ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1959

NATIONAL HEART INSTITUTE

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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ANNUAL REPORT OF
PROGRAM ACTIVITIES
NATIONAL HEART INSTITUTE
1959

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Mr. Karl E. Mundy
Off. of Research
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Bg. 1 Rm. 137
Nat'l Institutes
of Health
Bethesda, Md.

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REPORT OF 1959 NHI PROGRAM ACTIVITIES
REFERENCE SHEET

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Heart Information Section		
Geographic Disease Studies Section		
Epidemiology Section (Framingham)		
Field Epidemiological Research Section (Memphis)		
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Administration
(Organization)

The National Heart Institute, under the National Heart Act, is charged with the responsibility for: the conduct of research relating to the causes, prevention, and methods of diagnosis and treatment of diseases of the heart and circulation; assisting and promoting such research by other public and private agencies; coordinating all research results and promoting their application; providing training as necessary; and assisting local and State agencies.

The accomplishments of the Heart Institute administrative offices, from the standpoint of both program direction and administrative services provided, are most properly reflected in the achievements of the component units of the Institute: the Grants and Training Branch; the Intramural Research Branch; the Technical Services Branch, including the Heart Information Center, Epidemiology, Biometrics Research, and Geographic Pathology and the Heart Disease Control Program. Contributions to the research program by these units are described elsewhere in this report.

The administrative function is constantly being re-evaluated in terms of how well it assists the operating people to do their jobs adequately. Following is a list of the changes and developments in administrative areas during the year 1959.

1. Dr. James Watt, Director of the National Heart Institute, has been appointed Special Assistant to the Secretary for Aging by Arthur S. Flemming, Secretary of the Department of Health, Education and Welfare. Mr. Robert H. Grant, Executive Officer of the National Heart Institute, in a simultaneous action was appointed Director of the Department's Special Staff on Aging and Staff Director of the 1961 White House Conference on Aging.

Dr. Watt will act as advisor to the Secretary and Miss Bertha S. Adkins, the Under Secretary, who has responsibilities for Departmental policy and planning in the field of aging. He will continue in his position as NHI Director. Mr. Grant will assume full-time responsibility for the direction of the Department's two staffs on aging.

Mr. Philip Janus will be Acting Executive Officer for the Heart Institute during Mr. Grant's absence. He has been on the staff of the Executive Officer, Office of the Director, NIH. Mr. John Reed of the Financial Management Branch, NIH, has been detailed to assist Mr. Janus, with specific responsibility for NHI budget and fiscal activities.

2. Action has been taken to institute the transfer of Dr. Harold Dorn from the Division of Research Services to the Biometrics Research Section in the capacity of Chief of the Section. Due to space limitations, it has been necessary to physically locate the Biometrics Section in rented quarters in the Robin Building in Silver Spring, Md.

3. Dr. Willard H. Eyestone, formerly Chief of the Laboratory Aids Branch, Division of Research Services, has been appointed Assistant Chief of the Grants and Training Branch, NHI, for Primate and Veterinary Grant Programs. Dr. Eyestone will work in the Grants and Training Branch of the Heart Institute specializing in the establishing of research centers in the field of primate and veterinary medicine.

4. The budget shop within the Financial Management Branch of the Office of Administrative Management was recently decentralized. (OAM is the former Division of Business Operations.) As a result of this action, a budget examiner formerly assigned to the Heart Institute from NIH was permanently transferred to NHI. This examiner continues to be assisted in budget administration for the Institute by a budget assistant and a clerk-typist.

5. At the request of the Heart Institute, a senior member of the NIH budget section, Mr. John B. Reed, made a thorough and detailed study of the budget process. The study was completed near the end of the year. It is anticipated that the findings and recommendations contained within the study will be implemented in the forthcoming year.

6. The Heart Institute program established in 1958 whereby high school students with outstanding science records were allowed to work in our laboratories was found to be successful and was continued during the Summer of 1959. The students participating in this facet of the Departmental objectives to foster and encourage scientific talent, performed regular laboratory experiments corresponding to their maturity level, and served without pay. The regular Summer employment program for undergraduate science students and for medical students, the Student Trainee and Schedule A positions, was also in operation, as was the regular Commissioned Officer Student Training and Extern Program (COSTEP).

7. Mr. Charles H. Rogers, Chief of the Heart Information Section for the past two years, accepted a position as press officer for the Department of Health, Education, and Welfare. A replacement for his former position has not been designated.

8. The Grants and Training Branch of the Institute has undergone extensive reorganization. A new Administrative Officer has been designated; the responsibility for administration and processing of grant applications, a previously divided function, is now centered upon one person, with an assistant for research grants and another for fellowship grants and training; a new position to handle matters relating primarily to the National Advisory Heart Council was set up; due to space limitations, the Fellowship Unit moved to Building 3.

9. The name of the Memphis Epidemiology Section, established in 1958, was changed to the Field Epidemiological Research Section. This section continued its studies involving the incidence of various types of cardiovascular disease in the white and colored races; these studies are carried on in cooperation with the facilities of the staff of the University of Tennessee School of Medicine.

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BIOMETRICS RESEARCH SECTION

During the calendar year 1959 the Biometrics Research Section devoted all its attention to providing statistical services for the Heart Disease Epidemiology Study at Framingham, Massachusetts. At the beginning of the year the Section helped plan the content and format of the code sheets used for transferring into punch cards the information gathered about the patient in the sixth round of biennial examinations, which began in September 1958; and at the end of the year the Section participated in the planning of the printed forms used for recording information about the patient on Exam VII, which is due to start September 1960. Completed code sheets for Exam V were revised and edited by the Section for inconsistencies before the information was transferred into punch cards. From the information contained in punch cards for the four examinations now completed and the fifth examination three-fourths completed, the Section compiled tables and made analyses in response to requests by the doctors at Framingham in their study of factors associated with the development of clinical degenerative cardiovascular disease in a cross-section of the Framingham population.

Three papers presented at meetings in 1958 were published in 1959: "Blood Pressure and Its Relation to Coronary Heart Disease in the Framingham Study," in the Proceedings of the Council for High Blood Pressure Research, American Heart Association, April; "Some Methodologic Problems in the Long-Term Study of Cardiovascular Disease: Observations on the Framingham Study," in the Journal of Chronic Diseases, September; "Some Factors Associated with the Development of Coronary Heart Disease: Six-years' Follow-up Experience in the Framingham Study," in the American Journal of Public Health, October. In addition, "The 'Silent Coronary': The Frequency and Clinical Characteristics of Unrecognized Myocardial Infarction in the Framingham Study," was published in the Annals of Internal Medicine, June, 1959.

Since June 1957 the Biometrics Research Section has been without a Chief. With the coming of Dr. Harold F. Dorn into the National Heart Institute near the end of 1959, there should follow an expansion of the statistical operations performed by the Biomstrics Research Section.

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Heart Information Center

The Heart Information Center carries out a program of public and professional information relating to the heart and circulatory system and the diseases which affect them. This is a continuing operation with its general function specified in the National Heart Act.

Activities are conducted in response to broad informational-educational needs, with variance in emphasis, content, or type as indicated by current heart program objectives. A wide range of media is utilized. A brief summary of materials produced and activities conducted during 1959 follows.

Publications. Three new publications and five revised publications were issued during the past year. The complete text of the joint NHI-AHA meeting, A Report to the Nation on a Decade of Progress Against Cardiovascular Disease, was printed and widely circulated. Highlights of Heart Progress--1958, a selection of items of interest from research studies conducted and supported by the National Heart Institute, was released for the general public. Also issued was the third annual compilation of NHI grants, entitled Public Health Support of Cardiovascular Research, Training, and Community Programs. The five revised publications were all part of the PHS Health Information Series: 1) Heart Disease, 2) Rheumatic Heart Disease, 3) Coronary Artery Disease, 4) Hypertension, and 5) Varicose Veins.

Extensive requests for certain existing publications necessitated reprinting. The HIC went back to press on the following: The Food You Eat and Heart Disease, A Living Pump, Highlights of Heart Progress--1957, Program of the National Heart Institute--A Brief Summary, and William Harvey and the Circulation of the Blood. In addition, HIC edited a report of a 1959 conference at Princeton, N.J., called "Epidemiology of Cardiovascular Diseases: Methodology (Hypertension and Arteriosclerosis)." Two publications are currently in preparation: 1) a bibliography of scientific papers published in 1958 resulting from research supported by NHI grants, and 2) a handbook of terms and names related to heart disease.

Exhibits. Three new exhibits were completed during the year, two scientific displays and one for the general public. "Gas Chromatography in Medical Research: Microanalysis of Fatty Acids by Gas Chromatography," a four-part exhibit jointly sponsored by the NHI, Yale University School of Medicine, and Sinai Hospital, was created expressly for the 51st Annual Session of the American Society for Clinical Investigation and the American Federation for Clinical

Research. This was the only scientific exhibit selected for showing with the thirty-six industrial exhibits on display at the meeting.

"Cellular Physiology: Various Expressions of a Biological Process," a scientific exhibit, was completed by NIC for the Gerontology Branch in Baltimore. It was shown for the first time at the American Association for the Advancement of Science in Chicago where it received considerable attention by the press.

"Understanding the Heart and Circulation--From the Discovery of the Circulation to Modern Cardiology," an exhibit for the general public, was built last year and has already had four successful showings. For example, more than 400,000 laymen saw the exhibit during its 100-day schedule at the Oregon Centennial Exposition, and many thousands more are presently viewing the exhibit at the Cleveland Health Museum where it has been on continuous display since October.

Exhibits constructed during previous years, but still of interest, were also extensively scheduled. The joint exhibit of the AHA and the NHI, "Working Together to Keep Hearts Strong," was among those so used. New audiences were most receptive to this exhibit throughout the year, and when it was displayed at the APHA in October it received a Certificate of Merit from the Committee on Scientific Exhibits.

Staffing exhibits and consultation on exhibits continued to be important functions of the NEC. Cooperative exhibit projects with outside organizations increased and numerous exhibit services were provided to NHI scientists and others. One new exhibit for the general public is now in preparation: "Stop Rheumatic Heart Disease Before It Starts."

Reports. Reports constituted another important area of activity, with numerous reports of regular or special nature being prepared in whole or in part. These included reports prepared weekly containing items of interest on selected cardiovascular research advances and program developments for information of the Director, NIH, the Surgeon General, PHS, and others; the annual report of the Heart Institute for publication in the over-all annual report of the Department; reports itemizing selected informational activities for the Office of Research Information and the PHS Information Office; and an annual report on activities of the Heart Information Center. Among special reports were statements giving background, extent of problems, research opportunities, and promising areas of investigation in arteriosclerosis, hypertension, and congenital heart disease for the Secretary of DHEW; a statement on program goals of the Heart Institute containing projections of research expenditures; a report on Foreign Scientist Visitors and their handling in the Heart Institute; and a report on international findings in heart disease suggestive of opportunities for epidemiological research, for publication in "Patterns of Incidence of Certain Diseases Throughout

the World", a print issued by the Senate Committee on Government Operations. Other reports, mainly of an administrative nature, included a formulation of information plans for the year, a report on status of exhibits for Medical Arts Section, SRB; a report on available heart disease lay materials for the PHS information office; and one on personnel involved in preparation or dissemination of lay information.

The Heart Information Center also participated extensively in development of materials required for budgetary and appropriations hearings. Among such documents were the Director's Opening Statement for hearings on the Institute's fiscal 1961 appropriation; a compilation of highlights of research progress and program developments that occurred during the 1959 calendar year; a summary report on recent progress against heart disease for the House appropriations subcommittee; and editing of special material, such as the report concerning primate research centers.

Speeches and Articles. During the year the Heart Information Center assisted NHI staff and others with a total of 18 addresses and talks. This assistance included the production of 9 full texts, the collection of previously prepared material for one speaker, and the composition of 8 speech outlines. Among the speakers assisted were Senator Lister Hill; DHEW Secretary Arthur Flemming; PHS Surgeon General Dr. Leroy Burney; Dr. Aims McGuinness, Special Assistant for Health and Medical Affairs, DHEW; Dr. James Watt, NHI Director; and Dr. Luther L. Terry, Assistant Director, NHI. Of the 18 audiences addressed, 11 were professional groups, and 7 were composed chiefly of laymen.

Five articles were prepared for periodicals and other publications during 1959. Two were encyclopedia articles, one was for a medical journal, one was done for a popular health magazine, and a fifth was a guest contribution for a syndicated newspaper column. All articles either bore the Director's by-line or were credited to him. In addition to providing copy for articles on request from outside publications, the Center regularly contributed to the NIH Record.

Press Releases, Radio, Television, and Films. The Heart Information Center issued twelve press releases during the year: seven reporting research findings made in NHI laboratories; three announcing appointments to National Advisory Heart Council membership; one dealing with the AHA-NHI "Report to the Nation" and one with influenza vaccination. Five of the twelve were released at the annual meeting of the Federation of American Societies for Experimental Biology. HIC also provided copy for releases by Representative Fogarty and Senator Hill on "A Report to the Nation" and by St. John's University School of Law on an NHI grant-supported study of legal bases for awards in cardiac cases. In the media of radio and television, HIC arranged for the taping of "A Report to the Nation" for radio network use and, afterward, an

interview with Dr. Paul D. White and Dr. Howard A. Sprague for the Voice of America; provided material for a radio interview between Dr. Watt and Congressman Denton and for Dr. Watt's TV and radio appearance with Congressman Laird on "City Side"; and provided taped heart sounds for use by station WCAY. HIC also arranged for the filming of "A Report to the Nation" and edited and narrated the version recently released through NHI and AHA; and also assisted with the staging of NHI sequences for films produced by Kiplinger Magazine and USIA.

Services to Science Writers. Many services were given newspapers, wire services, magazines, and other outlets for health, medical, and scientific information, through working directly with science writers, reporters, and others. Background material was provided, special information obtained and furnished, or interviews arranged with Institute scientists and others in behalf of staff members of national publications, the press, house organs, and free lance writers. In addition to furnishing requested information and material, photographs were supplied or arrangements made for taking photographs in a number of instances.

Among publications serviced during the year were Medical News, Scope Weekly, Wall Street Journal, Drug Research Reports, U. S. News and World Report, Newsweek, Time, Science Newsletter, Changing Times Magazine, Business Week, Charm Magazine, and Drug Trade News. Cooperation was also given to science writers of various news services and organizations, including the Associated Press, United Press International, Science Service, Scripps-Howard, Voice of America, French News Agency, and those on the staffs of several large metropolitan papers. A press kit was prepared and distributed, and press facilities arranged, in connection with the "Report to the Nation" meeting; and a press room was set up and assistance given science writers in connection with the "Seventh Microcirculatory Conference", which was held at NIH. Material was also prepared in conjunction with press conferences to be held by the Secretary of HEW.

Inquiry and Reference Services. Provision and maintenance of inquiry and reference services was a continuing function, with many requests for information from the general public, individuals in medical and health professions, and organizations, being received and answered during the year. In addition to mail, telephone, and in-person inquiries received directly by the Center, inquiries were also referred to it for appropriate handling from other units of the Institute, the Service, the Department, other government agencies, members of Congress, and the White House. Many inquiries could be serviced by forwarding publications containing information pertinent to the request. Others, however, required special compilation of information, involving consultation with scientists, preparation of reading or reference lists, or searching out relevant data and material.

The collection of reprints accumulated by the Center, comprised of the papers which present findings of research conducted or supported by the National Heart Institute, has been very useful in facilitating reference and research reporting activities. The utility of this source material was further increased by subject indexing of the 1958 grantee publications, which was completed in connection with preparation of a bibliography covering that year.

Specialized Information Programs. Specialized programs were tailored to fit the needs of a wide variety of groups and individuals who asked to come or were invited to the National Heart Institute during the year. Such projects included a cardiovascular research nursing symposium for 73 graduate nurses; an information program especially designed for 10 Bucks County (Pa.) high school science students actively pursuing research interests; and a special program for 13 teachers of biology from Roanoke, Virginia. Among other occasions for which special planning and preparation were required were a Torch Lighting Ceremony in cooperation with the Montgomery County TB and Heart Association; the visit to NIH by the medical member of the Khruschev party; the visit by a group of Russian surgeons to the Heart Institute's Clinic of Surgery; and an NIH information officers meeting held under auspices of the Heart Information Center.

A unique communications event of the year, the Report to the Nation presented by the American Heart Association and the National Heart Institute on "A Decade of Progress Against Cardiovascular Disease," involved extensive participation of the Center in its organization and management. Also, programs were planned and arrangements made for the four Heart Seminars held during 1959, at which 26 representatives from State heart associations developed knowledge and familiarity with the operations, activities, and services of the NHI-PHS heart program.

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GEOGRAPHIC DISEASE STUDIES SECTION

In April 1959 a Conference on Methodology of Diet Appraisal was held at Princeton, jointly supported by the American Heart Association and the Heart Institute at which time the major problems encountered in collecting and evaluating dietary data in epidemiological studies of cardiovascular disease were studied exhaustively. Specific recommendations of this group have resulted in a number of studies, the intent of which is to provide data, methods or other information which will increase the effectiveness, accuracy, and comparability of dietary studies being done on a wide scale in this country on the epidemiology, pathogenesis, etiology and evolution of cardiovascular diseases. Among these studies:

1. Extension and revision of standard food composition tables is being carried out in cooperation with USDA and other agencies. One problem area is the large number of widely used, commercially prepared mixes and ready-to-eat foods of varied composition being placed upon the market each year. Another is the more accurate evaluation of mixed dishes. For these food tables increasingly greater attention is being given to the saturated and unsaturated fatty acid content of common foods.

2. Use of an electronic computer for processing dietary data has been arranged, using more reliable and more specific food values, such as those for fatty acids. This provides a speedier and more efficient method of handling such data, and this service will be offered first to grantees engaged in field studies in cardiovascular disease.

3. A collection, compilation, and critical evaluation of published and unpublished work on methodology for diet appraisal has been undertaken under the direction of Drs. James Hundley and Marjorie Whiting. In this connection Mrs. Rose Ernsberger has been assigned to the section to assist in the translation and compilation of recent Russian literature in the nutrition field.

4. A contract has been awarded to the University of Michigan for the development of new methods to determine dietary intake. The emphasis is on simplified procedures for large scale surveys that will provide data of sufficient accuracy to serve as a basis for more intensive follow-up studies. The study will combine the efforts of nutritionists, sociologists, epidemiologists, and statisticians.

5. Through a cooperative arrangement with FAO and USDA, a biochemist was assigned to FAO headquarters in Rome to assess the available data on food consumption of selected national populations throughout the world with special attention to the amount and type of fat consumed. Following this evaluation, attention will be directed to the design of country studies which will produce data pertinent to current research in cardiovascular disease.

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The Geographic Disease Studies Program has awarded a contract for the preparation of a guidebook for anthropologists, setting forth criteria and methods for collecting useful epidemiological data and specimens. Anthropologists specially trained to collect such data in the course of their regular studies may uncover valuable leads that can be exploited by epidemiologists in such fields as cardiovascular disease, diabetes, and cancer. The data collected including blood and urine samples, physical measurements, and other applicable data will be sent to NIH for medical and statistical analysis. When the findings prove promising, more exhaustive studies of the same population group will be undertaken by specialized epidemiological teams.

The first such study will be carried out among the Herrero, an isolated African group which has subsisted for generations on a diet derived chiefly from dairy products.

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For the future, as adequate staffing is obtained and information resources built up, the Center anticipates increased participation in various areas of chronic and metabolic disease.

Through contacts now being developed, it will be possible to assist other groups in obtaining cooperation from isolated homogeneous cultural groups suitable for study of specific problems. Rapport will be established with a number of such groups for which social and environmental data has already, or is currently being, collected. One such example is in the state of Chiapas, Mexico.

Negotiations are now under way to set up at the University of Minnesota Summer School a seminar where nutritionists and statisticians actively engaged in dietary appraisal for epidemiological studies can obtain consultation and exchange ideas regarding the problems they encounter. From this type of study it is anticipated that more extensive use of computers will result and that new ideas for the processing of dietary information will develop.

There is a proposal now under investigation for cooperation with the Rockefeller Institute in a pilot study of the fatty acid content of human adipose tissue. The role of the Geographic Disease Studies Program will be to expedite the selection, location and contact with isolated homogeneous cultural groups suitable for this particular study. Arrangements for analysis of composition of the diets of these populations will be planned to parallel the collection of biopsy material for analysis.

Technical Services - Epidemiology Section
Framingham, Massachusetts

The Heart Disease Epidemiology Study, NHI, Framingham, Massachusetts has now completed 10 years of study of a random selected adult population in a search for factors which may be related to the development of coronary heart disease and hypertension and to describe the natural history of these diseases.

Within another six months the study population will all have been observed for a minimum of eight years. By this time it is expected that a sufficient number of new cases of coronary heart disease will have developed to permit evaluation of many factors which it has not been possible to study heretofore because of the small numbers of cases involved.

The status of examinations carried out as of October 31, 1959 is attached.

Accomplishments during the year.

1. Examinations of the study population for the fifth and sixth times have continued at the rate of about 200 per month as indicated in the table.

Plans to complete Examination V and to prepare an analysis and report of the first eight years study have been started. The possibility of applying new machine operations for discriminate function analysis has been discussed and a pilot project using presently available data laid out in cooperation with Dr. Lee Cady at New York University.

Considerable data on pulmonary function has been accumulated on Examination V following methodology developed in cooperation with Doctors John R. Goldsmith, William Franklin and Benjamin G. Ferris of the Harvard School of Public Health. Analysis of this data should prove to be of great interest.

A special study of the relation of rheumatoid arthritis to the development of coronary heart disease was begun after a pilot study of its feasibility. This study is being carried out in cooperation with the Robert Breck Brigham Hospital in Boston, Mass. and will determine latex fixation test on the entire population as well as clinical and x-ray observations regarding joint disease. Follow-up observation will be made to test out the hypothesis of the relationship of these two diseases put forth by Dr. Sydney Cobb.

In order to test out the hypothesis that many persons with hypertension have developed this abnormality because of chronic pyelonephritis, a study has been started in cooperation with Dr. Edward Kass at the Boston City Hospital to determine the extent of urinary tract infection in our study populations and observe the relationship between bacteriuria and elevated blood pressure.

Technical Services - Epidemiology Section
Framingham, Massachusetts

Status of Examinations October 31, 1959

Number selected	6,507
Number examined	4,469 = 69%
Plus SK	740
Total examined	5,209

Number Examined Each Round

Round No.	Total	Sample	SK
I	5209	4469	740
II	4792	4052	740
III	4653	3935	718
IV	4541	3843	698
V	3750	3079	671
VI	1200	767	433

Number Not Examined Each Round

Round No.	Total	Sample	SK	
II	417	417	0	
	51	51		Deceased
	188	188		Examined later round.
	178	178		Lost
III	356	334	22	
	110	103	7	Deceased
	159	151	8	Examined later round.
	287	280	7	Lost
IV	668	626	42	
	161	141	20	Deceased
	95	85	10	Examined later round.
	412	400	12	Lost

The cooperative study with the Atomic Bomb Casualty Commission's Hiroshima project has been continued. Electrocardiograms taken on the Japanese in Hiroshima have been analyzed and tabulated.

A cooperative study with the Albany, New York group was carried out early in 1959 in our attempt to find out whether dietary interview now done in Framingham is comparable to the questionnaire used in Albany. Results of the study should soon become available.

Discussions were held with the authorities of the Framingham Union Hospital relative to the construction of a new building to house this Clinic. It is believed that satisfactory progress toward a lease of the new building is being made and that a much more efficient operation of this project can be brought about by the proposed move.

II. Assistance to other groups:

Visitors or written inquiries seeking advice or comparison with others' findings continue to be a time-consuming function of the Framingham Study. Some of the persons and groups for whom some service was performed during the year are listed below:

Dr. Knut Westlund, Ullevel Sykehus, Oslo, Norway
Dr. C. M. Fletcher, Postgraduate Medical School, London, England
Dr. Aubrey Kagan, Social Medicine Research Unit, London, England
Dr. Selck Mins, Australia
Dr. John Stokes, Wembley, Western Australia
Dr. Vladan Josiporic, Belgrade, Yugoslavia
Prof. Dr. Kasmery Rowinski, Ministry of Health, Warsaw, Poland
Dr. Z. Fajfar, World Health Organization, Geneva, Switzerland (Czechoslovakia)
Dr. Pincus and Dr. Freeman, Worcester Foundation, Shrewsbury, Massachusetts
Dr. Walter Wardwell, Department of Sociology, University of Connecticut, Storrs, Conn.
Division of TBC, Department of Health, Commonwealth of Massachusetts, Boston, Mass.
Dr. Julius Lester, Framingham Union Hospital, Framingham, Mass.
Dr. Edward Kass, Boston City Hospital, Boston, Mass.
Atomic Bomb Casualty Commission, Hiroshima, Japan

III. Staff Service Program: Assistance has been given to the following institutions:

Harvard School of Public Health, Instructor
Framingham Union Hospital, Lectures
Peter Bent Brigham Hospital, Physician in Out Patient Department
Cushing Hospital for the Aged, Lectures.

The association with Cushing Hospital has included extern training of three medical students, a foreign resident physician on an ECG-pulmonary function test technician, as well as a series of lectures to the nursing service of the hospital.

IV. Staff Education Program: (Courses Taken)

MPH in Bio-statistics (Completed by Dr. William Kannel and started by Dr. Nicholas Revotskie)

Recent advances in Cardiovascular Diseases (Mt. Sinai Hospital, New York City)
Spatial Vectocardiography and Vector Interpretation (Mt. Sinai Hospital, New York City)
Statistical Inference, Models, and Decision Making (Boston College)

V. Meetings attended:

Scientific Sessions, American Heart Association, Philadelphia, Pa.
Council for High Blood Pressure, American Heart Association, Cleveland, Ohio
Department of Public Health, New York City (Auspices of Columbia University)
Clinical Society, U. S. Public Health Service, Boston, Massachusetts
Subcommittee on Classification of Cardiovascular Diseases, Mt. Sinai Hospital New York City
Committee on Smoking and Cardiovascular Diseases, New York City
Tobacco Research Committee, New York City
American Association of Clinical Chemists, Cleveland, Ohio
Conference on Methodology in Epidemiologic Studies of Cardiovascular Diseases, American Heart Association, New York City and Princeton, N.J.

Publications:

Kagan, A., Gordon, T., Kannel, W.B., and Dawber, T.R. Blood Pressure and Its Relation to Coronary Heart Disease in the Framingham Study. Hypertension VII: Drug Action, Epidemiology and Hemodynamics Proceedings of the Council for High Blood Pressure Research, American Heart Association, November, 1958.

Joseph Stokes, III, M.D. and Thomas R. Dawber, M.D., FACP. The "Silent Coronary": The Frequency and Clinical Characteristics of Unrecognized Myocardial Infarction in the Framingham Study. Ann. Int. Med. 50: No. 6 1359-1369, June 1959.

Dawber, T.R., Kannel, W.B., Revotskie, N, Stokes, J.III, Kagan, A., and Gordon, T. Some Factors associated with the Development of Coronary Heart Disease. Six Years' Follow-up Experience in the Framingham Study. AJPH, 49: No. 10, 1349-1356, October, 1959.

Gordon, T., Moore, F.E., Shurtleff, D., Dawber, T.R. Some Methodologic Problems in the Long-Term Study of Cardiovascular Disease: Observations on the Framingham Study. Journ. of Chron. Diseases 10: No. 3 186, September 1959.

Investigators:

Thomas R. Dawber, Medical Director
William B. Kannel, Senior Surgeon
Nicholas Revotskie, Surgeon
Miss Georgiana Pearson, Dietitian
Abraham Kagan, M. D.
Joseph Stokes, III, M. D.

Dr. Robert B. McGandy, Heart Disease Control Branch, Bureau of State Services
Dr. Peter E. Barry, Heart Disease Control Branch, Bureau of State Services

FIELD EPIDEMIOLOGICAL RESEARCH SECTION

This section is concerned with measuring and comparing the frequencies and distributions of cardiovascular diseases among specific segments of populations. These measurements and comparisons are used in orienting the occurrence of disease by chronological demarcations and by the characteristics of patients and their ecology. Such studies begin in selected communities with defined and described disease and lead to identification of those portions of the population from which such disease arises. The aim of these studies is to identify those factors and conditions that cause disease.

Memphis Epidemiology Laboratory. Three distinct activities are in progress, with a major aim to explain recognized differences in cardiovascular morbidity and mortality between white and non-white elements of the population. Each activity starts with cases of a specific cardiovascular disease, subsequent efforts being made to determine and characterize the populations from which the disease stems.

One activity concerns itself with structual and biochemical measurements of hearts in a large autopsy population. A total of 50 hearts from white decedents have been contrasted with 150 hearts from non-white decedents, with respect to their relative frequencies of coronary occlusion and myocardial infarction, and to their relative contents of specific lipids in coronary arteries. Another phase of this activity employs fluorescent antibody and other microbiological techniques in search of infectious origins of specific cardiovascular diseases.

A second activity deals with secular and other changes in mortality rates of specific diseases through analyses of death certificates of Memphis residents. Certificates for 1917 through 1958 are being reappraised in the light of modern medical concepts as to probable cause of death.

The third activity comprises the initiation of studies in hospitals and outpatient clinics at the Medical Center, University of Tennessee. These studies stem from differences recognized between the races in the autopsy and death certificate analyses described above. Investigations using living populations are developing into an on-going classification and categorization of patients as to their kinds and severity of specific diseases.

New York City Studies. These activities are aimed at assessing the symptoms and physical activities antecedent to sudden death to identify those physical, emotional, and environmental factors that may predispose to or precipitate coronary heart disease. Study subjects include autopsied populations coming to medico-legal examination and patients with chronic diseases among the employees of the Bell Telephone Company of NYC. Information on decedents is obtained by home interview with surviving family members.

Proposed Activities. A series of population studies will be undertaken in quest of basic and contributing causes of cardiovascular diseases. These will include activities out of Framingham, Massachusetts, and Rochester, Minnesota.

Studies will be undertaken to extend and complement the basic Framingham Community project. The kinds and amounts of cardiovascular diseases will be determined and related to the specific populations from which they derive. These may include residents of Framingham and surrounding communities. Associations will be sought between patients with disease and such factors as the distinct socio-cultures, ethnic classes, and occupations of appropriate population subgroups. Specific hypotheses of causation will be tested through such comparisons.

A population of Rochester school children first examined in the mid 1930's for blood pressure levels and reactions to the cold-pressor test will be identified and reexamined by similar techniques. The frequency of hypertension and the frequency of altered reaction to a standard stimulus will be assessed. In addition, the prevalence and incidence of hypertension in Rochester will be gauged through blood pressure determinations on a probability sample of the population.

PHS - NIH
NATIONAL HEART INSTITUTE
Individual Project Report
Calendar Year 1959

GRANTS AND TRAINING BRANCH

Research Grants Program:

An appropriation of $36,468,000 was made for research projects in fiscal year 1960. As of December 1, 1959, 1,829 research grant applications have been recommended favorably by the National Advisory Heart Council in the amount of $30,073,897. After allowance is made for reserves already set up (primate and clinical research centers, Russian translation, etc.), $3,360,795 remains for further grant awards following the recommendations of the National Advisory Heart Council at the March 10-12, 1960 meetings. At this meeting, it is probable that more than 500 competing applications will be considered.

The objective of this program is to encourage and support research in cardiovascular and related areas. In order to attain this objective, the program planning must (1) be mindful of the needs of the scientist and find mechanisms to promote stability and security for the experienced investigator, (2) develop and support programs which will attract promising young scientists, (3) encourage research in fields which show particular promise, and (4) be alert to new trends and ideas in medical research and scientific methodology.

One of the problems encountered in handling this program during the past, which has been alleviated to great degree during this past year, is the lack of stability of support. This has been helped through the allowance of seven year commitment recommendations by the National Advisory Heart Council increasing the length of promise to pay, thus giving the outside institution scientist a firmer basis on which to begin a project with long range goals. A continuing problem and one that is not expected to be eased in the immediate future is the lack of available research space. This difficulty, well known to the NIH internal operation, is duplicated as the rate of research support increases at medical schools, universities and private research institutions throughout the country.

Research grants support inquiries into both the basic structure and function of the cardiovascular system and into the causes and methods of treatment of the major cardiovascular diseases. These projects include laboratory research, clinical studies of patients, and studies of general population groups - healthy as well as diseased - in order to learn more about the natural occurrence of these diseases. A trend already established but receiving impetus in 1959 is the support of collaborative research projects. This type of project generally involving a sizeable grant with long promise of support, allows for the collaboration of investigators, with various specialties from different departments within an institution, toward concentrated effort on a

specific problem. This activity could essentially be called a "research center", bringing together possibly, basic and clinical research people or various departments such as biology, biochemistry, and physics, working toward a common goal.

Methods of treatment and means of prevention of cardiovascular diseases have been improved during the past year through research supported by grants from the National Heart Institute. Some of these accomplishments, described in the following pages indicate the avenues of investigation being used by the thousands of scientists, technicians, and auxiliary personnel whose work is supported by research grants.

Arteriosclerosis

Research workers in the field of arteriosclerosis, and coronary heart disease in particular, are pursuing the answers to such cogent questions as: What dietary recommendations can the medical profession make to patients who have coronary heart disease? Can a practical food pattern be developed for the whole population that will be effective in reducing serum cholesterol levels and maintaining them at lowered levels to decrease the risk of coronary heart disease? What is the effect of "stress" on the development of the disease? And of special interest to industry, what are the chances for rehabilitation of the cardiac patient as a productive worker?

The answers to these and many other questions will come from research supported by NHI grants in the fields of basic research, animal experimentation, and clinical study.

It is generally agreed that blood lipids are deeply implicated in atherosclerosis. Studies are being made of the hows and whys of their action on arteries. A feeding experiment indicates that two highly unsaturated dietary fats--one poor in essential fatty acids and sterols and the other rich in these two factors--have similar effects on human serum lipid levels, implying that the unsaturation of dietary fat rather than its content of essential fatty acids and sterols is important in lowering serum lipids. Another study of patients maintained on a fat-free diet long enough to reduce plasma lipid levels to a plateau shows that the further lowering of plasma lipids is due to the addition of unsaturated fat to the diet rather than to the subtraction of other materials.

Big strides are being made in the development of technical devices and procedures for the separation and analysis of biologically important fatty acids. Capillary columns, for example, are found to possess an efficiency exceeding that of the conventional columns by several hundred percent. Availability of such methods will undoubtedly bring forth new and important information on the role of these fatty acids in health and disease.

The stress imposed upon man in his quest for economic or social "success" may be playing havoc with his health. A study of a group of patients with clinical evidence of coronary artery disease suggests that circumstances interpreted by the patients as stressful elevated the serum cholesterol level, when diet and exercise were held constant. These patients displayed remarkable similarity in personality growth, attitudes and behavior pattern, although they represented a varied occupational group. A more detailed study will be made of the psychodynamics involved. Related to this is the finding that among animals in one zoo, the increase over forty years in the frequency of arteriosclerosis was related more closely to population density (number of animals per cage) than to age at death or to characteristics of the diet. This suggests that "social pressure" among animals is a major factor in the occurence of arteriosclerosis.

Irradiation of the heart as a way of increasing the inter-coronary circulation is being investigated. For poor surgical risks, irritation of the heart by this means may be a good substitute for surgery. Follow-up studies on dogs six months after irradiation will determine whether the procedure results in the laying down of scar tissue which would adversely affect inter-coronary circulation. Use of substances which could be injected into the myocardium or placed on its surface to stimulate the formation of new vessels is being studied. Surgical methods for stimulating the development of collateral coronary circulation are being tried.

A variety of drugs has evolved for use in arteriosclerotic disease, but their usefulness is not yet established. Some are designed to cause a regression of atheromatous plaques. Soy phosphatides produce promising results in atheromatous rabbits. A pituitary hormone appears to stimulate mobilization of fat from adipose tissue.

The quest for drugs which will lower serum cholesterol is engaging the attention of many investigators. Use of nicotinic acid for this purpose has produced conflicting results, and additional controlled studies are indicated. This substance is also reported to promote fibrinolytic activity and it is being studied for use in the therapy of thrombo-embolic diseases. Consistent lowering of serum cholesterol levels following oral administration of neomycin--an antibiotic--is reported. This effect does not appear to be caused by a modification of the intestinal flora since other antibiotics do not lower the cholesterol level.

Hypertension

Although elevation of arterial blood pressure is the basic attribute of all forms of hypertension, the etiology of the various clinical types remains uncertain. "Essential" hypertension and hypertension secondary to pyelonephritis, for example, may be unrelated. On the

other hand, it is possible that such secondary hypertension occurs chiefly in persons who are highly susceptible to essential hypertension. Studies are underway to clarify these points. One in particular is aimed at ascertaining the occurrence of hypertension among relatives of known hypertensives, to study the physiological, socio-economic and environmental characteristics of individuals in whom hypertension has or has not developed, and ultimately to determine the effect of familial factors which will throw some light on the comparative roles of inherited and acquired factors in the pathogenesis of hypertension.

Disability and death due to hypertension are often more closely linked to atherosclerosis than to an elevation of arterial pressure per se. The experimental production of hypertension, the effects of dietary deficiency, nephrectomy, and the use of renal transplants in animals are methods being used to study the relationship between hypertension and arteriosclerosis.

A host of drugs for the alleviation of high blood pressure are being evaluated for clinical use. Their mechanisms and sites of action are being determined in animals by intricate techniques whereby central and peripheral activity can be distinguished, prior to therapeutic use on patients.

Blood pressure increases gradually with age, and the process by which this occurs is being sought. Variations in the blood and urine of hypertensive and normal subjects are being analyzed. Whether the hyperexcretion of electrolytes in secondary to the blood pressure elevation within the kidney or is a primary renal or metabolic defect is under consideration.

Rheumatic Fever and Rheumatic Heart Disease

Recent investigations into the etiology of rheumatic fever have solidified the concept that it usually follows Group A streptococcal infections. But the mechanisms by which the disease is initiated, the immunologic processes involved, the roles of environmental factors and of genetic predisposition continue to beg for clarification. Preliminary data from a study of streptococcal infection among school children indicate that a large percentage of children had positive cultures of the nasopharynx on one or more occasions during the school year. But the rate of clinically manifest streptococcal infection has remained relatively low. The significance of the positive cultures is being intensively studied by addition of serologic techniques to aid in the diagnosis of streptococcal illness and to detect the presence of coincident viral respiratory illnesses, for any viral-bacterial relationships in the problems of streptococcal virulence. Various types of drugs in the prevention and treatment of rheumatic fever are being evaluated. The use of intramuscular injections of penicillin in a three year study of prophylactic methods proved more effective than

either oral penicillin or sulfadiazine. The current studies will be expanded to verify dose relationships.

A direct approach to the correction of heart damage due to rheumatic heart disease is the replacement of diseased aortic and mitral valves by better and safer prosthetic ones with moving leaflets. But the problem of adequate fixation of the prosthesis is a pressing problem.

Surgery

In addition to the advances in surgery related to disease areas cited elsewhere, there is an active search for better methods of arresting and reviving the heart in connection with open heart surgery, such as the use of various combinations of drugs, perfusion of the heart with oxygenated and decalcified blood, or perfusion with cooled blood (hypothermia). Recent findings indicate that the hypothermic technic provides the advantages of cardiac arrest periods longer than those induced by chemicals, simplifications in the extracorporeal circulation apparatus, and reduction in the incidence of certain clinical complications such as cerebral edema. The requirement for large volumes of blood during open heart surgery may be reduced as a result of the experimental finding that perfusion of only a few vital organs rather than the entire body is sufficient to permit complete recovery after circulatory arrest for more than an hour. A surgical procedure still in the experimental stages promises to be of value in the alleviation of a serious type of congenital defect involving transposition of the major blood vessels leaving the heart. Of particular interest in the field of tissue and organ transplantation, is the apparently successful transplantation of a kidney between fraternal (not identical) twins which constitutes a major advance, and the continuing study of preoperative X-irradiation of patients to minimize graft rejection due to antibody formation.

Cerebrovascular and Peripheral Vascular Disease

The application of arterial radiographic visualization to the study of cerebrovascular disease has revealed a high frequency of occlusions of the internal and common carotid arteries. A large scale study is under way for the selection of operable patients in the hope of averting progress of occlusive disease leading to strokes. Investigators are also seeking a means to prevent the thrombosis of small extracranial blood vessels following surgery without the use of hazardous anticoagulants. If this is successful it may have application to the smaller blood vessels of the body such as the intracranial arteries or the coronary arteries.

Improved techniques and materials are being developed for the replacement of sections of peripheral blood vessels. Materials are

Peripheral Vascular Disease

sought which will maintain their patency, elasticity, and the ability to bend without kinking. The formation of scar tissue causing stiffness of porous tubes is a major cause of graft occlusion.

Pilot studies indicate the possibility of extending successful endarterectomy to full length leg arteries permitting the salvage of limbs otherwise doomed to amputation.

Nutritional factors are being studied for their effect on cerebral function as they are for their effect on arteriosclerosis. A study in hamsters has shown that large fat meals significantly alter the circulation resulting in aggregation of red blood cells, slowing of the circulation and elevation of blood viscosity. In addition alterations occur in the size and shape and sedimentation of the red blood cells. These changes cause alterations in the available oxygen in the cerebral hemispheres, which frequently produce convulsions. Clinically it appears that patients with cerebrovascular disease have a relatively high viscosity. On a low fat diet the blood viscosity falls to near normal in three to six months. Further study is being undertaken to confirm these results and to appraise the significance of these findings.

Epidemiology and Geographical Pathology

Because of the multitude of factors believed to be related to the development and course of cardiovascular disease an approach which studies the various factors as they naturally occur in population groups offers unique opportunities

Several factors in our mode of life are suspect. These include mental stress, physical activity, smoking habits and diet. These factors are not easily measured, and associations with disease do not necessarily indicate causal relationships. Many variables may be encountered, genetic and environmental, which may not be possible to control in a single study. But there is no doubt that the epidemiologic approach provides clues on causation for testing under more strictly controlled conditions, and a background against which laboratory experience can be assessed.

Studies are being done among unique racial groups, with similar ethnic origin, cultural background, dietary habits and geographic location, to determine any possible correlation with the incidence of cardiovascular disease.

One seven year study on 1800 men to determine the incidence of coronary heart disease in relation to various biological and social factors indicates that among white males significant differences occur according to relative weight, blood pressure levels, serum cholesterol levels and family history of heart disease. No differences in

Epidemiology and Geographical Pathology

incidence were observed according to physical activity of the job class, (professional, clerical, unskilled laborer) or to economic status.

Another study of the epidemiology of congenital heart disease is in progress, pursuing various lines: the relationship to meteorologic conditions, radiologic fall-out, pregnancy histories, blood types of children and mothers, virus content of serum and heart tissue, race, ethnic origin, income, education and housing.

Other Areas of Research

National Heart Institute grants are supporting research in many areas which do not lend themselves to the classifications described above. The more important groups of these are:

Instrumentation: Recent advances in the application of instruments to basic as well as to clinical problems in the cardiovascular area, include development of an apparatus for studying heart wall motion by means of ultrasound waves; a device for recording on magnetic tape the blood pressure and vessel circumference at multiple sites in the vasculature of intact living animals that will make possible valuable studies on blood pressure regulation; the improvement of extracardiac pacemakers which are small electronic devices attached to the body that rhythmically stimulate the heart to contract; an electronic instrument for the study of rapid mechanical changes in contracting muscle that promises to make available new knowledge about cardiac muscle function and the mode of action of cardiac drugs; a device that simulates in part the structure of the heart and in which various types of experimental prosthetic valves can be tested for their effects on flow rates, turbulence, pressure differences, etc. in the model system; and the further application of nuclear magnetic resonance spectroscopy to biochemical studies of molecular structure.

