversity of review and oversight activities of this office necessitates an expansion of professional staff. During FY 1980, a new Health Scientist Administrator, Dr. Marvin Kalt, was added to staff. He will concentrate in the areas of contract review, training, and applications dealing with cell and molecular biology.

Program Analysis

The Program Analysis Office is responsible for collecting, organizing, retrieving, analyzing, and interpreting NIA scientific and administrative data for information systems development and implementation. It is also responsible for providing technical information on a wide variety of Institute functions and issues in order to evaluate Institute program development and emphases. The office also serves in a consultative role to NACA, NIA staff, NIH, and other agencies and groups.

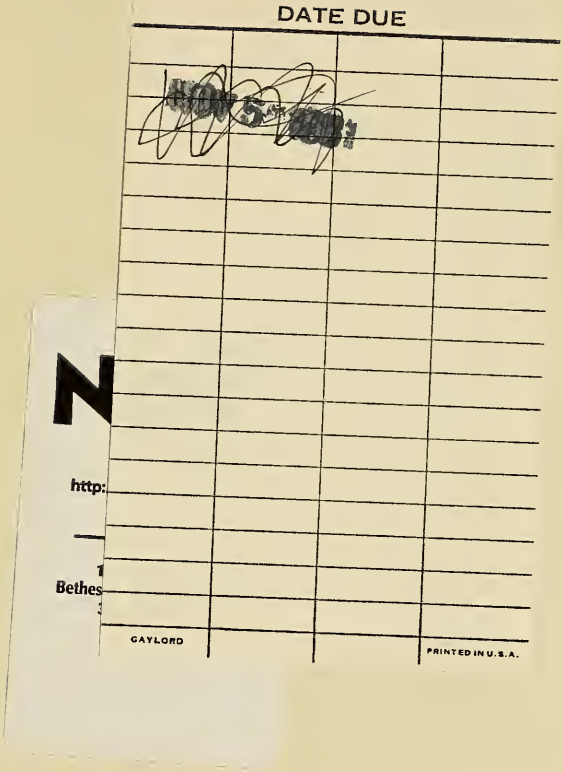
The Program Analysis office consists of the following four functional areas:

The Systems Design and Implementation group has determined the most effective application of EDP technology to meet the Institute's information requirements, has planned, developed, and managed the NIA Management Information System, and has established procedures for data collection, maintenance, and control within NIA and from outside the Institute. The Animal Models Inventory contract was completed and a final system version made ready for testing. This system permits automatic shipment scheduling, projection of animal population size and control. The Fiscal Year Ledger system provides current-year grant and contract expenditures and allows easier end-of-year fiscal reconciliation. A new discipline/specialty coding system for training grants and fellowships was developed in conjunction with NIH. An NIA data management security system to ensure the security of Institute data and equipment was established. A procedure for tracking responses to program announcements was developed. The graphics module for the NIA MIS was developed, allowing high-quality graphs and charts to be produced for summary data.

Data Management and Reporting has collected, coded and maintained administrative and programmatic data, written and modified computer programs, designed and produced reports, maintained files, and documented these programs and systems. Utilizing the ADRES system, created and maintained by the PAO, the Institute was able to get relevant and timely information. The reports were produced on a routine or ad hoc basis allowing questions posed by Congress, the general public or the Institute to receive readily available and comprehensive answers. Detailed, summary, and exception reports were provided for operations, management planning, evaluation, and for Council as needed. The PAO maintained a system for coding incoming applications by scientific content, program area, project officer, and Program Classification. These codes allow the ADRES system to be queried for numbers of grants or dollars funded in a given research area, lists of applications assigned to the Institute's project officers, etc., thus permitting rapid response to such questions from Institute staff, Congress, or the general public. The necessary tables for the program reviews to the Office of the Director were developed, and a comprehensive set of available standard report formats for use by NIA staff were compiled and distributed.

Scientific Classification has contracted for the development of an extramural program scientific data base. This contract will provide rapidly retrievable scientific classification of all NIA projects using program descriptors in the NIA ADRES system. It will also provide more complete aging

*U.S. GOVERNMENT PRINTING OFFICE: 1981-0-341-132/3501



National Institute on Aging
National Institutes of Health
Bethesda, MD 20205