Genetics: Heredity as a causal factor in cardiovascular disease is the subject of increasing research interest. Long-term studies on the genetic aspects of arteriosclerosis and hypertension are underway involving not only detailed investigations of selected disease-prone families but also broad surveys of population groups. Inherited abnormalities in the molecular structure of blood proteins, especially the hemoglobins, which have been known clinically for some time, are being investigated to determine the exact nature of the molecular abnormalities, their biochemical properties and the mechanisms by which they produce symptoms of disease. Additional knowledge on other blood disorders is accruing; for example, the pattern of inheritance of a deficiency in the Hageman (clotting) factor has been studied as well as its chemical properties when partially purified. Recent findings indicate the inheritance of certain blood defects is correlated with the transmission of non-cardiovascular genetic defects such as color blindness. An additional related topic currently under

investigation concerns the effect on the cardiovascular system of certain inherited disturbances in other body structures such as the muscular system.

Therapeutic evaluation: In the search for improved methods for the treatment of cardiovascular diseases, research effort will focus on the synthesis and evaluation of new drugs as well as the testing of known pharmaceuticals under new experimental and clinical conditions. In addition to the studies on drugs discussed in relation to arteriosclerosis, other workers in this field are investigating the use of hormones such as a synthetic compound related to estrogen that decreases serum cholesterol and has little or no feminizing power in males, the value of purified blood components in regulating the formation and lysis of clots that can occlude blood vessels, and in connection with arterial surgery, the identification of various chemicals that can adhere to the inner surface of the arterial wall and minimize clot formation.

Clinical trials on a new drug, guanethidine, indicate its ability to reduce blood pressure in renal and essential hypertension without the side effects accompanying other commonly used agents. Other advances in cardiovascular drug research that show promise include a compound that may be clinically useful in treating disturbances in the rhythmical contractions of the heart and the isolation and testing of plant substances of potential value for patients with heart failure.

Blood coagulation and thrombolysis: The clinical problems presented by blood coagulation disturbances are frequent, challenging and extensive, including the life-long difficulties of the hemophiliac (bleeder), gangrenous states in the limbs of the body due to obstruction of blood vessels, severe paralytic strokes caused by cerebrovascular occlusion and sudden death due to coronary thrombosis or pulmonary embolism. In studies on bleeding disorders due to the decreased amount of a clotting factor in the blood, attempts are being made to isolate the necessary factor from sources of normal blood plasma and to test its ability to remedy the defect. The coagulation disturbances associated with arteriosclerosis are the subject of considerable research activity in order to elucidate the factors that increase blood coagulability and impair clot dissolution and also to develop means of preventing or counteracting these disturbances. A system recently devised for observing coagulation in flowing human blood should make further important studies possible. In the area of anticoagulant drugs, the definitive characterization of an antithrombin isolated from blood now seems possible and several substances are reported to be of value in counteracting the danger of excessive levels of these drugs.

In addition to these studies concerning the prevention of clotting, research is actively continuing on the factors involved in

thrombolysis, the dissolution of clots. For example, investigators are attempting to isolate thrombolytic agents from natural sources such as soil fungi. Plasmin, an enzyme normally occurring in blood, has been used to dissolve blood clots experimentally produced in the coronary arteries of test animals. Since no injurious effects were noted in this study, clinical trials have been proposed using a plasmin preparation recently isolated in a sufficiently pure state for human use. New knowledge is also being gained about a number of substances, such as streptokinase, that promote clot lysis by increasing the production of active plasmin in the body. The development of a practical method for the use of streptokinase on patients with intravascular clots is under investigation.

Primate Research Centers

The need for national facilities for research on sub-human primates in the cardiovascular field has been of major concern to the National Advisory Heart Council for some time. During 1959, steps have been taken to achieve a solution to this problem. Attention was given to the alternatives of a single national primate research station or several national primate centers. Consideration was also given to the question of whether a station or center should have only a cardiovascular orientation or should be a facility for research on primates, more broadly conceived.

The Council's committee on the Organization and Establishment of Primate Research Centers, with staff assistance visited thirteen possible sites in various parts of the country. A number of places visited were judged by the Committee to have sufficient local interest and center possibilities. They were encouraged to submit formal applications and a number of these applications have been received. The applications will be reviewed initially by the National Advisory Committee of Primates in the Division of Research Grants and will receive final review by the National Advisory Heart Council in March 1960. Following the Congressional directive on the use of funds the $2,000,000 appropriated for this purpose in fiscal year 1960 and after competitive review, obligations will be made with a view toward the establishment of two centers based on the broad research objective approach.

While the long range goals of this primate program have previously been slanted toward the establishment of four to six smaller centers, serious thought is being given to the advisability of the establishment of at least one large national station. This larger unit would allow the development of several species of primates in sufficient numbers within one facility to permit both long and short term, comparative studies.

Training Grant Program:

An appropriation of $8,679,000 was made in fiscal year 1960 for research and clinical training grants at the undergraduate and graduate levels. As of December 1, 1959, 304 training grants have been awarded in the amount of $7,990,850. It is probable that approximately 42 training grant applications will be considered at the March 1960 meeting of the National Advisory Heart Council.

In the training program, training grants continue to assist in the development and expansion of research training opportunities in laboratories with strong on-going research programs. The National Heart Institute now supports over 300 training programs, with nearly all medical schools participating, and ranging from intensive programs in such fields as lipid chemistry technology and cardiac surgical research to broad inter-disciplinary training in clinical investigation. Noteworthy, as a result of the increased Congressional appropriation for fiscal year 1960, has been the development of multi-departmental programs where several laboratories with related research goals pool their training resources to offer broadly diversified, carefully structured research training opportunity. It is anticipated that more programs of this type will be developed in the coming year, especially programs which join preclinical departments (or even components of other colleges such as electrical engineering departments or biology departments) with clinical departments in order to more rapidly and authoritatively bring the research technologies of basic sciences into clinical cardiovascular research.

Two other programs in the training field where much needed further expansion will be possible as a result of the increased funds for training this year, are (1) training investigators in the exacting methods of drug evaluation; (2) development of programs in specially needed areas such as medical electronics, comparative cardiology, medical genetics, etc.

Finally, as a pilot program it is planned to select certain academically oriented and otherwise qualified hospitals in large communities where there are no medical schools, and develop programs which will encourage the growth of both basic and applied clinical research in the hospitals. This program has three aims: (1) to increase the numbers of loci of research in the country; (2) to improve the scientific and academic atmosphere of these institutions so that they may become nuclei from which medical schools or other graduate medical educational institutions may in time develop; and (3) to bring scientific medicine closer to the practicing physician.

Research Fellowship Program:

An appropriation of $2,663,000 was made in fiscal year 1960 for predoctoral, postdoctoral, special, and part-time medical fellowships. As of December 31, 1959, a total of 297 fellowships in the amount of $1,296,000 have been awarded. It is probable that about 324 competing applications will be considered during the monthly meetings of the National Heart Institute Fellowship Review Board during the remainder of the fiscal year.

The research fellowships program continues to be a vital and growing aspect of research training. It serves the important function of providing research training (1) when the trainee is seeking a unique or specially designed training sequence not provided by any of the formal training programs, (2) when young laboratories wish to develop training experience but are not in a position, yet, to set up comprehensive training programs, (3) when senior investigators want to refurbish or diversify their research background in the course of sabbatical or other tenures, (4) to support young men who are completing their training but whose independent research is not yet strong enough to win a more senior faculty position.

The purpose of the present program is to develop an increased number of predoctoral, postdoctoral and special research fellows in the cardiovascular areas. The significance of this objective is that the national reservoir of accomplished researchers and teachers will be increased to aid in meeting the future needs of staffing new medical schools, teaching and research institutions, thus moving towards the goal of improving the cardiovascular health of the nation.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services
Division of Special Health Services
Heart Disease Control Program

INTRAMURAL RESEARCH PROJECTS
as of July 1, 1959

TITLE	INVESTIGATOR	DIVISION NO.
Coronary heart disease in vegetarians	Marjorie Cantoni Olive Hayes	HD-10
The disabled consequences of coronary heart disease: Nature and causes	Herbert S. Caron	HD-11
Trends in prevalence of rheumatic heart disease among college students	Roy P. Sandidge Margaret Evans	HD-14
North Dakota coronary disease study	Philip E. Enterline William J. Zukel Jean Pekover	HD-16
Relationship between obesity in childhood and obesity in adult life	Sidney Abraham S. Leonard Syme Marie Nordsieck	HD-17
Overweight vs. obesity as related to cardiovascular disease	H. A. Tyroler Sidney Abraham	HD-18
Cardiovascular mortality variation by and State of birth	Philip E. Enterline Herbert I. Sauer	HD-19
Twenty-year follow-up of stethographically recorded functional murmurs in children	Carl Marienfeld B. Boone Marie Nordsieck	HD-20
The use of an electronic computer as a diagnostic aid	Arthur E. Rikli	HD-21
Serum cholesterol level of American Indians on five reservations in the United States	Sidney Abraham David C. Miller	HD-22 (Completed)
A preliminary study of occupational stress in relation to hypertension, hypercholesterolemia; and coronary heart disease	H. A. Tyroler S. Leonard Syme	HD-23
Development of methods for assessing the adequacy of cardiovascular diseases mortality statistics for epidemiological studies	J. Stamler I. Moriyama Herbert I. Sauer	HD-24
Program service statistics	Edith Jungblut Marie Nordsieck Herbert I. Sauer	HD-25
Epidemiology of cardiovascular disease in the Carolinas and in Georgia	Herbert I. Sauer Joseph P. Conte	HD-26 (New)

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-10

5. PROJECT TITLE
Nutrition Study of Seventh-Day Adventists at Washington Sanitarium.

6. INVESTIGATORS
Miss Marjorie Cantoni
Miss Olive Hayes

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
November 1955

9. OBJECTIVES
To compare the degree of coronary atherosclerosis in vegetarians and non-vegetarians of the same age, sex, and race.

10. PRINCIPAL RESULTS

1. The clinical laboratory at the Washington Sanitarium and Hospital completed the standardization of the modified Abell-Kendall method for serum cholesterol determinations. Assistance was obtained from the National Heart Institute Study at Framingham, Massachusetts, and an exchange of serum samples showed that the laboratories were in agreement.

2. The collection of pilot study information has been completed (22 diet interviews and 58 serum cholesterol determinations). The data are being reviewed to determine changes necessary to improve the collection of data on additional subjects.

3. Miss Olive B. Hayes, nutritionist, joined the staff May 25, 1959, and will be responsible for taking dietary interviews. She will assist Miss Marjorie Cantoni in developing a method of analyzing diet history information according to food groups and cultural eating patterns that may be related to the study of coronary artery disease.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-11 (contract)

5. PROJECT TITLE
The disabling consequences of coronary heart disease: Nature and causes.

6. INVESTIGATOR
Herbert S. Caron, Ph.D.

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
July 1955

9. OBJECTIVES
Coronary heart disease is believed to result in a great deal of disability beyond what may be attributed to physiological changes alone. The present study has the purpose of describing the nature and the approximate extent of such disability and determining the psychological factors associated with such disability.

10. PRINCIPAL RESULTS
In this study those patients who recovered fully following a coronary tended to be more depressed immediately after the attack than those patients who did not acknowledge their illness. It is hypothesized that depression and denial are alternative reactions to illness functionally related to recovery or disability. This hypotheses is being tested further under a contract with George Washington University.

11. PUBLICATIONS
A paper has been prepared for submission to the Journal of Chronic Disease.

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-14

5. PROJECT TITLE
Trends in prevalence of rheumatic heart disease among college students.

6. INVESTIGATORS
Dr. Roy P. Sandidge, Jr.
Miss Margaret Evans

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
January 1956

9. OBJECTIVES
To determine trends in prevalence of rheumatic heart disease as detected in entering freshman college students over a period of five years. Further, to determine the extent of use of prophylactic measures to prevent recurrences of rheumatic fever among this group.

10. PRINCIPAL RESULTS
During the first two years of the proposed five-year study, data were reported on 176,588 entering college freshmen. Analysis of the data showed that there were 2,866 students who had a definite history of rheumatic fever and/or definite rheumatic heart disease, for a rate of 16.2 per 1000 students examined. Only 288 of these students were following a regular program of anti-streptococcal prophylaxis at the time the examination was made.

11. PUBLICATIONS
A paper reporting the results of the first two years of the study was presented at the annual meeting of the American College Health Association in May 1959 and will appear in a subsequent edition of the Association's journal, Student Medicine. A second paper is now being written to be offered for publication in the American Journal of Pediatrics.

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-16

5. PROJECT TITLE
North Dakota Coronary Disease Study

6. INVESTIGATORS
Dr. William J. Zukel
Mr. Philip E. Enterline
Mrs. Jean Pekover

7. LOCATION OF PROJECT
Grand Forks, North Dakota, and six contiguous counties

8. DATE PROJECT INITIATED
September 1956

9. OBJECTIVES

1. To obtain the incidence of newly manifest clinical coronary disease defined as angina pectoris, coronary insufficiency, and myocardial infarction occurring within a six-county area (pop. 106,000) of North Dakota.

2. To compare the farming and non-farming population as to incidence of newly manifest coronary disease.

3. To attempt to correlate occupation, physical exercise, smoking habits, diets, and other factors with such new coronary disease manifestations.

4. To obtain a long-term prognosis of such new coronary disease manifestations by yearly follow-up and by checking death certificates at the Division of Vital Statistics, State Health Department.

5. To follow a cohort of males age 35+ from the general population to relate subsequent morbidity and mortality from coronary heart disease to certain base line environmental characteristics established by the Census.

10. PRINCIPAL RESULTS

Initial findings in 228 cases of coronary heart disease (CHD) reported during 1957 in males 35 years of age and over show that a lower incidence of CHD occurred in farmers than among other occupational groups; that there was a higher incidence of CHD in cigarette smokers than non-smokers; and that physical activity probably was also related to CHD. Comparison of recent dietary histories of cases and controls revealed no differences in mean caloric intake, total fat consumption, or other major dietary constituents. A paper on diet in relation to

10. PRINCIPAL RESULTS (Cont'd.) SHS-HD-16

coronary disease is being prepared for publication in the Journal of the American Dietetic Association. A paper presenting the clinical picture of coronary disease is also being prepared for publication. Additional analyses of these data are currently under way.

11. PUBLICATIONS

A paper on methodology was published in the February 1959 issue of the American Journal of Public Health. An abstract of a paper reporting preliminary findings appeared in the March 1959 issue of Public Health Reports and will be published shortly in the American Journal of Public Health. Both papers were presented at the American Public Health Association meeting last fall.

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-17

5. PROJECT TITLE
Relationship between obesity in childhood and obesity in adult life.

6. INVESTIGATORS
Mr. Sidney Abraham
Dr. S. Leonard Syme
Miss Marie Nordsieck

7. LOCATION OF PROJECT
Hagerstown, Maryland

8. DATE PROJECT INITIATED
August 1957

9. OBJECTIVES
To see if obese children tend to become obese adults, and to see if, in a group of obese adults, weight status as children is related to weight reduction problems as adults.

10. PRINCIPAL RESULTS
A list containing the names of 100 of the most overweight and 100 of the most average-weight children was drawn from records of 2,400 physical examinations performed in 1938. These selected individuals, now adults, were interviewed and measured (for height and weight) during the summer of 1958. Data from these interviews have been coded and processed and are currently being analyzed. These data show that childhood weight status is of major importance in determining weight status in adults and suggest that control measures to be effective should probably be aimed at overweight children rather than at adults.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-18

5. PROJECT TITLE
Overweight vs. Obesity as related to cardiovascular disease.

6. INVESTIGATORS
Dr. H. A. Tyroler
Mr. Sidney Abraham

7. LOCATION OF PROJECT
Asheville, North Carolina

8. DATE PROJECT INITIATED
May 28, 1957

9. OBJECTIVES
To evaluate the significance of body fat content as distinguished from overweight per se as a factor related to the development of cardiovascular disease, and as a factor related to serum cholesterol levels and blood pressure levels.

10. PRINCIPAL RESULTS
Total serum cholesterol levels of the study group are in agreement with the levels found in high serum cholesterol populations. Too few cases of cardiovascular disease have developed thus far to warrant extensive analysis.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-19

5. PROJECT TITLE
Cardiovascular Mortality by Geographic and Related Factors, particularly by Country and State of Birth.

6. INVESTIGATORS
Mr. Philip E. Enterline
Mr. Herbert I. Sauer

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
July 1957

9. OBJECTIVES
To compare cardiovascular mortality rates of (a) native whites and (b) foreign-born whites (total and by specific country of birth):
(1) for different sections of the United States;
(2) with countries from which most of the migrants to the United States have come;
(3) by State of birth of the native white
for the purpose of determining more precisely the differences in these rates, and to provide a basic framework from which to continue the search for factors responsible for these contrasts.

10. PRINCIPAL RESULTS
For foreign-born males in the Middle Atlantic States, 1950, the Italian-born have the lowest death rates for coronary heart disease, all cardiovascular diseases, and also for all causes. Those born in Ireland have the highest rates for all cardiovascular diseases and also for all causes.

Mortality rates by metropolitan areas and economic subregions present with more precision a pattern similar to that of death rates by State. Most of the metropolitan areas with high coronary death rates are near the ocean. The economic subregions, consisting of rural areas and small cities, show wide variation, with the lowest rates chiefly in the Great Plains area and with the highest rates generally near the coast. In general, the metropolitan areas have much higher rates than do the non-metropolitan areas.

Coronary heart disease death rates by State of birth generally follow patterns similar to those by State of residence. Those who

10. PRINCIPAL RESULTS (Cont'd.) SHS-HD-19

were born in New York who moved away have death rates almost as high as those who remained in New York. Likewise, those who moved away from North Dakota have rates almost as low as those who remained in North Dakota.

11. PUBLICATIONS

A paper, "Are Geographic Variations in Death Rates for the Cardiovascular Diseases Real?" has been accepted by the Journal of Chronic Disease. (Sauer and Enterline)

A paper, "Death Rates for Coronary Heart Disease in Metropolitan and Nonmetropolitan areas of the United States, 1949-51," has been submitted to Public Health Reports. (Enterline, Rikli, Sauer, and Hyman)

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-20

5. PROJECT TITLE
Twenty-year Follow-up of Stethographically Recorded Functional Murmurs in Children.

6. INVESTIGATORS
Dr. Carl J. Marienfeld
Dr. Bert Boone
Miss Marie Nordsieck

7. LOCATION OF PROJECT
Hagerstown, Maryland

8. DATE PROJECT INITIATED
April 1958

9. OBJECTIVES
To make a longitudinal evaluation using stethograms, electrocardiograms, and X-rays taken 20 years ago among Hagerstown, Maryland, school children. If internists are correct and functional murmurs are significant, they should still be present and associated with other manifestations of heart disease in the group of persons followed. If, on the other hand, the pediatricians are right and functional murmurs are normal for children and adolescents, then the murmurs should have largely disappeared, and not more than 5 cases of heart disease should be observed (based on an expected prevalence of 2%* in the general population with an allowance for sampling variation).

10. PRINCIPAL RESULTS
Of the 158 children with loud but apparently functional murmurs who were selected from among 4,000 who had phonocardiographs in 1938, 100 were located, and 97 have had examinations which included physical examination, laboratory studies, X-ray, phonocardiogram and electrocardiogram. All of these readings have been completed. Analysis will begin after September 1, 1959, and the work should be complete by December 31, 1959.

11. PUBLICATIONS
None

*Based on 3,300,000 selective service examinations of men between 18 and 37 years of age; conducted from 1940 through 1952.

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-21

5. PROJECT TITLE
The Use of an Electronic Computer as a Diagnostic Aid.

6. INVESTIGATOR
Dr. Arthur E. Rikli

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
December 1957

9. OBJECTIVES
To explore how an electronic computer might serve as a diagnostic aid to physicians.

10. PRINCIPAL RESULTS
The first phase of the work plan, the feasibility study, has been completed. Forty-five data points have been manually measured for 15 normal and 15 pathological subjects. IBM punch cards have been prepared. Tabulations of data are almost completed. Data analysis will follow.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-22

5. PROJECT TITLE
Serum Cholesterol level of American Indians on five reservations in the United States.

6. INVESTIGATORS
Sidney Abraham
Dr. David C. Miller

7. LOCATION OF PROJECT
Serum cholesterol data from Indian Health Survey

Crow, Montana
Yankton, South Dakota
Acoma, New Mexico
San Carlos, Arizona
Lac Court Oreilles, Wisconsin

8. DATE PROJECT INITIATED
July 1, 1958

9. OBJECTIVES
To see whether the mean level of serum cholesterol of clinically healthy Indians residing on five reservations in the United States is low or high in comparison to the mean levels generally accepted for the U. S. population.

10. PRINCIPAL RESULTS
The study showed that the serum cholesterol level of this American Indian Series was significantly lower than that of the Cleveland Clinic group, whose mean level is similar to that found in other surveys of American non-Indian populations.

11. PUBLICATIONS
Paper published in Public Health Reports, May 1959, by Sidney Abraham and David C. Miller, M.D.

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-23

5. PROJECT TITLE
A Preliminary Study of Occupational Stress in Relation to Hypertension, Hypercholesterolemia, and Coronary Heart Disease.

6. INVESTIGATORS
Dr. H. A. Tyroler
Dr. S. Leonard Syme

7. LOCATION OF PROJECT
Canton, North Carolina

8. DATE PROJECT INITIATED
November 1958

9. OBJECTIVES
The general objective of this proposed study is to examine the relationship of occupational stress to hypertension, hypercholesterolemia, and coronary heart disease. Specific objectives are:

(a) To study a set of theoretically defined "stresses" in order to see which of these "stresses" are related to specific physiological and medical conditions;

(b) To describe various occupational groupings (e.g., executive and unskilled manual workers) in relation to the "stresses" by which they are characterized.

10. PRINCIPAL RESULTS
This project is temporarily inactive pending the recruitment and orientation of a new medical director at the Champion Paper and Fibre Company. It is anticipated that the project will become reactivated during the coming fiscal year.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-24

5. PROJECT TITLE
Development of methods for assessing the adequacy of cardiovascular diseases mortality statistics for epidemiological studies.

6. INVESTIGATORS
Dr. Jeremiah Stamler
Dr. Iwao Moriyama
Mr. Herbert I. Sauer
and others

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
September 1958

9. OBJECTIVES
To make progress in outlining techniques for assessing the accuracy of cardiovascular death rates, particularly in groups in which routine autopsy verification of cause of death is not available. A minimum goal is to develop standards from which inferences could be drawn regarding differences in mortality rates at least to the degree that:

(a) The differences in the specified rates are great enough and meet specified tests in a way that they most probably represent real differences, even though proof may be lacking as to the exact magnitude of the differences.

(b) There is insufficient information available to determine whether the differences are real.

(c) These differences are consistent with specified hypotheses that the differences easily may be due entirely to differences in classification of cause of death or other data-collecting methods.

10. PRINCIPAL RESULTS
In addition to the three individuals listed under item 6 above, the members of this group project are:
Dr. Samuel A. Levine, Clinical Professor of Medicine, Harvard Medical School
Dr. James C. Roberts, Jr., Pathologist, East Tennessee Baptist Hospital

10. PRINCIPAL RESULTS (Cont'd.) SHS-HD-24

Dr. Morton D. Schweitzer, Assoc. Professor of Epidemiology, School of Public Health and Admin. Med., Columbia University
Miss Marjorie T. Bellows, Chief Statistician, American Heart Association
Mr. Herbert H. Marks, Assistant Statistician, Metropolitan Life Insurance Company
Mr. Robert W. Buechley, Associate Social Research Technician, Bureau of Chronic Diseases, Calif. Dept. of Public Health
Mr. Dean E. Krueger, Public Health Analyst, National Heart Institute

Invitations for Study Group membership were extended to individuals whose work is directly affected by or related to possible deficiencies in death data for the cardiovascular diseases. Individuals were selected to achieve a broad representation of views.

At its first meeting on May 1, the Group urged further study of:

(a) Representative groups of deaths, using autopsy and other methods for determining the adequacy and comparability of cause of death entered on the death certificate by the clinician.

(b) Deaths of middle aged persons (rather than all ages) - an emphasis made for both public health and scientific reasons. These are premature deaths, and in them cardiovascular diseases tend to be more specific than in deaths of elderly persons.

(c) Problems of counting deaths according to residence history, in a way to recognize the effect upon death rates of migration for health reasons and movement to rest homes and institutions.

(d) Approaches such as those given in the editorial, "Pitfalls in Interpreting Coronary Artery Statistics," American Heart Journal, November 1958.

Very substantial progress was made in agreeing upon methodological approaches. The objective is to assess methods for determining real differences or similarities in death rates, as basic to the discovery of any specific cause factors.

11. PUBLICATIONS
None

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-25

5. PROJECT TITLE
Program Service Statistics

6. INVESTIGATORS
Miss Edith Jungblut
Miss Marie Nordsieck
Mr. Herbert Sauer

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
January 1959

9. OBJECTIVES

To collect, analyze, and summarize various types of statistical information needed by the Heart Disease Control Program for planning, managing, and evaluating program activities in different phases of cardiovascular disease control work throughout the country. This includes:

1. Mortality statistics.
2. Morbidity statistics and estimates.
3. Program activity statistics such as casefinding, clinics, registries, and nursing.

10. PRINCIPAL RESULTS

Morbidity and control activities statistics have been obtained from various sources, including the National Health Survey and State and local health departments, and utilized to meet the needs of the Program and Division.

Tables and charts have been approximately completed and rough draft of text prepared for a publication, "Cardiovascular Mortality Data," to provide more recent mortality data than that presented in PHS Publication No. 429. Between 1949 and 1957, (a) cardiovascular mortality rates for white females age 45-64 show a definite decline while the white male rates remain on a high plateau, and (b) the number of CV deaths has increased substantially in the age groups 65 and over, apparently due to the increase in the population in these age groups.

11. PUBLICATIONS SHS-HD-25

A short article, "Recent Trends in Coronary Heart Disease Death Rates Among Middle-Aged White Males," has been accepted for publication in Public Health Reports (Enterline).

RESEARCH PROJECT REPORT OF PROGRESS

DATE: August 3, 1959

1. DIVISION
Special Health Services

2. BRANCH

3. PROGRAM
Heart Disease Control

4. DIVISION PROJECT NUMBER
SHS-HD-26

5. PROJECT TITLE
Epidemiology of Cardiovascular Mortality in the Carolinas and Georgia.

6. INVESTIGATORS
Herbert I. Sauer
Joseph P. Conte

7. LOCATION OF PROJECT
Washington, D. C.

8. DATE PROJECT INITIATED
April 1959

9. OBJECTIVES
Are the high cardiovascular mortality rates for the Carolinas and Georgia real? Are the extreme contrasts in rates for different parts of these States real?

(a) If they are not real, why not?
(b) If they are real, why?

10. PRINCIPAL RESULTS
Areas near the coast of Georgia and South Carolina have death rates for all causes for white males (age 65-74 as well as age 45-64) almost twice the rates for economic subregion 33 (western North Carolina and nothern Georgia) and death rates for coronary heart disease 3-1/2 times as high. While white female rates are much lower than white male rates, they tend to parallel the white male rates.

Preliminary discussions have been held with the Directors of Heart Disease Control and Vital Statistics in both Georgia and South Carolina as a basis for developing plans (a) to tabulate age-sex-race specific rates by county, so as to describe mortality patterns in these States with more precision, and (b) to check the accuracy of mortality data, especially in the areas with the highest and lowest rates.

In view of the demand for death rates for these areas, a paper is being prepared to present rates by metropolitan areas and economic subregion, and also showing long-term trends for these States.

11. PUBLICATIONS
See project number 19.

THE ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR

The National Heart Institute

Calendar Year 1959

by

Dr. Robert W. Berliner

The Heart Institute is charged with the responsibility for research aimed at the improvement of methods for the prevention and treatment of disorders of the cardiovascular system. The work of the intramural research branch can not be separated from this aim. There might, however, be wide differences of opinion as to how research efforts might best be distributed to serve this long-term goal most effectively.

The organization of intramural research in the Heart Institute is based on the premise that progress toward any goal in science is best made by the creative efforts of individuals motivated by their own intellectual curiosity toward the solution of problems that interest them. It does not seem appropriate to attempt to divide research into the categories of "applied" or "basic" since there would be little agreement on how such a classification could be made or even on how the groups would be defined. One suspects that the scientist himself would accept a classification that held that the man working on his own problems and following whatever leads may arise is doing basic research, while the man working on problems devised by someone else is doing applied research.

The problems which interest some individuals may have immediate practical significance; that which motivates others may not. Thus within the Heart Institute there are men interested in improving the treatment of hypertension, in the improvement of diagnostic technics and surgical procedures for the correction of anatomical defects of the heart, while others are concerned with exploring the mechanism of chemical reactions or the relation between the structure of a protein and its biologic function.

Only through both types of activity can the long-term goals be achieved and the best assurance of a maximum rate of progress is a high level of general scientific productivity. The major responsibility of those charged with the leadership of intramural research is to assure maximum productivity by the selection of men (for the promise of their areas of interest as well as their capacity to contribute) and the provision of an environment and facilities most conducive to scientific accomplishment and interdisciplinary collaboration.

The pages that follow reflect the scientific progress within the individual research groups of the Heart Institute in the last year largely as seen by the leaders of those groups.

Laboratory of Cellular Physiology and Metabolism

Section on Cellular Physiology

The work of the Laboratory of Cellular Physiology and Metabolism, Section on Cellular Physiology, continues to be aimed at elucidation of the structure of proteins, the relationships between this structure and specific function, and with the synthesis of proteins with respect both to biochemical mechanism and genetic control.

During the past year the work of the Section has been concerned with: 1) The development of methods for the study of protein structure and the application of these methods to ribonuclease, lysozyme and several other proteins. These studies also interlock with investigations on the relationships between structure and function in biologically active proteins; 2) Investigations on the genetic control of the biosynthesis of proteins of bacteriophage with emphasis on the enzyme lysozyme, a catalyst employed by the phage particles for rupturing the cell wall of their host bacterial cell; 3) Investigations of the secondary and tertiary structure of certain fibrous proteins and fibrous protein models; 4) Biosynthesis of proteins in the hen's oviduct and a detailed study of certain lipid substances which appear to be intimately involved with the biosynthetic process; and 5) Studies on the metabolism of triglycerides by adipose tissue and liver.

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1) The complete structure of ribonuclease has now been worked out in detail, through the combined efforts of Dr. Werner Hirs and his colleagues at the Rockefeller Institute and of Dr. Anfinsen and his colleagues of the Section on Cellular Physiology. Certain inconsistencies between the results of the two groups have been investigated in some detail and have been resolved in the past few months. These inconsistencies were concerned mainly with two of the 124 amino acid residues of ribonuclease whose positions in the polypeptide chain, as reported by Hirs et al., required inversion on the basis of the NHI data. This relatively minor point has been examined by a series of controlled proteolytic digestions and quantitative analyses. These detailed studies were of special interest since they involve the portion of the polypeptide chain which other studies suggest is involved in the active center of the enzyme.

In a continuation of earlier research it has been shown that all four disulfide bridges in ribonuclease can be cleaved by reduction with mercaptoethanol and that the resulting inactive product can be converted to the original native molecule by simple exposure to atmospheric oxygen.

Earlier uncertainties regarding the proper matching of half-cystine residues have now been resolved by the demonstration that the pairing of such sulfhydryl side chains is almost certainly identical with that found in the native protein and by the demonstration that regenerated protein is indistinguishable from native protein in immunochemical cross reactions. It has also been possible to show that approximately one-fifth of the polypeptide chain of the native molecule can be removed before the reduction-reoxidation procedure without destroying the "regeneratability" of the disulfide bonds in the remaining four-fifths of the protein. In terms of "genetic information," therefore, it seems possible to state that the information necessary for proper disulfide bridge formation is coded into only four-fifths of the molecule and that the rest of the chain must be present for other biological reasons. These studies are being continued with the aim of reducing the protein to a minimum size which will still permit reduction and reoxidation with the formation of an active regenerated substance. It is also planned to continue the work on stepwise reduction and stepwise reoxidation in an effort to prepare active intermediates which differ significantly in gross structure from the native enzyme. Preliminary studies have already indicated that several amino acids at the ends of the reduced, extended chain are superfluous from the standpoint of function and more drastic degradation is therefore indicated.

The species comparisons reported in last year's annual report which showed differences in structure between sheep and beef ribonuclease now have been extended to porcine pancreatic ribonuclease. Although the latter is superficially identical with the bovine enzyme in covalent structure it is totally non-reactive with anti-serum prepared against bovine ribonuclease (with first course serum, but reactive with second and third course serum). These immunological observations indicate the necessity for a more detailed study of comparative structure since they suggest that there may be some drastic, but not obvious, difference, perhaps in the nature of the pairing of disulfide bridges. Highly purified preparations of ribonuclease have also been made from spinach leaves and B. subtilis, although not in sufficient quantities or purity for attempting structural analyses. The species comparisons will be continued since they should lead to information on common denominators of structure which would inferentially suggest the location and nature of the active center of ribonucleases in general.

The variation of protein structure among various species is also being studied by preparing lysozymes from several bacteriophages and from the egg whites of a broad spectrum of birds. These studies, being carried out in part in collaboration with Professor Charles Sibley at

Cornell, are at the moment mainly concerned with the development of simple, reproducible methods for the isolation of lysozyme from egg whites and such a method is now essentially free of difficulties. It involves adsorption of the very basic lysozyme protein onto the cation exchanger XE-64, followed by elution and purification on columns of the same resin. Comparisons of structure can then be made on the purified proteins by separation of peptides produced by tryptic and chymotryptic digests on paper sheets, using chromatographic and electrophoretic methods -- the so-called "fingerprinting" technique. Preliminary results already indicate that the lysozymes from species to species will vary considerably less in structure than ovalbumins from the same species, supporting the hypothesis that enzymes can, in general, suffer less change during evolution than proteins whose functions are more concerned with storage or cellular architecture.

2) A major effort is being made to determine whether or not there exists a direct correspondence between the arrangement of genetic subunits in specific genes and the structure of the protein controlled by this particular gene. Since lysozyme from bacteriophages is a relatively small and easily isolated protein and since mutant forms of bacteriophages should be relatively easy to isolate and subject to genetic mapping, this protein has been chosen for special study. A number of mutant forms of bacteriophage T2 and T4 have been isolated which, during growth, release lysozymes of varying heat stability into the surrounding medium. It is the present plan to make genetic crosses of these mutants for the purpose of gene mapping and to isolate the lysozymes in pure form for direct comparison of structures. A major aspect of the work at the moment involves the study of the sequential structure of both bacteriophage and egg-white lysozymes in order to provide baselines for future studies on the relationships between structure and function. It is also planned to investigate whether or not nonlethal mutations will, as might be predicted, only occur in those areas of the lysozyme molecule that are not essential for activity.

3) The gross molecular structure of myosin, the protein unit of the muscle contractile mechanism, has been under active study by Drs. Harrington and Mihalyi. It was observed that the molecular weight of the myosin particle was decreased from 619,000 to 206,000 by concentrated guanidine solutions which tend to rupture hydrogen bonds and break protein polymers into smaller units. The conclusions reached from ultracentrifugation studies were supported by sedimentation, diffusion and viscosity measurements. These results, when considered together with observed length and width and with the optical rotatory and X-ray diffraction properties of the myosin molecule, led to the conclusion that myosin

is made up of three identical polypeptide chains, each wound into an α-helix and with the three strands twisted together to form a rope-like structure. Studies of the primary sequence of the myosin unit chain are now in progress. These should reveal whether all three are oriented in the same direction. The details of primary structure may then be used to explain the secondary and tertiary coiling and folding. Efforts are also being directed at determining the mechanism by which myosin subunits polymerize to form the aggregates characteristic of myofibrils. A second group of fibrous protein or protein-like molecules that have been thoroughly investigated are the collagens and collagen-like proline-glycine copolymers. Various proteolytic enzymes have been employed as specific probes of the secondary structure (that is, the internal coiling) of these long polypeptide chains and it appears that here, as in the case of myosin, these long molecules are made up of alternating amorphous and semi-crystalline regions with differential sensitivity to proteolysis. The kinetics of proteolytic digestion suggests that neighboring charged groups strongly influence the susceptibility of sensitive bonds to hydrolysis.

4) In the biosynthesis of proteins in the hen's oviduct, certain lipid components appear to be associated with an extremely active pool of amino acids. Dr. Hendler has separated these on alumina-silica columns in quantities for direct chemical study. When oviduct tissue or the bacterium E. coli is incubated with radioactive amino acids, the first metabolic pool to become labeled is a class of organic soluble substances which carry amino acids and peptide-like compounds. Whether or not the bond between the amino acids and peptides and the lipid moieties is covalent has not been established. The combined information on these interesting compounds suggests that they may be involved in the biosynthetic processes taking place in the so-called endoplasmic reticulum, which, it has been suggested, may be associated with protein synthesis. A study on the dissociation of this endoplasmic reticulum into its lipid components and ribosomal granules and the subsequent separation of these various components on ion exchange columns has been undertaken in collaboration with Drs. Peterson and Kuff of the National Cancer Institute. It is believed that information for making particular proteins is contained in the configuration of the nucleic acids of the ribosomal granules. On this basis one should expect that these granules will be heterogeneous and that it may be possible to fractionate them into classes, each responsible for a particular protein or group of proteins.

5) Work on triglyceride metabolism and on the nature of the heparin-induced lipoprotein lipase which have been under investigation

in this laboratory for a number of years has been continued by Dr. Korn. Lipoprotein lipase has been subjected to further purification with the purpose of determining whether heparin is an integral part of the enzyme.

Another group of long-chain polysaccharides associated with the yeast cell wall has also been investigated. These experiments, carried out by Dr. Korn in collaboration with Dr. D. H. Northcote at Cambridge University, have led to the isolation of fractions of yeast cell walls much more highly purified and better characterized than has previously been obtained. The studies on cell wall chemistry serve as models for the study and understanding of other conjugated proteins. The techniques for handling large conjugated proteins are relatively similar, whether the conjugated material is lipid or carbohydrate, and these studies should serve, therefore, as excellent background for the projected investigations of lipoproteins.

Dr. Rodbell has continued his investigation of the metabolic processes involved in the removal of chylomicrons of plasma. Rat epididymal adipose tissue does not distinguish between rat chylomicrons and synthetic fat emulsions with respect to uptake and metabolism, suggesting that chylomicron proteins are not essential for fat uptake or metabolism. Inhibition of lipoprotein lipase did not substantially reduce fat uptake, suggesting that this enzyme is perhaps necessary for chylomicron metabolism but not for transport into cells. C^{14}-labeled triglycerides were taken up from blood by the parenchymal cells of rat liver and these triglycerides were found to be associated with the microsomes and nuclei of the liver cells. The triglycerides are then converted to phospholipids and triglycerides characteristic of normal liver fats. These studies, in general, have implicated the microsomes in the absorption of exogenous fat by liver cells and it would appear that the endoplasmic reticulum may serve both as a channel for the entry of exogenous triglycerides as well as the site for metabolism and transformation.

Section on Metabolism

1. Studies on the basic physiology of fat absorption and fat transport.

a) Considerable progress has been made in studies of the metabolism of adipose tissue and the nature of its responses to hormonal factors. Last year it was reported that epinephrine added in vitro would stimulate the release of free fatty acids from adipose tissue. It has now been shown

that glucagon and ACTH added in vitro also stimulate release of free fatty acids. Further studies revealed that all three of these hormones lead to an increase in the levels of active phosphorylase in adipose tissue and stimulate the uptake of glucose. It is of interest that this activity in the adipose tissue is quite analogous to the activities of these hormones on other peripheral tissues. Epinephrine and glucagon increase phosphorylase activity in the liver (but not in the adrenal) and ACTH increases phosphorylase activity in the adrenal (but not in the liver). Preliminary results suggested that these hormones might effect the observed increase in rate of release of fatty acids by inhibiting the synthesis of triglycerides. For this reason studies on the mechanism of triglyceride synthesis in adipose tissue were initiated. A cell-free system which will incorporate fatty acids into triglyceride has been derived from rat epididymal fat pads. This system carries out the first reported triglyceride synthesis in adipose tissue homogenates. The system requires α-glycerophosphate as a precursor and glycerol will not substitute for this requirement. ATP and Coenzyme A are required, presumably for the activation of the free fatty acids. Diglycerides of very high specific radioactivity have been isolated and are probably intermediates. Unlike the system in liver the adipose tissue homogenate does not accumulate phosphatidic acid but the requirement for α-glycerophosphate suggests that this is nevertheless an intermediate in the synthetic pathway. These studies are being pursued in the hope that with a better understanding of adipose tissue metabolism it may be possible to demonstrate the site at which the several hormones discussed above interact with the enzymatic mechanisms controlling fat deposition and release.

Heparin is known to lead to a marked increase in the levels of lipoprotein lipase in the serum. Studies completed this year show that addition of heparin to adipose tissue in vitro leads to a striking outpouring of lipoprotein lipase from the tissue into the medium. Also of interest is the finding that the levels of lipoprotein lipase in the adipose tissue of fasting rats is considerably lower than the level found in the tissues of carbohydrate-fed rats. Thus, the levels of lipoprotein lipase, rather than paralleling the rate of release of fatty acids, vary inversely with the rate of release of fatty acids. These results suggest that the role of lipoprotein lipase may be in the uptake of fat rather than in its release.

b) A study of the fatty acid composition of the chylomicron fat in patients fed large meals of different types of fat has been carried out. It was found that the pattern of fatty acids in the chylomicron resembles very closely the pattern of the dietary fat used. These results are

clearcut and disagree with results reported by Dole of the Rockefeller Institute, who claimed that there were large differences between the composition of fats fed and the fat in the chylomicrons during absorption. The disagreement may stem from the failure of the latter investigator to completely remove low density lipoproteins from chylomicrons prior to analysis.

Administration of carbohydrate by mouth or intravenously reduces considerably the rate of absorption of fat, as shown by studies carried out in rats with cannulated thoracic ducts.

c) Kinetic studies on the utilization of injected C^{14}-fatty acids have been continued and analysis of these results shows that at least 50% of the fat utilized during fasting is transported through the serum as free fatty acid. Injection of epinephrine raises the net turnover of free fatty acids. During exercise there is a marked increase in net fatty acid utilization but the fraction accounted for by transport through the FFA fraction falls considerably.

2. Studies of dietary and hormonal factors determining serum lipoprotein levels.

a) Adrenal control of lipoprotein levels. It has been shown that injection of epinephrine in oil not only elevates the plasma levels of free fatty acids (FFA) but also leads to an elevation of lipoprotein levels. The FFA response occurs early and is transient; the rise in lipoproteins does not occur until 12 to 24 hours after epinephrine injection. Studies completed this year showed that adrenalectomy or hypophysectomy abolished both the FFA and the lipoprotein responses to epinephrine. Pretreatment of the operated animals with cortisone or with ACTH, respectively, restored their ability to respond to epinephrine with both a rise in FFA and in lipoproteins. Administration of cortisone to normal dogs exaggerated the lipoprotein response to epinephrine injection. When the animals received extra cortisone as much as an 80% rise in serum cholesterol was obtained with three daily injections of epinephrine.

These results suggest a physiologic basis for the hypercholesterolemia of stress. It is well known that animals and patients under stress demonstrate hyperactivity of both the adrenal medulla and the adrenal cortex. This pattern of hormone production would appear to be adequate to explain elevations of both FFA and cholesterol (lipoproteins). Studies are currently in progress to evaluate the response of patients to exogenous epinephrine and cortisone.

b) Studies on the effects of dietary fat on cholesterol excretion have been completed. Of major interest was the observation that a surprisingly large fraction of the cholesterol excreted in feces in man appears there in the form of cholesterol itself (35 to 80%). This is in contrast to the pattern in rats and other laboratory animals in which practically all of the cholesterol excreted appears in the feces in the form of bile acids. A study of eight patients fails to reveal any consistent effect of unsaturated fats on the rate of excretion of intravenously administered C^{14}-cholesterol in the feces. The mechanism by which dietary fats modify cholesterol levels has not been established. The effect may be on a redistribution of cholesterol within the body but this has not been established in man.

Parallel with the studies on cholesterol excretion, bile acid turnover studies have been done under various dietary conditions and in various clinical conditions. These studies were carried out in collaboration with Dr. Sven Lindstedt from Sweden. Results of the study are not yet complete. These collaborative studies are continuing in order to determine whether there are systematic differences in bile acid turnover in various forms of hypercholesterolemia.

c) Studies on the production of lipoproteins by rat liver slices in vitro were continued and definitive identification of alpha-1-lipoprotein was obtained. This was done by preparing lipoproteins in vitro from a complete mixture of C^{14}-amino acids, purifying them, digesting with trypsin and chymotrypsin, and chromatographing the mixture of peptides in two dimensions. It was found that all of the peptides derived from the alpha-1-lipoprotein coincided with peptides derived from alpha-1-lipoprotein prepared from normal rat serum. The identification of the beta-lipoproteins with serum beta-lipoproteins was inconclusive.

It was shown that the rate of cholesterol synthesis is not apparently a rate-limiting reaction in lipoprotein synthesis. Liver slices taken from cholesterol-fed rats (in which the rate of cholesterol synthesis is markedly suppressed) incorporated labeled amino acids into the protein moiety of lipoproteins at a normal rate. Conversely, accelerating the rate of cholesterol synthesis by injection of Triton did not increase the rate of synthesis of lipoprotein protein.

d) In collaboration with investigators at the University of Maryland a study of modified milk fat was carried out. Dr. Shaw and his co-workers in the dairy department at Maryland University were able to alter the iodine number of milk fat by appropriate changes in feed. However, the changes were relatively small (increase in iodine number

from 30 to 48) and no significant difference in the effects of fat of these two types was demonstrable in patients. Thus, it appears that unless a more radical change can be effected this approach to the problem of dietary fat will not be suitable.

e) The technique previously described for incorporating cholesterol into lipoproteins has proved valuable for the incorporation of other non-polar molecules. In particular, the technique serves to incorporate carcinogenic hydrocarbons so that these can be administered intravenously in known quantities and their metabolism studied. Studies of these hydrocarbons have been hampered because of their insolubility and the resultant uncertainty in evaluating absorption and distribution.

3. Studies on the metabolism of cholesterol and therapeutic agents useful in lowering serum cholesterol levels.

a) A new inhibitor of cholesterol biosynthesis produced by the Wm. S. Merrell Co. (MER-29) has been studied in animals and in man. This compound: 1- [p-(β -diethylaminoethoxy)-phenyl] -1-(p-tolyl)-2-(p-chlorophenyl) ethanol was shown by Dr. Blohm to suppress markedly the incorporation of radioactive acetate into cholesterol and to lower the serum and tissue levels of cholesterol in rats. Studies in this laboratory with the collaboration of Dr. Erich Mosettig and Mr. Thompson of the Arthritis Institute have now established the probable site of action of the drug. It has been shown that 24-dehydrocholesterol accumulates in the liver of rats fed MER-29. It may account for as much as one-half of the total sterol in these livers. 24-dehydrocholesterol (desmosterol) has previously been shown to be a precursor of cholesterol in the rat. It differs from cholesterol only in having an additional double bond at the 24, 25 position in the side chain. Presumably it is converted to cholesterol by a simple reduction step. It will be of interest to explore the mechanism by which this new drug blocks this last step in cholesterol synthesis.

Clinical studies confirmed the work of others in that there was some lowering of serum cholesterol levels, although this was not marked. Some patients were studied on a diet free of cholesterol but this did not appear to magnify the response of the drug. It was possible to show that 24-dehydrocholesterol appears in the serum of treated patients in significant amounts. Because this sterol gives a lower color yield in the Lieberman-Burchard reaction the apparent drop in serum cholesterol obtained using the usual methods is misleading. While there is a slight decrease in total sterol it is smaller than would appear from the usual analyses. It will be important to evaluate the atherogenic potential as well as other metabolic effects of 24-dehydrocholesterol before extending clinical trials.

b) A kinetic study of the distribution of C^{14}-cholesterol among the various tissues of the animal organism (rat and rabbit) has shown that every tissue, including brain tissue, takes up radioactive cholesterol from the serum. By extending the studies over a long time period it was shown for the first time that the specific radioactivity of the slowly metabolized cholesterol pools (brain, muscle, kidney) contained cholesterol of a higher specific radioactivity than that in the serum in the latter stages of the experiments. A simple mathematical model satisfactorily accounts for the observed results on the basis of isotopic exchange.

4. Studies on hypoalbuminemia and the mechanisms responsible for it.

a) A new clinical syndrome, exudative enteropathy, or protein-losing gastro-enteropathy, has been described. This is a condition characterized by loss of plasma proteins into the intestine with a resultant lowering primarily of the level of serum albumin, but also that of other serum proteins as well. The patients in this category have previously been described as having "idiopathic hypercatabolic hypoproteinemia." By the use of a non-metabolizable polymer of molecular size comparable to that of albumin it has been shown that these patients lose into the G. I. tract much larger amounts of injected macromolecules than do normals. The fate of the polymer, polyvinylpyrrolidone, which is of molecular size comparable to that of serum albumin, probably reflects quite well the fate of circulating albumin molecules.

The diagnostic test using I^{131} labeled PVP is technically simple. Many medical centers have received samples prepared at NIH and a large number of cases has already been uncovered. A commercial firm is planning to produce labeled polymer for routine clinical use.

b) Biopsies of intestinal mucosa were obtained in 6 cases of protein-losing gastroenteropathy. In 5 of these a common lesion consisting of markedly dilated lymphatics within the villi was demonstrable. This, combined with the fact that many cases have had chylous effusions, suggests that there may be a common etiology somehow associated with the lymphatic system.

c) Dr. Gordon carried out similar studies in patients with Asiatic cholera in Bangkok. There was no evidence of excessive loss of protein into the intestinal tract. This negative finding is not consistent with the generally accepted concept that there is serious desquamation of the intestinal mucosa in cholera.

5. Studies on the mechanisms of protein synthesis and degradation.

a) Conclusive evidence of the incorporation of amino acid analogues into crystalline proteins was obtained and published. A comprehensive review of "The Specificity of Protein Biosynthesis" was prepared and published in ADVANCES IN PROTEIN CHEMISTRY. Preliminary studies that have revealed the presence in mammalian tissue of peptides apparently conjugated to nucleotides were completed and published. This phase of the laboratory program has now been, temporarily at least, discontinued.

6. Basic studies on the structure of proteins and the nature of the clotting process.

a) Investigations of the fundamental mechanism of fibrin formation were continued with particular reference to the proposed role of tyrosine residues. By the application of highly sophisticated spectrophotometric methods it was shown that the tyrosine residues not titrated in fibrinogen are probably not involved in hydrogen bonds but rather masked by some sort of hydrophobic bonding. These studies are being continued, using careful kinetic analysis of pH changes in order to clarify the mechanisms of the fibrinogen-fibrin transformation.

7. Studies on the disturbed metabolism of lipids in nephrosis and on the immunochemical mechanisms involved.

a) A comprehensive study of the serum lipid pattern in patients with nephrosis has forced a revision of the usual concept that only the very low density lipoproteins are elevated. Many patients were found to have the most marked elevation in the β_1-lipoprotein fraction. It was observed that during improvement due to steroid therapy the lipoprotein pattern undergoes shifts toward the higher density β-lipoproteins. These findings refute the theory of Gitlin that the defect in nephrosis is a deficiency in the conversion of very low density lipoproteins to higher density lipoproteins.

b) It has been repeatedly shown that infusion of albumin lowers the lipid levels in nephrosis. Surprisingly it now appears from studies on nephrotic rats done in this laboratory that infusion of inert macromolecules such as dextran and polyvinylpyrrolidone also causes a decrease in lipid levels.

c) Intravenous infusions of glucose generally cause some decrease in serum cholesterol level and little change in serum triglyceride levels. In three cases of nephrosis, however, glucose infusion caused a marked rise in triglyceride levels and in low density lipoprotein levels. The significance of these results is not yet determined but will be investigated further.

8. Development of techniques for radioassay in the liquid scintillation spectrometer.

The new approach described in last year's report has now been in use for over a year and has proved to be a very valuable adjunct in radioassay. It has been shown that the method has a wide range of applicability. It has been effectively used for assay of tritium, C^{14}, Ca^{45}, and it has been shown in pilot studies that it is applicable for counting P^{32} and I^{131}. The method has received wide acceptance, particularly for the assay of weak beta emitters. Studies are being continued to determine whether the method can be used for pure gamma emitters and weak x-ray emitters.

Section on Enzymes

The activities of the Section on Enzymes have been directed toward elucidation of the following diverse fundamental biochemical processes: 1) the metabolism of heterocyclic compounds, 2) the metabolism of three carbon compounds, 3) cellular differentiation and protein synthesis, 4) anaerobic oxidative phosphorylation and electron transport, 5) nucleotide decomposition, 6) the metabolism of onium compounds, 7) the metabolism of isoprene derivatives, and 8) the metabolism of amino acids.

I. The Metabolism of Heterocyclic Compounds.

a) Riboflavin degradation (Drs. E. R. Stadtman, P. Z. Smyrniotis, and L. Tsai). Previous studies in this laboratory have shown that the oxidative dissimilation of riboflavin to ammonia and CO_2 by an aerobic bacterium involves the intermediary formation of 1-ribityl-2, 3-diketo-1, 2, 3, 4-tetrahydro-6, 7-dimethylquinoxaline (compound I) and 3, 4-dimethyl-6-carboxy-α-pyrone (compound III). Evidence has now been obtained showing that 3, 4-dimethyl-2, 3-quinoxalinediol (compound II) and oxamide are intermediates in the conversion of compound I to compound III. The conversion of riboflavin to compound I involves a cleavage of the pyrimidine

ring (ring C) with a stoichiometric formation of urea and CO_2. This transformation is of special interest since from the overall chemical point of view it can be represented as a simple hydrolytic process; however, it occurs only in the presence of molecular oxygen. The conversion of compound I to compound II involves a cleavage of the N-ribityl linkage. This cleavage is also of unique interest since it too requires molecular oxygen. Although the exact fate of the ribityl moiety is still unknown, the oxygen requirement is not restricted to oxidative degradation of the side chain since oxygen is required also for the cleavage of the N-hydroxyethyl, N-methyl, and N-acetaldehyde analogues. The further degradation of compound II to a mixture of oxamide and the α-pyrone derivative is obviously a complicated process. This conversion is inhibited by arsenite, iodoacetate and hydroxylamine and is activated by various oxidizable substrates such as ethanol, lactate, pyruvate and glucose. Further studies on the mechanism of the individual reactions in these various transformations are in progress.

b) Phenazine-1-carboxylic acid biosynthesis (Dr. M. Levitch). The bacterium Pseudomonas aureofaciens Kluyver offers a unique opportunity to investigate the biosynthesis of the heterocyclic phenazine ring system since this organism produces unusually large quantities (1.0 gm/liter) of phenazine-1-carboxylic acid during growth. Further insight into the mechanism of this biosynthetic process has been sought by measuring the incorporation of isotope carbon into phenazine-1-carboxylic acid when the bacterium is grown in a medium supplemented with various C^{14}-labeled compounds. Of numerous compounds tested, the most effective precursors are acetate, bicarbonate, alanine, serine and methionine. Methods for the stepwise degradation of the phenazine derivative to permit a determination of the distribution of labeled carbon from the various precursor compounds is now in progress. It is hoped that the results of these studies will suggest an intelligent approach to the problem of phenazine biosynthesis at the enzyme level.

c) Alkaloid biosynthesis (Drs. E. Kravitz and P. R. Vagelos). Studies on the biosynthesis of opium alkaloids by tissue preparations of the poppy plant, Papaver somniferum, have been undertaken as an additional effort to obtain basic information on the biochemistry of heterocyclic compounds. The poppy plants were supplied by the USDA Plant Industry Station in Beltsville, Maryland. Progress to date has been restricted to the development of optimal experimental conditions for the in vitro synthesis of alkaloids by tissue slices and in the development of procedures for the isolation and separation of the various alkaloids produced. Alkaloid synthesis has been followed by measuring the incorporation of isotopic carbon into the alkaloid fraction after incubating the

plant preparations with methyl-C^{14}-methionine or β-C^{14} serine, which were introduced by the vacuum infiltration technique. It has been found that with 4 to 5 week old plants the isotope is incorporated predominantly into narcotine and papaverine, whereas in older plants labeled morphine and codeine were also produced (identification of the various alkaloids is still tentative). Studies with tissue slices derived from various parts of the plant have revealed that the roots are by far the most active sites of alkaloid synthesis. In future studies efforts will be made to develop cell-free preparations of roots that are capable of catalyzing alkaloid synthesis.

An ion exchange procedure using Dowex-1-OH^- and Dowex-50-H^+ has been devised for the separation of the major opium alkaloids.

In addition to the above investigation, studies have been initiated to investigate the biosynthesis of the ergot alkaloids by the fungus Claviceps purpura.

II. The Metabolism of Three-carbon Compounds.

a) Propionic acid oxidation (Drs. P. R. Vagelos and W. Sly). In previous studies, Dr. Vagelos has shown that cell-free enzyme preparations of the bacterium, Clostridium kluyveri, catalyze the oxidation of propionate by a pathway involving the intermediary sequential formation of propionyl CoA, acrylyl CoA, β-hydroxypropionyl CoA, malonylsemialdehyde CoA, and malonyl CoA. Further studies on this metabolism have led to the discovery of a curious exchange reaction between the carboxyl group of malonyl CoA and added $C^{14}O_2$. This exchange is absolutely dependent upon the presence of catalytic amounts of an acyl CoA derivative of a saturated fatty acid having 4 to 16 carbon atoms. In view of the fact that malonyl CoA and CO_2 have been shown recently to be involved in the biosynthesis of fatty acids, it appears probable that the observed exchange reaction represents one step in fatty acid synthesis. The exact mechanism of this reaction is therefore of immediate interest and is under further investigation.

The enzyme catalyzing the TPN-coupled oxidation of malonylsemialdehyde CoA to malonyl CoA has been partially purified and is under further study.

Incidental to these investigations has been the development of a good general method for the chemical synthesis of β-ketothiolesters.

b) The role of biotin and vitamin B_{12}-coenzyme in propionate metabolism (Dr. E. R. Stadtman in collaboration with Mr. P. Overath and Prof. F. Lynen in the Max Planck Institute fur Zellchemie, Munchen, Germany). Previous studies by Flavin et al., with animal enzymes, and studies by Whitely, Carson, Wood and Delwiche, with enzymes derived from propionic acid fermenting bacteria, have established an intermediary role of succinyl CoA and methylmalonyl CoA in the metabolism of propionic acid. A consideration of the fact that propionic acid formation represents the major metabolic process catalyzed by bacteria belonging to the genus Propionibacteria and the observation that these organisms possess unusually high concentrations of vitamin B_{12} coenzyme and biotin, have prompted an investigation to determine if these vitamins are involved in propionic acid metabolism. Propionic acid metabolism in cell-free extracts of Propionibacterium shermanii was measured by the overall incorporation of 1-C^{14}-propionate into succinate. After treatment with protamine and charcoal and then dialysis, cell-free extracts lose their ability to catalyze the incorporation of labelled propionate into succinate. This ability is restored by the addition of catalytic levels of acetyl CoA and a light-labile factor present in boiled extracts. The latter factor is completely replaced with low concentrations (10^{-8}M) of pure dimethylbenzyimidazole-B_{12}-coenzyme (supplied by H. A. Barker). A role of biotin in the propionate exchange system is indicated by the fact that the reactivated enzyme is completely inhibited by avidin but not by avidin which has been pretreated with biotin. In light of the recent report that the succinyl-CoA isomerase activity of rat liver is lowered in B_{12} deficiency, the above findings form the basis of a working hypothesis that the propionate - succinate exchange is the net result of two vitamin coenzyme linked reactions: 1) the B_{12}-coenzyme dependent isomerization of succinyl CoA to form methylmalonyl CoA, and 2) the reaction of methylmalonyl CoA with biotin-enzyme to form a biotin-enzyme-CO_2 complex and propionyl CoA. The reversible exchange of propionyl CoA with free labeled propionate and reversibility of the other postulated reactions could account for the observed results. This hypothesis is under investigation.

c) Propionic acid fermentation by Clostridium propionicum (Dr. H. Goldfine). In continuing studies on the anaerobic fermentation of various three carbon compounds it was found that cell-free extracts of C. propionicum convert pyruvate, lactate and serine predominantly to acetate and CO_2, whereas α-alanine and β-alanine are converted mainly to propionate. Evidence was obtained supporting the conclusion that propionate formation from β-alanine proceeds by the following pathway: β-alanine ⟶ β-hydroxypropionate ⟶ β-hydroxypropionyl CoA ⟶ acrylyl CoA ⟶ propionyl CoA ⟶ propionate.

The first step, i.e., the conversion of β-alanine to β-hydroxypropionate involves the release of ammonia and is obligately dependent upon the presence of catalytic amounts of pyruvate and α-ketoglutarate. The latter observation and the demonstration that β-alanine serves as an amino group donor to form α-alanine and glutamate from the corresponding α-ketoacids, together with the further discovery that the DPN-linked oxidative deamination of α-alanine occurs only in the presence of α-ketoglutarate, supports the conclusion that the formation of β-hydroxypropionate from β-alanine occurs by a transamination of the amino group of β-alanine to pyruvate, thence to α-ketoglutarate, and then the release of the amino group as free ammonia by the action of glutamic dehydrogenase. As yet no evidence has been obtained for the formation of malonyl semialdehyde as the expected intermediary in β-alanine transamination.

III. The Biochemistry of Cellular Differentiation and Protein Synthesis.

a) Amino acid and protein metabolism in the slime mold (Dr. B. K. Wright, Mr. G. McNeil and Miss Minnie Anderson). Studies on the turnover of amino acids and protein during cellular differentiation of the slime mold *D. discoideum* have been continued. It has been found that the differentiation process is associated with a net decrease in protein content, but that active protein synthesis, as measured by the incorporation of S^{35} methionine into the protein fraction, occurs throughout all stages of development. At preculmination the methionine in protein is replaced by the endogenous pool S^{35}-methionine at a rate of about 7% per hour. A unique feature of this metabolic system is the discovery that the size of the "free" endogenous methionine pool is not influenced by changes in the exogenous methionine concentration. Although fixed in size at any given stage of development, the endogenous methionine pool can nevertheless undergo exchange with exogenous S^{35}-methionine, and the extent of this exchange (i.e., the specific isotope content of the pool methionine at equilibrium) is a linear function of the exogenous S^{35}-methionine concentration. This curious phenomenon remains as yet unexplained. The results suggest the possible existence of a heterogeneous endogenous methionine pool, in which the exchangeability of various parts is differentially influenced by the external methionine concentration.

Following momentary exposure of the organism to S^{35}-methionine, the separation of cellular proteins into various arbitrary classes by means of solubility in ethanol and by DEAE column chromatography has revealed marked differences in the rates of isotope incorporation into the various protein classes. From such studies evidence has accumulated which indicates that methionine molecules in various parts of the amino acid pool

are "fixed" with respect to the proteins into which they are incorporated. It appears that, on the average, pool methionine molecules which exchange readily with exogenous S^{35}-methionine are most readily incorporated into certain protein fractions which attain a relatively high specific radioactivity, whereas pool methionine molecules exchanging poorly with exogenous S^{35}-methionine are preferentially incorporated into protein fractions attaining a relatively low specific radioactivity. It is evident from the results obtained that the slime mold is particularly well suited for further studies on the biochemistry of protein metabolism.

b) The chemotactic hormone, acrasin (Dr. B. K. Wright and Mr. G. Liddel, in collaboration with Dr. E. Heftmann of NIAMD). Acrasin is the chemotactic hormone involved in initiation of aggregation at the onset of differentiation. A sterol with acrasin activity has been isolated from D. discoideum as a pure crystalline compound and has been identified as Δ^{22}-stigmasten-3B-ol. As judged by the lack of hormone activity in other fractions during purification and by the fact that the recovered acrasin accounts for most of the hormone activity of the crude cellular extract, it is concluded that this sterol is the major active compound present after acid hydrolysis. Since it is not as active as crude acrasin, attempts to isolate a conjugated form of this sterol are in progress.

IV. Anaerobic Oxidative Phosphorylation and Electron Transport (Drs. E. B. Brown and E. R. Stadtman). The reduction of crotonyl CoA to butyryl CoA by reduced pyridine nucleotide is associated with a standard free energy change of -14,000 calories and it has been postulated that this oxido-reduction system may be coupled with phosphorylation. In preliminary reports from another laboratory evidence has been presented to support the conclusion that ATP is produced during the reduction of crotonyl CoA to butyryl CoA by extracts of Clostridium kluyveri. Results of the present studies on this enzyme system suggest that the observed phosphorylation may not be associated with the reduction of crotonyl CoA per se but that it is derived indirectly by a dismutation of crotonyl CoA to butyryl CoA and acetyl CoA, followed by the formation of ATP from the latter compound via acetyl phosphate.

V. Nucleotide Decomposition (Drs. E. B. Brown and E. R. Stadtman). Four separate ferrous iron-dependent nucleotidases were identified and partially purified from cell-free extracts of C. propionicum. Two of these enzymes are mononucleotidases sharing a remarkable degree of resistance to heat but differing in their sensitivity to versene inhibition; the other pair of enzymes are heat sensitive dinucleotidases separable on the basis of versene sensitivity. Successive action of the

di- and mono-nucleotidases catalyzes the irreversible decomposition of diphosphopyridine nucleotide to adenosine, nicotinamide mononucleotide and two equivalents of orthophosphate.

VI. The Metabolism of Onium Compounds.

a) The anaerobic fermentation of choline (Drs. H. Hayward and T. C. Stadtman). An organism capable of deriving its carbon, nitrogen and energy for growth from the anaerobic dissimilation of choline was previously isolated from the soil and was shown to catalyze the conversion of choline to one mole of trimethylamine and one-half mole each of acetate and ethanol. This organism has now been identified as a new species belonging to the genus Vibrio and has been given the name Vibrio cholinicus. Studies with cell-free extracts of the organism have shown that choline degradation involves the intermediary formation of acetaldehyde which then undergoes a dismutation to form ethanol and acetate. By means of sedimentation in an ultracentrifuge, crude sonic extracts have been separated into a particulate fraction and a soluble fraction, both of which are needed to catalyze the decomposition of choline. The enzymes catalyzing the dismutation of acetaldehyde are present in the soluble fraction. This dismutation is catalyzed by the joint action of a TPN-specific ethanol dehydrogenase and an acetaldehyde dehydrogenase. Although the detailed mechanism of acetaldehyde oxidation has not been elaborated, it is significant that no dismutation occurs in the absence of TPN, ADP or a sulfhydryl compound; moreover, the reaction is markedly stimulated by the addition of ferrous iron or other divalent cations and by coenzyme A.

It has been further established that the dissimilation of choline by crude extracts is associated with the esterification of orthophosphate to form ATP. The possibility that this phosphorylation is associated with electron transport is suggested by the fact that phosphorylation is inhibited by 2, 4 dinitrophenol in concentrations known to uncouple oxidative phosphorylation.

Preliminary, as yet inconclusive, evidence has been obtained that betainaldehyde is an intermediary in choline degradation.

The discovery that cell-free extracts of this organism contain large amounts of a cytochrome pigment that is spectrally similar to animal cytochrome c was previously reported. A functional role of this cytochrome in choline metabolism is suggested by the observation that cell-free extracts catalyze its reduction in the presence of choline.

b) The metabolism of sulfonium compounds (Dr. C. Wagner). The hydrolysis of sulfonium compounds at neutral pH is associated with a standard free energy change of about 21,000 calories. In an effort to investigate the possibility that cleavage of the sulfonium bond can be energetically coupled with cellular metabolism (as for example by the synthesis of ATP), an organism has been isolated from the soil that is capable of growing anaerobically with dimethyl- β-propiothetin as the major source of energy and carbon. Preliminary studies have been made to determine the nature of the fermentation process. It has been found that the decomposition of propiothetin is accompanied by the formation of propionic and acetic acids.

VII. The Metabolism of Isoprene Derivatives.

a) Cholesterol degradation (Dr. M. G. Horning in collaboration with Prof. S. Bergstrom and Dr. H. Danielsson at the Karolinska Institute, Stockholm). Incubation of human red blood cells with cholesterol-4-C^{14} results in the formation of several degradation products which have been separated into three major classes, acids, diols, and triols, by means of reverse phase chromatography. Although neither cholic acid or chenodeoxycholic acid could be detected in the acid fraction, one of the acids produced appears to be identical with a compound formed when cholesterol is incubated with liver mitochondria. This compound may be a di- or tri-hydroxy coprostanic acid. 3β, 5α, 6β-trihydroxycholestane was identified as a component of the triol fraction and one of the diols was identified as 7 β-hydroxycholesterol. These results indicate that blood may have an important role in the metabolism of cholesterol. Future studies will be directed toward a more detailed analysis of this metabolic process at the enzyme level.

b) Isoprenoid degradation (Dr. W. Seubert). In order to facilitate studies on the biochemistry of polyisoprene metabolism, an aerobic bacterium has been isolated from the soil that can utilize a simple diisoprene derivative, citronellol, as its sole carbon and energy source for growth. This organism has been identified as a new species belonging to the genus Pseudomonas and has been designated Pseudomonas citronellolis. In addition to citronellol, higher analogues, such as farnesol and farnesoic acid, and the cyclic isoprenoid, β-ionone, are also utilized for growth. Results of various experiments to ascertain the mechanism of isoprenoid dissimilation have shown: 1) Citronellic acid accumulates in the culture medium as a transient intermediate during growth of the organism on citronellol; 2) In the presence of various isoprenoids, arsenite-inhibited resting cell suspensions catalyze the incorporation of $C^{14}O_2$ into acetate. This incorporation does not occur in the absence

of isoprenoids or when the isoprenoids are replaced by straight chain saturated fatty acids; 3) Incubation of arsenite-inhibited cell suspensions with $C^{14}O_2$ and citronellic or farsenoic acids leads to the accumulation of β-keto-acids; 4) Cell-free extracts catalyze the incorporation of $C^{14}O_2$ into acetate in the presence of either dimethylacrylyl CoA, geranionyl CoA or farnesyl CoA; 5) Extracts catalyze the incorporation of $C^{14}O_2$ into acetoacetate in the presence of dimethylacrylyl CoA. These observations provide indirect support for the working hypothesis that the oxidation of polyisoprenoids involves a stepwise degradation of the terminal isoprene moiety by reactions analogous to those involved in the oxidation of isovaleric acid. Thus the oxidation of farnesol would be visualized to occur by the following reaction sequence:

Farnesol $\xrightarrow{I}$ farnesoic acid $\xrightarrow{II}$ farnesyl CoA $\xrightarrow{III}$ geranyl-acetoacetate $\xrightarrow{IV}$ geranionyl CoA $\xrightarrow{V}$ dimethylallylacetoacetate $\xrightarrow{VI}$ dimethylacrylyl CoA $\xrightarrow{VII}$ acetoacetate $\xrightarrow{VIII}$ 2 acetyl CoA.

According to this mechanism a fixation would occur at steps III, V, and VII.

VIII. The Metabolism of Amino Acids.

a) Threonine biosynthesis (Dr. M. Flavin and Mr. C. Slaughter). The enzyme, threonine synthetase, catalyzes the conversion of O-phosphohomoserine to threonine and orthophosphate. This novel reaction involves the elimination of an O-phosphoryl group from the alpha position of homoserine and the simultaneous introduction of an hydroxyl group into the beta position. In a continuation of studies on the mechanism of this reaction, the enzyme has been purified 500-fold from Neurospora extracts and some of its properties have been determined. Activity of the purified enzyme requires the presence of added pyridoxal phosphate. A further insight into the reaction mechanism has been obtained from studies with H_2O^{18} and D_2O carried out in collaboration with Dr. Tetsuro Kono of the McCollum-Pratt Institute. When the reaction is carried out in the presence of H_2O^{18}, O^{18} is incorporated into threonine but not into phosphate. O-phosphothreonine is not decomposed. From these observations it must be concluded that the phosphate group of phosphohomoserine is removed through cleavage of the C-O bond by an elimination reaction rather than by hydrolysis. When the reaction is carried out in the presence of 100% D_2O, two atoms of deuterium are incorporated into threonine, one in the α-position. On the basis of these results it is tentatively proposed that threonine biosynthesis involves the intermediary formation of a Schiff base of vinylglycine and pyridoxal phosphate. The

further observation that only 0.1 atom of solvent hydrogen per mole is incorporated into threonine when the reaction is carried out in H^3_2O, indicates a high degree of discrimination against tritium. Tritium ions add to the postulated vinylglycine intermediate at only 2 to 3% of the rate of proton addition.

b) The reductive deamination of glycine. (Dr. T. C. Stadtman) Further studies have been made with the soluble enzyme system form Clostridium sticklandii that catalyzes the reduction of glycine to acetate and ammonia by 1,3-dimercaptopropanol, with the simultaneous esterification of orthophosphate to form ATP. Attempts to purify the enzymes involved have revealed that a minimum of four proteins are essential for catalysis of the overall reaction; hence the mechanism is more complicated than was anticipated. The conversion of glycine to acetate and the coupled phosphorylation are inhibited by antimycine A. This and other indirect evidence supports the belief that a quinone derivative is involved. Analysis of the lipid fraction of C. sticklandii failed to detect the presence of tocopherols or quinones of the vitamin K or Coenzyme Q types. During the course of these experiments, it was found that the characteristic reddish orange color of extracts of C. sticklandii is due to the presence of remarkably high concentrations of the adenine vitamin B_{12} coenzyme. Nutritional studies revealed that the concentration of the B_{12} coenzyme is a function of the rate of one-carbon metabolism by the cell. For example, supplementation of the culture medium with formate (which is fermentated largely to acetate) results in a much higher synthesis of the B_{12} coenzyme. The potential significance of the B_{12} coenzyme in one-carbon metabolism was further indicated by the discovery that two species of methane producing bacteria are exceptionally rich sources of the coenzyme. No evidence could be obtained to indicate that the B_{12} coenzyme is involved in the reduction of glycine to acetate. Evidence to the contrary was obtained by showing that two other strains of Clostridia capable of catalyzing the reduction of glycine to acetate, do not contain appreciable amounts of the B_{12} coenzyme.

c) The fermentation of γ-aminobutyric acid (Mr. J. Hardman and Dr. T. C. Stadtman). Previous studies in this laboratory have shown that the fermentation of γ-aminobutyrate by Clostridium aminobutyricum involves the intermediary formation of succinic semialdehyde and γ-hydroxybutyrate. The overall conversion of γ-aminobutyrate to form γ-hydroxybutyrate by cell-free extracts of this bacterium was found to be an oxidation-reduction process in which α-ketoglutarate and glutamate have catalytic roles. The reaction involves a transamination of the amino group of γ-aminobutyrate to α-ketoglutarate with the formation of succinic semialdehyde and glutamate; the latter compound then

undergoes oxidative deamination by a DPN-specific dehydrogenase to form α-ketoglutarate and DPNH. The oxidation of glutamate is finally coupled with the reduction of succinic semialdehyde to form γ-hydroxybutyrate. The enzyme catalyzing the latter reaction has been purified about 20-fold from crude extracts. It is a DPN-specific dehydrogenase showing marked substrate specificity. It appears to be a zinc-sulfhydryl enzyme. A requirement for two proximal sulfhydryl groups is suggested by the observation that the enzyme is inhibited by concentrations of arsenite that selectively react with disulfhydryl compounds. Aging of the purified enzyme results in a drastic loss of enzyme activity which is curiously restored by the addition of AMP.

d) Anaerobic metabolism of the dibasic amino acids, ornithine and lysine (Dr. V. Tarantola). Although ornithine was originally reported by Stickland and Woods in their classic studies on the Stickland reaction to undergo reductive deamination, it would appear that the mechanism does not involve a direct reductive deamination. The final reduced and deaminated product, δ-amino valerate, produced by extracts of Clostridium lentoputrescens, appears rather to be formed via a pathway involving a preliminary formation of a keto acid derivative of ornithine by a transamination followed by ring closure and reduction to proline. A further reductive ring cleavage of proline by proline reductase and 1, 3-dimercapto propanol yields δ-amino valerate.

A new clostridium, as yet unidentified, has been isolated from swamp mud that is capable of growing on lysine as a single amino acid substrate. The products, ammonia, acetate and butyrate indicate that the organism probably catalyzes the same coupled oxido-reduction reaction originally discovered in C. sticklandii. The new organism is unique in that it can grow as a result of this fermentation and thus may be much more suitable as experimental material for study of this interesting cleavage of the lysine molecule. In C. sticklandii the formation of acetate and butyrate from lysine requires lipoic acid but the enzyme system proved to be very unstable.

Laboratory of Chemistry of Natural Products

The work of the Laboratory during the past year may be summarized in four categories: 1) studies in the structural chemistry of naturally occurring substances, particularly the Amaryllis alkaloid group, 2) the development of new methodology for lipid studies, and the application of new methods to lipid problems, 3) studies of the callicrein-callidinogen-callidin system, with particular regard to isolation procedures and the development of assay methods, and 4) work on reactions related to biochemical transformations involving amine oxides and hemiacetals.

The work in structural chemistry continued and extended past studies. A new ring system, not previously known to exist for either a synthetic or a natural substance, was found for the alkaloids montanine, coccinine and manthine. The structural relationships between these compounds and other members of the Amaryllis alkaloid group was established through a transformation linking the new series with another of the known groups. Since stereochemical relationships are of profound importance in determining the direction of biogenetic reactions, and usually lead to major variations in degree of physiological action, the previous structural studies were extended through the determination of the configuration and stereochemical relationships of the ethanophenanthridine compounds. The C:D ring fusion is cis. Of interest is the fact that two groups of compounds of opposite stereochemical configurations occur in this field. These are based on the (+)- and (-)-crinane series. A number of additional structural determinations were made; structures were established for 1-acetyl-lycorine, epicrinine, nerbowdine, 6-hydroxycrinamine, criwelline and haemanthamine.

This work provides a considerable amount of new information about alkaloid structures, and about naturally occurring ring systems. The isolation work was mostly concerned with the new compounds discovered during the year, but attention was also given to the problem of galanthamine isolation. This substance was introduced into Russian clinical medicine last year as a new and superior drug for the treatment of myasthenia gravis. Earlier chemical work in Russia led to the proposal of an incorrect structure for this compound; the correct structure has since been established through work in Japan and in this laboratory. Current studies have resulted in the development of a compound of still greater activity. Pharmacological studies of these substances are being carried out by Dr. R. L. Irwin (NINDB) and Dr. B. Holmstedt (Karolinska Institute, Stockholm).

Other alkaloid studies included those of the Lunasia and Cassia compounds. Degradative studies of cassine were combined with instrumental data to lead to a proposed structure; the substance is a reduced pyridine compound with a long alkyl side chain. The Lunasia work was terminated with several structural determinations.

Present work in this category is directed toward completion of the Amaryllis structural studies, with particular regard to stereochemical problems, and to the development of biosynthetic investigations.

2) Until very recently most work in the lipid field was characterized by the use, out of necessity, of poor and inexact methods. This circumstance, more than any other, was responsible for the very slow rate of development in lipid chemistry and lipid metabolism over the last thirty years. The apparent relationship of lipid metabolism to atherosclerosis, and the modification of human serum cholesterol levels by dietary fatty acids, has stimulated efforts to improve the methodology of lipid chemistry. In general, two broad areas of work were involved. The problem of identifying and measuring amounts of individual fatty acids was the more difficult of the two. Preliminary experiments showing that this could be done by gas-liquid chromatography were carried out in England in 1952, but five years later gas chromatographic methods were still ineffective for biological applications. Preliminary studies indicated that the existing detection methods were not satisfactory and a continuing exchange of information with several U.S. laboratories was started in an effort to develop instruments and separation methods that would provide both the sensitivity and resolution required for work with lipid. In this laboratory the characteristics of the argon detector were studied, and conditions for linearity defined. A study of liquid phases and of coating procedures was also carried out. Since the success of the method rests on the proper functioning of the column as well as the detection system, a new method for coating liquid phases was developed, and new procedures were worked out for preparing polyester liquid phases with desirable separation factors and high stability. Perhaps the most important factor involved in the use of polyester phases lies in the removal of exchange catalysts. From studies made in various ways it is believed that the polyester procedures developed here have solved this problem.

The development of column chromatographic procedures for the separation of lipid classes has also been pursued. Existing methods were evaluated and suitable modifications were developed so that using silicic acid columns the following classes can now be separated effectively: hydrocarbons, cholesterol esters, triglycerides, cholesterol,

cephalins, lecithins, sphingomyelins, and lysolecithins (work in another laboratory has shown that monoglycerides may also be separated, when present).

Several laboratory studies using these and related methods are in progress. A detailed study of the lipids of severely atherosclerotic patients has been started. This work is in collaboration with Dr. Michael DeBakey (Baylor University) and Dr. B. G. Creech (Methodist Hospital, Houston). The characterization extends to serum lipids, the lipids of the arterial block tissue, and adipose tissue.

A method for the qualitative and quantitative estimation of the long chain base fraction of sphingolipids has been devised. This work disclosed the presence of a new long chain base in human sphingomyelin.

A comparison of the lipids (serum and aortic) of rabbits fed cholesterol with those of normal rabbits is in progress; this is in collaboration with Dr. D. B. Zilversmit, who carried out the feeding experiments.

Current work involves the continuation of methodological studies and the extension of new methods to laboratory problems. The chief new area of methodology work is capillary chromatography. The highest degree of resolution obtained so far at 180-200° for fatty acid work is about 50,000 theoretical plates. While this is far beyond that obtainable by any other method, it seems likely that a separating capacity of 300-500,000 theoretical plates can be reached. Further, with this resolving power it should be possible to separate steroidal substances, if suitable liquid phases and modified techniques can be developed. This problem is under study in several laboratories. The extension to steroids is needed to study cholesterol formation and degradation more effectively. A high degree of resolution is also needed to separate positional and stereoisomers for unsaturated fatty acids.

3) The callicrein-callidinogen-callidin system has been studied with particular regard to isolation and assay procedures. This work was carried out in collaboration with Dr. S. J. Sarnoff and Dr. M. E. Webster. Methods were developed for the preparation of purified fractions of callicrein (plasma, pancreatic and urinary) and for callidinogen. The objective of the work is the characterization of these substances which may be of great importance in the functioning of the circulatory system. The problem is made difficult by the complexity of the protein and polypeptide mixtures which must be dealt with. A new assay method (Dr. Webster) is expected to be of considerable help.

Current work is concerned with the perfecting of additional chromatographic purification methods.

4) Amine oxide studies were resumed through an investigation of the catalyst requirements for the reaction. This work was started by Dr. J. C. Craig at the University of Sydney, Australia, and is now being continued at the same laboratory. A variety of iron complexes were used. It was found that both α-hydroxy and α-amino acids were excellent complexing agents, and that the nature of the iron complex was important in determining the effectiveness of the rearrangement reaction. The data suggested that the mechanism involved a sequence of one electron shifts, with the transient formation of iron IV. The overall reaction leads to the removal of a methyl group from nitrogen and it provides a model for the reaction of biological demethylation.

A study of hemiacetal formation for long chain compounds was carried out. This work arose from observations on plasmalogens and related lipid substances in which an aldehyde reaction is clearly involved. It was found that long chain aldehydes (palmitaldehyde) were highly reactive substances, and that both trimer formation and hemiacetal formation proceeded readily. The formation of esters from hemiacetals was also studied. These are models for studying lipid aldehyde reactions. Dr. Craig will continue this work at the University of Sydney.

Laboratory of Chemical Pharmacology

Development of New Drugs

Drugs for Arthritis, Gout, and Muscular Spasm -- a Resume

Largely because of studies in this laboratory with phenylbutazone and zoxazolamine, three derivative drugs for treatment of arthritis and gout and one for muscle spasm are now available. Two of these were introduced in the past year. The four drugs are: 1) Oxyphenbutazone (in arthritis); 2) Sulfinpyrazone (Anturan) (uricosuric agent); 3) Zoxazolamine (Flexin) (uricosuric agent), 4) Chlorzoxazone (Paraflex) (for treatment of muscle spasm). Two other potent phenylbutazone derivatives with prolonged uricosuric action, a para-methylsulfone derivative of phenylbutazone and a keto derivative of oxyphenybutazone, may be of value as long acting uricosuric agents.

Additional studies of phenylbutazone analogues confirm the view that these compounds act in the ionic forms and that an alkyl side chain is necessary for antirheumatic activity. Our continuing search for a non-toxic antirheumatic agent will be guided accordingly.

Reserpine Analogues

Last year, studies with reserpine, in collaboration with Ciba Pharmaceuticals, indicated that the trimethoxybenzene ester linkage might be unnecessary for activity. A [illegible] reserpine has been found to release peripheral amines selectively, [illegible] increasing brain amines. It is now in clinical trial for treatment of [illegible].

Biogenic Amines

Drugs Acting Through Release of Brain Amines

Whether the tranquilizing action of reserpine is due to loss of brain norepinephrine (NE) or is associated with inability of brain to bind serotonin (HT) is a crucial question. Dr. Brodie and his associates have shown that small doses of a reserpine analogue, dimethylaminobenzoyl methylreserpate (Su 5171), deplete stores of brain NE considerably, without releasing stores of brain HT appreciably and without eliciting sedation. Larger doses of Su 5171 elicit sedation only if it affects 50% or more of brain serotonin binding sites.

Animals subjected to cold (or other stress), and then given reserpine, are not sedated; brain NE but not brain HT is depleted. In contrast to effect on reserpine, stress had no effect on chlorpromazine action. Hypophysectomized rats, subjected to cold and then given reserpine, are sedated and brain HT stores are released, indicating that a pituitary substance is needed for "stress" to prevent reserpine action.

These results support the view, for which further pharmacologic evidence has been obtained, that "automatic" functions of brain are integrated by antagonistic neuronal systems: an adrenergic system (blocked by chlorpromazine); and a serotonergic system (stimulated by reserpine). If reserpine, as postulated, acts centrally by stimulating a neuronal system (trophotropic) which integrates parasympathetic with somatomotor and psychic functions, then the drug should increase parasympathetic output from the CNS. Reserpine elicits profuse salivation from the cannulated salivary gland, by a central parasympathetic action. Previous studies have shown that reserpine-induced miosis and enhanced light reflex are a reflection of central parasympathetic stimulation. Since chlorpromazine does not increase central parasympathetic tone but instead decreases sympathetic tone, the two drugs must act centrally on opposing autonomic systems.

Drugs Acting Through Release of Peripheral NE

Studies with syrosingopine (Singoserp) show that this compound can release peripheral NE, without releasing brain amines. This confirms view that reserpine lowers sympathetic tone not by a central action, but by depleting peripheral NE and suggests that this drug may be useful in hypertension since it is likely to be effective in doses that do not produce depression.

Studies with Guanethidine (Su 5864, a Ciba product) show that this drug probably lowers blood pressure by depleting peripheral NE but not brain NE. It presumably acts by a different mechanism than does reserpine since it does not release serotonin, and releases NE more slowly than does reserpine.

Drugs Acting Through Blocking the Metabolism of Amines (Monoamine Oxidase (MAO) Inhibitors)

Toxicity - Certain MAO inhibitors, especially hydrazine derivatives of phenylethylamine, produce, in dogs, degenerative lesions in the inferior olivary nucleus and in the pyriform lobe accompanied by neurological symptoms. JB 516 (Catron) produces such effects in dosage as low as 0.5 mg per kg daily. Preliminary [illegible] suggest similar results in cats, but not in rabbits and monkeys.

Effect on Brain Amines - Previous studies using MAO inhibitors have established a rapid turnover for brain HT but a slow turnover for NE; however, excitation elicited by MAO inhibitors is temporally related to rise in brain NE. Turnover of dopamine has also been found to be extremely rapid (50% in 15 minutes), indicating that MAO is important in the metabolism of this catecholamine in brain.

Further evidence favors the view that the chief role of MAO is not to metabolize physiologically released NE but to regulate amounts of NE and HT stored in neurons. Thus, the two enzymes, O-methyltransferase and MAO, may have quite different roles, the former modifying released catecholamines, the latter regulating the amount in storage.

Mechanism of Action of the Anti-depressant Drug, Imipramine (Tofranil)

This drug, structurally related to chlorpromazine, is an antidepressant but is not a MAO inhibitor. It does not elicit excitation in normal man or in animals, but acts only in the depressed individual. Studies in this laboratory have shown that Tofranil blocks a number of the central actions of reserpine (but not of chlorpromazine). It blocks the ability of reserpine to potentiate alcohol and barbiturates in mice and rats and to produce sedation, ptosis and miosis in rats. It does not, however, interfere with release of brain amines by reserpine. Preliminary evidence indicates that chlorpromazine, despite its depressant action, also has a delayed action in blocking reserpine. These findings are tentatively explained by the working hypothesis that phenothiazines and related compounds may have two actions: central adrenergic blocking and central serotonin blocking. Chlorpromazine exerts both effects, with antiadrenergic predominating. Tofranil exerts both actions with antiserotonin predominating.

Mechanism of Uptake of Catecholamines by Brain Tissue

In previous studies it has been shown that platelets take up HT and catecholamines through active transport, by a mechanism blocked by reserpine. Similar studies are being undertaken to ascertain whether the uptake of amines by brain also involves active transport. On incubation in vitro with plasma, brain slices including hypothalamus, thalamus, rhinencephalon and pituitary, but not cerebellum, take up epinephrine. In contrast, brain slices from animals pretreated with reserpine do not concentrate epinephrine.

Studies on Distribution and Role of NE, HT, and Their Synthetic Enzymes in Nervous Tissue

a) Micromethods for the estimation of 0.04 γ of NE and 0.20 γ of HT were developed.

b) The constancy of the ratio of 5HTP to DOPA decarboxylase activities throughout the cat brain indicates the same decarboxylase acts on both substrates. The concentration of amines and enzymes is low not only in cerebellum and cortex but also in those sensory nuclei which are situated in the brain stem. In contrast, they are high only in those parts of brain associated with "automatic behavior," e.g., reticular formation, hypothalamus and rhinencephalon.

c) Levels of brain amines are being related to gross behavior and drug action at various ages. At birth the level of NE in rats is only about 20% of adult level, while the HT level is about 40% adult level. The levels increase with age and the results suggest an association of brain amine levels and development of behavioral patterns. Studies on guinea pigs are underway, since these animals are born more fully developed than rats.

d) Reserpine decreases by 90% the NE content of the superior cervical ganglion in cats. The post-ganglionic response to a preganglionic electrical stimulation is markedly enhanced. This suggests that normally NE may have a role in the ganglionic response to acetylcholine.

Histamine Studies

The development of a specific and simple fluorometric procedure for histamine assay in tissues should help in studies of this substance whose physiological role and biosynthesis are still unknown. Application of the method to brain has shown that contrary to many reports, there is little histamine present and this may be associated with non-nervous vascular tissue. By use of compounds that inhibit MAO but not diamine oxidase, it has been shown that MAO has no role in metabolizing histamine in vitro and presumably in vivo. It has also been found that the reserpine releases histamine from rabbit platelets but from no other tissue. Evidence indicates that this release may be mediated by free HT.

Passage of Drugs Across Membranes

Blood-Brain Barrier

Kinetic data from a study of the penetration into CSF of 20 drugs with diverse structures and physical proper[illegible]s now provide considerable

confidence in the assumption that the blood-brain barrier acts as an inert lipoid boundary to drugs. Only the undissociated forms of the drugs appreciably penetrate the CSF and at rates determined by their heptane/water partition ratios. However, certain parts of brain, including both lobes of the pituitary, the intercolumnar tubercle and the area postrema have no blood-brain barrier to N-acetyl-4-aminoantipyrine, sulfaguanidine, radioactive sodium and labelled epinephrine. In contrast to their slow passage into CSF, water-soluble substances such as sucrose and phenol red readily leave the CSF. They may leave via the arachnoid villi and the rate may be related to the turnover of CSF.

Penetration of Drugs into Cells

The penetration of a number of drugs from plasma into red cells has been studied. Substances generally cross this boundary much more rapidly than blood-intestinal or blood-brain barrier, perhaps because of large surface/volume relationship. The relative rates of entry of organic bases seem to be determined by lipid solubility, but the passage of organic acids does not fit the lipoid barrier pattern. Instead organic electrolytes follow a pattern not unlike that shown by the simple mineral ions. Thus, sulfonic acids penetrate many times faster than quaternary ammonium ions, and acids at the steady state may occupy with a fraction of total volume of cell water in contrast to bases which occupy the total volume of cell water.

Passage of Purines and Pyrimidines Across the Intestinal Tract

A transport mechanism for the absorption of uracil and thymine is present in the intestinal mucosa. Active transport has been shown in vitro using everted intestinal sacs. The mechanism requires oxygen and is saturated at low concentrations of pyrimidines. Thymine transport is blocked by uracil, hypoxanthine and other pyrimidines and purines.

Drug Metabolism

Substances (Antimetabolites) Metabolized by Relatively Specific Enzymes of Intermediary Metabolism

Studies on the metabolism and mechanism of action of the antitumor agent, 6-chloropurine, have been continued. Last year the isolation of a new substance, 6-chlorouric acid, was reported. Studies this year show that the purine skeleton of 6-chloropurine is incorporated into the adenine and guanine of both RNA and DNA. Furthermore, 6-chloropurine inhibits the turnover of both RNA and DNA, as followed by p^{32} in vivo, in liver slices, and in isolated liver nuclei.

Substances Acted on by Extremely Non-specific Enzymes Not Involved in Intermediary Metabolism

Further studies have been made on the oxidative enzymes in liver microsomes.

a) Microsomal Sulfoxidase. The enzyme system that oxidizes 4,4'-diaminodiphenyl sulfide and chlorpromazine to the corresponding sulfoxides has been definitely characterized as another microsomal enzyme (a sulfoxidase) requiring TPNH and O_2.

b) Nicotine. The key step in oxidation of nicotine is not cotinine as suggested in last report, but hydroxylation of the carbon atom next to nitrogen in side chain to yield a cyclic aldehyde (hydroxynicotine). This supports the view that the first step in demethylation is hydroxylation of the methyl group. Elucidation of this reaction may indicate how ring systems are split in the body.

c) Mechanism of Microsomal Drug Oxidation. This mechanism involves direct utilization of oxygen and may share the same donor of "active" oxygen as cholesterol. A similar mechanism may be involved in the hydroxylation of steroid rings to form corticoids. In previous work it was shown that TPNH is oxidized in the presence of drug to yield H_2O_2. Two drugs, 4,4'-diaminodiphenyl sulfide and p-ethoxyacetanilide, do not affect the rate of TPNH oxidation, but cause a decrease in formation of H_2O_2 equivalent to the formation of their metabolites. This supports the view that TPNH and O_2 react in microsomes to form a "hydroxyl donor." In the absence of drug substrate, a part of the donor participates in normal hydroxylation reactions (e.g., cholesterol hydroxylation), and the rest breaks down to H_2O_2. In the presence of drug, a part of the "hydroxyl donor" is used by a number of nonspecific "drug enzymes" to hydroxylate foreign compounds.

d) Induced Enzyme Formation. Administration of certain drugs, e.g., phenylbutazone, aminopyrine, 3-4, benzpyrene, phenobarbital, increases the ability of rats to metabolize the same or closely related drugs. This increased activity is also shown in vitro by liver microsomes. This effect may explain, in part, the tolerance to barbiturates. A particularly interesting observation is that barbiturates lower coumarin anticoagulant levels in man. The tolerance of hyperthyroid subjects to drugs prompted a study of effects of thyroxin on drug metabolism. Pretreatment of rats with thyroxin decreases the duration of zoxazolamine paralysis and increases the activity of liver microsomal enzyme that metabolizes the drug.

e) Biochemical Evolution. It was shown in previous studies that the appearance of drug metabolizing enzymes is associated with adaption to living on dry land. In continuing studies of the ontogenetic development of drug enzymes, we find that liver microsomes in chicken embryos, unlike those in the mammalian embryo and the tadpole, contain enzymes that oxidize drugs and which require TPNH and O_2. The possession of these enzymes by the chicken embryo may be related to its non-aqueous milieu.

Studies in Biochemical Behavior

Preliminary experiments in rats on the temporal relation between pituitary stimulation and various responses were undertaken. Typical stimuli were cold, intradermal formaldehyde, ethionine, reserpine, ethanol, dibenamine, carbon tetrachloride, 3-methylcholanthrene and Tofranil. (No responses are obtained in hypophysectomized animals.) 1) Adrenal ascorbic acid is rapidly reduced, returning to normal in about 5 hours. With persistent stimuli (cold and methylcholanthrene), adrenal ascorbic level returns to higher than normal value. This probably reflects the induced, increased synthesis of ascorbic acid in the rat, since in guinea pigs which do not make ascorbic acid, the adrenal levels remain low in persistent stress. 2) Plasma corticosteroid levels are maximal in 1 to 4 hours and then return to normal. With persistent stress, the high levels are maintained. 3) Plasma-free fatty acids (FFA) usually are maximal in 1 to 4 hours and then return to normal, except in persistent stress. Anomalous results are obtained with electroshock which lowers FFA and with Tofranil which produces a delayed response. 4) Although many stimuli cause a rise in FFA only some of them result in deposition of liver triglyceride. This suggests that something must also happen in liver to cause fatty deposition. 5) The activity of tryptophane peroxidase (TPO) in liver is increased by all stimuli. 6) A chemical picture typical of "stress" is elicited by the "anti-stress" compounds, reserpine and chlorpromazine.

Specific Studies

a) Reserpine. It has been reported that reserpine pretreatment inhibits the responsiveness of pituitary-adrenal axis to stressful stimuli. Surprisingly, reserpine depletes adrenal ascorbic acid in the rat, increases the level of circulating corticoids and increases the level of liver tryptophane peroxidase. No effects are observed in hypophysectomized rats. Preliminary results indicate that the effect on pituitary may be related to change in brain amines.

b) 3-Methylcholanthrene. This compound in rats causes prolonged pituitary stimulation lasting up to 48 hours. The effects are depletion of adrenal ascorbic acid, increase in plasma corticosteroids, increase in plasma FFA, increase in liver TPO and antagonism of reserpine sedation. Other carcinogenic agents do not elicit this marked response. 3-Methylcholanthrene affected the pituitary-adrenal axis in doses as low as 0.1 μg/kg. The mechanism of this effect is under study.

c) Effect of Various Pituitary Stimuli on Various Body Enzymes. The most sensitive index of pituitary stimulation found thus far is the increase in liver TPO. Not only is TPO increased by stimuli previously mentioned but by certain barbiturates. None of the stimuli is effective in hypophysectomized animals. Of interest is the finding that tryptophane in doses said to act as specific inducer of TPO, also decreases adrenal ascorbic acid, and increases plasma corticoids and FFA.

The effect of many drugs in stimulating the biosynthesis of L-ascorbic acid in rats, and which has been shown in this laboratory to result from a stimulation of glucose metabolism via glucuronic acid, l-gulonic, etc., is also a pituitary response, which occurs in adrenalectomized rats, but not in hypophysectomized animals. Drugs that increase ascorbic acid synthesis also increase the activity of liver microsomal enzymes. Whether this effect on liver microsomes is an adaptive response to reduce toxicity to foreign compounds or is a more generalized response remains to be seen. Other enzymes that are stimulated by drugs are those in liver that convert galactose to glucuronic acid.

d) Effect of Drugs on Triglyceride Mobilization and Deposition. Triglyceride deposition in rat liver is produced by CCl_4, ethanol, and ethionine. The fatty acids appearing in the neutral fat of liver appear to be mobilized from fat depots (collaborative work with Dr. Marjorie Horning), but ethanol may also stimulate their synthesis in liver. Triglyceride deposition may be blocked by large doses of adrenergic blocking agents.

Isolation of Cardiotonic Substances from Mammalian Tissues

In addition to lysolecithin, previously reported, other active factors, all acidic lipids, have been obtained from mammalian tissues. These include: 1) Beef blood factor – probably cis vaccenic acid. 2) Beef heart factor – an acidic factor which, from infrared spectra, may be a lactonized hydroxy fatty acid. 3) Rabbit serum factor; infrared also suggests that this is an unsaturated lactonized hydroxy acid.

It is believed that these substances may influence the passage of ions through membranes, as a result of which the muscle contractility may be increased.

Studies with Ascorbic Acid

Ascorbic acid is synthesized in rats from glucose through D-glucuronic acid and L-gulonic acid. During the past year evidence has been obtained for an enzyme system in rat liver which synthesizes D-glucuronic acid through uridine nucleotide precursors. The occurrence of such reactions in the intact animal was confirmed by experiments showing that D-galactose is a considerably better precursor of L-ascorbic acid than is D-glucose

Information has also been obtained on the metabolism of L-ascorbic acid. An enzyme system in guinea pig and rat tissues which decarboxylates L-ascorbic acid has been purified and two sugar acids, L-lyxonic and L-xylonic acids, have been identified as products of the reaction. A strain of yeast has been adapted to grow on L-ascorbic acid as its sole carbon source; this observation may furnish a useful tool for studying the mechanisms involved in ascorbic acid metabolism

Laboratory of Technical Development

Gas Chromatography

Considerable effort has been devoted to the development of methods of increasing the sensitivity and versatility of gas chromatography techniques. The radiofrequency (RF) discharge detector system has been thoroughly evaluated and shown to be applicable to both standard columns and capillary columns and to provide a high sensitivity. Comparisons of the RF detector with argon ionization detectors showed an approximately 10-fold greater sensitivity for the RF discharge detector. Variations in conditions produce changes in the baseline with the RF detector whereas similar variations yield, with the argon detector, changes in sensitivity with consequent errors in the size of the peaks but with a stable baseline. This makes the records produced with the argon detector superficially more attractive since the errors are less obvious. At present it is simpler to use the argon detector but improvements in the radiofrequency excitation source may change this. Studies of the radiofrequency discharge as a source of ionization which can be measured downstream from the gas discharge and to improve the radiofrequency excitation systems are continuing.

The need for a satisfactory system for the radioassay of C-14 labeled compounds was pointed out by the Committee on Lipid Analysis and a program was undertaken to solve this problem. The method developed consists of capturing the effluent in short sections of column using anthracene crystals coated with one of several liquid phases shown not to interfere with the counting. These short columns permitted direct radioassay by the usual automatic scintillation counting techniques. The efficiency of this system is very close to theoretical limits. In addition to the above noted gas chromatographic techniques, Dr. Karmen participated in several studies in cooperation with other laboratories, notably in the studies on chylomicron composition after fat ingestion and a study of binding of unesterified fatty acids to various serum proteins with Drs. Bragdon and Shafrir, respectively. An exploration of the application of zeolite molecular sieves to analysis of respiratory gases was undertaken as a project for one of the high school teachers on summer training. Although the attempt to produce a zeolite sieve to separate all of the respiratory gases in one step was not successful, the study suggested several other potential uses of these sieves.

In consideration of the fact that it would be particularly advantageous to have a detector for gas chromatography columns that would not only be sensitive but would also give information as to the molecular

weight of the gas, a gas chromatographic detector based on the measurement of sound velocity was developed. From a consideration of the parameters contributing to the change in velocity, it would appear that if the quantity were known an estimate of the molecular weight could be obtained from the sonic velocity curve. The sensitivity of ordinary methods of sound velocity measurement suggested that the method would be of no use unless some high sensitivity system for sound velocity measurement were developed. An ingenious electronic circuit system was devised by Mr. Noble, which far exceeded expectations as to sensitivity. The method of frequency multiplication and phase comparison available with this apparatus makes possible sensitivities which can be utilized only if suitably stable sample chambers can be developed. Test runs using this sound velocity system showed it to possess sensitivity exceeding that of the thermal conductivity system. The relative complexity of the electronic equipment and the requirement for a split stream differential system can be matched by an expectation of very high sensitivity and an extremely small cell volume. The system in current operation has a cell volume of 0.08 ml and this can be reduced several fold without sacrifice of the capability of the instrument. This equipment in its present form, with a Linde sieve type 13X, can yield an analysis of 5 µl of air for oxygen and nitrogen content in 0.4 minutes.

Microanalysis by Excitation in Low Pressure Helium Discharge

Several aspects of the helium discharge and its ability to excite spectral emission were investigated. It was found that the discharge would excite molecular and atomic emission from volatile organic compounds. This suggested the possibility of analysis of the spectra of the glow as the fractions appeared from the chromatograph column. The emission spectra were taken and studied for several types of compounds. The complexity of the spectra produced by mixtures of gases does not encourage specific analysis by this means. The analysis of the spectra, however, showed a relatively clear zone above 500 millimicrons, so that it was concluded that the presence of organic materials in blood and urine specimens would not produce interfering bands that would overlap the region used for the determination of the alkali metals. The work on emission spectroscopy by means of helium discharge for the determination of alkali metals is now continuing after a period during which the apparatus was modified and these spectra explored. The apparatus is now improved with stabilized methods of measurement, stable excitation sources and a new chamber allowing volatilization of the sample on a heated platinum wire. In addition, preparation of measuring pipettes has been facilitated by the use of a commercially available quartz tubing 33 µ I. D. available under the name of Santo tube from Monsanto.

Errors in Catheter-recording Systems and Their Correction

The completion of Mr. Noble's work on a precise variable frequency hydraulic pressure generator has made possible accurate analysis of the performance of various intracardiac pressure measuring systems. It has been shown that pressure curves taken by cardiac catheterization, as it is normally performed, are distorted in shape and time. As these errors may be wrongly interpreted as due to the vascular system rather than the instruments, several efforts have been directed toward the development of a catheter tip transducer. A solution developed by Mr. Noble makes it possible to compensate electrically for the errors introduced by the catheter. The simple electronic circuit can be adjusted for the specific catheter so that all of the errors due to the changing characteristics due to aging of the catheter and due to the particular pressure transducer used are compensated at once. Tests of the efficacy of the system are being run in collaboration with Dr. Guy Barnett of the Section on Cardiodynamics. The current method utilizes the recently developed hydraulic pressure generator to make and confirm the compensation, but a relatively simple method of setting the compensator can be developed using a simple transient pressure pulse applied to the catheter after it is in place.

Computer for Analysis of Overlapping Distributions

The analog computer for the analysis of overlapping distribution functions was completed during the year and tested for its ability to resolve overlapping peaks. The utility of the instrument will be more completely evaluated by application to resolution of absorption spectra by Dr. Hayes, who has prepared and provided for this purpose some specific compounds with known absorption spectra. In addition, the application of the instrument to the automatic analysis of infrared spectra is anticipated.

Evaluation and Quantification of Valvular Regurgitation

An investigation of methods of quantitating mitral valve regurgitation has been undertaken by Dr. Peter Frommer in collaboration with the Cardiology Section of the Surgery Branch. Methods have been reviewed and the possibilities of the dye dilution technique utilizing radiopaque dye and serial x-ray films are under consideration. The exploration has been facilitated by the use of a simple analog computer which provided easily changed parameters with idealized curves produced to indicate the effects of backflow and dilution. From the examination of

the properties of the analog system, it would appear that the radio-opaque dye dilution technique has promise but puts some stringent requirements on the instrumentation and clinical procedures. The analog system proved so convenient and informative in the evaluation of partial mixing and regurgitation that a paper on the method is in preparation.

Flow Meter Development

Dr. Frommer has guided the investigation of the possibility of utilizing for flow measurement the Coriolis force produced when a fluid is made to gain and lose angular momentum as it passes through a rotating loop of pipe. This type of flow meter would measure actual mass flow, be independent of viscosity and provide a convenient form of differential flow meter. A rather large model system was constructed and was sufficiently promising to encourage further work on more compact designs. This was a summer project almost entirely performed by a summer student employee.

Investigation of nuclear magnetic resonance phenomena in the measurement of blood flow and their potentialities in analytical instrumentation is continuing. Mr. Kudravcev has constructed several types of nuclear resonance apparatus and circuits have been developed which yield a considerable reduction in the size and in the instability noted in previous equipment. It still appears feasible to evolve a flow meter system sensitive to volume flow using nuclear resonance principles. The possibility that such a device can be made without requiring intimate electrical contact may compensate for the complexity of the apparatus. Application to pulsating flows has not yet been extensively examined. If the volume flow measurement should prove impractical, the velocity technique may have value. Considerable information has been gained with regard to construction of simplified nuclear magnetic resonance systems and this will presumably be useful in the development of analytical devices should development of a practical flow meter prove impossible. The analytical characteristic of the system may also be applied to flow measurement as a method for determining the concentration of a nuclear "dye" as it goes past the detector. Fluorine, for example, is easily detected and could be used with present apparatus in a dye dilution system. Highly fluorinated materials such as freon could conceivably be utilized.

Theoretical Analysis of Transport

Dr. Stephenson has evolved several contributions to the theoretical analysis of transport in biological systems. This work has resulted in two papers in the Bulletin of Mathematical Biophysics, the subject

This theoretical material has been helpful in the analysis of Dr. Fredrickson's experiments on fatty acid metabolism. The data have been analyzed and programmed for computation on the IBM. The investigation of the physics of the ultra-rapid freezing of water in biological materials is continuing. A fuller understanding of the process may lead to information on the structure of biological material and aid in the design of methods for preservation of material by freezing and drying, both for banking of biological materials and the preparation of material for electron microscopy.

Phosphorescence Analysis

An investigation of the application of phosphorescence to the analysis and characterization of biological materials is being pursued by Dr. Hayes with the loan of the phosphorimeter from the American Instrument Company. One of the first problems was to find a solvent that would be suitable for investigation of water soluble materials. A survey of materials that would form a satisfactory glass at a temperature at which phosphorescence observations could be made was undertaken, and it was found that propylene glycol at minus 80° formed a satisfactory glass. At the temperature of liquid nitrogen, however, the solvent would crystallize and spoil the optical properties of the system. Accordingly, liquid gases for the cooling of the sample were surveyed to find one which would provide temperatures between those available from carbon dioxide and liquid nitrogen. Nitrous oxide was found satisfactory. Utilizing the new system, a survey of biological compounds of biological interest is currently in progress. Evaluation of phosphorimetry as a method for analysis and characterization of materials will be pursued.

Ultrasonics

Dr. Weissler's investigation of the effects of ultrasonic irradiation of hemoglobin has shown that the products of ultrasonic irradiation of water participate in the destruction or conversion of the hemoglobin molecule, and that additives in the form of dissolved gases or chemical agents modify the form of degradation. This work suggests the possibility of modifying the destructive effects of ultrasonic irradiation when utilized for liberation of biological materials from cells and tissues by high powered ultrasonic disintegration. In addition the selection of the environment may provide for selective isolation of particular enzymes by protecting one at the expense of the other.

Laboratory of Cardiovascular Physiology

Studies on the Heart

1. **The Diastolic Pressure - Myocardial Segment Length Relation in the Ventricle. Observations on the Contribution of Atrial Systole.** The relation between left ventricular diastolic pressure and the simultaneously recorded changes in the length of a segment of left ventricular myocardium was intensively studied. The shape of this curve laid the basis for a clearer understanding of the circumstances under which atrial systole will increase the [illegible] in relation to the elongation of the fibers of the ventricular musculature. Further, when considered together with the curve relating pressure to stroke work it gave added support to the position that in any given metabolic state the force of contraction of the ventricle is a function of the fiber length from which the contraction begins. These observations show the importance of atrial systole for ventricular filling.

2. **The Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on the Relation Between Left Ventricular Diastolic Pressure and Myocardial Segment Length.** The full curve relating ventricular pressure to myocardial segment length [illegible] the lack of any change in this relation with sympathetic or vagal stimulation. These observations made it clear that the family of curves relating filling pressure to stroke work is accompanied by a family of curves relating initial fiber length to stroke work. A corollary of this [illegible] under sympathetic stimulation, the ventricle will produce more work from any given initial fiber length as well as from any given diastolic pressure. This conclusion [illegible]

In the course of these studies [illegible] out concomitant sympathetic stimulation [illegible] especially if the stroke volume is high) that the ventricle does not have sufficient time to acquire its "normal" diastolic pressure-length relation. The addition of sympathetic stimulation at the same heart rate so condenses systole that it permits the same amount of diastole that would have occurred at a lower heart rate. The technical and conceptual advances in these studies have helped to bring into clearer focus the importance of the simultaneous positive inotropic effect on the ventricle when tachycardia is induced by the sympathetic outflow.

3. **The Regulation of the Ventricle's Contraction: The Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on Atrial and Ventricular Dynamics.** With the advances described above it was possible to systematize understanding of the means by which the central nervous

system can produce acute changes in the performance of the heart other than by the well known effects on rate.

The pertinent observations may be stated as follows:

1. At constant heart rates efferent stimulation of the vagus nerve exerts a profound depressant effect on the strength of the atrial contraction and can thereby influence ventricular filling and ventricular stroke work; mean atrial and thus venous pressure are elevated at any given level of cardiac work or cardiac output during vagal stimulation despite the fact that the vagal stimulation used does not alter the performance characteristics of the ventricle. The effects of vagal stimulation are blocked by atropine.

2. Stellate ganglion stimulation or norepinephrine infusion augments the strength of atrial contraction and thus the atrial contribution to ventricular filling. The augmented atrial contraction takes place in a shorter period of time.

3. Stellate ganglion stimulation or norepinephrine infusion increases the external work and power produced by the ventricle from any given filling pressure and fiber length.

4. There is a family of curves [illegible] the relation between end diastolic fiber length and [illegible] as a family of curves representing the relation [illegible] work.

5. When taken together [illegible] sympathetic effects on [illegible] a reasonably comprehensive [illegible] central nervous system [illegible] of the heart.

On the basis of these observations [illegible] and his colleagues propose what they refer to as "the law of the innervated heart" as follows:

1. If the effective catechol amine stimulus remains constant, the contraction of the ventricle varies with its end diastolic pressure and fiber length. If the end diastolic pressure and fiber length remain constant, the contraction of the ventricle varies with the effective catechol amine stimulus.

2. The central nervous system has available efferent neuronal pathways to the heart by means of which it can vary ventricular end

diastolic pressure and fiber length while keeping the effective catechol amine stimulus constant, means by which it can increase the effective catechol amine stimulus, or both.

4. The Regulation of Ventricular Contraction by the Carotid Sinus: Its Effect on Atrial and Ventricular Dynamics. The role of carotid sinus baroreceptors in circulatory regulation was re-evaluated and showed that a dominant aspect of the carotid sinus regulatory activity is to augment or diminish the contraction of the ventricle. The basis for this conclusion is as follows:

1. Carotid hypotension diminishes venous distensibility. The net effect of such a change, if it alone occurs is an increased ventricular end diastolic pressure and fiber length and thus an augmented ventricular contraction. Splenic contraction would have the same effect.

2. Carotid hypotension augments and shortens the atrial contraction. The net effect of such an atrial augmentation, if it alone occurs, is an increased ventricular end diastolic pressure and fiber length and thus an augmented ventricular contraction.

3. Carotid hypotension directly augments the work produced by the ventricle from any given end diastolic pressure or fiber length and produces more complete systolic emptying.

4. Carotid hypotension augments ventricular power as well as contractility, since it shortens the systolic time for any given amount of work produced, provides more rapid relaxation and thus diminishes filling impedance. If this alone occurs, it provides for a longer interval of diastolic filling than would otherwise occur and thus produces an augmented ventricular contraction, a factor which becomes especially important at high heart rates.

5. The catechol amines secreted by the adrenal medulla in response to a lowering of carotid sinus pressure would be expected to re-enforce the effects enumerated under 1 through 4 above.

In each experiment, over the range of aortic pressures and flows observed, the increase of ventricular stroke work was several times the simultaneously observed increase in total peripheral resistance when carotid pressure was lowered. Thus rather than acting primarily to safeguard blood flow to the vital organs such as the brain and heart, the baroceptor acts not unlike a voltage regulating element which causes an increased input into an electronic system so as to maintain a constant

hypertension augments ventricular

systolic emptying

any given end diastolic pressure or

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voltage when the current requirements of the system it is supplying are increased. Thus the carotid sinus helps to regulate the blood flow to all the tissues.

5. A Comparison of the Hemodynamic Effects of Pacing the Atrium and Ventricle at the Same Rate. By causing the atrium to contract while the atrioventricular valve is closed, the importance of the atrial contribution to ventricular filling was further shown.

By closing the door, so to speak, on the atrium during atrial systole and thus depriving the ventricle of the filling pressure and fiber length it would otherwise have achieved, the ventricular end diastolic pressure was significantly lowered and the external work produced was thereby lessened. It was further observed that the amount of work produced by the ventricle from any given end diastolic pressure was lower during ventricular pacing than during atrial pacing. Analysis of high speed ventricular pulse contours showed that the total ventricular effort is appreciably less concerted and synchronous. Thus, when the first fibers are contracting, the flaccidity of those which are not as yet activated tends to impose the same hydraulic limitations as a ventricular aneurysm, e. g. diminish the effectiveness of the contraction. Similar considerations apply to the last contracting fibers.

6. The Analysis of Coronary Sinus Blood for Catechol Amines Before, During and After Sympathetic Stimulation of the Heart. The concentration of norepinephrine in coronary sinus blood rises sharply during cardiac sympathetic nerve stimulation. An interesting lead, however, is the massive outpouring, after the cessation of sympathetic stimulation, of a substance which assays chemically as norepinephrine by the Weil-Malherbe and Bone technique. This is probably not norepinephrine since the heart's action does not conform to the presence of large amounts of the active substance at that time.

7. The Influence of the Vigor of Atrial Systole on Closure of the Mitral Valve. In the dog with heart block, between two and five atrial A-waves, and their reflections on ventricular diastolic pressure, can be studied in the absence of the disturbances ordinarily produced by ventricular activity. As evidenced by the staircase pattern or its absence in the ventricular diastolic tracing, it can be determined whether the mitral valve has closed after any given atrial systole. The "non-closing" atrial systoles are transformed by sympathetic stimulation into "closing" atrial systoles. The greater speed of the decline in atrial pressure seem after the strong atrial A-wave causes the leaflets of the mitral valve to close, since the increased rate of change of atrial pressure after a strong atrial systole produces an initially higher velocity of refluxing blood and thus is likely to close the valve.

8. Auto-Regulation of the Performance Characteristics of the Ventricle. The ventricle of the isolated heart, beating at a constant rate and free of reflex or hormonal regulatory influences, requires no longer a time to put out any given stroke volume against a high aortic pressure than against a low one. If the work of the ventricle is increased solely by increasing aortic pressure, the ventricular function curve is much steeper than if the ventricular work is increased solely by increasing stroke volume. When oxygen consumption is increased by increasing aortic pressure while holding stroke volume constant, there is a greater relative increase in coronary blood flow and a narrowing of the arteriovenous O_2 difference. Conversely, when O_2 consumption is increased by increasing stroke volume while holding mean aortic pressure constant, the increase in coronary blood flow is less marked and there is a widened arteriovenous O_2 difference.

Re-examination of these phenomena showed that the effects of raising aortic pressure by increasing the resistance to left ventricular ejection produced alterations in the pulse contours which were in every respect similar to those observed after the administration of norepinephrine.

There is a body of evidence suggesting that the responsiveness to catechol amine stimulation is a function of the biochemical environment of the stimulated effector organ. It is proposed that increased aortic pressure, by increasing coronary flow relative to O_2 utilization, so alters the biochemical environment of the myocardium as to render it more responsive to catechol amines and thus increases the effective catechol amine stimulus. This hypothesis is to be investigated further.

Studies on Circulatory Regulation - Exercise

The total flow to both lower extremities was metered, before and during exercise, while the arteriovenous O_2 difference, arterial and venous pO_2 and pH were recorded. Prior to exercise, three types of sympathetic stimulus were applied: a) the reflex increase in sympathetic tone consequent to lowering carotid sinus pressure; b) the emphatic sympathetic stimulation resulting from stimulating the central cut end of the vagus nerve; and c) the injection of constricting doses of norepinephrine into the arterial line supplying the lower extremities. These were then repeated during simulated exercise induced by electrical stimulation of the muscles of both lower extremities. In a second type of experiment the blood flow to each lower extremity was separately metered so that one extremity, the resting extremity, could act as the control while the opposite extremity was exercised.

The results of both types of experiments make it clear that with augmented muscular activity, the vascular bed of the active area can disregard a sympathetic stimulus to which it would ordinarily be responsive. This might be termed the functional sympathectomy of activity. The basis of this phenomenon is not yet known but it does not appear to be pO_2.

All of the studies in this laboratory have been greatly facilitated by the improvement of instrumental and recording techniques, developments which have been largely carried out in this laboratory with some advice and assistance from members of the Laboratory of Technical Development.

Abdominal Pressoreceptors

It was earlier observed that splanchnic or pancreatic vascular hypotension will produce tachycardia and a pressor response in the cat. In current experiments the lower abdominal aorta, near the bifurcation, is perfused at constant flow and the femoral arterial pressure recorded. The increased femoral artery pressure in the area perfused at a constant flow which takes place when the coeliac and superior mesenteric artery are occluded suggest that a reflex increase in peripheral vascular resistance takes place. It remains, however, to exclude the possibility that the observed elevations of pressure in this vascular bed are not due to the influence of collateral channels.

Studies on the Callicrein System

The objective of these investigations was to obtain an increased understanding of the physiological significance of this system for circulatory regulation. Callicrein, a hypotensive proteinase, acts on callidinogen, an alpha-2 globulin in plasma, to release a polypeptide called callidin which produces the observed vasodilatory effect. Since various callicreins are found in the urine, pancreas, plasma, saliva, etc., and these callicreins may be differentiated by differences in their susceptibility to proteolytic inhibitors, it is proposed that the callicreins are limited to local vasomotor regulation, the magnitude of the effect being determined by the activity of the tissue or organ which is involved.

During the past year semi-quantitative methods have been developed for the biological determination of some of the various components, e. g. callicrein, callidinogen, callidin, and two plasma callicrein inhibitors.

The callidin inhibitor can be estimated by measuring the rate of destruction of callidin following its liberation from callidinogen. However, a quantitative method for the measurement of this inhibitor remains to be developed. With the techniques currently available, plasma and urine from patients with orthostatic hypotension show a greater deviation from their normal controls than do plasma and urine from patients with essential hypertension.

Plasmin, the proteolytic activity of serum found in the euglobulin fraction, has definitely been distinguished from plasma callicrein. Other factors in plasma which have not yet been distinguished from plasma callicrein are the glass activated proteinase of Anderson and co-workers, Hageman's factor and the permeability factor of Miles and Wilhelm. Studies on comparison of these factors have been initiated and no differences have yet been found.

Because plasma callicrein was inhibited in vivo with diisopropylfluorophosphate, a known inhibitor of esterolytic activity, it was thought possible that the callicreins might be capable of digesting synthetic esters. Tosyl L-arginine methyl ester (TAME) has been found to be a substrate for the various callicreins. Crude urinary callicrein attacks this substrate at the same rate of activity as does highly purified hog pancreatic callicrein (obtained from Dr. Mortya, Japan), and it is possible that urinary callicrein may be the mild esterase excreted in the urine. The addition of acetone to human plasma, a procedure which causes the activation of plasma callicrein, activates at least two esterases, one inhibitable with soy bean trypsin inhibitor (SBI) and the other not. To date correlation between SBI inhibitable proteinase and plasma callicrein has been consistent. It is possible that [illegible] (callicreinase) responsible for the [illegible] precursor (calli[illegible] teinase inhibitor [illegible] TAME esterase [illegible] studies will be required to determine whether acetone causes the activation of more than one proteinase inhibitable with SBI.

Reflexly Induced Changes in Renal Function

During stimulation of the isolated stellate ganglion of the anesthetized dog there is a diuresis which is not related to the changes in arterial blood pressure. The diuresis is characterized by a rapid onset, little or no change in urine solute or chloride concentration, an increase in glomerular filtration rate and a rapid cessation with the "stoppage" of stimulation. Also, the diuresis occurs in the presence of a lowered left

atrial pressure. It was further found that cervical vagotomy diminishes the extent of the diuresis whereas vagotomy at the level of the diaphragm does not appear to modify it. From the results of these experiments it was suggested that the increased urine flow during stimulation of the stellate ganglion could be attributed to a peripheral vasodilatation elicited via the baroreceptors in response to the changes in dynamic pressure effected by stellate stimulation. Vagotomy effectively deprived the organism of a portion of the pressure stabilizing system thus lessening the extent of the reflex changes induced by stellate stimulation and thus lessening the extent of the changes in renal vasomotor tone, glomerular filtration rate and urine flow.

Laboratory of Kidney and Electrolyte Metabolism

Five major areas of research are being pursued in the Laboratory of Kidney and Electrolyte Metabolism. These include: 1) studies on the mechanism of electrolyte excretion and acidification of the urine in intact animals; 2) studies on the mechanism of urinary dilution and concentration; 3) studies of electrolyte and water transport in isolated systems, both living and artificial; 4) studies of the control of aldosterone secretion in dogs; and 5) studies of a mammalian cardiotonic protein system.

Drs. Orloff and Burg have completed their examination of the effect of the cardiac aglycone, strophanthidin, on electrolyte excretion in the chicken. As noted in the previous annual report they were able to elicit unilateral natriuresis in the chicken by injecting strophanthidin into the renal portal venous system. Associated with the diminution in Na^+ reabsorption they also observed interference with both K^+ and H^+ secretion. These effects have since been shown to be inhibited to a considerable degree by the simultaneous administration of K salts. The effects of strophanthidin are unlike those observed following the injection of other pharmacological inhibitors of Na reabsorption. In the latter instance a fall in Na reabsorption is generally attended by reciprocal, rather than parallel changes in the secretion of K and H ions. On the basis of these data, as well as the results of studies of the kinetics of K^+ transport in renal cortical slices of rabbits, to be summarized below, it has been concluded that strophanthidin exerts its renal effect by inhibiting a contraluminal [illegible] pump. The latter pump is thought to be similar to that present in many cells and presumably involved in the maintenance of the characteristic intracellular concentrations of Na and K. By interfering with the normal functioning of the pump, and K uptake on the contraluminal border, the intracellular concentration of both ions as well as of H^+ is depressed.

The thesis that strophanthidin affects a hypothetical contraluminal exchange system derives further support from other studies of Drs. Orloff and Burg. These workers reexamined the effect of various inhibitors, including strophanthidin, on K transport in rabbit renal cortical slices. The methods used were described in detail in the previous report. They confirmed their earlier findings that strophanthidin reduces intracellular K and raises that of Na; and that the effect is due in part at least to specific interference with K influx. These changes are consistent with an effect on linked Na-K exchange, analogous to that observed in red cells, muscle, etc. by other workers.

Drs. Orloff and Burg were unable to demonstrate an antagonistic effect of adrenal steroids and strophanthidin on electrolyte transport as had been described by others. The effects of the aglycone on electrolyte excretion are not altered by potent adrenal steroids (including aldosterone); the strophanthidin effects on K^+ and Na^+ accumulation in cortical slices of both normal and adrenalectomized animals are not altered by salt-active steroids; nor is the inhibition of PAH accumulation by strophanthidin altered by aldosterone.

Drs. Kahn, Brenes, Earley and Orloff are investigating the effects of the infusion of ammonium salts on electrolyte excretion in the chicken. They have confirmed an earlier finding of this laboratory that the infusion of a series of ammonium salts into the leg veins elicits a profound natriuresis. The mechanism of this effect is being actively studied.

Drs. Jaenike and Berliner have concluded studies utilizing a modification of the stop flow technique described originally by Malvin and Wilde. The technique involves occlusion of the ureteral outflow in the anesthetized dog for a variable period of time, release of the occlusion and collection of serial urine samples. The composition of these samples is thought to reflect to a considerable extent the composition of the "stopped" intraluminal fluid at various levels in the nephron. The modifications introduced were designed to permit critical examination of distal tubular functions by effectively eliminating renal pelvic dead space (filling it with mineral oil) and isotopically labelling distal convolution fluid (subcapsular injection of K^{42} during stop flow). The data confirm that vasopressin markedly affects the permeability to [illegible] of the distal convolution and further that [illegible] is also abstracted in this area. These results are consistent with a view of the mechanism of urinary dilution and concentration previously described by Dr. Berliner and his associates. Utilizing this technique Drs. Jaenike and Berliner have also been able to reaffirm the view that K^+ and Na^+ are exchanged in the distal system and have clarified a number of factors influencing this process.

These investigators are at present studying the effect of vasopressin on the permeability of the collecting system to urea in the dog. The data support the interpretation that as vasopressin enhances bulk flow of water along its osmotic gradient out of the collecting system into the interstitium, urea movement is accelerated in the same direction. This concept is of considerable significance [illegible] to the role of urea in the concentrating mechanism. [illegible] urine to plasma concentration ratio [illegible]

a time when flow of H_2O out of the collecting system into the medulla is high, and is considerably lower when no vasopressin is present and there is limited movement of water (and of urea).

Dr. Bray has investigated the osmotic pressure of rat kidney slices by a modification of a technique which depends on the direct observation of the relative thawing time of previously frozen slices. The presence of a progressive increase in osmotic pressure from cortex to inner medulla in kidney slices of either dehydrated or non-dehydrated rats has been confirmed. The cortex, which, for the most part, is isosmotic, contains some tubules which are definitely hypotonic. The hypotonic tubules are not seen in the outer medulla. In contrast, the inner medulla which is considerably more concentrated, contains no detectably hypotonic structures. The collecting ducts in the inner medulla generally seem less concentrated than surrounding structures. In animals undergoing a water diuresis, the concentration gradient is less marked; the cortex resembles that of dehydrated animals as does the outer medulla. The inner medulla on the other hand is considerably less concentrated than in dehydrated rats but the smaller structures (loops, capillaries) are definitely hypertonic and as expected, the collecting ducts are distinctly hypotonic.

A clinical situation pertinent to the problem of urine concentration and dilution is being studied by Drs. [illegible] and Orloff. Nephrogenic diabetes insipidus, a disease characterized by inability to elaborate a hypertonic urine due to insensitivity to [illegible] has been reported to respond to a limited degree to chlorothiazide, a diuretic agent. This observation has been confirmed [illegible] action of the drug is under study.

Dr. Hoffman is continuing his investigation of the characteristics of electrolyte transport in human red cell ghosts (hemolyzed red cells). He has established that the ghost system transports Na^+ in exchange for K^+ essentially as does the intact red blood cell. Thus electrolyte transport in ghosts has three components. 1) active transport; 2) passive transport, and 3) exchange diffusion. Active transport of Na out of cells requires the presence of K in the extracellular phase and is blocked by strophanthidin. Although this is similar [illegible] in intact human cells, the kinetics of the process [illegible] in contrast to the red cell findings, that a single [illegible]

In view of the precise [illegible] it has been possible to utilize [illegible] the metabolic intermediates [illegible]

has been able to show that electrolyte is immediately and completely dependent upon the availability of adenosine triphosphate, further any reaction capable of generating ATP stimulates the transport system as does ATP alone. It appears that the pump itself is, or has as an intimate component in its structure, ATPase. These observations together with related findings in other laboratories constitute a major advance toward an understanding of the mechanism of active electrolyte transport.

In association with Dr. Hoffman, Dr. Sidel and Dr. Ryan are exploring the possibility of measuring the rate of flow of H_2O across the red cell membrane under an osmotic gradient utilizing a rapid flow system developed by Dr. Tosteson, a former member of this laboratory. The method allows for rapid and continuous separation of extracellular fluid from a flowing system of suspended cells. They are also examining the effect of various liquid membranes (organic solvents) on the movement of Na and K from aqueous phases separated by the solvent membrane. They have established that the addition of cephalin to the membrane confers a slight degree of specificity to the movement of Na and K from one aqueous phase to the other. Other parameters are being studied.

Dr. Cotlove in association with Dr. Hogben (now at George Washington University) has examined the kinetics of chloride transport across isolated frog gastric epithelium. They have established that chloride movement is more rapid across the luminal than the nutrient membrane of the epithelial cell. By the use of tracers and an inhibitor which affects only active transport and exchange diffusion (DNP) they have concluded that the drug interferes with luminal transport only, presumably implicating this to be the site of linked carrier-mediated transport. Dr. Cotlove has also continued his examination of the so-called "true" chloride space described in detail in the last report.

Dr. Davis and his associates have extended their studies on the mechanism of aldosterone secretion in dogs and have restudied the role of the pituitary in this process. Although administration of a low sodium diet or constriction of the thoracic inferior vena cava stimulates hormone secretion in normal animals, no such effect was observed following hypophysectomy. Administration of ACTH reproduces the capacity to respond to vena cava constriction with an increase in the aldosterone content in adrenal vein blood. That this effect of ACTH is permissive is indicated by studies in unanesthetized dogs. The stress of anesthesia and operation induces ACTH secretion and a high corticosterone output in the unanesthetized animal. In the unanesthetized trained animal, corticosterone output is low, indicating that ACTH secretion is not increased. Nevertheless

caval constriction stimulates aldosterone output just as in anesthetized animals, without an increase in corticosterone secretion. It is probable that ACTH secretion is normal in the secondary hyperaldosteronism of caval constricted animals, and that ACTH merely serves to support aldosterone production at a high level rather than initiate its secretion.

Attempts to characterize the precise mode of stimulation of aldosterone secretion are continuing. It is now apparent on the basis of work reported in the past by this group that an unknown trophic hormone is involved in the increased secretion of aldosterone in animals with constrictions of the inferior vena cava. Efforts to isolate and identify this hormone are being carried out in collaboration with Dr. Titus.

Chronic denervation of the cervical common carotid, the carotid sinus, and the cervical plexus of the external and internal carotid had no significant effect on short-term sodium balance or aldosterone excretion in normal dogs [illegible] constrictions of the vena cava. However, in one dog acutely denervated [illegible] constriction and cervical carotid denervation increased aldosterone excretion without affecting sodium balance. [illegible] denervation of the abdominal arteries (coeliac, superior mesenteric and inferior mesenteric) increased arterial pressure [illegible] Although as yet inconclusive these [illegible] with the possibility of neural receptors somewhere in the [illegible] studies of this nature are being pursued. Neither [illegible] understanding has been shown to appreciably affect [illegible] and animals with such lesions appear to respond [illegible] aldosterone output in [illegible]

One [illegible] cardiotonic [illegible] than in the past. Recent [illegible] of the physiological significance [illegible] in man and animals. The concentration of the cardiotonic [illegible] is distinctly elevated in a small group of patients with uncomplicated hypertension and in patients with aortic stenosis. It is markedly diminished in a considerable proportion of patients suffering from congestive heart failure of unknown origin (that is not due to the usual causes, such as hypertension, rheumatic heart disease, etc.). The activity virtually disappeared from the plasma of patients subjected to extracorporeal circulation and returned to normal in all but one who developed irreversible vascular collapse. These preliminary data [illegible] the tentative hypothesis that the system has specific cardiotonic function in man, the activity increasing in a compensatory fashion in disease [illegible] associated with increased isometric tension of the left ventricle, and [illegible] diminished in severe myocardial insufficiency. Animal studies are in progress in an attempt to delineate the [illegible] of the system in a more precise manner.

Laboratory of Clinical Biochemistry

The problem of amine formation and metabolism has been reinvestigated to determine the nature of the decarboxylation of the various amino acids and the routes of metabolism of the resulting amines. By the use of column and paper chromatography it was possible to demonstrate that human urine from normal individuals (particularly following administration of monoamine oxidase inhibitors) contains at least 25-30 amines. In addition to those already known, it was possible to identify ortho-tyramine, meta-tyramine, and phenylethylamine. The presence of so many amines suggested either that there must be many individual amino acid decarboxylases or that the available enzymes were not very specific. The enzyme system, 5-hydroxytryptophan (5HTP) decarboxylase, when purified was found to decarboxylate DOPA, 5HTP, tryptophan, phenylalanine, tyrosine and histidine, at rates in the order listed. Further work is proceeding on the assumption that a single enzyme, analogous to L-amino acid [illegible] the lack of specificity, is responsible for catalyzing all these reactions. This finding is of great significance from a [illegible] practical standpoint. First, it means that a large number of diverse [illegible] metabolically formed amino acids continually give rise to a spectrum of amines. The amount of a given amine which appears in the [illegible] dependent upon the affinity of the decarboxylase for its precursor amino acid, the concentration of the amino acid in the tissues, competing amino acids, and finally on its rate of production by competing enzymes. The latter two factors may [illegible] amounts. As for amine metabolism, [illegible] mechanisms are available. Although [illegible] localized, to some extent, [illegible] specific enzymes [illegible]. Thus histamine [illegible] methyl pherase. [illegible] they are introduced into the circulation. Oxidation [illegible] the major route of metabolism. However, it would appear from studies of Dr. Udenfriend and his associates that metabolism of norepinephrine in brain, heart and other organs is largely due to MAO. The complexity of the effects produced by inhibitors of amine metabolism is no longer surprising when account is taken of the fact that serotonin and norepinephrine are but two of the many amines affected. The marked central effects of tryptophan and tryptamine in patients receiving MAO inhibitors comprise but one example of the significance of the [illegible] of amines.

In view of all these factors, an investigation of the biochemical effects of inhibitors of amino acid decarboxylation in patients was undertaken in collaboration with [illegible].

α-Methyl DOPA was found most effective in this respect and following its administration tyramine formation was found to be markedly diminished; the excretion of tryptamine and serotonin was also decreased and it appears that there may have been some detectable effects on the formation of norepinephrine. Simultaneous studies with purified mammalian decarboxylase indicate that α-methyl DOPA inhibits formation of all aromatic and cyclic amines including histamine. The finding that α-methyl DOPA is a potent antihypertensive agent (see report from Section of Experimental Therapeutics) is most interesting and gratifying. However, the biochemical findings indicate that the mechanism of its action is not yet clear and it does not appear to be attributable entirely to diminished formation of norepinephrine.

In view of these interesting and important findings, studies of a number of additional aspects of amine metabolism have been undertaken. Conversion of tryptamine to 6-hydroxytryptamine was shown to be catalyzed by liver microsomes and TPNH. It may be that ortho-tyramine may be formed from phenylethylamine in a similar manner. The mechanism of formation of meta-tyramine is not yet clear but may involve intermediate formation of meta-tyrosine through the action of phenylalanine hydroxylase. Methods have been developed for the determination of tryptamine and tyramine in tissues and in urine. Studies with kynuramine, the amine derived from kynurenine, have led to a simple and direct spectrophotometric procedure for assaying MAO. The aldehyde formed from kynuramine cyclizes so readily to 4-hydroxyquinoline that in the crudest preparations spectrophotometry can be used as a rapid and direct assay for oxidative deamination. This method may be expected to facilitate markedly steps leading to purification and characterization of MAO.

In studies of catecholamine metabolism the enzyme dopamine β-oxidase was shown to be present in very high concentration in hypothalamus and caudate nucleus as in adrenal medulla. However, little if any was found in the higher centers of the brain. The mechanism of the dopamine β-oxidase catalyzed reaction has been under investigation in collaboration with Dr. Witkop's laboratory. One of the results of these studies was the demonstration that dopamine gives rise to 2, 4, 5-trihydroxyphenylethylamine upon chemical oxidation and that this product appears in the urine when dopamine-C^{14} is administered to animals and patients with pheochromocytoma.

Studies on the mechanism of uptake of serotonin by platelets have continued. Using saline media it has been possible to obtain additional evidence that this process is one of active transport. Requirements for

K^+ and $PO_4^=$ were demonstrated, the demonstration of inhibition by digitoxin being further evidence of a K^+ requirement. A relationship between serotonin uptake and glycolysis was also shown, including marked inhibition by fluoride.

Mechanisms whereby amino acids penetrate into various mammalian cells are also under study. It has been possible to show that L-tyrosine is taken up from blood into brain in vivo by a process of facilitated transport. The evidence is that the L-isomer penetrates rapidly and several times faster than the D-isomer and more rapidly than non-amino acid congeners. The uptake of L-tyrosine is markedly inhibited by other aromatic amino acids including tryptophan and fluorophenylalanine but is not inhibited by alanine, histidine or lysine. Although tyrosine is rapidly taken up into muscle too, this process is not inhibited by other amino acids. Using rat diaphragm muscle it has thus far not been possible to detect any evidence of active or facilitated transport of tyrosine, the amino acid entering by diffusion only. These findings are contrary to conclusions put forward by Christiansen and others. The studies will be extended to other tissues and to other amino acids.

There have been some interesting findings relating to γ-aminobutyric acid. Transamidination has been shown to occur in brain and to yield γ-guanidinobutyric acid. A peptide containing γ-aminobutyric acid and histidine and possibly another amino acid has been found in brain. In beef brain it is present in amounts as high as several milligrams per cent. Histidyl-γ-aminobutyric acid has already been synthesized by Dr. L. Cohen of Dr. Witkop's laboratory to help in the investigation of structure.

Studies on the metabolism of amino acids unique to collagen have been continued. It was shown that ascorbic acid which influences collagen formation does not do so by influencing hydroxyproline formation. Ketoproline increases hydroxyproline levels in tissues. Investigation of this phenomenon showed that ketoproline inhibits hydroxyproline metabolism by liver and by bacteria, and is itself converted to hydroxyproline. A mammalian enzyme system which catalyzes this conversion in the presence of DPN was studied and found to be distinct from other dehydrogenases. Studies in this laboratory have corroborated the reported presence of ketoproline in actinomycin and have shown that the ketoproline is of the L configuration. The metabolism of another amino acid found only in collagen was also investigated. Hydroxylysine was found to be metabolized by achromobacter and liver microsomes to 5-hydroxypipecolic acid and 1-amino-5-hydroxyadipic acid. A sensitive and specific method for measuring hydroxyproline in tissues was developed. It has been applied to studies on urinary hydroxyproline in

man and animals. Using proline-C^{14} it was shown that in rats urinary hydroxyproline becomes labelled. The rate of disappearance of C^{14} from urinary hydroxyproline in adult rats indicates three components. The first may represent a rapid conversion of proline to free hydroxyproline; the second represents a hitherto unknown peptide material with a fairly rapid turnover (half life $\overline{\text{ca}}$ 15 days); the third is typical of the slow turnover of collagen hydroxyproline which is known to obtain in adult animals.

Section on Experimental Therapeutics

The principal areas of investigation in this section may be grouped for descriptive purposes as 1) studies on vasoactive substances, 2) metabolism of amino acids in man, and 3) action and metabolism of drugs.

1) Vasoactive Substances: Accumulated experience in this laboratory on the differential assay of urinary catecholamines in pheochromocytoma (18 cases) plus a review of such assays reported in the literature (60 cases) indicates the procedure has usefulness in localization as well as diagnosis of the tumor. With exception of two tumors of the Organs of Zuckerkandl, excessive excretion of epinephrine represented a tumor in the adrenal area. In collaboration with Dr. J. Pisano (LCB) methods were developed for measuring the m-O-methyl metabolites of epinephrine and norepinephrine. In studies on the urine of 15 patients with pheochromocytoma, the free catecholamines accounted for 0.4 - 6.7%, the methoxy-catecholamines for 17 - 42% and 3-methoxy-4-hydroxymandelic acid for 57 -78% of the total excretion of catecholamines plus metabolites. It is felt that catecholamine assays may be supplanted by measurements of metabolites for the initial chemical detection of pheochromocytoma.

A method for estimating catechol-O-methylpherase activity in man has been developed. It consists of administering the d-isomer of isoproterenol and determining the percentage of the dose excreted in the urine as the O-methyl metabolite. Use of this technique along with methods for measuring monoamine oxidase (MAO) activity in vivo have revealed no differences between normal subjects and those with primary hypertension. This suggests that if an abnormality in the metabolism of norepinephrine in hypertension exists, the defect lies in biogenesis of the amine or in some other mechanism for inactivation such as protein-binding.

The further study of amines excreted in the urine of patients receiving MAO inhibitors has led to conclusive identification of m-tyramine and phenylethylamine. In two cases of phenylketonuria the rise in urinary phenylethylamine upon the administration of MAO inhibitors was considerably greater than normal while the rise in m-tyramine excretion was subnormal. The first finding represents the only demonstration of excess amounts of a centrally-active compound in phenylketonuria and the latter suggests that the defective hydroxylation of phenylalanine in this condition may involve the meta as well as the para position.

The cardiovascular actions of various amines are being investigated systematically in dogs and in patients. Many different amines increase contractile force and potentiation of the effect of these amines has been observed during MAO inhibition in the case of phenylalkylamines which lack a B-hydroxyl group and/or alkyl substitution on the amino group. These factors also determine susceptibility as substrates for MAO. Most attention has been given to norepinephrine, serotonin, dopamine, tryptamine and tyramine. Curiously, the administration of Ritalin produced an effect opposite to that of MAO inhibitors, with potentiation of the cardiac effects of norepinephrine and serotonin but not of dopamine, tryptamine and tyramine. In hypertensive subjects, potentiation of pressor responses to dopamine by MAO inhibitors was closely related to the degree of enzyme inhibition (as measured by urinary tryptamine) whereas potentiation of the pressor effects of norepinephrine and methoxamine occurred only when there was also sympathetic blockade as manifested by orthostatic hypotension.

In cooperation with the Clinic of Surgery, extensive studies of cardiac contractile force responses in man have been done using the strain gauge arch technique. The findings were similar to those in the dog and thus resolve such old arguments between pharmacologists and clinicians as to whether norepinephrine is a cardiac stimulant in man and whether cardiac glycosides increase the contractile force of the "normal" human heart. The answer to each question is yes.

2) Metabolism of Amino Acids in Man: The method of assay for hydroxyproline (OPR) in tissues and urine has been simplified considerably so that it is now possible to measure OPR routinely. A more extensive survey of connective tissue disorders has begun and arrangements have been made to perform a broader study of Marfan's Syndrome in 48 affected families under the care of Dr. V. McKusick of Johns Hopkins Hospital. Study of the specific activity of urinary OPR in rats after injection of C-14 proline has given a measure of the turnover rate of OPR and presumably also of body collagen. Similar studies in patients appear feasible.

Recently, the compound α-methyl-dihydroxyphenylalanine (α-MDOPA) has become available for clinical studies. This substance has been found in LCB and elsewhere to be an effective inhibitor of various amino acid decarboxylases in vitro and in vivo in laboratory animals. Administration of the compound (2.0 gm/day) to human hypertensives has been shown also to produce decarboxylase inhibition as indicated by a decrease in the excretion of tryptamine and tyramine following standard

loading doses of tryptophan and tyrosine respectively. In the course of this work, a hypotensive response was also observed (see next section). αM-DOPA is only the first of several decarboxylase inhibitors to be studied and thus attempts are being made to develop procedures for use as indices of decarboxylation. Patients with pheochromocytoma, carcinoid syndrome, phenylketonuria and urticaria pigmentosa may prove helpful in these studies because of the exaggerated formation of amines in these conditions. By the same token, specific decarboxylase inhibitors may be useful therapeutic agents in these disorders.

3) Action and Metabolism of Drugs: Five different MAO inhibitors have been evaluated for biochemical and pharmacologic activities in human hypertensives: iproniazid (Marsilid), 1-phenyl-2-hydrazinopropane (JB-516, Catron), dl-phenylcyclopropylamine (SKF-385), nialamide (Niamid) and phenylethylhydrazine (Nardil). Although administration of each of these agents produced postural hypotension, a precise correlation could not be made between the degree of enzyme inhibition and hypotension. However, the dose of each drug required to produce an increase of urinary tryptamine to levels of 500 - 700 µgm/day was of a magnitude similar to that reported to give optimal psychiatric effects. JB-516 is still the most potent and consistently effective hypotensive agent to be found among this group of drugs. Studies at George Washington University Hospital in 30 patients over a period of 6 months confirmed its effectiveness particularly when used in combination with chlorothiazide. However, the development of visual toxicity (diminished acuity as well as color perception) which has been only slowly reversible in three cases seriously limits the use of this agent to the management of hypertension. The daily dose which may be administered safely is less than 12 mg/day in our opinion and this amount is insufficient for control of the blood pressure in many hypertensives. The d and l isomers of JB-516 have been found to be equally effective for reducing the blood pressure but it is not yet known whether one of them will be devoid of visual toxicity. Complete absorption of JB-516 has been shown through the unusual experiment of demonstrating equivalent effects on urinary tryptamine excretion by single oral and intravenous doses of the drug.

Studies on the actions of MAO inhibitors on the autonomic nervous system in dogs have shown sympathetic (but not parasympathetic) ganglionic blockade with harmine and iproniazid but not with several other inhibitors.

The finding that αM-DOPA produces lowering of the blood pressure in patients with hypertension is under active investigation. A

uniform and significant decrease in both supine and standing blood pressure has been observed in several cases during short term studies.

Since June 1959 studies on anti-fibrinolytic agents have been conducted in collaboration with Professor J. Waldenstrom and associates of Malmo, Sweden. Several aliphatic amino acids were found to be inhibitors of plasminogen activation in vitro, the most potent being Δ-aminovaleric acid, Δ-aminolevulinic acid and E-aminocaproic acid (E-ACA). Administration of the latter compound to two patients with pathologic fibrinolysis (secondary to leukemia and cirrhosis) has shown it to be effective in man. A method for chemical assay of E-ACA in urine was developed and about 60% of a single dose (5 gm.) given intravenously or orally to patients was found to be excreted within 12 hours. The hypothesis that the early stages of atherosclerosis are related to deficient fibrinolysis is an attractive one and for this reason a continued interest in synthetic and naturally-occurring inhibitory substances is contemplated.

Section on Cardiodynamics

It is the ultimate objective of this section to study the physiological behavior of the cardiopulmonary system of human subjects as they go about their usual daily activities and as they are subjected to various physiologic, psychic, pharmacologic and other stressful interventions, both in health and disease. The measurement of a large number of physiologic variables under conditions most nearly simulating normal activity has made the development of new methods of instrumentation of prime importance. Further advances of this broad approach will depend largely upon 1) development of new highly specialized instrumentation and 2) the development of some new sophisticated biophysical and physiologic approaches. The activities of the Section have been determined largely by these needs.

The accurate measurement of pressures has remained a problem of high priority in the Section since new approaches developed in this laboratory both in the vascular and the pulmonary field have placed ever increasing demands on the accuracy of the measurements. A paper reviewing progress in this field will appear shortly. This section has worked with the Laboratory of Technical Development to develop an electrical pressure correction device which will instantaneously and continuously correct dynamic pressure errors in various pressure manometer systems.

A major advance in the field of blood flow measurement was achieved when the catheter-computer instantaneous gradient method for the instantaneous and continuous measurement of blood velocity was developed for use in the human. This has opened the way to the measurement of the power output of the heart from moment to moment, the kinematics of cardiac ejection, the power loss at diseased valves, and the distributed impedances and junctional admittances in the vascular tree. Measurements of this kind will be necessary to evaluate the abnormal physical properties and energetics of the vascular system in myocardial disease, coronary attenuation, arteriosclerosis, hypertension and related conditions. To date nineteen studies involving the use of the catheter-computer method for instantaneous blood velocity have been performed without major complication and the data are undergoing analysis. The resources of the mathematical and computer section NIH have been called upon for processing of some of these data.

Various electrical analogs of the vascular system are being devised and tested with the use of a Donner Analog Computer. The relationship between the vascular visco-elastic properties and the transmitted

pressure and flow wave are being studied in animals. Preliminary results indicate among other things that under certain circumstances it should be possible to infer the character of the central pulse from measurement of peripheral pressure pulses. The significance of this is two-fold: 1) Pressure pulse data may be useful in indirect determination of the visco-elastic properties of intact human vascular systems by the use of a relatively simple computer unit. 2) It may be possible to compute the instantaneous blood flow from the heart using peripheral pulse information.

The implication of this latter possibility is important in that it may make it possible to measure cardio-circulatory function under circumstances close to those of normal activity. The development of miniaturized transducers, amplifiers, telemetering systems and tape recording will be necessary for the ultimate realization of this goal. To this end pilot studies using a tape recorder have been started to investigate the feasibility of multi-channel tape recording in the physiologic application. Progress to date has suggested that many improvements must be made; however, the approach appears entirely practical and the acquisition of an improved system of this type will be necessary for the continuation of this work.

Pilot studies in animals, using the blood velocity catheter, are in progress to determine the effect of impairment of the coronary circulation. Acute attenuation of the coronary circulation either by ligature or embolization of the coronary has produced dramatic and reproducible changes in the blood velocity curve. Methods are being developed for producing chronic coronary insufficiency in dogs. Pilot studies in dogs are in progress to determine the pressure-flow relationships in various parts of the systemic and pulmonary vascular bed so that inferences regarding flow can be made from the blood velocity curve. When these studies are completed, the problem of systemic and pulmonary vascular impedance can be better evaluated. Preliminary data indicate that although there is a marked divergence between the shape of the pressure and velocity curves in the aorta, their shapes become almost identical as either the systemic or pulmonary capillary bed is approached. This indicates that the arteriolar-capillary-venular bed probably behaves as a pure resistance without significant reactance.

Vascular resistance in the pulmonary bed is being studied both by the catheter blood velocity technique and by conventional dye dilution curve methods to establish comparison of the methods and to establish the effect of intrathoracic pressure and various pharmacologic agents on resistance. These studies are being carried out both in man and in animals. A new method was developed for simplifying and making more reproducible the calibration of dye dilution curves.

Since the direct measurement of the intrathoracic pressure in man is hazardous and in itself alters the normal function of the lung, studies are in progress to establish the relationship of intrathoracic pressure to intraesophageal pressure. The determination of intrathoracic pressure is necessary for determination of the transmural stress on the intrathoracic structures in most of the foregoing studies.

New methods of studying the mechanical behavior of the lung were developed. Detailed studies of the unified pressure-flow-volume-time relationship of the living human lung were carried out in normal, cardiac and emphysematous subjects. A relationship between the maximum achievable expiratory flow and degree of lung inflation was discovered which theoretically has far-reaching physical and physiological implications of importance.

Studies are being undertaken jointly with the Section on Experimental Therapeutics to assess the changes in flow and resistance occurring with the administration of inotropic and vaso-active agents in normal controls and patients with various disease states. The new approach to indicator dilution curves makes it possible to do a sizeable number of these determinations with markedly reduced amount of time and effort.

Other studies are being undertaken to determine the incidence and character of arrhythmias in patients after closure of atrial septal defects. These are done as a joint project with the Clinic of Surgery, NHI. Study of the characteristics and possible mechanisms of formation of ectopic beats during right and left heart catheterization is being pursued by members of the Section in association with Dr. Albert Kistin of the Beckley Memorial Hospital, Beckley, West Virginia. This includes development of better intraesophageal and intracardiac electrodes.

Studies of coronary flow dynamics in human patients are to be undertaken in an attempt to develop methods that are valid and clinically more useful. The analysis of nitrous oxide gas by newer techniques including gas chromatography has been explored and has promise. It also appears worthwhile to try to assess the possible use of isotope tracer substances with external counting as a means for finding some index of myocardial blood flow.

Section on Clinical Endocrinology

The activities of the Section on Clinical Endocrinology may be grouped into four general areas, as follows: 1) studies on the function and metabolism of steroids and their role in disease states; 2) studies of the abnormalities in water metabolism found in various disease states; 3) studies of calcium metabolism, with special reference to the effects of parathyroid hormone, the effects of vitamin D, and the effects of calcium on renal function; and 4) studies on the permeability of arteries to large molecules.

Steroid Metabolism

Studies in the area of steroid metabolism include further exploration of the control of aldosterone secretion, clinical studies in hyperadrenal corticism, and measurement of relation of steroid function to structure.

Afferent pathways mediating control of aldosterone secretion were explored. The decreased aldosterone secretion which occurs upon release of constriction of the inferior vena cava had been found to require the presence of the vagus nerves. It is likely that they arise in the area of the auricles and the great vessels. Studies were carried out to define the pathways required for the increased secretion of aldosterone which occurs upon application of caval constriction. It was found that constriction of the carotid arteries was also an effective stimulus to increased secretion of aldosterone. Exploration of the carotid arteries in the dog revealed the presence of an area with baroreceptor function in the region of the thyrocarotid arterial junction. This baroreceptor function could be abolished by denervation of this area. It was found that denervation in this area also abolished the rise of aldosterone secretion following constriction of the carotid arteries as well as that which follows constriction of the inferior vena cava. Analysis of the results suggests that an important stimulus to increases of aldosterone secretion depends upon arterial pulse pressure, and that the intracarotid pulse pressure is the most important variable.

In patients with aldosteronism, direct measurements of blood volume, pulse pressure, and potassium balance have been carried out. Extracellular fluid volume was changed by loading subjects with sodium or depleting them of sodium. Changes in intravascular volume were produced by the infusion of albumin. Finally, the action of aldosterone on the renal tubules was blocked with the use of aldosterone antagonists.

With these measurements, it was hoped that primary aldosteronism, with autonomous secretion from a tumor, or unexplained "primary" hypersecretion from hyperplastic glands, could be distinguished from secondary aldosteronism. (Numerous studies, previously reported, support the view that changes in intravascular volume have a major role in the control of aldosterone secretion in man, as in the dog with experimentally produced aldosteronism.) It was found that measures which changed intravascular volume would induce changes in aldosterone secretion in patients with secondary aldosteronism, but not in patients with primary aldosteronism. The effect of aldosterone-blocking agents was more complex. Whereas in most cases of primary aldosteronism aldosterone secretion did not rise when the effect of the hormone was blocked, exceptions were seen when large increases in serum and total body potassium followed the use of the blocking agents. Under these circumstances, aldosterone secretion would rise in primary aldosteronism, as it regularly did in secondary aldosteronism.

It was found that potassium deprivation would lower aldosterone secretion, and restoration of the deficit would elevate aldosterone secretion, as previously reported. In a certain controlled balance studies, it was shown that this phenomenon could be produced without changes in the blood volume, as measured directly by dye dilution methods.

Two patients with [illegible] disease were studied in an attempt to elucidate the relationship between aldosterone secretion and potassium loss. It was found that the secretion of aldosterone would change markedly in response to changes in sodium intake and, thus, could not be considered to be "[illegible]" or "autonomous." The degree of potassium [illegible] from the secretion of aldosterone. [illegible] have hyperplastic adrenal cortices. [illegible] following subtotal resection.

Studies on the aldosterone secretion in patients with postural hypotension have been continued. In view of the evidence cited above, to the effect that arterial pressure has an important role in the control of secretion, it was considered to be worthwhile to measure the efficiency of the control of secretion in as many subjects with this disorder as possible. Subjects were classified according to the location of lesion, with the use of a series of tests, including mental arithmetic (which may reveal a normal efferent system and locate the essential lesion in afferent pathways) and peripheral nerve block or vasoconstrictor agents (which may reveal an inactive, hypersensitive efferent system and locate the lesion in efferent pathways). It was found that, whereas patients with afferent lesions may show an inability to secrete aldosterone with sodium depletion, patients with efferent lesions usually retain this property

Patients with Cushing's syndrome have been studied for relative dependence of hydrocortisone secretion upon blood levels of hydrocortisone or hydrocortisone analogs. These studies were done to clarify the locus of the essential lesion in Cushing's syndrome. All patients with hypersecretion of hydrocortisone were tested first with moderate doses of hydrocortisone analog. Under these conditions patients with Cushing's syndrome were found to excrete tetrahydrocortisone in unaltered quantities. They could be distinguished clearly from patients with ovarian disorder who may, at times, show hypersecretion of hydrocortisone, but a ready fall of secretion with the suppressive steroid. The patients with Cushing's syndrome [illegible] subjected to suppressive doses four times as large. The response of the urinary steroids to this procedure allowed the separation of the patients into two groups: patients whose hydrocortisone secretion was not suppressed had adrenal cortical adenomas; with one exception, those who did show suppression had hyperplasia of the adrenal cortex. Subjects with Cushing's syndrome are being further studied by measuring the response to agents which block the 11-hydroxylation of steroids. It is hoped, in this way, to distinguish Cushing's syndrome of pituitary origin from that of hypothalamic origin.

Methodology has been developed for fractionation of 17-ketosteroids, and patterns are being determined in patients with Cushing's syndrome, patients with the adrenogenital syndrome and patients with ovarian disease. In this way, it is hoped that more can be learned about the specific enzymatic defect in the various disorders. In particular, patients with the adrenogenital syndrome may be classified in this way, as regards the presence or absence of 11-hydroxylase.

The factors influencing the protein-binding of steroids in vivo have been further studied. The [illegible] bound could be greatly increased by administration of [illegible] but this does not alter the metabolic effects attributable to hydrocortisone; it appears probable that the metabolic activity of circulating hydrocortisone depends solely upon the "free" fraction. A number of steroids were tested for their ability to bind to serum proteins. Studies of the effect of fasting and of surgical trauma on the binding of steroids are now in progress.

Studies of the relation of steroid structure to steroid function have been continued, both in metabolic balance studies in man and in acute studies of renal sodium and potassium excretion in the adrenalectomized dog. It is hoped, with such studies, to achieve an understanding of the essential features in steroid structure responsible for the various metabolic effects, and also to allow prediction of structural changes which might enhance the activities of steroids.

Studies of Disturbed Water Metabolism

Studies have been carried out to define the abnormality in water metabolism in a number of diseases in which there is limitation of free water clearance, water retention, and hyponatremia. In patients with cirrhosis, it was found that the antidiuresis resulting from "physiologic" doses of pitressin were not more marked and did not last longer than the effects produced in normal subjects. Furthermore, all subjects could excrete free water after a water load, albeit in minimal amount. The defect in free water clearance in these subjects was attributable to excessive sodium reabsorption in the proximal tubules, and it was shown that they could excrete normal or even increased amounts of free water when proximal sodium was "delivered" to distal sites with the use of mannitol. A similar effect could be produced with infusion of sodium chloride and under these circumstances the increase of free water occurred even without increase of solute excretion. Preliminary results have been obtained in patients with cardiac failure and a similar mechanism appears to be responsible for the defect in free water clearance.

In Addison's disease there is a defect of free water clearance in the presence of large amounts of sodium in the urine. It was shown that a marked increase in free water clearance could be obtained by expansion of total extracellular fluid volume with sodium chloride or of intravascular volume with albumin. In the absence of any steroid therapy, these studies are being pursued to determine whether steroid therapy has an additional effect and whether hemodynamic changes alone would explain the defect in free water clearance or antidiuretic hormone hypersecretion is also involved.

A further patient with hypercalcemia from pancreatic carcinoma has been studied. The study showed that sodium was, in part, dependent upon sodium intake but related to a much greater extent to water intake. The findings support the view that the syndrome results from sustained inappropriate secretion of antidiuretic hormone and does not result from renal or adrenal disease.

Studies of Calcium Metabolism

Studies on the essential metabolic abnormalities in primary hyperparathyroidism have been continued. With the use of a rigorously controlled test of the effect of phosphorus deprivation together with a low calcium intake, a number of new patients with hyperparathyroidism have been discovered. The adequacy of phosphorus deprivation has been estimated from the extent of the decline in urinary phosphorus. When the

phosphorus deprivation is adequate, patients with hyperparathyroidism have shown in all cases a rise of urinary calcium and, in most cases, a fall of serum phosphorus. The addition of calcium loads distinguishes patients with hyperparathyroidism from those with hypercalciuria of other origin in that the latter but not the former, show a corresponding increase in urine calcium. The addition of glycogenic steroids serves to differentiate patients with hyperparathyroidism and "idiopathic" hypercalciuria on the one hand from those with sarcoidosis on the other. In patients with sarcoidosis studied thus far the urinary calcium has fallen with glycogenic steroid.

Calcium metabolism in [illegible] has been studied with the use of balance techniques. These patients have further been subjected to all the tests currently in use in this laboratory for hyperparathyroidism. This includes the determination of the Tm of phosphorus and the response of serum and urine phosphorus to a standard calcium infusion during a period when a constant diet is given. The balance studies have been so designed that the effects of vitamin D (to which these patients are said to be hypersensitive) and the effects of glycogenic steroid (which are said to block the effects of vitamin D in this syndrome) could be assessed separately and together. In this way, we are exploring the hypothesis that the defect in calcium metabolism in sarcoidosis is essentially one of hyperabsorption of calcium, that this results from abnormal sensitivity to vitamin D, and that the effects are reversible with steroids.

An attempt to prepare [illegible] vitamin D is in progress. This is to be used to determine the tissue distribution of vitamin D. The effects of [illegible] will be assessed in a preparation of rabbit intestine and [illegible] parathyroidectomized and maintained on calcium-[illegible].

The defect in the renal concentrating mechanism in all types of hypercalciuria has been studied with renal function techniques. We have confirmed that this defect is pitressin resistant. It has been found that it may occur in the presence of hypercalcemia or hypercalciuria without hypercalcemia. It has been shown to be independent of solute load. A quantitative measure of the extent of the defect may be obtained by measuring TcH_2O in the manner of Zak, Brun and Smith. With the use of this technique it has been shown that the defect may be produced in patients with "essential" hypercalciuria within a period of two weeks by high calcium feeding, and that it may be eliminated by a similar period of calcium deprivation. In patients with hyperparathyroidism it was possible to return urinary calcium to normal with versene and sodium phosphate. With

this treatment the concentrating defect did not improve. These studies will be extended to determine whether hypercalcemia alone is sufficient to produce the defect and to determine the points of similarity of this defect with that resulting from potassium depletion.

Studies of Aortic Permeability to Large Molecules

Studies of the movement of proteins and lipid through arterial walls have been continued in rabbits and dogs. Labeled albumin and cholesterol have been administered and the rate of accumulation and loss from the aortic wall has been measured as a function of depth of penetration and at a variety of hydrostatic pressures in the aorta. Comparable studies on animals with the [illegible] to hypothyroidism have been instituted. The development of atherosclerosis, as judged from these studies, [illegible] to depend more upon a decreased rate of removal from more distal areas than upon increased rate of deposition of cholesterol and protein in the [illegible] from available data, as a function of [illegible] aortic wall.

Surgery Branch

There has been a continuing interest in methods for the precise detection and localization of circulatory shunts. The usefulness for this purpose of various inert gases as well as the application of indicator-dilution curves has been previously demonstrated. In a study of the use of indicator-dilution techniques in determining the drainage pathways of the pulmonary veins in the [illegible] it has been found that, by appropriate injection and sampling, the presence or absence of anomalous pulmonary [illegible] drainage can be correctly determined. The validity of the method [illegible] studied. The injection of a [illegible] chamber with sampling at a [illegible] pulmonary or tricuspid valvular [illegible] the magnitude of the regurgitant [illegible] to-right shunt by means of [illegible] required injections into the [illegible] this technique were found [illegible] pling was carried out [illegible] intravenous injection of [illegible] correct site of entrance [illegible] A study was also made [illegible] on indicator-dilution [illegible] regurgitation. [illegible] as measured by [illegible] pressor drugs [illegible]

An important [illegible] ogy has been [illegible] as an oxidation [illegible] incorporated [illegible] can be recorded [illegible] cipal advantages have been [illegible] experience to date it appears that [illegible] tion curves are virtually [illegible] through a photoelectric densitometer. Similarly, the [illegible] tions of cold saline for recording [illegible] vestigated further. Difficulty with the method in the past has been [illegible] the establishment of a stable base line, but a new electronic circuit has, in pilot studies, apparently obviated this disadvantage. Curves recorded after the injection of cold solutions with thermistor sensing in a peripheral artery have the same advantages as those described for dilution curves.

It has been previously shown in several laboratories that when a gamma-emitting substance, such as radioactive diodrast, is injected intravenously the passage of the isotope through the heart can be detected with a scintillation counter placed over the patient's chest. Such curves have been recorded in more than 100 patients admitted to the service and it has been found that the contour of the precordial dilution curve is indicative of the presence or absence of a left-to-right circulatory shunt. The method is considered a valuable one in the screening of patients and may obviate the necessity of postoperative catheterization in patients who have been subjected to operation for the correction of such shunts.

Finally, the principle of isotope dilution has been applied in the study of abnormal communications between the systemic and portal venous systems. In both patients and animals a solution of radioactive krypton^{85} was injected into the spleen. When no abnormal communications existed, the appearance of the gas in expired air was greatly prolonged. When esophageal varices or a patent portacaval anastomosis were present, however, the gas appeared immediately and in high concentration. It is probable that this method is a safer and more sensitive one than portal venography for assessing patients with portal hypertension and varices both before and after operative treatment.

An important group of studies has centered around the clinical use of the artificial heart and lung machine. The instrument itself has been further refined to permit constant observation of the oxygen tension in blood returned to the patient, precise control of the volume of blood returned to the patient, and yet another electronic device has been developed which maintains constant the volume of blood contained within the patient and the extracorporeal circuit. All of these improvements have resulted in clinical procedures more closely approximating the normal physiologic state. A detailed investigation has also been made of the bacteriology of the heart-lung machine. These studies indicated that in virtually all instances bacterial contamination of the apparatus occurs, but that it could be minimized by special assembly techniques and the installation of bacterial filters at all points where room air has access to the circuit.

In the early experience with open heart operations, flaccid paralysis of the heart was often induced by the injection of solutions of potassium citrate into the coronary bed. It was noted in several patients that effective ventricular contraction did not resume after cardiac arrest and two studies of this phenomenon were undertaken. In an experimental study it was shown that left ventricular function was severely impaired after the administration of either potassium

citrate or acetylcholine, but that intermittent occlusion of the ascending aorta without a chemical agent had no demonstrable effect on ventricular function. In 15 of 19 patients who had been subjected to potassium citrate arrest a distinctive type of myocardial necrosis, most prominent in the left ventricle, was found. In the hearts of 19 other patients in whom this agent had not been employed no lesions of this type were discernible These physiological and anatomic observations have led to the abandonment of the technique of elective asystole in the course of cardiovascular operations. Intermittent aortic occlusion can be employed in virtually all procedures in which aortotomy is not necessary. When the aortic valve must be exposed for long periods it has been found that effective myocardial contraction can be maintained for periods of nearly two hours by direct perfusion of the left coronary artery. This technique is thus employed whenever operations upon the aortic valve are necessary.

When the heart is divorced from the peripheral circulation in the course of cardiopulmonary bypass there is a unique opportunity for studying the effects of various drugs and procedures on myocardial contractility and the central and peripheral effects of these agents can be separately assessed. In nearly 30 patients a myocardial strain gauge arch has been placed at the beginning of the thoracotomy and in the course of the operations injections of various pharmacologic agents have been made and their effects on myocardial contractility studied. It has been found that acute digitalization increases the contractility of the nonfailing heart. The effects of various vasopressor agents have been compared. Norepinephrine and epinephrine produced identical increases in cardiac contractile force but vasoxyl gave no such response. It has also been possible, with the strain gauge arch to study the effects of various anesthetic agents such as fluothane, demerol and muscle relaxants, drugs given commonly in the course of cardiac operations. Studies of contractile force have further indicated the safety of aortic occlusion and coronary perfusion in the course of open procedures.

The development of methods for catheterization of the left side of the heart by the transbronchial, the percutaneous and the retrograde arterial routes has been described in previous reports. In the past year the transseptal method of left heart catheterization developed in this laboratory has been employed in more than 100 patients. In this technique the left atrium is entered by a needle passed from the right atrium in the course of right heart catheterization. The method provides opportunity for the prolonged measurement of pressures in the left side of the heart with the patient in a comfortable basal condition. No complications have been encountered in this limited experience. Preliminary experiences also indicate that selective angiocardiography, with injection

into the left atrium through the transseptal needle, is a convenient and useful method in the study of patients with both congenital and acquired lesions.

Mitral commissurotomy, in the past, has usually been performed with dilatation of the valve with the finger inserted from the left atrium or with a knife passed from this approach. More recently, however, the valve has been opened by means of a dilator inserted from the apex of the left ventricle, the commissurotomy being controlled by a palpating finger passed from the left atrial appendage. A detailed study of the results of operation in patients operated upon by the latter method indicate that a superior hemodynamic result almost invariably can be obtained. Twelve patients have been operated upon for the second or third time for mitral stenosis. In no patient could restenosis of the valve be documented and it is felt that in most instances the obstruction was residual rather than recurrent. In 8 of the 12 patients a good hemodynamic and clinical result was obtained by an effective repeat operation. In the course of both open and closed operations for the correction of mitral stenosis and mitral regurgitation, valves have been encountered which are so badly damaged that a corrective procedure has been either unsatisfactory or impossible. A prosthetic mitral valve, suitable for entire replacement of the diseased valve, has been designed and preliminary studies of its usefulness have been carried out in animals. The present, and most promising, model is constructed of urethane foam, reinforced with plastic mesh cloth. Studies of the effects of various plastics and plastic surfaces on the coagulation mechanisms of the blood have also been initiated. Such information will probably be of paramount importance in selecting the material for fabrication of prosthetic mitral, as well as aortic, valves.

In normal patients or animals general body hypothermia at temperatures of 28-30° permits total arrest of the circulation for periods of only 10 or 12 minutes. If the metabolic demands of the body could be further reduced by abolition of the thyroid gland this safe period of circulatory interruption might be extended. In an investigation of this possibility dogs were rendered myxedematous by the injection of I^{131}; survival could be regularly obtained after 20 minutes of circulatory interruption. Another experimental study concerning hypothermia has been an evaluation of the effects of quinidine on myocardial function. This drug is commonly administered during general hypothermia to prevent arrhythmia. Preliminary results thus far indicate that quinidine itself has a depressant action on myocardial function.

The major problem of total replacement of the heart is immunologic. Even if means can be found to obviate these difficulties, however, many technical problems remain. In acute studies in animals the transplanted heart is completely denervated and this is considered, in most instances, the cause of death. An experimental study of the totally denervated heart in situ is underway and with refinement of the operative technique chronic survivors have been obtained. An attempt to determine the optimal method for storing an excised heart prior to reimplantation is also in progress. The comparative value of perfusion with blood and various physiologic solutions is being studied and attempts are being made to determine whether the beating or arrested heart is most suitable for long term preservation.

No adequate operation is available for the treatment of patients with complete atresia of the pulmonary artery or true truncus arteriosus. An experimental study has been made of methods for total replacement of the pulmonary artery by the insertion of a plastic graft into the outflow tract of the right ventricle and suturing the other end of the graft to one or both pulmonary arteries. Death has occured in some animals for technical reasons, but in the majority of survivors the prosthesis has been proved patent at the time of sacrifice or when angiographic studies were carried out.

Eight patients have now been studied in whom obstruction to outflow from the left ventricle was caused not by discrete narrowing in the region of the valve or subvalvular region, but by massive left ventricular hypertrophy of unknown etiology. An attempt has been made to reproduce this lesion in the experimental animal. Constrictions of the ascending aorta were made either by excision of a portion of the wall of the aorta or by banding it with a tape of plastic material. Large pressure gradients between the left ventricle and aorta have been created and serial cardiac catheterizations are being carried out to determine the progression of the lesion. The studies have not been in progress long enough to determine if massive left ventricular hypertrophy can be induced by this technique.

Considerable effort (in the Section of Cardiology) has been directed toward elucidating the manner in which various hemodynamic factors modify the heart's performance. The relationship between left ventricular end-diastolic pressure and circumference has been systematically investigated in the dog. It was observed that with tachycardia, ventricular end-diastolic pressure rose at a constant end-diastolic circumference. At a constant heart rate, acutely induced hypothermia had a similar effect, and it is suggested that abbreviation of the phase of ventricular filling by both of these interventions is responsible. On the

other hand, deterioration of the heart's performance, i.e. acute heart failure, resulted in an augmentation of end-diastolic circumference at any end-diastolic pressure. This appears to be a true alteration in ventricular distensibility. However, alterations in aortic pressure and cardiac output did not modify ventricular distensibility. The latter observations indicate that myocardial oxygen consumption is not primarily dependent on the end-diastolic size of the heart.

In studies on the circulatory responses to acutely induced hypervolemia in man, it was observed that a striking augmentation of cardiac output occurred only when the activity of the autonomic nervous system had been reduced by ganglionic blockade. This investigation shows that in studying the validity of Starling's law of the heart in man, circulatory changes occurring secondary to activity of the autonomic nervous system, rather than of the heart itself, must be excluded. Indeed, in several subjects a consistent relationship between ventricular end-diastolic pressure and stroke work was observed in the presence of ganglionic blockage and under these circumstances, Starling's law quite clearly operated. In a similar investigation of the relationship between left ventricular end-diastolic fiber length, end-diastolic pressure and the force of ventricular contraction in patients with mitral valve disease and atrial fibrillation, studied at the time of operation, further evidence of the applicability of Starling's law of the heart to man was obtained.

As part of a continuing investigation on the pharmacology of digitalis glycosides, it was demonstrated both in dogs and in patients on cardiopulmonary bypass that these agents are potent vasoconstrictors. In addition, a venoconstrictor action in the dog was found. These observations, when taken together with the studies on myocardial contractile force in man, described above, permit a more rational explanation of the effects of digitalis on the normal human heart. In a study of the effects of acute digitalization on left ventricular dynamics, it was shown that the elevated left ventricular end-diastolic pressure of myocarditis may be lowered, but that this did not occur in patients with aortic stenosis. It is suggested in the latter group of patients that the hypertrophied ventricular wall alters ventricular distensibility and in this manner elevates end-diastolic pressure; the latter then does not reflect the presence of myocardial insufficiency, as it does in other diseases. Finally, increased digitalis requirements were demonstrated when hypothyroid patients were rendered euthyroid or when euthyroid patients were rendered hyperthyroid. Since catecholamine depletion by reserpine blocked this antagonism between thyroid and digitalis, the increased digitalis requirements associated with augmented thyroid activity are believed to be related to increased sensitivity to endogenous [illegible]

A preparation has been developed in the dog on complete cardiopulmonary bypass which permits the simultaneous determination of the capacity of the vascular bed and of the distensibility of the venous bed. This preparation will permit a study on the effects of a variety of cardiovascular reflexes and drugs on these two important hemodynamic parameters.

In a continuing study on the factors which modify the distribution of blood it has been demonstrated in normal control subjects that exercise results in an increase in intrathoracic blood volume and that morphine apparently decreases intrathoracic blood volume.

Gerontology Branch

The program of the Gerontology Branch is concerned with 1) a description of physiological and psychological changes that take place with increasing age in humans and 2) investigations of the basic biology of aging.

Human Physiology

Longitudinal Studies. Age differences in physiological and psychological characteristics of normal people still living successfully in the community are being evaluated by Dr. Shock, Dr. Falzone and Mr. Norris. Subjects ranging from 32 to 99 years have agreed to return to the laboratory for retesting every 18 months for the remainder of their lives, so that age changes can be observed in individual subjects. Re-test schedules began in October 1959, so that no serial analysis of individual records is possible. However, a preliminary comparison has been made of some tests on the first 100 subjects with previous results obtained on hospital subjects. The outside subjects are larger in size than the hospital group. However, they do not differ significantly in body composition or in basal metabolism from the hospital subjects. Their pulmonary function is better maintained than in hospital subjects and they fail to show an age decrement in concentrating capacity of the kidney as measured by 12 hours of water deprivation, although their 12 hour endogenous creatinine clearance falls with age at a rate similar to that of hospitalized subjects.

In addition to re-testing subjects in the series, new subjects will be added to the program, with special emphasis on men over age 70. A number of new tests of intellectual functions and personality characteristics will be administered to all subjects. A special test of attitudes toward aging, developed in this laboratory, is being administered to these subjects.

Renal Studies. A method for estimating the glomerular clearance of unbound hemoglobin in the human has been developed by Dr. Lowenstein and Dr. Faulstick and hemoglobin/inulin clearance ratios of 0.020 to 0.065 have been found in preliminary experiments. The disappearance of the hemoglobin-haptoglobin complex from the blood follows first order reaction kinetics over the time period of 0-120 minutes. Age differences in this function are being assessed and the validity of this test as an index of the functional capacity of the reticulo-endothelial system in the intact human is being assessed.

Comparison between the 12 hour endogenous creatinine clearance and short term inulin clearances have been made in the same subject by Dr. Oursler and Mr. Yiengst. Creatinine clearances are on the average 30% higher than inulin clearances, but the correlation is high, so that endogenous creatinine clearances can be used as an index of renal function where intravenous infusions and bladder catheterizations are impractical. Studies on age changes in renal function by serial measurements on the same individual will be continued. The studies on permeability of the glomerular membrane will be continued using infusions of dextran.

Body Composition. The helium chamber method for determining body volume has been perfected by Mr. Norris and Dr. T. Lundy. Estimates of body composition, based on density measurements as well as total water content, show a decrement with increasing age in community residing subjects that is of the same order of magnitude as hospital patients. Lean body mass, or body water determinations are being used rather than calculated surface area as a basis for the normalization of measurements such as basal metabolism.

Response to Standardized Exercise. Measurements of age differences in the cardiovascular, respiratory and metabolic responses to exercise have been continued by Mr. Norris and Dr. Falzone. Maximum work output and rate of recovery of vascular and pulmonary displacements are lower in old than young subjects. Mechanical efficiency is lower in old subjects at high and low rates of work, but is essentially the same for old and young at intermediate rates. The factors involved in the reduced efficiency of the aged are being investigated.

Psychological Studies

Although an increase in reaction time with age has been previously demonstrated, Dr. M. Davidoff and Dr. G. Suci have shown a linear relationship between the rate at which information can be handled and the age of the subject. This relationship appears when stimuli are considered in terms of information theory. Further investigations of this phenomenon in different sense modalities will be followed. Other studies by Dr. F. Hugin and Mr. Norris show that slowing of responses with age appears in tasks that involve the central nervous system, and that the slowing is a reflection of central rather than peripheral changes in the nervous system. Dr. W. Surwillo is continuing his studies on the relationship between EEG frequency and spinal reflex time, to determine central effects on reflex time.

Previous studies, showing that short term memory for visual comparisons is more easily interfered with in young than old subjects, have been extended to include auditory discriminations of time intervals by Dr. Davidoff. Other studies of memory for verbal material show that the poorer memory of older subjects can be improved by reducing the length of the verbal sequence to be recalled and increasing the redundancy or degree of relationships between the words in the series. Thus, rote memory is impaired to a greater degree than memory for logical sequences in older people.

Basic Biology

Cellular and Comparative Physiology. The activities of the Cellular and Comparative Physiology Section involve 1) the description of cellular and organismic changes in humans and appropriate experimental animals during the aging process and 2) the measurement of the effects of environmental and physical manipulation on the performance and mortality of experimental animals.

Dr. Bodenstein has demonstrated clearly in his studies of regeneration in cockroaches that the capacity to regenerate lost parts (e.g. legs) is not lost even when the animal would normally no longer demonstrate such capacity. Adult cockroaches will regenerate legs to replace those amputated if supplied with growth hormone through transplantation of the prothoracic gland from a young (molting) animal. This demonstrates that the cells of tissues of adult cockroaches still possess the capacity to replace lost parts in the proper humoral environment

Dr. Konigsberg has shown that contractile-striated skeletal muscles will differentiate from embryonic cells in tissue culture and that the process includes the following phases: a) Cell division and multiplication in culture. (The cells which will differentiate into muscle are, at this stage, not distinguishable from other dividing cells in the culture.) b) Cell fusion. During this phase, the cells form large multiple nucleated fibers or straps. Evidence from time lapse photography and electron microscopy makes it highly probable that these are indeed cells with cytoplasmic continuity. During this period, measurements of DNA per nucleus made in collaboration with Dr. Strehler indicate that there is no nuclear division following fusion. c) Differentiation of contractile fibers from the multinucleate straps. During the early stages of this process striations are visible only in glycerinated preparations. Later they become visible even in non-glycerinated preparations observed under phase contrast microscopy.

These findings furnish an ideal tool for the study of differentiation, factors affecting it and the possible dedifferentiation and redifferentiation which have implications regarding the continued capacity of cells to furnish replacement parts in adults or aged vertebrate animals provided that the factors limiting this process are known and modifiable in vivo (as they are, for example, in the roach studies).

Drs. Mildvan and Strehler have continued their study of the chemical and physical properties of heart age pigment granules. Approximately 75% of the weight of the particles consists of an insoluble material with chemical and infrared absorption characteristics consistent with protein. Further elucidation of its nature and of the adherent as incorporated pigment is being undertaken by Drs. Hendley and Strehler.

Dr. Strehler has continued his study of the effect of environmental factors on the longevity of Drosophila melanogaster. It appears clear that the aging of Drosophila is not a result of denaturation - since aging does not possess a high activation energy. This conclusion follows from the fact that the survivors of flies exposed to high temperature shocks sufficient to kill half of them, are not aged as measured by their subsequent mortality behavior. Similarly, it has been shown that aging in Drosophila is not the result of mutation since exposure of animals to 4500 R actually doubled their life expectancy. Heavy water, on the other hand, in 20% or 40% concentration reduced the longevity by about a factor of two.

Studies with Campanularia flexuosa hydranths have demonstrated that there is no decrease with age in the following physiological functions: food catching ability, rate of ingestion of food, rate of digestion, rate of egestion, and amount of food which can be handled. Low temperatures extend the life span in a fashion similar to that observed with Drosophila melanogaster. X-radiation even in enormous doses (50,000 R) does not inhibit the continuation of development of hydranths which has already begun. Moreover, the animals receiving moderate doses of radiation lived twice as long as their controls - in agreement with the results on Drosophila outlined above.

Nutritional Biochemistry and Tissue Enzymes. The determination of various enzymatic activities as well as DNA and protein nitrogen in the liver, kidney and heart of ten 1, 3.5, 12 and 24 month old rats failed to establish a simple classification of enzymes into groups which follow similar age changes. Dr. Barrows has found that although the concentrations of the various enzymes in the tissues of one month old rats were different from those of older animals, the only change which

could be associated with senescence was the higher cathepsin activity in the liver and kidney of the aged rat. No evidence for an impaired protein synthesis in senescent rats was found by the depletion-repletion method and only slight differences were observed over the age span of 3.5 to 24 months.

A preliminary age study carried out on young and old rats subjected to unilateral nephrectomy showed similar hypertrophy of the remaining kidney in both young and old rats. Whereas the increase in total DNA, d-amino acid oxidase and alkaline phosphatase approximated the increase in organ weight, the total succinoxidase and pyrophosphatase showed a greater increment. The degree of hypertrophy estimated by any of these measurements failed to indicate any age differences.

Future experiments will include further studies on oxidative phosphorylation in order to find a system which will be adequate for an age study.

Intermediary Metabolism. One of the major research activities has been concerned with the mechanism of oxidative phosphorylation associated with α-ketoglutarate oxidation. The oxidative reactions in the electron transport chain immediately concerned with the synthesis of primary high energy compounds have not been identified. Preliminary work led to the hypothesis that the critical step might be reduction of a disulfide compound to a product with vicinal dithiols and simultaneous phosphorylation of one of the thiols utilizing the strain energy of the cyclic disulfide. Transfer of phosphate to an acceptor would lead to a dithiol compound. Studies to test the hypothesis are in progress but have not yet provided definitive conclusions.

In α-ketoglutarate oxidation, the primary high energy compound is an unidentified acyl enzyme complex. The present aim is to identify the complex and to study the mechanism of its formation. Resolution of the α-ketoglutaric dehydrogenase complex and purification of the components was necessary in order to permit use of stoichiometric amounts of highly purified enzyme for direct isolation of the acyl enzyme complex. One of the resolved components has been purified and shown to be a flavoprotein with flavin adenine dinucleotide as the prosthetic group. This flavoprotein which catalyzes the terminal transfer of electrons from the reduced thioctyl to DPN, seems to be identical with Straub diaphorase.

There are claims in the literature that the morphology and oxidative properties of mitochondria change with age. It is of considerable interest in this connection to determine whether mitochondria have a defined life span at the end of which the entire unit disintegrates or whether components within the mitochondria turn over at different rates. It is proposed to label three different components of mitochondria - lipids, proteins and cytochrome C - and follow the decrease in the labeled component with time. The results may be useful in deciding between the two alternative possibilities.

Biophysics. Evidence has been found suggesting that the amino group on the adenine ring of ATP is involved in the interaction of ATP with the muscle enzyme myosin, and that this interaction is accompanied by a conformation change of this enzyme. Studies in this laboratory have shown that Cu^{++}, Cal^{++}, and Zn^{++} interact with myosin in a manner superficially similar to that of PCMB (parachloromercuribenzoic acid, a sulfhydryl reagent). Furthermore, these metals and PCMB are found to interact with myosin during the course of incubation with myosin. ATP appears to prevent some of this time dependent interaction.

Cultures of a bleached variety of Euglena gracilis B have been established and it is hoped that these organisms will prove useful as a tool for biophysical and biochemical studies on the effect of "age" on single cell systems. Preliminary results have shown that such cultures may be kept well over one month at constant cell density. Experiments have now been begun to study the effects of aging under various well defined conditions on subsequent growth. Since these organisms are sensitive to steroid hormones Dr. Buetow has tested their response to vitamin D, and has found that growth appears accelerated by this vitamin. This is the first time an effect of this vitamin has been found in other than a mammalian system.

After the biochemical characterization of the mitochondria from Euglena has been completed, studies will be undertaken to determine the effects of aging on a variety of mitochondrial functions, such as stability, turnover, permeability, etc.

Molecular Biology. Important findings in the elucidation of the structure of catalase are that the four heme groups are symmetrically placed in the molecule, and that they can be reversibly removed from the molecule without affecting their site of attachment to the protein.

A method is being developed for detecting the position of nucleosides in a nucleic acid chain by selective complex formation with metal ions.

Catalase that has been cleaved into quadrants, each containing one heme, has no anomalous rotatory dispersion, indicating that the heme is symmetrically surrounded by protein; intact catalase, on the other hand, containing four hemes, has an asymmetric iron atom. The heme has been removed from catalase, yielding the apoenzyme; the latter can apparently be recombined with hemin, to reform the catalase molecule.

Horse hemoglobin is split along different axes by acid and base treatment. Human hemoglobin, like horse hemoglobin, has asymmetric iron in the reduced, oxidized, and oxygenated forms.

Vitamin B_{12} exhibits anomalous rotatory dispersion due to the presence of an asymmetric cobalt atom. The rotatory dispersion curve is unaffected by substitution for cyanide on the cobalt atom, and it is not greatly affected by reduction of cobalt. The rotation is markedly influenced, however, by changes in the three-dimensional structure of the molecule.

A correlation has been made between the electronic configuration of transition metal ions and their ability to catalyze the aconitase and enolase reactions. It has been shown that nickel, cobalt, and iron catalyze the aconitase reaction in the absence of enzyme. The mechanism of formation of the Schiff base intermediates in transamination reactions has been further elucidated by the finding that a carbinol amine intermediate is not formed in such reactions.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Study of Movement of Proteins and Lipids Through Arterial Walls and Similar Tissues

Principal Investigator: Duncan, L.E., Jr., M.D.

Other Investigators: Buck, K., and Lynch, A. (Technical)

Man Years:

Total:	2.75
Professional:	1.00
Other:	1.75

Patient Days: None

Objectives:

An understanding of the processes involved in the movement of proteins and lipids into and out of arterial walls and similar tissues.

Major Findings:

The work has been carried out in rabbits and dogs. Since no method existed for determining the transfer rates of substances into and out of tissues when their transfer rates are slow, our early studies were devoted to working out such methods. In these early studies the movement of labeled albumin through rabbit aorta and tissues which were in some way morphologically similar to aorta was studied.

Following this the movement of labeled albumin into and out of the aorta of the dog was studied. The larger size of this artery made a more detailed analysis possible. The aorta was divided into a number of areas and each area was split into inner, middle, and outer layers. The data support the concept that proteins move from blood in the aortic lumen across the intimal endothelium into the inner layer of the aorta. A striking gradient of inflow rates for albumin was found. In the proximal aorta near its origin from the heart, albumin moves into the aortic wall very rapidly. This inflow rate decreases progressively down the length of the aorta. The outflow rates do not exhibit any such gradient but are the same along the length of the aorta. Thus, the concentrations of albumin in the proximal aortic wall are higher than they are in the distal aortic wall.

Knowledge of the factor causing this gradient would be a matter of some interest, since it appears that this factor is a major determinant of the rate of movement of proteins into arterial walls. The gradient of inflow rates is not caused by differences in lateral blood pressure since this is the same along the length of the aorta. However, since the diameter of the aorta decreases progressively along its length, the circumferential tension to which the aortic wall is subjected would tend to decrease proportionately as described by Laplace's law. Current theory holds that proteins pass through endothelium by moving through pores in the intercellular cement which joins the endothelial cells rather than by passing through the bodies of the cells themselves. It appears possible that the greater circumferential tension in the proximal aorta separates the endothelial cells more widely there than elsewhere and thus permits proteins to flow through more rapidly.

Since the alternative possibility existed that gradient of rates was in some way related to the pulsatile nature of the blood pressure or to respiratory variations in intrathoracic pressura, the passage of labeled albumin into aortas removed from the body and filled with plasma under a normal mean arterial pressure was studied. The results of this study exclude phasic pressure variations as a cause of the gradient.

Preliminary results indicate that the rate of passage of albumin into the aortic wall is diminished when blood pressure is reduced by hemorrhage and increased when pressure is increased by section of the carotid sinus nerves.

In the normal dog we have found that labeled cholesterol passes into the aortic wall with a similar gradient of rates. The similarity of the gradient and the magnitude of the rates support the concept that in the normal dog cholesterol enters the aortic wall as part of normally occurring lipoprotein molecules.

We have also studied the rate at which cholesterol is deposited in the aortas of dogs fed thiouracil and cholesterol. At the end of a month of such treatment there is a gradient in the concentration of cholesterol deposited along the length of the aorta which corresponds to the gradient observed for albumin. The similarity of the gradients supports the view that atherosclerosis is produced by penetration of intact lipoprotein into arterial walls. Thus these results lend credence to the "filtration theory" of atherogenesis.

At the end of 5 months on thiouracil and cholesterol, the concentration of cholesterol in the abdominal aorta is higher than that in the thoracic aorta. It thus appears that the well known tendency for atherosclerosis to be worse in the abdominal aorta is due, not to rapid entrance of cholesterol into the abdominal aorta but rather to its slow removal from that site.

Significance to Heart Research:

These studies lend strong support to the "filtration theory" of atherosclerosis. They illustrate the importance of quantitative information on the rates at which protein and lipid enter and leave arterial walls and provide the beginnings of systematic knowledge in this area.

Proposed Course of Project:

We are trying to develop satisfactory methods for studying the passage of lipoproteins into arterial walls and for characterizing those that accumulate there. We are continuing to study the factors influencing the passage of albumin into arterial walls.

Part B. included Yes

Part B:

Publications

1. Leroy E. Duncan, Jr., Jerome Cornfield, and Katherin Buck, Circulation of Iodinated Albumin Through Aortic and Other Connective Tissues of the Rabbit. Circulation Research 6: 244, 1958.

2. Leroy E. Duncan, Jr., Jerome Cornfield, Katherin Buck, Circulation of Labeled Albumin Through the Aortic Wall of the Dog. Circulation Research 7: 390, 1959.

3. Leroy E. Duncan, Jr., and Katherin Buck, Passage of Labeled Cholesterol into the Aortic Wall of the Normal Dog. Circulation Research 7: 765, 1959.

Serial No. NHI - 2

1. Gen. Med. & Exptl. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Factors controlling free water clearance in patients with Addison's disease, orthostatic hypotension, cardiac failure, and cirrhosis.

Principal Investigators: Bartter, F.C., M.D., John R. Gill, Jr., M.D., and Harold P. Schedl, M.D.

Other Investigators: Gann, Donald S., M.D., Norman H. Bell, M.D., J.P. Thomas, M.D., G. Saucier, M.D., C. S. Delea and G. Smith

Man Years:

Total:	0.3
Professional:	0.2
Other:	0.1

Patient Days: 110

General Purpose:

To investigate the machanism of the defect of free water excretion in Addison's Disease, orthostatic hypotension, cardiac and renal failure, and cirrhosis. To attempt to evaluate the role of intravascular volume in the defect in free water excretion in Addison's Disease and orthostatic hypotension. To determine the role of proximal tubular sodium reabsorption in the free water defect in cardiac failure and renal disease.

Major Findings:

It was possible to correct the free water defect in Addison's disease by expansion of the extracellular fluid volume with sodium chloride or intravenous albumin in the absence of any adrenal hormonal therapy. Similar results have been obtained in patients with orthostatic hypotension.

Patients with cardiac and renal failure are being tested for a defect in free water excretion by administration of a water load (5% fructose). If the defect is present, attempts are being made to

correct it by administration of 5% mannitol infusions. The studies on the defect in free water clearance in the patients with cirrhosis, and in control patients depleted of sodium, have now been completed. This work has been accepted for publication under the title "An Explanation for and Experimental Correction of the Abnormal Water Diuresis in Cirrhosis", Schedl, H.P. and Bartter, F.C. This will appear in the February, 1960 Journal of Clinical Investigation.

Proposed Course of Project:

The data on patients with Addison's Disease have been presented and are being prepared for publication. Experiments are continuing with the other groups of patients.

Part B. included Yes

Part B.

Publications

1. Gill, John R., Jr., Gann, D.S., Delea, C.S., and Bartter, Frederic C. Correction of the Defect in Water Excretion in Untreated Addisonian Patients by Volume Expansion alone. Clin. Res. 1959, 7, 254.

2. Schedl, H.P., Bartter, F.C. An Explanation for and Experimental Correction of the Abnormal Water Retention in Cirrhosis. To be published in the Journal of Clinical Investigation February, 1960.

Serial No. NHL-3

1. Gen. Med. & Exp. Therap
2. Clinical Endocrinology
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Role of ADH in salt and water metabolism

Principal Investigators: Saucier, G., M.D., and Bartter, F.C., M.D.

Other Investigators: Delea, C.S., Diller, E., Berkant, D.

Man Years:

Total: .20
Professional: .10
Other: .10

Patient Days: 90

General Purpose:

To investigate the effect of ad libitum and controlled water intake on sodium excretion in patients under the effect of Pitressin. It has been postulated that in patients with bronchogenic carcinoma hyponatremia can result from sustained inappropriate secretion of antidiuretic hormone (ADH). It has also been denied that ADH alone would induce the changes of the syndrome. The present studies were designed to evaluate the relationship of the water intake to the sodium loss, and the role of voluntary water intake.

Major Findings:

A marked negative sodium balance has been observed in normal subjects on water ad libitum while under the effect of Pitressin. There was no conscious objection to, or refusal of water. A similar negative sodium balance could be induced and repeated in water intoxicated subjects at different levels of serum sodium by controlling the water intake.

Proposed Course of Project:

To repeat this study with graded stepwise expansion of body fluids.

Part B. included Yes

Part B.

Publications

1. William B. Schwartz, Daniel Tassel and Frederic C. Bartter. Further Observations on Hyponatremia and Renal Sodium Loss Probably Resulting from Inappropriate Secretion of Antidiuretic Hormone. Accepted for for publication in the New England Journal of Medicine.

Serial No. MHL- 6
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Structure-function relationships in steroids.

Principal Investigators: Bartter, F.C., M.D., Saucier, Guy, M.D.

Other Investigators: C. S. Delea, E. Diller, and D. Berkant

Man Years

Total:	2.0
Professional:	.5
Other:	1.5

Patient Days: 200

General Purpose:

1. To correlate activities of steroids with their structure.

2. To correlate effects of steroids on sodium and potassium balance with those on the "aldosteroid index" in the dog.

Major Findings:

Steroids with halogen atoms in position 6 have been supplied by Syntex Company. These have been assayed in adrenalectomized dogs for acute effect on sodium retention and potassium excretion. They are being tested in normal subjects and in subjects with Addison's disease for effect on metabolic balances of sodium, potassium and nitrogen.

Preliminary results indicate that 1) 6 halogenation may increase the early potassium loss resulting from steroids and the nitrogen-losing effect of steroids, 2) 6 halogenation decreases the sodium-retaining properties of steroids. 3) Effects of chloride substitutions are consistently less marked than the effects of fluoride substitution.

These studies are being pursued and completed.

Proposed Course of Project:

It is contemplated that other structural changes in the steroid nucleus will be systematically investigated in future studies.

Part B, included yes

Part B:

Publications:

1. Schadl, E.P., M.D., Catherine Delea, and Frederic C. Bartter, M.D. Structure-Activity Relationships of Anabolic Steroids: Role of the 19-Methyl Group. J. of Clin. Endocrinology & Metabolism. 19, August, 1959, 921-925.

Serial No. NMI- 5

1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Effect of Chronic Hypercalciuria on Renal Concentrating Mechanism and Sodium Conservation.

Principal Investigator: Gill, John R., M.D.

Other Investigators: Bartter, F.C., M.D., and Smith, G.

Man Years

Total: .3
Professional: .2
Other: .1

Patient Days: 100

General Purpose:

1. To study the effect of hypercalciuria, parathyroid hormone, and Vitamin D on the ability of the kidney to concentrate.

2. To determine the effect on the concentrating defect of restoring urinary calcium to normal in hypercalciuric patients.

3. To determine whether the concentrating defect is associated with inability to retain sodium.

It has been reported that patients with hypercalcemia and hypercalciuria may lose the ability to concentrate urine even in response to pitressin. The present studies were instigated to determine what factors are essential for the development of this defect.

Major Findings:

The studies described in the previous annual report have been extended and completed. It was found that phosphate loading would lower serum and urine calcium to normal in patients with hyperparathyroidism, but that this procedure would not produce a restoration of concentrating ability. It will not be possible to separate the effect of hyperparathyroidism from those of phosphate loading until cases are found in which urine and serum phosphorus can be lowered without the use of phosphate.

These studies have been written up and accepted for publication in the Journal of Clinical Investigation. The title will be "On the Impairment of Renal Concentrating Ability in Prolonged Hypercalcemia and Hypercalciuria in Man", by Gill, John R. and Bartter, F.C.

Part B. included No.

Serial No. NIH-6

1. General Med. & Exp. Therapy.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Vitamin D

Principal Investigators: Bell, Norman H., M.D. and Chen, Philip S., Jr., M.D.

Other Investigators: None

Man Years

Total: .10
Professional: .10
Other:

Patient Days:

Major Findings:

An effort was made to obtain tritium labelled Vitamin D_3 by the gas exposure technique for metabolic and *in vitro* studies. Several trials resulted in complete failure; small amounts of Vitamin D_3 were devoid of incorporated tritium. The radioactivity present (from Tracerlab or New England Nuclear) in the irradiated Vitamin D_3 mixture was associated with a more polar compound running faster on the reversed phase Kodicek paper chromatographic systems. A further effort was made by tritiating Vitamin D_3 dinitrobenzoate by the Wilzbach technique. A labelled compound was obtained; however, when the ester was hydrolyzed, the Vitamin D_3 obtained had no activity and the radioactivity which was present remained with the unhydrolyzed ester. These studies are still in progress.

Part B. included No

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Calcium Metabolism in Sarcoidosis

Principal Investigators: Gill, John R., M.D., Bell, Norman H., M.D. and Bartter, Frederic C., M.D.

Other Investigators: C. Dales and Staff

Cooperating Units: Vitamin D blood level determinations have been performed by the Food and Drug Administration

Man Years

Total:	.80
Professional:	.50
Other:	.30

Patient Days: 140

General Purpose:

Patients with sarcoidosis frequently have hypercalciuria, either with or without hypercalcemia. It has previously been suggested, but not established, that the hypercalciuria is secondary to an increased calcium absorption resulting from a hypersensitivity to vitamin D. It has further been hypothesized that steroids having carbohydrate activity will block this vitamin D sensitivity and correct the hyperabsorption of calcium.

Major Findings:

Fifteen patients having sarcoidosis have been found to have hypercalciuria. Studies thus far have included measurement of PO_4 Tm, response to calcium infusion and to phosphorus deprivation. Three patients have been studied by balance techniques including calcium, nitrogen and phosphorus. Studies previously carried out have demonstrated a response to meticorten with an increase in fecal calcium either with or without a change in urine calcium. Studies have been extended in two of the patients to include response to small dosages of vitamin D. In one patient a clear hypersensitivity was demonstrated; in the second, the results were less conclusive. In each of the latter three patients repeated vitamin D blood levels (bioassay) were found to be normal.

Proposed Course of Project:

Collection of sufficient data, bearing on the points outlined above, to produce a definitive conclusion.

art B. included Re

Serial No. AMI- 3

1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Action of Parathyroid Hormone

Principal Investigators: Fronove, Pepita, M.D., Bell, M.H., M.D.
Barter, Frederic C., M.D.

Other Investigators: C.S. Delea, G. Smith, E. Diller

Man Years

Total:	1.40
Professional:	.80
Other:	.60

Patient Days: 400

General Purpose:

1. To study the physiology of the parathyroid hormone.

2. To clarify the diagnosis of hyperparathyroidism.

Major Findings:

1. Tm of phosphorus. In six (6) normal subjects the Tm of phosphorus was greater than 3 mg/min., whereas, in eleven (11) cases of hyperparathyroidism the Tm was less than 3 mg/min. in 7 and greater than 3 mg/min. in four (4) patients. The latter are, therefore, within normal limits. This portion of the project has been discontinued.

2. Calcium infusion test: This was evaluated according to the response of serum and urinary phosphorus to the infusion of calcium, 15 mg/kg, over a 4-hour period. Of 14 cases of confirmed hyperparathyroidism, one has shown a fall of urinary phosphorus on the infusion day of greater than 10% below that of the control day, and one has shown a rise of urinary phosphorus on the post-infusion day reaching the normal range of greater than 110% of the phosphorus excretion of the control day. Thus far, no patient with hyperparathyroidism has shown both the normal fall and the normal "rebound" rise with the infusion. The serum phosphorus rises in patients as well as in normals.

3. Amphojel test. This test continues to be the most valuable diagnostic test for hyperparathyroidism. Fourteen patients with confirmed parathyroid tumor or hyperplasia had "positive" results. A positive result is taken to mean a rise of urinary calcium to or beyond 250 mg. per day during ten days on a low calcium, low phosphorus diet with amphojel.

This test is being extended by the addition of 1) prednisolone treatment with patients showing "positive" tests. This addition distinguishes patients with hyperparathyroidism from those with sarcoidosis and 2) addition of calcium to the intake in those patients showing a "negative" test. This addition has enabled us to classify certain patients as suffering from "hyperabsorption hypercalciuria".

Proposed course of Project: The studies in hyperparathyroidism are being summarized and prepared for publication. Evaluation of the tests in all types of patients with hypercalcemia or hypercalciuria is being continued.

Part D. included No

Serial No. NHI- 9

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Ultrafiltration studies of steroid protein binding.

Principal Investigator: Bartter, F. C., M.D., Chen, P.S., Jr., M.D. Schedl, H.P.

Other Investigators: Mills, I.H., H. Smith, C.S. Delea, D. Berkant

Man Years

Total:	2.03
Professional:	.86
Other:	1.17

Patient Days: 30

General Purpose:

Many steroids are bound to plasma proteins. The steroids differ in the extent to which they are bound and in the strength of the bond. At least two different plasma proteins are involved in steroid binding. Under certain physiological conditions, the degree of binding (and presumably the amount of binding protein) undergoes change. The present studies are designed to study protein binding of all steroids, to study the factors which alter protein binding, and to evaluate the proposition that only the unbound fraction is physiologically active.

Major Findings:

The studies in the last progress report have been pursued. The effect of Nilevar, of fasting and of surgical trauma on plasma binding of hydrocortisone were further tested. More recent results with Nilevar suggest that it may have a minimal effect on steroid binding. The effects of surgical trauma have not been evaluated.

The studies on the renal clearance of hydrocortisone have been prepared forpublication and accepted for the Journal of Clinical Endocrinology and Metabolism. The effects of estrogen have been summarized in a paper which has been accepted in Journal of Clinical Endocrinology and Metabolism.

We are preparing, at present, a paper summarizing the results of binding studies with all other steroids.

Part B. included Yes

Part B.

Publications:

1. Mills, I.H., Schedl, H.P., Chan, P.S., Jr., and Bartter, F.C. The Effect of Estrogen Administration on the Metabolism and Protein Binding of Hydrocortisone.

2. Chan, P.S., Jr., Schedl, H.P., and Bartter, F.C. Studies on the Plasma Binding of Steroids. In Press.

Serial No. MHI-10

1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Fractional of 17-Ketosteroids in the urine of patients with adrenal Diseases

Principal Investigators: Saucier, G., M.D., Bartter, F.C., M.D.,

Other Investigators: Berkant, D., and Diller, E.

Man Years

Total: .6
Professional: .4
Other: .2

Patient Days 60

General Purpose:

To determine the pattern of urinary 17-Ketosteroids in patients suffering from Cushing's syndrome, hyperplasia, and the Stein-Leventhal syndrome and to compare this pattern with that induced by an 11β hydroxylase blocking agent. To determine the pattern of urinary 17-Ketosteroids in children given ACTH and blocking agent.

Major Findings:

A large amount of work has been done to work out the method and standardize the multiple steps involved in extraction, hydrolysis, fractionation and quantitative determination of steroid. Some preliminary findings indicate that the method is of interest and could provide clues as to the mechanism of enzymatic determination of adrenal dysfunctions.

Proposed Course of Project:

Complete a series of cases, studied both with and without hydroxylase blocking agents.

Part B. included No

Serial No. MHI-11
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Pathophysiology of Cushing's Disease

Principal Investigators: Bartter, F.C., M.D., Sawicr, C., M.D.

Other Investigators: Gann, D.S., M.D., Delea, C.S., Diller, E., Berkant, D., M.D.

Man Years: Patient Days: 90

Total:	.65
Professional	.15
Other:	.50

General Purpose:

To establish a pre-operative diagnosis of the hypophyseo-adrenal dysfunction in Cushing's disease and Cushing's syndrome and Stein-Leventhal syndrome by way of dynamic tests of adrenal function.

Major Findings:

Using stimulation with standard ACTH infusion, suppression with two levels of Dexamethasone and blocking with SU4885, we have investigated patterns of responses in normal subjects and in patients suffering from Cushing's disease, hypopituitarism and hypoadrenalism. The ACTH infusion does not differentiate well between Cushing's of pituitary origin, Cushing's syndrome resulting from adrenal tumor, and Stein-Leventhal syndrome. The suppression test is a better indicator of pituitary control of adrenal function, and in our hands has been of value in differentiating Cushing's syndrome from the Stein-Leventhal syndrome.

The suppression test is also of some help to differentiate between bilateral hyperplasia and adenoma of adrenals as a cause of Cushing's syndrome.

Proposed Course of Project:

To attempt, by way of the blocking te- endogenous ACTH, to differentiate the pituitary of bilateral adrenal hyperplasia.

Part B, included No.

Serial No. NME- 12

1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Aldosterone Metabolism in Autonomic Nervous System Dysfunction with Postural Hypotension.

Principal Investigator: Bartter, Frederic C., M.D., and Gann, Donald S., M.D.

Other Investigators: Thomas, J. Picton, M.D., Gill, John R., Jr., M.D., C.S. Delea, D. Berkant, E. Diller, H. Henderson C. Kirby, G. Smith

Man Years: Patient Days: 48

Total:	0.3
Professional	0.2
Other:	0.1

General Purpose:

Stimuli such as potassium loading or deprivation, ACTH, and contraction and expansion of ECF or intravascular volume consistently affect aldosterone secretion in normal subjects. The present study is an attempt to determine whether patients with postural hypotension and autonomic insufficiency have similar mechanisms for control of aldosterone secretion.

Major Findings:

In 5 patients with autonomic insufficiency and postual hypotension, localization of the lesion was attempted by measurement of response to Valsalva maneuver and to mental arithmetic, response of skin temperatures to peripheral nerve block, response of pupils and blood vessels to small doses of epinephrine, norepinephrine, and other sympathicomimetic drugs. With metabolic balance techniques, 24-hour urinary excretion of aldosterone was studied during potassium loading, during sodium depletion, with expansion of intravascular volume with albumin, and with ACTH. All subjects tolerated potassium poorly, showing abnormal rises in serum potassium and small rises in aldosterone secretion. Response to salt deprivation varied markedly, as did the excretion of aldosterone; it appears likely that the location of the lesion determines the degree of impairment of the response.

Proposed Course of Project:

These studies are being continued until it is possible to determine the exact nature of the defect in aldosterone secretion in patients with postural hypotension.

Part B. included No

Serial No. NHI-13

1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

On the Nature of Potassium Losing Renal Disease

Principal Investigators: Bartter, Frederic C., M.D., and Gill, John R., M.D.

Other Investigators: Delea, C.S., Biller, E., Middleton, M., Henderson, H., and Berkant D.

Man Years: Patient Days: 75

Total: 1.
Professional: .5
Other: .5

General Purpose:

Hypokalemia associated with urinary potassium loss appears in a number of syndromes. The present studies were instituted to determine what features these syndromes have in common, and in particular the role of aldosterone in the renal potassium loss. Although for the purpose of the present study patients with primary aldosteronism have been excluded, the studies point out the difficulty of establishing clearly the difference between primary and secondary aldosteronism.

Major Findings:

Two patients with alkalosis, hypokalemia and renal potassium loss have been restudied on balance regimen. In one, results clearly indicated that sodium deprivation could increase aldosterone secretion and that the use of aldosterone blocking agents could also increase aldosterone secretion. In view of the very serious hypokalemia in this subject and the evidence of normal renal function the adrenals were explored and a subtotal adrenalectomy was done. This resulted in a partial cure of the hypokalemia. The fundamental cause of the hypersecretion of aldosterone remains unexplored.

Proposed Course of Studies

Comparable studies are being carried out on the other subject with hypokalemia and alkalosis.

Part B. included No

Serial No. NHI - 14
1. Gen. Med. & Expt1. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

The Role of Adrenal Cortical Steroids in the Salt Retention of the Edematous State.

Principal Investigators: F.C. Bartter, M.D., D.S. Gann, M.D., J. P. Thomas, M.D.

Other Investigators: G. Saucier, M.D., J.R. Gill, Jr., M.D., N.H. Bell, M.D., V. Petersen, M.D., C.S. Deles, A.G.T. Casper, H. Henderson, H. Smith, G. Kelly, E. Diller, G. Smith, M. Middleton.

Man Years: Patient Days:

Total:	4.0
Professional:	2.0
Other:	2.0

General Purpose:

To evaluate the role of the adrenal cortex in the sodium retention of edema:

1. To investigate the stimuli to aldosterone secretion
2. To evaluate the role of aldosterone in pathological sodium retention
3. To elucidate the mechanism of action of aldosterone on the kidney.
4. To evaluate the action of agents with a potential influence on the secretion, or action of aldosterone.

Major Findings:

1. The effect of potassium in elevating aldosterone secretion and of potassium depletion in lowering aldosterone secretion was investigated in normal subjects. It was shown that aldosterone secretion could be elevated upon loading and decreased with potassium depletion without concomitant reciprocal changes in intravascular volume. Direct measurements of blood volume were done with Cr_{51} and I_{131}.

1. In studies with dogs, it was shown that denervation of the thyro-carotid arterial junction prevents the increases in aldosterone secretion in response to caval or carotid constrictions. The data indicate that the primary para meter mediating the increase in aldosterone secretion in response to these stimuli is dimished intracarotid pulse pressure.

2. The role of aldosterone and other steroids in edema has been studied in edematous patients. Correlation with changes in blood volume have been obtained, and the effects of various steroids, diuretics and blocking agents have been evaluated. The effect of aldosterone blocking agents and of Δ^1 analogs of hydrocortisone have been measured, with especial reference to 1) changes in GFR, 2) effects on K metabolism, 3) effects on diuresis, and 4) effects on aldosterone secretion.

In studies with dogs, the effective variables in the production and maintenance of edema and the regulation of blood volume have been investigated. Denervation of the thyro-carotid arterial junctions modifies the ability of dogs to maintain edema.

3. Clearance and balance studies have been carried out in normal and Addisonian patients and in adrenalectomized dogs to investigate the mechanism of action of the spironolactone group of aldosterone blocking agents as well as the effects of certain new analogs of hydrocortisone on sodium and potassium metabolism.

4. The spironolactone group of drugs was used in patients with primary and secondary aldosteronism. Patients in the latter group consistently showed increases in urinary aldosterone, while patients in the first group had variable responses. These drugs may be of some value in distinguishing between primary and secondary aldosteronism.

Proposed Course:

Studies are being pursued along all these fronts.

art B. included Yes

Part B.

Publications

1. Bartter, F.C., Mills, I., and Gann, D.S. Increase in Aldosterone Secretion by Carotid Artery Constriction in the Dog and its Prevention by Thyro-carotid Arterial Junction. Am. Soc. for Clin. Inv., May 1959 meeting. (Abstract)

2. Bartter, F.C., Mills, I., and Gann, D.S. Increase in Aldosterone Secretion by Carotid Artery Constriction in the Dog and its Prevention by Thyro-carotid Arterial Junction. Submitted to Journal of Clin. Invest. for publication.

3. Gann, D.S., Mills, I., and Bartter, F.C. Hemodynamic Parameter Mediating Aldosterone Increase in the Dog. Presented at 40th meeting of Endocrine Society, p. 52 (Abstract)

4. Gann, D.S., Mills, I., and Bartter, F.C. Hemodynamic Parameter Mediating Aldosterone Increase in the Dog. Submitted for publication.

5. Bartter, F.C., Gill, J.R., Jr., and Gann, D.S. Mechanisms Controlling the Secretion of Aldosterone. Proceedings of the 40th Annual Meeting of American College of Physicians, Chicago, April 1959.

6. Bartter, F.C. Secondary aldosteronism. Progressiin Clinical Endocrinology (In Press)

7. Bartter, F. C. Searle Symposium on Aldosterone Antagonists, Chicago, October 1959.

8. Bartter, F.C., I. H. Mills, E.G. Biglieri, and C. Delea. Studies on the Control and Physiologic Action of Aldosterone. Recent Progress in Hormone Research, Vol. XV, 1959, Academic Press Inc., New York.

1. [illegible]en. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Clinical Investigation of Action and Metabolism of Drugs.

Principal Investigator: Louis Gillespie, Jr., M.D.

Other Investigators: John A. Oates, M.D., Albert Sjoerdsma, M.D., Ph. D., David Horwitz, M.D., and Luther L. Terry, M.D.

Man Years:
Total: 1.2
Professional: 1.0
Other: 0.2

Patient Days: 4500

Major Findings:

1. Drug Therapy of Hypertension. Monoamine Oxidase Inhibitors: Studies begun in 1958 to assess the antihypertensive effects of an expanding group of agents known as monoamine oxidase inhibitors has continued during this present year. The measurement of monoamine oxidase inhibition in man was initially accomplished by the Serotonin Conversion Test (described previously). Improved methodology for measuring urinary tryptamine (discussed elsewhere) has permitted measurement of the endogenous urinary excretion of this amine in man, and rises of tenfold or greater have been observed in patients during the administration of a monoamine oxidase inhibitor. The latter method of estimating inhibition of this enzyme in man is thought to be more sensitive and accurate than the Serotonin Conversion Test for a number of reasons.

During this year a total of twenty-six hypertensive patients have been thoroughly studied with regard to both their alterations of urinary tryptamine excretion and lowering of blood pressure with one or more of several monoamine oxidase inhibitors. Five compounds have been most thoroughly examined: iproniazid (Marsilid), 1-phenyl-2-hydrazinopropane (JB-516, Catron) DL-phenylcyclopropamine (SKF-385), nialamide (Niamid), and phenelzine (Nardil). These observations have permitted certain conclusions about comparative clinical dose levels of the various agents, demonstrating that the dose levels of the various agents purported to achieve a psychiatric effect (i.e. anti-depressive) appear to have approximately equivalent enzymatic effects as indicated by changes in urinary tryptamine. Although each of these agents will produce postural

hypotension if administered in sufficient dose, a precise correlation could not be established between degree of enzyme inhibition and postural hypotension.

A. JB-516 (Catron): Clinical evaluation of the use of this agent in the treatment of hypertension has continued. A parenteral form of this agent has become available for investigational use. Intravenous administration of Catron to patients results in an initial rise in blood pressure, predominantly systolic which is thus similar to the effects of amphetamine and the results observed in dogs. By measuring the rise in urinary tryptamine it was found that single doses of the drug have approximately equal enzymatic effects by the oral or intravenous route, suggesting rather complete absorption from the intestinal tract.

The initial clinical impressions of the efficacy of this agent have continued essentially unchanged in a larger group of clinic patients followed for an extended period. None of the effects of parasympathetic blockade have been observed. No evidence of tolerance has been encountered in patients, some of whom have received the drug for a period of 12 months. The potentiation achieved by the addition of a thiazide derivative remains apparent and useful. Only occasional examples of euphoria have been observed and for the most part this has been a pleasant contrast to the often observed depressive effect of other antihypertensive agents.

In the original group of 12 clinic patients receiving JB-516, 6 developed reversible loss of red and green color discrimination while on higher doses of the drug (i.e 25 - 50 mg/day). Subsequently, two patients receiving only 12 mg. of JB-516 daily, but in combination with hydralazine (another hydrazine compound), developed not only loss of color vision but also a diminution of visual acuity associated with central scotomata. These patients were withdrawn first from JB-516 and later from the hydralazine and followed closely with repeated eye examinations. Improvement was not noted until both hydrazine compounds had been withdrawn, and then recovery to pretreatment levels of vision was accomplished over a period of about four months in one patient, while the remaining patient appears to have stabilized with residual damage to the optic tracts and now has a mild degree of optic atrophy. Quite recently, under controlled hospitalized conditions, a single patient has been studied at progressively increasing doses of JB-516 for the purpose of determining the limits of monoamine oxidase inhibition achievable. At slowly progressive doses over a period of six weeks, a level of 63 mg/day was reached, and after five days at this level this patient suddenly lost color vision completely and a marked diminution of visual acuity was recorded. This case is the most severe degree of visual acuity toxicity which we have encountered yet and rather clearly indicates that the patient has a retrobulbar neuritis. This patient was receiving no other drugs at the time, and currently the effects of large doses of pyridoxine and ACTH in reversing the toxicity are being evaluated.

In view of the severity and frequency of visual toxicity being encountered in patients receiving larger doses of JB-516, further study of this drug as an antihypertensive or anti-anginal agent has been discontinued in hospitalized and clinic patients.

B. Dextro- and levorotatory isomers of JB-516: These isomers of the original compound became available for study from two pharmaceutical firms. Their individual effects on both blood pressure and monoamine oxidase inhibition have been studied in 4 hospitalized patients and 6 clinic patients. The isomers appear to produce the same degree of postural hypotension. It was postulated that the levorotatory form might have less central stimulating effect, but such has not been conclusively demonstrated. Further, it was hoped that one of the isomers might lack visual toxicity but studies to date are not conclusive in this respect.

C. George Washington Hospital Hypertension Clinic: The cooperative clinical evaluation of monoamine oxidase inhibitors in hypertension has continued under the direction of Dr. Irene Tamagna and Dr. Harold Orvis. Patients from this clinic have continued to be admitted to our inpatient service. Dr. David Horwitz of our group has been working in the hypertension clinic at George Washington throughout this year, maintaining liason between the respective groups. During this year the antihypertensive effects of JB-516 alone and in combination with chlorothiazide have been evaluated, and more recently an evaluation of the isomers of this compound has also begun, the results of which are not yet completely known. However, preliminary assessment reveals similarly effective therapeutic results as previously observed in our outpatient group.

D. SU-5864 (Guanethidine): This is a new compound developed by CIBA Pharmaceutical Products, Inc. The compound has some similarities to the rauwolfia derivatives but is considerably more potent, resulting in an effective postural lowering of the blood pressure without remarkable side effects. This compound is being evaluated in 6 to 10 patients currently.

E. Alpha Methyl Dihydroxyphenylalanine: This compound, synthesized as an analogue of the amino acid precursor of noradrenalin, dihydroxyphenylalanine (dopa) has been studied in the laboratory as a potential competitive inhibitor of amino acid decarboxylation. Initially, it was planned to administer this compound to several patients merely to attempt to measure any metabolic alterations in man, and such a study was begun following adequate animal toxicity studies. The first patient to receive the compound was a hypertensive and demonstrated a quite significant lowering of both the recumbent and standing blood pressure. The effect was manifest on the first day of administering the compound, and the pressure returned within twenty-four hours to pretreatment levels after discontinuing the drug. These studies have now been extended to a total of five patients, and all have responded similarly. It appears that the predominant effect on blood pressure may be an orthostatic effect, but recumbent pressure is also significantly lowered. None of the laboratory parameters of toxicity have been abnormal to date. It appears that this drug may be metabolized only very slightly in the body, depending on urinary excretion to terminate its effect. Further, there is an initial suggestion that because of this latter effect, smaller doses at longer intervals can be given to patients with renal impairment. At present a routine dose schedule has not been established. Comparatively, in the hospitalized patient, this pressure-lowering effect appears to be as marked as is achievable with

other antihypertensive agents. No parasympathetic side effects have been observed. The only consistent side effect has been a drowsiness or sedative effect observed during the first two to three days of therapy, subsiding subsequently. At the present time outpatient evaluation is beginning although only one patient is under study. During the evaluation of the hypotensive effects of alpha methyl dopa in hospitalized patients, appropriate metabolic studies are being performed simultaneously and are discussed in detail elsewhere.

2. Interests in unilateral renal disease: As an obvious sequella of processing a considerable number of hypertensive patients for pharmacologic studies, questions relating to the diagnosis and therapy of unilateral renal disease have arisen. When to perform the more difficult diagnostic tests, which tests to perform and what surgical correction can be performed are the major questions. During the past year renal arteriograms have been performed in twelve patients, and retrograde ureteral catheterization bilaterally with function studies (Howard test) has been performed in five patients. Dr. Thomas Stamey, urologist from the Johns Hopkins Hospital, has a specific interest in this area and collaborates with our group, performing the rather difficult Howard test when indicated. During the past year we have detected two cases of unilateral renal disease - one being corrected by nephrectomy, the second by renal vascular surgery. Both of these cases are examples of renal arterial lesions and are currently being followed in the clinic for long-term results.

Proposed course of Projects:

The clinical and chemical evaluation of alpha methyl dopa and other alpha methyl analogues of amino acids represent our major and most pressing interest currently. Alpha methyl dopa appears to have considerable therapeutic potential at this early data and deserves thorough clinical trial. The addition of an inhibitor of another enzyme to already available inhibitors of monoamine oxidase greatly enlarge the type of investigation which may be pursued towards better understanding of a relationship between these metabolic alterations and lowering of the blood pressure. Laboratory methodology in determining many of the amines and their metabolites has been refined to a degree which also permits many sophisticated experiments in humans.

In order to further extend our clinical investigations, the outpatient clinic is being enlarged selectively to include patients satisfying the criteria of severe hypertension, intelligence, and compulsive cooperativeness. Such an enlargement of the clinic is not contemplated to interfere with our continuing working relations with Drs. Tamagna and Orvis at the George Washington Hypertension Clinic. It is hoped that when sufficient data has been compiled that broader clinical evaluation of alpha methyl dopa will be instituted in this latter clinic.

Since the number of hypertensive patients being admitted to inpatient study as well as being followed on an outpatient basis has increased markedly during the past two years, we are aware of a growing collection of clinical data and are taking steps to process such data so that it may be available for future reference. This concept has particular reference to such diagnostic studies as the tetraethylammonium infusion test for separation of renal from essential hypertension, renal arteriography and retrograde ureteral catheterization studies.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part B:

Publications:

1. Gillespie, L., Jr., Terry, L. L. and Sjoerdsma, A. The application of a monoamine oxidase inhibitor, 1-phenyl-2-hydrazinopropane (JB-516) to the treatment of primary hypertension. Am. H. J. 58:1, 1959.

2. Sjoerdsma, A., Gillespie, L., Jr., and Udenfriend, S. A method for measurement of monoamine oxidase inhibition in man: Application to studies on hypertension. Ann. N. Y. Acad. Sc. 80:969, 1959.

3. Gillespie, L., Jr. Discussion of clinical toxicity studies of a monoamine oxidase inhibitor. Ann. N. Y. Acad. Sci. 80:954, 1959.

4. Sjoerdsma, A., Oates, J. A. and Gillespie, L., Jr. Quantitation of monoamine oxidase inhibition in man with various monoamine oxidase inhibitors. In preparation.

5. Orvis, H. H., Tamagna, I. G. and Thomas, R. E. Evaluation of two monoamine oxidase inhibitors (Iproniazid and JB-516) in the therapy of arterial hypertension. Am. J. Med. Sci. 238:336, 1959.

Serial No. NHI-16
1. General Med. & Exp. Ther
2. Experimental Therapeutic
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Effects of Dopamine, Norepinephrine and Monoamine Oxidase Inhibitors in Man: A Phase of Studies on Vasoactive Substances.

Principal Investigator: Horwitz, David, M.D.

Other Investigators: Goldberg L. I. M.D., Ph. D., Sjoerdsma A., M.D., Ph. D. National Heart Institute Nursing Service (Technical Assistance).

Man Years:
Total: 1.0
Professional: 0.85
Other: 0.15

Patient Days: 500

Major Findings:

1. Metabolic and hemodynamic effects of dopamine in man: Dopamine infusions have been administered to nine hypertensive and six normotensive patients, confirming that it is a pressor agent causing a rise of systolic pressure in the presence of an essentially stable diastolic pressure and pulse rate. Its pressor effects are evident at infusion rates 30 to 300 times those of norepinephrine administered to the same patients. A slight increase in blood glucose levels occurred at infusion rates producing cardiovascular effects.

Dye dilution studies have been performed in three patients. These reveal that dopamine produces a striking increase in cardiac output, a moderate fall in peripheral resistance, and negligible change in O_2 consumption.

2. The influence of monoamine oxidase inhibitors on the effects of infused amines in man: The possibility was entertained that clinical effects of MAO inhibitors in man were mediated through changes in the levels and potency of endogenous amines. Initial studies were designed to evaluate changes in the response to infusions of the predominating amines of the sympathetic nervous system, the humoral mediator, norepinephrine and its precursor amine, dopamine.

Seven hypertensive patients were given intravenous infusions of dopamine and norepinephrine before and during therapy with monoamine oxidase inhibitors. Three inhibitors were used, JB-516, SKF-385 and Nialamid. During inhibitor

therapy, the pressor effects of dopamine were strikingly potentiated so that 5-11% of the control dose reproduced the pressor response observed in the pre-inhibitor state. There was an accompanying prolongation of effects from 4 to 9 minutes to as long as an hour. This potentiation appeared to be related to MAO inhibition as determined by increases in urinary tryptamine excretion.

Norepinephrine showed a different pattern of potentiation. The potentiation was of lesser magnitude, was unaccompanied by prolongation of effect and occurred only after the development of postural hypotension. Additional studies with methoxamine, an amine which is not degraded by MAO showed that the effect of this amine was augmented to approximately the same extent as that of norepinephrine. In similar fashion to norepinephrine, methoxamine was potentiated only after the development of postural hypotension. It is concluded that the potentiation of these two amines is not directly related to MAO inhibition.

No evidence of potentiation of the rise in blood sugar levels produced by dopamine was found after MAO inhibition.

3. Effect of a potent monoamine oxidase inhibitor JB-516 on angina pectoris: The reported effectiveness of the MAO inhibitor, iproniazid, in angina suggested that JB-516 might prove similarly useful. Fourteen patients with angina were screened for inclusion in such a study. Of these five proved suitable candidates. A single blind technique was used, alternating varying dose levels and a placebo. Two patients showed good responses; one patient did not respond; the response in one patient was uninterpretable because he responded to placebo and drug and one patient is still being evaluated.

Because of the limited number of suitable angina patients and the great variability of the disease, the objective of the study has been limited to obtaining an impression of potential usefulness of the drug in angina, rather than pursuing a conclusive evaluation.

Proposed course of Project:

1. Effects of amines of various structures on the blood unesterified fatty acids (UFA) in man: Initial observations revealed no rise in UFA levels upon infusion of dopamine in amounts producing a pressor response. A pronounced UFA response to the structurally-related amines, norepinephrine and epinephrine, has been well documented by other workers. The effect of amines of varying configuration upon the blood UFA response in man will be studied.

2. Pressor and UFA responses to norepinephrine in hypertensive and normotensive patients: Hypertensive patients have been reported to show an increased pressor response to norepinephrine when compared with normotensive

patients. Our amine studies of the past year have confirmed this. It has not been determined whether this difference in response is attributable to differences in vessels, receptors, or the pattern of degradation of the humoral mediator, norepinephrine. A study has been initiated to determine whether a non-vascular response to norepinephrine, the UEA response, differs in hypertensive and normotensive patients. In view of the heightened atherogenesis in hypertensives, any indication of a difference of fat metabolism in normotensives and hypertensives would be of great interest.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part B:

Publications

1. Horwitz, David, Goldberg, L. I., and Sjoerdsma, Albert. Increased blood pressure responses to dopamine and norepinephrine produced by monoamine oxidase inhibitors in man. In preparation.

Serial No. NHI-17
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title:

Studies on the Amine Pathways of Indole, Phenol and Phenyl Amino Acid Metabolism in Man.

Principal Investigator: John A. Oates, M.D.

Other Investigators: A. Sjoerdsma, M.D., Ph. D., John B. Jepson, Ph. D. (LCB) Sidney Udenfriend, Ph. D. (LCB), Perola Zaltzman and Walter Lovenberg.

Man Years: Patient Days: 1000
Total: 1.2
Professional: 1.0
Other: 0.2

Major Findings:

1. Tryptamine: Utilization of urinary tryptamine as an index of monoamine oxidase (MAO) inhibition in man has continued, and has proved in general, to be the most satisfactory and sensitive index of MAO inhibition applicable to clinical studies. The inhibitory potencies of various drugs have been compared. The possibility of a correlation between the monoamine oxidase inhibition and the hypotensive effects of these drugs has been investigated, but the data at this juncture does not allow any definite conclusion regarding such a correlation.

Studies on the formation and metabolism of tryptamine in man have been extended. It was found that tryptamine was a superior substrate for monoamine oxidase, none of this compound being excreted unchanged following an infusion of 25 mgm. Such an infusion in conjunction with a MAO inhibitor does allow the excretion of tryptamine (about 4% of the infused dose), but even so most of the infused amine is still accounted for as urinary indoleacetic acid, indicating a lack of major alternate pathways of breakdown for tryptamine. One patient when infused with tryptamine in the presence of a MAO inhibitor developed a syndrome of "ethanol-like" intoxication very similar to that produced by tryptophan plus MAO inhibition, suggesting that this mental alteration produced by tryptophan may be effected via tryptamine. It was found that oral loads of tryptophan would produce up to 5-10 fold increments in urinary tryptamine.

Serial No. MMI-17
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title:

Studies on the Amine Pathways of Indole, Phenol and Phenyl Amino Acid Metabolism in Man.

Principal Investigator: John A. Oates, M.D.

Other Investigators: A. Sjoerdsma, M.D., Ph. D., John B. Jepson, Ph. D. (LCB) Sidney Udenfriend, Ph. D. (LCB), Perola Zaltzman and Walter Lovenberg.

Man Years:
Total: 1.2
Professional: 1.0
Other: 0.2

Patient Days: 1000

Major Findings:

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Because of the above infusion data, it has been assumed that this increase in tryptamine after tryptophan load is due almost entirely to decarboxylation of tryptophan in the kidney, allowing direct excretion of the amine. It was conceived that this type of amino acid loading test should afford a good index of renal amino acid decarboxylase activity in man, and a program was initiated to study the decarboxylase inhibition produced by various pharmacologic agents.

2. Aromatic amino acid decarboxylation: In vitro studies in the Laboratory of Clinical Biochemistry and elsewhere have demonstrated a number of compounds which inhibit aromatic amino acid decarboxylation. Among these were Hydralazine, 1-phenyl-2-hydrazinopropane (JB-516) and Alpha-Methyl-Dihydroxyphenylalanine (CMD), the latter being the most potent.

To measure this enzyme's inhibition in man, the tryptophan loading test alone was applied initially. Subsequently, it was found that 3-5 fold increases in urinary tyramine could be measured following an oral tyrosine load, thus affording an index of tyrosine decarboxylation as well. Following preliminary evidence that hydralazine was a weak decarboxylase inhibitor in therapeutic doses, it was decided to study the inhibition of tryptophan and tyrosine decarboxylase by CMD in hypertensive patients. The first patient studied with 2.0 gm. CMD/day showed a decrease in renal tyrosine decarboxylation to 20% of control, and to 50% of control values for tryptophan decarboxylase. In addition, a depressor effect of the drug was noted which disappeared 24 hours after substitution of a placebo. A BP lowering (discussed in detail elsewhere) has been a consistent effect of CMD in all of the 3 hypertensive patients studied to date. Some sedation accompanied depressor doses of the drug.

Current work in the LCB and this laboratory suggests that at a given tissue concentration of CMD, tyrosine decarboxylation is the most profoundly inhibited of the decarboxylations studied to date. In vitro, inhibition of the decarboxylation of tryptophan, phenylalanine, Dopa and 5-hydroxytryptophan is produced by CMD, the latter two amino acid decarboxylations being the least susceptible to the inhibitor.

Preliminary studies on the absorption of tyrosine from the human gastrointestinal tract and on uptake of tyrosine into brain in animals suggest that CMD does not act by interfering with amino acid transport.

3. Phenylketonuria: By utilizing JB-516 to inhibit monoamine oxidase in phenylketonuric patients, it was possible to isolate phenylethylamine from their urines in milligram quantities. This was accomplished by ether extraction from alkaline urine and chromatography in a two way system. In normal subjects phenylethylamine could be detected only in trace quantities when a phenylalanine load was given during MAO inhibition. The structural similarity of phenylethylamine to amphetamine suggests that this observation is of some interest. Clinically, the only changes seen in phenylketonuric patients on short term MAO inhibition were increased hyperreflexia and tremor, and some staggering of gait which was rapidly reversible.

Orthotyramine was also demonstrated in phenylketonuric urine. This seemed to be present in greater quantities than in the normal. On the other hand, metatyramine was not readily demonstrable in phenylketonuric urine, suggesting that meta-hydroxylation of phenylalanine may be dependent on the same enzyme as para-hydroxylation.

Studies of tyrosine uptake into the cerebrospinal fluid of phenylketonurics showed no decreased uptake compared to normals, and evaluation of tyrosine decarboxylation by use of tyrosine load tests showed no difference from the increased tyramine seen in non-phenylketonurics. These findings are preliminary evidence that phenylalanine does not compete with tyrosine for transport or for the decarboxylase enzyme in phenylketonuric patients.

It has been shown that the transamination of phenylalanine in phenylketonuria is dependent on the presence of α ketoglutarate. Recent in vitro studies have shown that pretreatment with steroids produces an acceleration of α ketoglutarate formation from the glutamic-pyruvate transaminase reaction. Also steroids have been shown to increase the rate of tyrosine transamination.

The effect of steroids on the transamination of phenylalanine was tested in one patient with phenylketonuria. Dexamethasone was given for 6 days and the blood phenylalanine and urine phenylpyruvic acid measured by Dr. B. LaDu (NIAMD). Only minimal changes occurred in either the phenylalanine or in the corresponding acid, suggesting that steroids have little effect on phenylalanine transaminase in phenylketonuria.

4. Studies on other urinary amines: A system for separation of amines from urine by extraction or on an ion exchange column has been developed and studies of these amines by two way chromatography applied. These methods of separation and identification were prerequisite to the identification of the amines studied in phenylketonuria. Monoamine oxidase inhibition produces an increase in the quantity of a number of amines thus demonstrated, many of which are as yet unidentified.

5. Method of assay of urinary serotonin: Current methods of assay of serotonin in urine are applicable only to measuring supra-physiologic amounts, such as are seen in some carcinoid patients. Physiologic or pharmacologically induced changes in urinary serotonin cannot be measured, nor can a decrease in serotonin such as might occur with decarboxylase inhibitors be accurately determined even in carcinoid patients. Consequently, preliminary work has been completed which demonstrates the applicability of a weak carboxylic acid type of ion exchange resin to the separation of serotonin from urine. The mechanics of applying this finding to an assay procedure are being investigated.

Proposed course of Project:

1. Tryptamine: The tryptamine assay continues to be utilized here (and elsewhere) in clinical studies of MAO inhibitors. Because of the finding

of elevated urinary tryptamine in a patient with hyperthyroidism, an attempt will be made to use tryptamine infusion and endogenous tryptamine excretion as technics for studying the role of MAO in the pathophysiology of thyrotoxicosis.

Further infusions of tryptamine in conjunction with MAO inhibition will be done to elucidate the possibility that this amine may play some role in the alterations of brain function seen following tryptophan ingestion by patients on MAO inhibitors.

2. Decarboxylation: The effects of CMD on hypertension are being studied intensively. The biochemical effects of this compound on all of the aromatic amino acid decarboxylases will be studied in man. Norepinephrine synthesis is being studied currently in a patient with malignant pheochromocytoma. Five-hydroxytryptophan decarboxylase inhibition will be tested with infusion of this compound. In addition, patients with the carcinoid syndrome are being studied regarding the effect of CMD on serotonin production as well as the effect on clinical manifestations. Tyrosine decarboxylation will probably be used as the major index of the biochemical effect of CMD and other decarboxylase inhibitors in patients owing to its sensitivity to such inhibitors. Phenylalanine decarboxylase inhibition is being studied in phenylketonuri[illegible]. An attempt will be made to admit patients with urticaria pigmentosa to allow investigation of histidine decarboxylase.

Other α methyl amino acids are currently being synthesized and studied for toxicity. The most promising of these will be studied in hypertensive patients regarding both their depressor effect and their relative inhibition of decarboxylation. The effect of hydralazine alone and in combination with the α methyl compounds should be further investigated.

3. Phenylketonuria: The only projected study of phenylketonuria regards the use of CMD as an agent to decrease phenylethylamine formation.

4. Studies on other urinary amines: Identification of many of the amines demonstrated by the chromatography system is necessary. The study of the pattern of amine excretion will be applied to various medical disorders.

5. Method of urinary serotonin assay: It is planned to complete the development of a method to assay urinary serotonin in physiologic quantities and to apply this to clinical studies on MAO inhibitors, tryptophan metabolism and to the investigation of decarboxylase inhibitors.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part B:

Publications

1. Oates, J. A. and Zaltzman, P. Discussion of clinical measurement of MAO inhibition. Annals, N.Y. Acad. of Science 80:977-978, Sept. 1959.

2. Sjoerdsma, A., Lovenberg, W., Oates, J. A., Crout, J. R. and Udenfriend, S. Alterations in the pattern of amine excretion in man produced by a MAO inhibitor. Science 130:225, 1959.

3. Sjoerdsma, A., Oates, J. A., Zaltzman, P. and Udenfriend, S. Identification and assay of urinary tryptamine: Application as an index of MAO inhibition in man. J. Pharm. & Exptl. Therap. 126:3, 217-222, July 1959.

4. Jepson, J. B., Lovenberg, W., Zaltzman, P., Oates, J., Sjoerdsma, A. and Udenfriend, S. Amine metabolism studied in normal and phenylketonuric humans by MAO inhibition. In press.

Serial No. NHI-18
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Metabolism of Hydroxyproline and Collagen.

Principal Investigator: Darwin Prockop, M.D.

Other Investigators: Sjoerdsma, A., M.D., Ph. D. and Udenfriend, S., Ph. D.

Man Years:
Total: 1.1
Professional: 0.9
Other: 0.2

Patient Days: 300

Major Findings:

1. Over the past several years studies in this laboratory have shown that some patients with Marfan's Syndrome excrete increased amounts of bound hydroxyproline (OPR) in their urine. Since collagen is the only body protein which contains OPR, the excretion of OPR in Marfan's Syndrome probably reflects a basic defect in the metabolism of collagen.

2. An extension of the initial studies has shown that the daily urinary excretion of OPR is independent of dietary intake of substances other than gelatin. Patients and normal controls on low protein or protein free diets excrete approximately the same amount of hydroxyproline as the same subjects on normal diets. Up to the present we have been unable to demonstrate any abnormal metabolism of oral OPR in Marfan's Syndrome.

3. Experiments in which mixtures of amino acids were fed to patients and normal controls showed that feeding OPR as the free amino acid increased the free OPR excretion in the urine. The amount of free OPR excreted was increased if OPR was fed with proline or glutamic acid, apparently because the latter two amino acids decrease the renal tubular reabsorption of OPR.

4. Experiments in which gelatin was fed to patients and to normal controls showed that the amount of urinary OPR peptides is increased by feeding OPR in the form of a protein. This appears to be one of the first demonstrations that complete peptides can be absorbed from the gastro-intestinal tract and then excreted directly into the urine.

5. We have recently developed a simple method for the analysis of OPR in tissues and urine, which makes it possible to analyze OPR routinely on a large number of samples.

6. A modification of our method for OPR has enabled us to measure the specific activity of OPR by a simple, rapid technique. We have used this technique to measure the specific activity of urinary OPR after a single injection of C14-proline into rats. The results have given us a measure of the turn-over rate of hydroxyproline. Since hydroxyproline is found only in collagen, such results may also be used as an indication of collagen turn-over. There is a dramatic difference in the specific activity excretion curves for young and old animals, and the curves indicate that the young animals have a very active metabolic pool of collagen which is absent in older ones. The turn-over time for the active pool in young animals is more rapid than anyone had previously suspected for collagen.

Proposed course of Project:

1. With our new method for OPR we have undertaken a comprehensive study of patients with Marfan's Syndrome and a number of other heritable disorders of connective tissue. The study is being conducted with Dr. Victor McKusick of Johns Hopkins and will include urinary OPR measurements in 48 families in which Marfan's Syndrome has been documented. We also hope to include OPR measurements in a few acute inflammatory disorders of connective tissue such as rheumatoid arthritis and periarteritis nodosa.

2. The technique for measuring the specific activity of hydroxyproline should enable us to make a biochemical study of many experimental conditions affecting connective tissue. Among the conditions we hope to study are serum sickness, lathyrism, carrageenin tumors, starvation, and changes with aging.

3. The amounts of radioactive material required for our rat experiments are small, and with a few modifications of our technique, it may be possible to use tritium-labeled proline to perform similar measurements on patients.

4. We have recently initiated experiments on the enzymatic synthesis of hydroxyproline using specifically labeled compounds (see report from Laboratory of Clinical Biochemistry).

5. Studies on collagenase, the enzyme which destroys collagen, are currently under consideration (see report from Laboratory of Clinical Biochemistry).

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part B:

Publications

1. A. Sjoerdsma, J. D. Davidson, S. Udenfriend and C. Mitoma. Elevated excretion of hydroxyproline in Marfan's Syndrome. The Lancet, p. 994, Nov. 8, 1958.

2. C. Mitoma, T. E. Smith, J. D. Davidson, F. M. DaCosta and A. Sjoerdsma. Improvements in methods for measuring hydroxyproline; Application to human urine. J. Lab. Clin. Med. 53:970-976, 1959.

3. D. J. Prockop, C. Mitoma and A. Sjoerdsma. Observations on hydroxyproline metabolism in man. Federation Proc. 18:1, 1790, March 1959.

Serial No. NHI-19
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Inhibitors of the Fibrinolytic System

Principal Investigator: A. Sjoerdsma, M.D., Ph. D.

Other Investigators: Inga M. Nilsson, M.D. and Prof. Jan Waldenström, Medical Clinic, General Hospital, Malmö, Sweden.

Man Years
Total: 1.5
Professional: 1.5
Other: 0

Patient Days:

Major Findings:

1. Introduction: Project was initiated June 11, 1959 on arrival in Sweden. The fibrinolytic system of man is the subject of increasing interest. Pathologic fibrinolysis is not an infrequent complication of various diseases, including carcinoma of the prostate, abruptio placentae, polycythaemia vera, Boeck's sarcoid, leukemia, cirrhosis of the liver, etc. Bleeding due to fibrinolysis is also a significant complication of cardiac surgery with extracorporeal circulation. The two major areas of interest with regard to cardiovascular disease have been: 1) "anticoagulant" therapy using a plasminogen activator such as purified streptokinase and 2) the concept that the initial step in atherogenesis consists of intimal deposition of fibrin and thus might logically be related to deficient fibrinolysis. While it is known that an inhibitory system for plasminogen activation and plasmin activity exists, the nature of the active substance(s) has not been determined. The discovery of effective inhibitors would thus be of physiologic as well as therapeutic interest.

2. Epsilon-aminocaproic acid (E-AC): This aliphatic amino acid has been reported to be an effective inhibitor of plasminogen activation in vitro (Ablondi et al: Arch. Biochem & Biophys. 82:153, 1959 and Alkjaersig et al: J. Biol. Chem. 234:832, 1959). After preliminary toxicity studies in dogs and rabbits, the drug was administered to patients. No toxic effects were observed with single doses of 6.0 gm. given orally or intravenously. Using the fibrin plate technique of Astrup, all patients with abnormal bleeding at the General Hospital were screened for fibrinolysis. Pathologic fibrinolysis was discovered in 3 cases (Diagnoses: leukemia, cerebrovascular accident and cirrhosis of liver). In each case, the administration of 6.0 gm. of E-AC intravenously completely abolished fibrinolysis for 6 to 24 hours.

Severe hemorrhage in the leukemic case was controlled by administering 6.0 gm. E-AC every 4-6 hours orally for a period of 2 weeks. These findings suggest that E-AC will prove useful in the control of bleeding due to spontaneous (various diseases) or induced ("kinase" therapy and following cardiac surgery) fibrinolysis.

3. Metabolism of E-AC: Considerable effort was expended in developing a method to measure the urinary excretion of E-AC after its oral and intravenous administration. This was accomplished through the findings that 1) E-AC could be separated from other amino acids in urine using high voltage paper electrophoresis at pH-2; and, 2) even though reaction with ninhydrin is considered to be specific for α amino acids, an intense blue color was obtained with E-AC using 0.5% ninhydrin in butanol (pH 6) which could be converted to a stable red color on spraying with a copper reagent; by elution of the spots from paper with methanol, as little as 1 µgm. was easily measured at 508 mµ in a spectrophotometer. Using this procedure it was found that 60-80% of the drug is excreted unchanged in the urine within 12 hours, after either oral or I.V. administration. Thus, absorption from the gut is complete and multiple doses within a day are essential for optimal effects.

4. Other inhibitors: The inhibitory effect of E-AC and structurally-related compounds on plasminogen activation was tested in an improved clot system at 37°C. Each tube contained the following components in order of addition: 0.1 ml. plasminogen (3.1 mg/ml), 0.5 ml. 0.15 M Tris buffer pH 7.4 (vehicle for inhibitor), 0.2 ml. 0.5% bovine fibrinogen, 0.1 ml. streptokinase (50 u/ml) and 0.1 ml. thrombin solution (20 NIH units/ml). Control lysis times were 10-15 minutes and 10^{-3}M E-AC produced an average lysis time of 50 minutes. Other compounds tested included: β-alanine, α, β and γ-aminobutyric acid, putrescine, Δ-aminovaleric acid, Δ-aminolaevulinic acid, glutamic acid, ornithine, arginine, cadaverine, norleucine, lysine and ω-aminocaprylic acid. Δ-aminovaleric acid, Δ-aminolaevulinic acid, γ-aminobutyric acid and ω-aminocaprylic acid had activity approaching that of E-AC. Putrescine, ornithine, cadaverine and lysine were active at 5×10^{-2}M. Optimal activity would appear to reside in amino acids with a carbon chain of 4 to 7 C's with the amino group in the omega position. Of the more active compounds, only Δ-aminolaevulinic acid and γ-aminobutyric acid are known to occur normally. The former compound is an intermediate in the succinate-glycine cycle and is the precursor of porphobilinogen. Excessive amounts exist in certain "prophyrias", particularly that associated with lead intoxication. However, none of the patients with porphyria in Sweden have been observed to have a thrombotic tendency. Interest in γ-aminobutyric acid has been directed toward its unique localization to the brain and possible roles as a neurohormone. Whether it might play a role in the local dissolution of cerebral thrombi or be involved in a more general way in fibrinolysis associated with cerebral injury is open to speculation.

While it has not been reported to be a normal body constituent, δ-aminovaleric acid has been found in the salivas of patients with suppurative periodontal disease (Fosdick and Fletz, J. Dental Res. 32: 87, 1953), presumably due to putrefaction. This condition is a difficult dental problem. It is conceivable that the production of an inhibitor of the fibrinolytic system in the mouth is an important factor in perpetuating the disease.

Proposed course of Project:

It is hoped that some of these studies can be continued in Bethesda in 1960. Major points are as follows:

1. Inhibitor therapy: Further evaluation of E-AC in pathologic fibrinolysis. Patients with bleeding following extracorporeal circulation in the Clinic of Surgery might be suitable candidates. The 5 and 7 carbon analogues require evaluation.

2. Study of normal inhibiting system: It is likely that the system in the blood which controls fibrinolysis normally includes compounds of the type described here. This should be investigated in patients with thrombotic tendencies. Gut sterilization studies may prove revealing since bacteria are known to produce Δ-aminovaleric acid, also relationships to atherosclerosis, dietary fat, etc.

3. Study of inhibitors in cases of periodontitis: Studies on the saliva of such cases is currently under investigation.

4. Papers on the treatment of pathologic fibrinolysis with E-aminocaproic acid, method of its assay in urine and the discovery of new inhibitors are in preparation.

Part B included Yes No

Serial No. NMI-20
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Studies on Vasoactive Substances

Principal Investigator: A. Sjoerdsma, M.D., Ph. D.

Other Investigators: J. R. Crout, M. D., L. Gillespie, Jr., M.D., J. A. Oates, M. D., P. Zaltzman (Visiting Scientist), W. Lovenberg (Graduate student), J. Pisano, Ph. D. (LCB) and S. Udenfriend, Ph. D. (LCB). Technical: C. G. Muellenberg.

Man Years:
Total: 1.9
Professional: 1.0
Other: 0.9

Patient Days: 500

Major Findings:

These have largely been delegated to other projects due to my leave of absence from Bethesda (6-59 - 12-59).

1. Catecholamine metabolism: Through the persistence of Dr. Pisano (LCB), methods for assay of the m-o-methyl metabolites have been developed and applied with success to pheochromocytoma by Dr. Crout. Eight urine specimens on patients with pheochromocytoma were sent to Sweden. Analyses for 3-methoxy-4-hydroxymandelic acid by the electrophoretic technique of Studnitz showed close agreement with the NIH assays.

Dr. Crout has perfected a technique for study of O-methylation in man using infusions of d-Isuprel.

2. Biogenic amines in edible fruits: project completed.

3. Monoamine Oxidase (MAO) Inhibition: Urinary tryptamine has afforded a simpler index of this than the Serotonin Conversion Test. The enzymatic effectiveness of several agents has been established in patients and a gross correlation between the chemical effect and orthostatic hypotension observed (see Oates & Gillespie reports).

4. New amines in human urine: The hypothesis that undiscovered amines may be found in the urine of patients whose MAO has been blocked with a drug has proved fruitful. Phenylethylamine, m-tyramine and O-tyramine may now be added to those previously listed.

5. Decarboxylase inhibitors: α-methyl dopa was mentioned in last year's report. It has now undergone preliminary chemical and clinical studies in human hypertensives with promising results (see Gillespie and Oates reports).

Proposed course of Project:

1. Catecholamine metabolism: Use will be made of our techniques for measuring the two major enzymatic processes in catecholamine degradation in evaluating the pharmacologic effects of new drugs on blood pressure.

2. Continue identification of urinary amines in MAO blocked patients. A study of urinary amines in various diseases is in order.

3. Investigate new decarboxylase inhibitors in hypertension and secreting tumors.

4. Search for other amines in the sympathetic nervous system. The blood pressure effects of MAO inhibitors is not adequately explained by present concepts. The possibility of a transmitter in addition to acetylcholine at sympathetic ganglia should be studied. It is hoped that one of our visiting scientists will explore this problem in 1960.

Serial No. NMI-21
1. Gen. Med. & Exper. Therap
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title:

Catecholamine studies, A phase of studies on Vasoactive Substances.

Principal Investigator: J. Richard Crout, M.D.

Other Investigators: A. Sjoerdsma, M.D., Ph. D., D. Horwitz, M. D.
S. Udenfriend, Ph. D. (LCB), J. Pisano, Ph. D (LCB)
and C. Creveling (LCB).

Technical: D. Watts and D. Caton (summer student).

Man Years:
Total: 2.3
Professional: 1.3
Other 1.0

Patient Days: 500

Major Findings:

1. Studies of catecholamine metabolism in patients with pheochromocytoma have been continued. Using methods for the assay of the norepinephrine metabolites 3-methoxy norepinephrine (NMN) and 3-methoxy 4-hydroxymandelic acid (MHMA) developed by Dr. John Pisano (LCB), we have studied the urine of 15 patients with pheochromocytoma. Studies by others have indicated that these two compounds plus the free and conjugated catecholamines account for at least 90% of the metabolites of infused radioactive norepinepharine and epinephrine. The present study constitutes the first total quantitation of catecholamine metabolites in pheochromocytoma. Free norepinephrine and epinephrine were found to account for 0.4-6%, NMN for 17-42%, and MHMA for 57-78% of the total excretion of the catecholamines and their metabolites. These values are in reasonable agreement with those reported by others after the infusion of radioactive catecholamines.

The methods for NMN and MHMA were modified so as to be suitable for routine use in the usual hospital laboratory in the diagnosis of pheochromocytoma. The normal range given by these methods is being evaluated at the moment. It appears that either method is useful in diagnosing pheochromocytoma, and both represent a significant advance over the fluorimetric catecholamine assay in terms of ease of performance. However, a number of interfering substances in urine preclude the use of these methods at present for quantitative assay within the normal range.

2. The observation that monoamine oxidase (MAO) inhibition potentiates the vasopressor response to infused catecholamines in man (separate report by Dr. Horwitz) has led to a study of the mechanism of this effect. To determine whether this potentiation is due to an impaired overall metabolism of the infused amine, blood levels of infused catecholamines and the rates of disappearance from the blood have been determined in seven hypertensive patients both on and off MAO inhibition (in collaboration with Dr. D. Horwitz). To date no significant difference has been found between patients on and off MAO inhibition in the rates of disappearance of these amines from blood indicating that the mechanism clearing these compounds from plasma is not dependent upon the integrity of the MAO system. The blood level of infused amine was found to be correlated with the infusion rate and not the pharmacologic response; thus, the potentiated vasopressor response to infused dopamine during MAO inhibition occurred at very low infusion rates and low blood levels. This indicates that the potentiation of the response to dopamine during MAO inhibition occurs at or near the adrenergic receptor site. These studies suggest that MAO plays a considerable role in terminating the action of infused dopamine at the receptor site, but leave in doubt whether this is also true for norepinephrine. Further studies using other drugs such as ganglionic blocking agents to potentiate the effect of infused amines may help to clarify the function of MAO in limiting the action of norepinephrine at the tissue level.

3. D-Isuprel is an excellent substrate for catechol O-methyl transferase, the other major enzyme in catecholamine metabolism. Since it is much less active pharmacologically than L-Isuprel, measuring the urinary excretion of methylated Isuprel after a d-Isuprel infusion has provided an excellent method of measuring catechol O-methylation in man. In the small series to date no difference in catechol methylating ability has been found between hypertensives and non-hypertensives. No effective, non-toxic *in vivo* inhibitor of this enzyme is available as yet.

4. Although the pathways of metabolism of infused catecholamines are fairly well known, the relative importance of the two major enzymes (MAO and catechol O-methyl transferase) in degrading endogenously formed norepinephrine at the tissue level is not clear. Studies designed to clarify this problem are being carried on in collaboration with Dr. S. Udenfriend and Mr. C. Creveling of LCB. The influence of enzyme inhibitors (Marsilid and JB-516 for MAO and pyrogallol for catechol O-methyl transferase) on the level of norepinephrine in the brain and heart of the rat has been studied. MAO inhibitors have proven to be considerably more effective than pyrogallol in elevating brain and heart norepinephrine and in allowing injected norepinephrine to accumulate in rat heart. This suggests that at the tissue level MAO may be the more important enzyme for the degration of endogenous norepinephrine. Continuation and expansion of these studies is anticipated.

5. In collaboration with Dr. H. Weisbach (LCB) we have begun a study of aldehyde dehydroganase, the enzyme involved in oxidizing endogenous aldehydes

to free acids. The oxidation of the normally occurring aldehyde derivatives of serotonin, norepinephrine, tryptamine, etc. by this enzyme has not been studied previously. To date the enzyme has been partially purified from hog kidney, but the definitive evaluation of its role in amine metabolism remains under investigation.

6. The laboratory has continued to perform urinary catecholamine assays in selected patients not under the care of our group. Some of these patients represent pheochromocytoma suspects, and others are involved at the NIH in experimental studies in which the determination of urinary catecholamines is felt to be of research value. This work is usually of a service nature and not a major interest of the laboratory, but requires 1-2 days a week of a technician's time.

7. The recent discovery of the antihypertensive effect of α-methyl Dopa, a decarboxylase inhibitor, has prompted some preliminary investigation to determine whether this pharmacologic action is accompanied by measurable inhibition of norepinephrine synthesis in man. In hypertensive patients the compound interferes with the assay for urinary dopamine and norepinephrine, precluding such a study. In a study conducted in one patient with malignant pheochromocytoma (in collaboration with Dr. William Baker of the Massachusetts General Hospital) these technical difficulties were overcome. No change in the blood pressure or the excretion of norepinephrine metabolites occurred at dosages which have proven to be useful in lowering the blood pressure of hypertensives, but larger doses did produce a decrease in both the blood pressure and the excretion of metabolites. Further studies of the metabolism and fate of α-methyl Dopa in man are in progress.

Proposed course of Project:

1. Study of the excretion of catecholamine metabolites in patients with pheochromocytoma is nearing completion and further work in this area, other than to maintain an interest in new cases of pheochromocytoma which come to our attention, is not anticipated.

2. Infusion studies in man to explore the rate of degradation of catecholamines and the pharmacologic response under the influence of various potentiating agents (enzyme inhibitors, ganglionic blocking agents, reserpine, Ritalin) will continue.

3. Studies of the *in vivo* effect in animals of various drugs on the endogenous level of norepinephrine in various organs (principally heart and brain) will continue. It is hoped that this area can be expanded to include some evaluation of the transport and binding in tissue of injected catecholamines.

4. Further work in the purification and characterization of mammalian aldehyde dehydrogenase will continue. This is the major unstudied enzyme of those involved in amine degradation, and a study of its properties is long overdue.

5. The laboratory will continue to assay selected outside specimens for catecholamines as a service to other groups. The performance of these assays by the NIH clinical chemistry laboratory would be gratefully welcomed, but to our knowledge no plans for setting this up there are in progress.

Part B Included: Yes NO

Part B:

Publications

1. Crout, J. R. and Sjoerdsma, A. Catecholamine excretion after banana feading. J. Pharm. and Pharmacol. 11:190-191, 1959

2. Crout, J. R. and Sjoerdsma, A. The clinical and laboratory significance of serotonin and catecholamines in bananas. New Eng. J. of Med. 261:23-26, 1959.

3. Sjoerdsma, A., Lovenberg, W., Oates, J. A., Crout, J. R., and Udenfriend, S. Alterations of the pattern of amine excretion in man produced by a monoamine oxidase inhibitor. Science 130:225, 1959.

4. Crout, J. R., Pisano, J. J. and Sjoerdsma, A. Catecholamine metabolism in pheochromocytoma, abstract submitted to Clinical Research for publication in December 1959.

5. Crout, J. R. and Sjoerdsma, A. Catecholamine assays in the localization of pheochromocytoma. In preparation.

Serial No. MNI-22
1. Gen. Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

Studies of the Cardiovascular Effects of Pharmacologic Agents and Amines in Experimental Animals and Man.

Principal Investigator: Goldberg, Leon I., M.D., Ph. D.

Other Investigators: Sjoerdsma, A., M.D., Ph. D., DaCosta, F. and Gatgounis, J. (Medical College of South Carolina), Bloodwell, R. D., M.D. (HS), Braunwald, E., M.D. (HS) and Morrow, A. G., M.D. (HS)

Man Years:
Total: 1.6
Professional: 0.8
Other: 0.8

Patient Days:

Major Findings:

1. Effects of monoamine oxidase inhibitors on the cardiovascular actions of endogenously-occurring amines: A study of the effects of five endogenously occurring amines on heart contractile force and arterial pressure of the anesthetized dog and the influence of monoamine oxidase inhibitors on these effects has been concluded. The amines increased heart contractile force (by strain gauge arch technic) in the following order according to potency by weight: norepinephrine > serotonin > dopamine > tryptamine > tyramine. After stable amine responses were obtained a monoamine oxidase inhibitor was administered and the amines were reinjected. Six inhibitors of different chemical structure and pharmacologic activity were used in the study: JB-516 (1-methyl-2-phenyl-ethyl hydrazine HCl), JB-835 (4-phenyl-2-butyl-hydrazine), RO-50700 (2-benzyl-1-picolinylhydrazine), Isoniazid (1-isonicotinyl, 2-isopropylhydrazine) and two harmala alkaloids, harmine and harmaline. The effects of dopamine, tryptamine and tyramine on heart contractile force were markedly augmented and prolonged after administration of each of the inhibitors. The effects on blood pressure were also potentiated, but the results were more difficult to quantify because of variable pressor and depressor actions of most of the amines. The effects of norepinephrine were not significantly affected by the inhibitors. Both the pressor and inotropic effects of serotonin, however, were reduced by each inhibitor, except RO-50700. As serotonin is considered to produce part of its pressor and positive inotropic action by stimulation of sympathetic ganglia, the possibility was considered that inhibitors decreasing the effects of serotonin had ganglionic blocking activity. Several MAO inhibitors have been found to produce postural hypotension in man, but not in animals. The "anti-serotonin" action of MAO inhibitors may, therefore, be useful in screening MAO inhibitors for clinical trial.

2. Differential effects of Ritalin and monoamine oxidase inhibitors on the cardiovascular actions of endogenously occurring amines. Previous studies with Ritalin have demonstrated that this compound potentiates the pressor effects of serotonin and norepinephrine. As these amines were not potentiated by MAO inhibitors it was considered worthwhile to compare the effects of Ritalin and MAO inhibitors on the actions of several endogenously occurring amines on heart contractile force and blood pressure of the intact anesthetized dog. Studies in 10 dogs confirmed previous studies in that the inotropic and pressor effects of norepinephrine and serotonin were markedly augmented after administration of Ritalin. Additionally, it was found that the actions of dopamine, tryptamine and tyramine were markedly reduced after Ritalin. These effects were opposite to those produced by MAO inhibitors. The mechanism of action of Ritalin is not known, and the fact that this substance produces effects opposite to the MAO inhibitors may be helpful in formulating concepts of structure activity relationships and receptor responses. In addition to the possible theoretical implication of this study, the differential effects of MAO inhibitors and Ritalin have provided a useful aid in bioassay, for differentiating amines such as dopamine from norepinephrine or tryptamine from serotonin. The former amines are particularly difficult to separate chemically and this technic was used for this purpose in ascertaining the norepinephrine and dopamine content of urine after ingestion of bananas (Crout, J.R., and Sjoerdsma, A., N. Eng. J. of Med. 261:23-26, 1959).

3. Structure-activity relationships of sympathomimetic amines and potentiation by monoamine oxidase inhibitors. In order to determine the sympathomimetic amine structure which is potentiated by monoamine oxidase inhibitors a large number of amines have been studied in collaboration with Mr. John Gatgounis of the Department of Pharmacology of the Medical College of South Carolina. Results of studies thus far have shown that phenylethylamine and meta-tyramine are potentiated by MAO inhibitors in addition to the endogenous amines reported in Item 1. Epinephrine, synephrine, phenylephrine, methoxamine, amphetamine, methamphetamine, isoproteranol, hydroxyamphetamine and phenylpropanolamine were not potentiated. These results indicate that presence of a beta-OH group or an alpha carbon on the amine side chain prevents potentiation. As these are the same radicals which prevent action of monoamine oxidase in in-vitro biochemical studies, such results lend support to the concept that augmentation of amines by MAO inhibitors is due to MAO inhibition.

4. Effects of monoamine oxidase inhibitors on the sympathetic ganglia. In order to study the effects of monoamine oxidase inhibitors on the sympathetic ganglia 20 experiments have been conducted with the dog stellate ganglion and the cat nictitating membrane preparation. With each preparation, the preganglionic and postganglionic fibers were electrically stimulated and the effects on heart rate (in the dog) and nictitating membrane (in the cat) are determined. If a drug is a ganglionic blocking agent, the effects of

preganglionic stimulation are eliminated or reduced, whereas the post-ganglionic effects are unaltered. With the conventional ganglionic blocking agents such as hexamethonium and tetraethylammonium, the parasympathetic ganglia are also blocked. Preliminary results of the present study have indicated that it is possible to decrease or totally block preganglionic stimulation with two inhibitors, iproniazid and harmine, without affecting the parasympathetic nervous system. No ganglionic blocking was found with other inhibitors including JB-516, Nardil and nialamide. It would appear, therefore, that the ganglionic block produced by harmine and iproniazid may not be useful as a screen for clinically active drugs. However, the production of preganglionic block without parasympathetic block by these compounds has important theoretical implications, suggesting that neurotransmitters other than acetylcholine may be involved in transmission through sympathetic ganglia.

5. Effects of drugs on myocardial contractility in man. The effects of drugs and surgical procedures on myocardial contractility were studied by means of the Walton-Brodie strain gauge arch (Proc. Soc. Exper. Biol. & Med. 84:263, 1953) in 50 patients undergoing cardiac surgery with cardiopulmonary bypass. The strain gauge, which is sutured directly to the right ventricle, has been extensively used to determine the direct effects of drugs in experimental animals, has been found to be equally applicable in man. T. D. Darby et al (Ann. Surg. 147:596, 1958) previously demonstrated the advantage of this technic in monitoring cardiac contractility during open heart surgery in 10 patients, but the present investigation is the first study of the direct effects of drugs in man. Prior to institution of cardiopulmonary bypass, sympathomimetic amines were administered to 18 patients. Results have shown that catechol neurohormones, norepinephrine, and epinephrine, produce almost identical increments in heart contractile force of short duration. Aramine and Wyamine produced similar increments in myocardial contractility as norepinephrine and epinephrine, but the effects persisted for at least 30 minutes. Methoxamine, on the other hand, had no effect on heart contractile force and therefore, increased blood pressure entirely by peripheral vasoconstriction. These studies have demonstrated that the effects of sympathomimetic amines in man are the same as those previously reported in the dog (Goldberg, L. I. et al, J. Pharmacol & Exper. Therap. 108:177, 1953) and have confirmed the hypotheses that "pressor amines" may differ in their effects on myocardial contractility. With the information obtained in this investigation, therapy of hypotension with sympathomimetic amines may be more rationally planned. After institution of cardiopulmonary bypass acetylstrophanthidin in full digitalizing doses was administered via the heart pump to 12 patients with atrial septal defect, but without cardiac failure. In each patient marked prolonged increments in heart contractile force were observed. These results demonstrate for the first time direct positive inotropic effect of a digitalis preparation in man and indicate that digitalis increases myocardial contractility in the absence of congestive heart failure. In addition to studies

digitalis and sympathomimetic amines the effects of anoxic cardiac arrest, partial coronary perfusion, anesthetic agents and antiarrhythmic agents have also been studied. There were no complications either during surgery or in the post-operative period related to the use of the strain gauge arch.

Proposed course of Project:

1. Structure activity relationships of amines and monoamine oxidase inhibitors and Ritalin. These studies will be continued to determine which structures are necessary for potentiation by MAO inhibitors and Ritalin to determine whether the opposing effects of these agents on the action of endogenous amines can be extended to other amines.

2. Further studies of the ganglionic blocking effect of monoamine oxidase inhibitors. Additional studies of the effects of monoamine oxidase inhibitors on the sympathetic ganglia are planned. Since several of the MAO inhibitors have direct effects on heart rate and the nictitating membrane, it may be worthwhile to study the electric potentials of the nerves rather than the effects on the end organs. Preliminary studies of this nature are planned in order to determine whether more extensive investigations in this direction would be fruitful.

3. The pharmacological effects of alpha-methyl dopa. In view of the recent finding in this laboratory that the decarboxylase inhibitor alpha-methyl dopa has a hypotensive action in man, animal studies are being planned to ascertain the mechanism for this clinical action. Preliminary studies in the dog indicate that this compound does produce hypotension in that animal and therefore, studies of mechanism of action in the dog would probably have clinical implications. This study would include determination of relative direct cardiac and peripheral effects of the agent, effect on sympathetic ganglia and the effects on the actions of sympathomimetic amines and amino acids (such as DOPA). As alpha-methyl dopa seems to have an effect on the central nervous system, studies may be included to determine the effects of this compound on sympathetic nerve impulses.

4. Direct effects of drugs on myocardial contractility in man. This project in collaboration with the detailed studies of Clinic of Surgery and Section of Anesthesia will be continued to include anesthetic agents, anti-arrhythmic drugs and further investigations of digitalis and sympathomimetic amines.

Part B included Yes No

Part II

Publications

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Intracardiac and Proximal Intrathoracic Electrocardiography

Principal Investigator: Joseph C. Greenfield, M.D.

Other Investigators: Samuel M. Fox III, M.D., Thomas N. Lynn, Jr., M.D.
Albert Kistin, M.D., Beckley Memorial Hospital.

Cooperating Units: None

Man Years (Calendar year 1959): 0.1

Patient Days (Calendar year 1959): 40

Project Description:

Objective: To obtain a better understanding of the genesis and spread of activation in arrhythmias both spontaneous and resulting from standard catheterization procedures.

Major Findings: The use of intra-esophageal electrodes has recently been introduced and three studies performed. Some useful data was obtained in two.

Four more studies of intracavitary potentials in right ventricular conduction disturbances have provided interest but no fresh information.

Proposed course of project: Continue as outlined above as material is available.

Part B included: Yes No
x

Serial No. NHI-24
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Length Changes in the Aorta.

Principal Investigator: Dali J. Patel, M.D., Ph.D.

Other Investigators: Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 1

Patient Days (Calendar year 1959): 0

Project Description:

Objectives: Most studies dealing with distensibility characteristics of the aortic wall have ignored changes in length, mainly because there was no satisfactory means of measuring these changes in the living dog. With the electrical caliper that has been developed in this laboratory by Mr. Wallace, it has been possible to study these changes. A complete distensibility study requires that diameter length and intravascular pressure be known at every instant in the cardiac cycle and it is the purpose of this study to evaluate this problem further.

Major Findings: Preliminary findings indicate:

(1) With inspiration the thoracic aorta elongates and the abdominal aorta shortens. With expiration, the reverse is true.

(2) During systolic phase of a cardiac cycle, the thoracic aorta elongates and the abdominal aorta shortens.

(3) The magnitude of the change in length in the thoracic aorta is roughly about 1/20 of the change in diameter under control condition.

Proposed course of project: Pursuance of the above studies.

Part B included:	Yes	No
		x

PHS - NIH
Individual Project Report
Calendar Year 1969

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Patel, Dali J., Schilder, D. P., and Mallos, A. J.
Mechanical Properties and Dimensions of the Major Pulmonary Arteries.
J. of Appl. Physiol. In Press.

Serial No. HHI-25
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Clinical and Experimental Electrocardiography.

Principal Investigator: Thomas N. Lynn, Jr., M.D.

Other Investigators: Samuel M. Fox III, M.D. and Joseph Greenfield, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 0.4

Patient Days (Calendar year 1959): 100

Project Description:

Objective: To correlate physiologic and electrocardiographic data with particular emphasis on the elucidation of influences causing delayed depolarization in the right ventricle.

Major Findings: A study was made of patients who had a decreased right ventricular stroke volume after surgical relief from a left-to-right shunt. The vast majority of these had a reduction of delayed forces presumably in the right ventricular wall.

In 7 cases of such delay an attempt was made to occlude ostium secundem type defects with a balloon catheter after a standard right heart diagnostic catheterization. In those hearts with significant depolarization delay the defect was of such size and/or the pressure difference between the atria became so great that the balloons, even up to a size of 35 mm diameter, were carried through.

The performance of a valid Valsalva maneuver usually resulted in a diminished right ventricular activation time.

Proposed course of project: In patients with a suggestion of increased right ventricular diastolic filling who otherwise appear to need saphenous catheterizations it is hoped some change can be elicited by a 15 to 20 second occlusion of the inferior vena cava by drawing a balloon down into the ostium from the right atrium.

A paper by Dr. Lynn is in preparation.

Part B included:	Yes	No
		x

Serial No. NHI-26
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Influence of Acute Coronary Ligation on the Left Ventricular Ejection Velocity in the Dog.

Principal Investigator: Guy O. Barnett, M.D.

Other Investigators: Alexander Mallos, B.S., Samuel M. Fox III, M.D.
Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 0.5

Patient Days (Calendar year 1959): 0

Project Description:

Objective: The method of velocity approximation of Fry and Mallos was used to give further insight into the hemodynamic changes following acute coronary artery ligation. This will be used as background information for the further study of the hemodynamic changes following the production of chronic coronary insufficiency in the dog.

Methods Employed: In the thoracotomized, anesthetized dog, acute coronary insufficiency was produced by transient ligation of the left anterior descending coronary artery. The instantaneous aortic velocity was measured through a double-lumen catheter placed in the ascending aorta. Left ventricular and central aortic pressures were measured with catheter manometer systems. "Myocardial contractile force" was measured by the strain gauge arch method of Brodie. An epicardial EKG was obtained from the area of myocardium made ischemic by the ligation.

Major Findings: Following acute coronary artery ligation, there were significant changes in the contour of the ejection velocity before any change occurred in left ventricular and diastolic pressure, aortic pressure, or in the standard electrocardiographic leads. These changes were reversible only if the ligation was removed within 1-2 minutes. This material was presented at the 32nd Scientific Session of the American Heart Association in Philadelphia in October 1959.

Part B included: Yes No
X

Serial No. NHI-27
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Changes in Aortic Ejection Velocity Following the Production of Chronic Myocardial Infarction.

Principal Investigator: Guy O. Barnett, M.D.

Other Investigators: Joseph Gresnfield, M.D., Alexander Mallos, B.S., Samuel M. Fox III, M.D., Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 0.5

Patient Days (Calendar year 1959): 0

Project Description:

Objectives: Aortic ejection velocity characteristics will be recorded in the unanesthetized dog before and following the production of myocardial infarction. Changes in aortic velocity will be correlated with changes in the blood pressure, electrocardiogram, stroke volume and cardiac output.

Methods Employed: Myocardial infarction will be produced either by bead embolization or by acute or chronic coronary artery ligation. Aortic ejection velocity will be approximated by the technique of Fry and Mallos. Cardiac output will be measured by the standard indicator-dilution technique.

Proposed course of project: Pursuance of the above studies.

Part B included:	Yes	No
		x

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Measurement of Relationship of Diameter to Pressure in the Aorta.

Principal Investigator: Guy C. Barnett, M.D.

Other Investigators: Alexander Mallos, B.S., and Allan Shapiro

Cooperating Units: None

Man Years (Calendar year 1959): 0.3

Patient Days (Calendar year 1959): 0

Project Description:

Objectives: In order to calculate the instantaneous aortic flow from the instantaneous velocity, it is necessary to evaluate the instantaneous aortic diameter (flow = velocity x diameter).

Methods Employed: In the anesthetized thoracotomized dog, the instantaneous aortic diameter was obtained using straingauge calipers specially devised by Mr. Mallos. The aortic pressure was monitored by a catheter manometer system. Data were gathered during periods when the pressure was changed both by changing the blood volume and by the administration of nor-epinephrine.

Major Findings: The experimental data showed a linear relationship between mean diameter and mean pressure - a relationship which was not changed by the administration of nor-epinephrine. The relationship of diameter to pressure during each cardiac cycle was also evaluated. It would appear that the use of the mean systolic diameter would be satisfactory in the computation of flow.

Part B included: Yes No
x

Serial No. MHI-29

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Computation of the Aortic Ejection Velocity from the Central Aortic Pulse Pressure.

Principal Investigator: Joseph Greenfield, M.D.

Other Investigators: Guy O. Barnett, M.D., Alexander Malles, B.S., Donald L. Fry, M.D., Samuel M. Fox III, M.D.

Cooperating Units: None

Man Years (Calendar year 1959)
0.5

Patient Days (Calendar year 1959):
0

Project Description:

Objectives: To compare the method of Fry and Malles for measurement of the instantaneous aortic ejection velocity with the aortic ejection velocity computed from the central pulse wave.

Methods Employed: The instantaneous aortic ejection velocity will be measured by the method of Fry and Malles. The central pulse contour will be recorded and from this the instantaneous aortic velocity will be computed using an operational amplifier. The open chested anesthetized dog will be used and cardiac output will be varied utilizing various drugs. The two methods will then be compared as to the similarity of the velocity contours.

Proposed course of project: Pursuance of the above studies.

Part B included:	Yes	No
		x

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Development and Evaluation of Basic Instrumentation for Application in the Field of Blood Flow and Pressure Measurement.

Principal Investigator: Donald L. Fry, M.D.

Other Investigators: Samuel M. Fox III, M.D., Donald P. Schilder, M.D., Guy O. Barnett, M.D., Dali J. Patel, M.D., Joseph Greenfield, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 2

Patient Days (Calendar year 1959): 50

Project Description:

Objectives:

It is the purpose of this project to develop various basic instruments and techniques for studying the mechanical properties of the cardiovascular and cardiopulmonary system. The specific inquiries are directed toward (a) the relationship of the pressure gradient to the flow in cylindrical tubes, (b) the distribution of velocity across a cylindrical tube in pulsatile flow, (c) the visco-elastic properties of the living vascular system with special reference to the pressure versus diameter relationships in the pulmonary artery and the aorta, (d) the measurement of intrathoracic pressure.

Methods Employed:

The relationship of the pressure gradient to pulsatile flow and the velocity profile across a cylindrical tube in pulsatile flow was studied in a recently developed mechanical flow generator in which the true flow is monitored for comparison with flow inferred by various other techniques. The velocity profile across the tube may be observed by injecting strands of Evans Blue dye and observing their change in shape with time. The instantaneous diameter as well as the elongation

strain of blood vessels under the various influences of pressure and drugs may be studied with an electrical recording caliper newly developed in this section. The method of determining intrathoracic pressure consists in the development of an improved intraesophageal balloon and tube system having special properties.

Major Findings: Experimental studies with the mechanical flow generator have revealed that the relationship between the instantaneous pressure gradient in a cylindrical tube and the instantaneous pulsatile flow bear relationships to one another which may be predicted mathematically with reasonable accuracy. The velocity profile examined by the introduction of blue dye strands into the pulsatile flow indicate that in the range of physiological frequencies the velocity profile across the tube is blunt with most of the fluid shear occurring at the boundaries of the flow. The more peripheral shells of flow appear to be in phase with the pressure gradient while the more central portions of the flow appear to be in quadrature with the pressure gradient. This is qualitatively in agreement with mathematical prediction. Studies are in progress to quantify this observation with high speed motion pictures.

Preliminary data on the electrical recording calipers used to measure strain in the vascular system have shown a good dynamic response and a low mechanical impedance. Several studies have been finished and many are in progress evaluating the visco-elastic properties of the vascular bed with this device. The results of these studies have been presented at the recent Heart Meetings and manuscripts are in preparation.

The intraesophageal balloon for estimating intrathoracic pressure has been perfected and is being currently used in projects evaluating intrathoracic hemodynamics as well as evaluating the role of stress distribution as a possible factor in the pathogenesis of pulmonary emphysema.

Part B included:	Yes	No
	X	

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Schilder, Donald P., M.D., Hyatt, Robert E., M.D., and Fry, Donald L., M.D. An Improved Balloon System for Measuring Intraesophageal Pressure. J. of Appl. Physiol. In Press.

2. Fry, Donald L., M.D. The Measurement of Pulsatile Blood Flow by the Computed Pressure Gradient Technique. IRE Transactions on Medical Electronics. In Press.

3. Fry, Donald L., M.D. Certain Aspects of Hydrodynamics as Applied to the Living Cardiovascular System. IRE Transactions on Medical Electronics. In Press.

4. Fry, Donald L., M.D. Panel Discussion of Methods of Flow Estimation by Pressure Sensing Techniques. IRE Transactions on Medical Electronics. In Press.

5. Fox, Samuel M. III, M.D., Mallos, Alexander, B.S., Cooper, Theodore, M.D., Ph.D., Fry, Donald L., M.D. Comparison of the Differential Pressure Catheter-Computer Technique with Other Methods for the Measurement of Instantaneous Pulsatile Blood Velocity. The meetings of the American Heart Association, San Francisco, 1958.

6. Fry, Donald L.,M.D., Noble, F.W., and Mallos, A.J.,B.S. An Evaluation of Modern Pressure Recording Systems. Circ. Research 5:40, 1957.

7. Fry, Donald L.,M.D., Hyatt, R.E., M.D., McCall, C.B. and Mallos, A.J.,B.S. Evaluation of Three Types of Respiratory Flowmeters. J. of Appl. Physiol. 10:210, 1957.

8. Fry, Donald L.,M.D., Noble, F.W. and Mallos, A.J.,B.S. An Electric Device for Instantaneous and Continuous Computation of Aortic Blood Velocity. Circ. Research 5:75, 1957.

9. Fry, Donald L.,M.D., Mallos, A.J.,B.S. and Casper, A.G.T.,B.A. A Catheter Tip Method for Measurement of the Instantaneous Aortic Blood Velocity. Circ. Research 4:5, 1956.

Serial No. MAI-31
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Pathogenesis of Chronic Diffuse Obstructive Pulmonary Emphysema.

Principal Investigator: Donald P. Schilder, M.D.

Other Investigators: Dali J. Patel, M.D., Ph.D., and Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 0.5

Patient Days (Calendar year 1959): 10 140

Project Description:

Objectives: Theoretical considerations have been developed that indicate that an abnormal stress distribution within the structure of the lung may be the major factor in the production of the disruptive lesions of emphysema. The abnormal stress distribution could be the result of either congenital or acquired abnormality of the smaller terminal air passages.

Major Findings: Progress has included the development of an improved type of intraesophageal pressure measuring system so that simultaneous pressures may be measured at several levels in the esophagus to evaluate the longitudinal stress distribution on the lung surface. Preliminary observations have indicated that this approach to measuring abnormal stress distribution is feasible and that there is a variable difference in intrathoracic pressure along the esophagus in the normal and diseased individual during certain respiratory maneuvers.

Proposed course of project: To study a number of normal and emphysematous subjects repeatedly in an effort to determine the reproducibility and magnitude of any such pressure differences.

Part B included:	Yes	No
	x	

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Schilder, Donald P., M.D., Kyatt, Robert E.,M.D., and Fry, Donald L., M.D. An Improved Balloon System for Measuring Intraesophageal Pressure. J. of Appl. Physiol. In Press.

Serial No. NHI-32
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of the Valsalva Maneuver on Pulmonary Vascular Resistance.

Principal Investigator: Donald P. Schilder, M.D.

Other Investigators: Dali J. Patel, M.D., Ph.D., Samuel M. Fox III, M.D., Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 0.5

Patient Days (Calendar year 1959): 5 75

Project Description:

Objectives: To determine the effect of the Valsalva maneuver on the pulmonary vascular resistance and to better define the mechanisms producing cough syncope.

Major Findings: Indicator dilution techniques have proved to be unsatisfactory for measuring the rapid and brief changes in flow that occur with such maneuvers and the pulmonary capillary "wedge" pressure has not been a valid reflection of pulmonary venous pressure under such circumstances. The catheter tip method of measuring pulmonary blood velocity has been applied to man successfully and left atrial pressure rather than pulmonary capillary wedge pressure will be required in this study. Preliminary studies indicate that the pulmonary vascular resistance does increase during the Valsalva maneuver.

Proposed course of project: Additional observation in several patients will be required to complete the project.

Part B included:	Yes	No
		x

Serial No. NHI-33
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pharmacodynamic Studies of the Pulmonary Circulation

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1959): 0.1

Patient Days (Calendar year 1959): 50

Project Description:

Objective: To determine in various states of disordered pulmonary function the characteristics of flow, distribution and the response to possibly useful agents.

Major Findings: Three patients with pulmonary vascular obstructive syndromes were given 1.5 to 2 mgm of Reserpine (Serpasil, Ciba) with a comparison being made between indices of Flow, Resistance, Oxygen Consumption, Stroke Volume and A-V Oxygen Difference by way of combined Fick blood gas and Dye indicator dilution techniques. No highly significant change occurred.

Two patients with patent ducti with reversed flow had multiple dye dilution studies done to try to determine the possible benefits of acetylcholine, priscoline, and reserpine and relatively pure oxygen inhalation. Only oxygen produced a consistent implied reduction in pulmonary vascular resistance.

Proposed course of project: Continuation along the same lines, and the addition of radio-isotope and velocity catheter studies should add to the understanding of the clinical picture and disturbed physiologic state.

Part B included: Yes No
X

Serial No. NHI-34

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Derivation and/or Improvement of Methods for Study of the Human Circulatory Response to Exercise and Other Maneuvers.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: Susan Lenkei, M.D., Thomas N. Lynn, Jr., M.D. Joseph C. Greenfield, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 0.5

Patient Days (Calendar year 1959): 100

Project Description:

Objectives: To find methods requiring little time and/or patient inconvenience that will give a useful assessment of circulatory function.

Major Findings: A grossly simplified yet accurate method of calibration for dye dilution studies has been worked out. Multiple studies on patients have demonstrated its reliability.

Proposed course of project: Using dye dilution techniques try to better establish the "normal" relations at rest and exercise of indices of Oxygen Consumption, Flow, Stroke Volume, Resistance and calculated A-V Oxygen Difference.

Continue to try to improve methods and particularly try to make use of external isotope counting techniques.

Part B included: Yes No

x

Serial No. NHI-35
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Coronary Flow

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1959): 0.1

Patient Days (Calendar year 1959): 20

Project Description:

Objectives: Because of the great need for a clinically useful index of coronary flow various attempts have been made to find satisfactory approaches.

Major Findings: The canine work during calendar year 1958 in which total coronary sinus drainage was attempted with a balloon catheter has pointed up severe and previously foreseen limitation which probably makes it inappropriate to pursue this further. Another approach using coronary sinus indicator dilution methods has shown feasibility in canine work (8 experiments) but has not been checked against known flows.

Special catheters have been made up for trial in humans but they will be tested in dogs against known flows first.

Proposed course of project: Coronary perfusion of a known amount will be used as a test of how good an index of total flow this method provided.

If a satisfactory correlation exists, trials in human subjects will be made along with the effects of vaso active agents such as nitroglycerine, aminophylline and digitalis.

Part B included:	Yes	No
		x

Serial No. NHI-36
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies Concerning the Determination and Significance of the Instantaneous Pulsatile Blood Velocity.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: Donald L. Fry, M.D., Guy Otto Barnett, M.D., Joseph C. Greenfield, M.D., Alexander Mallos, B.S., Dali J. Patel, M.D., Ph.D., Donald P. Schilder, M.D.

Cooperating Units: None

Man Years (Calendar year 1959): 2

Patient Days (Calendar year 1959): 300

Project Description:

Objectives: To assess the clinical and physiologic significance and the effect of therapeutically useful agents on the ejection velocity contour of the left and right ventricles, and the flow patterns of the aorta, pulmonary arteries and distal vessels.

Methods Employed: The catheter-computer technique has been brought forward in calendar year 1959 so that catheters of poorer qualities can be made useable and the system of maintaining a clear line has been simplified. Exploration of new gauges and techniques and the manufacture of better stopcocks have aided considerably.

Progress: A total of 15 patients have now had meaningful studies with the last four or five being of relatively high technical validity. A reference system has been considered as a means of classifying the ejection contours and a mathematical analysis has been undertaken of patient material through the NIH computer section.

Proposed course of project: A tape recorder and set of electronic circuits useful in obtaining better catheter response characteristics are being worked upon. When optimally functional, further studies will be made on pre and post-operative patients using various therapeutic agents. A study during surgery will also be undertaken.

Part B included:	Yes	No
		x

(Manuscripts on basic technique and initial findings are in preparation.)

Serial No. HHI-37
1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Myocardopathies: Definition of the physiologic disturbance and the response to therapeutic agents.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: None

Cooperating Units: None

n Years (Calendar year 1959):	Patient Days (Calendar year 1959):
0.2	500

Project Description:

Objective: As in the title with correlations of the clinical and hemodynamic parameters and in some cases the pathologic as revealed from myocardial biopsy to be done by the Surgical Clinic, N.H.I.

Major Findings: One case of myocarditis apparently due to the mumps organism has been followed to an inevitable death with post mortem. This is the first such case with this type course.

Various therapeutic trials were found to be of interest but of no major avail.

Corticosteroids were found useful in one other patient with ideopathic myocarditis on which a biopsy appears indicated.

On 3 other patients an approximation of aortic blood velocity has revealed low peak velocities with delayed and markedly reduced acceleration throughout left ventricular ejection.

This method of assessing the form of ventricular ejection has proven a more sensitive than the determination of the change in mean stroke volume or ventricular work upon stress or resulting from therapeutic manouvers.

Proposed course of project: As before with more refined techniques and better correlation.

Part B included:	Yes	No
		x

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Effect of age on the turn-over of mitochondria.

b. Principal Investigators: D. R. Sanadi (25% time)
Martin Fletcher

Other Investigators: None

Technical Assistance: None

Objectives: To determine whether mitochondria turn over as a unit or whether components of mitochondria turn over at different rates; to determine the effect of age of the animal on the half-lives of mitochondrial components.

Methods Employed: The proteins of mitochondria will be labeled with methionine-S^{35}, lipids with acetate-C^{14} and cytochrome C with Fe^{59}. The rate of loss of the components with time will be measured to determine half-life.

c. Progress During Past Twelve Months: The project was started in October. The methods for isolation of cytochrome C and the different protein fractions have been standardized.

d. Direction of Current Research: As outlined in Objectives and Methods Employed.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by D. R. Sanadi
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age changes in the intact rat.

   b. Principal Investigator: Donald A. Olewine (3/4 time)

   Other Investigator: Charles H. Barrows, Jr.

   Technical Assistance: Lawrence Valentine (L. D. 7/14/59)
   Lorenzo Lee (1/2 time) (E.O.D. 4/13/59)
   James Clemmons (E.O.D. 10/22/59)

   Objectives: The objectives of this program are: (1) to determine mortality rates of rats at different ages, (2) to determine gross pathology at death by means of a standardized autopsy, (3) to establish the pattern of body weight loss occurring prior to death in the senescent rat, and (4) to develop a procedure for blood vessel intubation and bladder catheterization in order that agewise physiological studies on rats may be carried out.

   Methods Employed: (1) Rats in our animal colony are of the Sprague-Dawley strain obtained at the age of one month from central animal quarters at Bethesda.

   (2) All cages were checked for dead animals in the early morning and late afternoon. At these times autopsies were performed and all information recorded on a standardized autopsy form. Presence of gross pathology was noted and graded using defined criteria.

   (3) All animals exhibiting signs of middle ear disease were immediately sacrificed.

   (4) Body weights were followed at weekly intervals beginning at about age forty weeks.

   c. Progress During Past Twelve Months: (1) Mortality. In both sexes 50% mortality is below the expected 104 weeks (males--70 weeks; females--85 weeks) which we have experienced with the McCollum strain used in other experiments. The sex difference in the age at 50% mortality is in agreement with previous findings. Mortality also shows a sex difference until the 78th week (males--0.40; females--0.24).

   (2) Findings at Autopsy. The principal finding at death in 413 animals is lung disease (based on gross examination)

regardless of age or sex (males 82% and females 78%). During the first year in both sexes middle ear disease accounts for 25% of the deaths but in the following year this drops to 3%. The incidence of mammalian tumors is 8% in females autopsied between ages of 52-78 weeks. In the next 26 weeks (79-104 weeks) this increases to almost 40%.

These findings indicate that, in our hands, this particular Sprague-Dawley strain is not as disease resistant as other strains used in this laboratory. The severity of respiratory infection is such that relatively few animals survive to 104 weeks (males--16%; females--22%). Such low survivor rates preclude the use of such animals for gerontological research. The high tumor incidence (40%) in older females is also undesirable. Consequently, plans are underway to introduce additional different strains of rats into the colony.

(3) Body Weights. It was felt that a knowledge of the patterns of body weight loss in the senescent rat would be of value in the following: (a) in predicting the time of death of an animal, (b) in providing a clue as to the time of onset of the unknown events which bring about the death of the animal.

Body weight curves compiled from weekly weighings after age 40 weeks show a variety of shapes. In general, they can be placed under two categories: (a) a gradual decline in body weight over a period of 5-8 weeks, and (b) sharp fall in weight over 1-2 weeks. In almost all instances death was preceded by a very rapid fall in weight 2-3 days before death. So far, attempts to correlate weight curves with autopsy findings have been unsuccessful.

(4) Intubation. Techniques have been developed so that intravenous infusions into the left external jugular vein and arterial blood samples from right common carotid artery can be made with a minimum of disturbance to the animal. By intubating these vessels with plastic tubes, blood has been withdrawn successfully three weeks postoperatively.

Of 15 animals intubated in this manner all have shown a postoperative loss in body weight which is not regained for 3-4 weeks, and half of these animals exhibit apparent neurological symptoms. In order to avoid these complications, arterial intubations in the future will be directed toward the aorta.

Several attempts to place a permanent catheter in the bladder have met with varying success. In one instance the bladder has been successfully flushed through the catheter one week postoperatively.

d. Direction of Current Research: (1) Techniques are currently being developed for the study of renal function in the senescent rat. Use of the rat in such a study has definite advantages since its tissues are readily available for examination for disease, cell loss, and enzymatic change. Such data may provide direct information as to the cause of functional change in the intact animal.

(2) As yet spontaneous activity and its relation to food intake and body weight has not been studied in the senescent rat. Since animals on a restricted dietary intake are believed to be more active and have longer lifespans than ad lib fed animals, activity itself may have an important relation to longevity. Also, the senescent loss in muscle mass may be partly due to a disuse atrophy resulting from a reduction in activity with age. Therefore, experiments are being planned for studying both spontaneous activity and the effects of forced exercise in the rat.

(3) Improvements in the operation and management of the animal quarters are being planned in an effort to reduce the incidence of lung disease in the rat colony.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Donald A. Olewine
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Action of vitamin D_3 at the cell level.

b. Principal Investigators: D. E. Bustow (40% time)

Other Investigators: None

Technical Assistance: M. Eileen Hogan (60% time)

c. Progress During Past Twelve Months: This is a new project. Cultures of a colorless Euglena have been grown in the presence of vitamin D_3 or ergosterol in various concentrations. There is an increased growth rate over the control cultures in those cultures containing vitamin D_3. Cultures grown in the presence of ergosterol show a decreased growth rate as compared to the control cultures.

d. Direction of Current Research: Attempts are underway to determine which concentration(s) of vitamin D_3 is (are) most stimulatory to growth of this Euglena. The action of vitamin D_3 on this single cell system will then be investigated.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: D. E. Bustow
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Effects of age on single cell cultures.

b. Principal Investigators: D. E. Buetow (40% time)
J. J. Blum (40% time)

Other Investigators: None

Technical Assistance: M. Eileen Hogan (40% time)

c. Progress During Past Twelve Months: This is a new project. Preliminary work has shown that it is possible to maintain single cell cultures (i.e., protozoa) at a constant cell count simply by removing the nitrogen and/or carbon source from the growth medium.

d. Direction of Current Research: Attempts are underway to determine how long such cultures may be maintained in a state of non-growth (i.e., "aging"). Biophysical and biochemical characteristics of such "aging" cultures will be investigated.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications: None

Prepared by: D. E. Buetow
November 4, 1959

Serial No. NMI-42

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Characteristics of mitochondria in aging protozoan cultures.

b. Principal Investigators: J. J. Blum (20% time)
D. E. Buetow (20% time)
D. R. Sanadi (10% time)

Other Investigators: None

Technical Assistance: H. L. Carr (30% time)

c. Progress During Past Twelve Months: This project has just been initiated. Preliminary work has shown that it is feasible to prepare intact mitochondria from protozoa.

d. Direction of Current Research: We are presently surveying the biochemical properties of these mitochondria, and are attempting to establish the pattern of similarities and of differences compared to mitochondria from the usual sources (e.g., liver).

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: J. J. Blum
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age changes in the regenerative potential of various organs of the rat.

b. Principal Investigators: Charles H. Barrows, Jr. (1/4 time)
Donald A. Olewine (1/4 time)

Other Investigator: J. A. Falzone, Jr.

Technical Assistance: Lois Roeder (1/4 time)
Frances Beran (L. D. 9/18/59) (1/4 time)

Objectives: The general purpose of this study is to determine whether (1) the amount of tissue produced during regeneration is different in senescent as compared to younger animals and (2) the tissue produced is biochemically different in the two age groups. Specific problems investigated during this period have been: (1) studies of kidney tissue following unilateral nephrectomy in rats of different ages, and (2) preliminary investigations of enzyme changes during liver regeneration following partial hepatectomy.

Methods Employed: (1) Unilateral nephrectomies were performed by the removal of one kidney by the dorsal route. The concentration of various enzymes, DNA and protein nitrogen were determined immediately after the removal of the kidney. Following various lengths of time, the animals were sacrificed, the remaining kidney removed and analyzed as described above.

(2) Partial hepatectomies by the method of Anderson were carried out on a limited number of animals. The concentrations of various enzymes, DNA and protein nitrogen were determined in the liver tissue removed. After 1, 2 or 3 days, the animals were sacrificed and the remaining liver tissue was analyzed as described above.

c. Progress During Past Twelve Months: (1) A comparison of the organ weights and the concentrations of various enzymes of the left and right kidneys in a limited number of animals demonstrated no differences which exceeded 10%. In the first preliminary experiment, the animals (four) were sacrificed one month following unilateral nephrectomy. The remaining kidney was found to be 30% heavier than the control. This was approximated by the total DNA, d-amino acid oxidase

and alkaline phosphatase. However, the total succinoxidase and pyrophosphatase exceeded slightly these other increments. In a preliminary age study carried out on 10 young (12 month old) and 6 old (22 month old) rats that were allowed to survive two months following unilateral nephrectomy, the remaining kidney was found to be 36% heavier than the control and the same pattern of enzymatic changes were observed as described above. No marked age differences were found.

(2) The total liver weight following 1, 2 and 3 days of regeneration was found to be 40%, 63% and 87% respectively of the initial weight, on the assumption that removal of the frontal lobe represented 70% of the initial liver weight. One of the difficulties encountered was an area of necrosis adjacent to the ligature. This represented approximately 10% by weight of the total liver following regeneration. In this preliminary study, this area was completely disregarded. The concentrations of succinoxidase and cholinesterase of the liver following one day of regeneration were reduced to 66% and 17% respectively of the initial concentrations, but gradually increased to approximately 90% after 3 days. On the other hand, the activities of alkaline phosphatase and cathepsin and the concentration of DNA were increased in samples of regenerated liver. It is interesting to note that the pattern of change in the concentrations of liver enzymes during regeneration is similar to that observed during protein-free feeding.

d. Direction of Current Research: (1) An agewise study using an adequate number of animals will be carried out to confirm the results of the preliminary experiment which indicated a lack of an effect of age on renal hypertrophy following unilateral nephrectomy.

(2) Experiments will be continued on the biochemical changes in liver following partial hepatectomy. When adequate methods are obtained, the effect of age on the regeneration of liver tissue following single and repeated partial hepatectomies will be carried out.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Charles H. Barrows, Jr.
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Measurement of reticuloendothelial clearance rate in man utilising hemoglobin-haptoglobin complex.

b. Principal Investigators: J. Lowenstein (1/4 time)
D. Faulstick (1/4 time)

Technical Assistance: M. Sellmayer (1/2 time)

Objectives: An attempt has been made to evaluate the magnitude of reticuloendothelial clearance in man utilising the colloidal haptoglobin-hemoglobin complex. The evidence for reticuloendothelial participation in the clearance of this complex from the blood stream is based on anatomic evidence (in animals by necropsy and in humans by body surface counting)[1, 2] and the similarity between the kinetics of blood stream clearance noted for hemoglobin-haptoglobin complex and such well described systems as colloidal carbon, chromic phosphate and heat denatured serum albumin[3, 4, 5, 6].

Methods Employed: Varying amounts of sterile autogenous hemoglobin solution (from 2-5 grams) were infused into 15 ambulatory male subjects hospitalized for minor surgery or residing on the Gerontology Ward at Baltimore City Hospitals. Following this loading dose, a sustaining solution of hemoglobin (2 grams/hour) was instituted in conjunction with studies on renal excretion of unbound hemoglobin.

Blood samples were drawn at intervals of 15 minutes for 2-3 hours and at hourly intervals thereafter but serum haptoglobin was fully saturated during loading.

Total serum hemoglobin was analyzed by the method of McCall[7]. Free- and haptoglobin bound-hemoglobin were separated by paper electrophoresis of serum samples in .0445 M phosphate buffer at pH 7 and the relative amounts of the two components evaluated, after staining with benzidine and hydrogen peroxide, by photometric analysis (Spinco Analytrol (R)). The values for hemoglobin-haptoglobin complex were plotted as a function of time.

1. Jandl, Greenberg, Yonemoto and Castle: J. clin. Invest., 35: (8), 842, 1956.

2. Jandl, Castle and Jones: J. clin. Invest., 36: (10), 1428, 1957.

3. Bennecerraf: Ed Brauer (Editor), Liver Function. A.I.B.S., Washington, 1958, p. 205.

4. Biozzi, Benacerraff and Halpern: Brit. J. Exp. Path., 34: 441, 1953.

5. Dobson: Physiopathology of the Reticuloendothelial System, Blackwell, 1957, p. 80.

6. Halpern, Biozzi, Benacerraf and Hillemand: C. R. Soc. Biol., 150: 1307, 1956.

7. McCall: Analytical Chem., 28: (2), 189, 1956.

c. Progress During Past Twelve Months: It has been observed that hemoglobin-haptoglobin complex disappears from the blood stream at a first order rate.

Values for the rate constant K and dose, D, have been obtained in 15 normal male subjects. The product K x D averaged 0.637 and the rate constant, K, averaged .00363. These values may be compared with the series of Halpern, et al. studying the clearance of heat coagulated human serum albumin[6]. Utilizing a dose of 10 mg./kg. body weight, K x D averaged 0.381 and K, 0.0381 in 10 subjects.

d. Direction of Current Research: An attempt will be made to elucidate further the mechanism and characteristics of the clearance of hemoglobin-haptoglobin complex in the normal and in various disease states.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: J. Lowenstein
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Structure of the interphase nucleus.

b. Principal Investigator: J. A. Falzone, Jr. (1/4 time)

Other Investigators: None

Technical Assistance: Wilbert Parson (1/4 time)

Objectives: (1) To determine the structure of interphase chromosomes; (2) To define chemical factors producing chromosome condensation; (3) To count chromosomes at interphase and (4) Age comparisons of results from above objectives.

Methods Employed: Simultaneous phase microscopy and chemical treatment of isolated nuclei and chromosomes; polarization microscopy; histochemistry.

c. Progress During Past Twelve Months: (Duration of present study = 6 months.) It is known that nuclei isolated in 0.25 M sucrose are normally hyaline in appearance but will shrink and become 'granular' if the medium is altered by reduction of pH or elevation of ionic strength. Polyvalent cations (Ca^{++}, Mg^{++}, protamine, histone) are particularly effective. These effects are reversible and can be attributed to the poly-anion nature of DNA. We have confirmed these results in rat liver with the following modifications: (1) The 'granulation' produced by the above agents is extremely 'regular' in appearance and resembles 'prophase' more than fixation artifacts if: (a) spherical nuclei are not examined with an oil immersion objective which has too shallow a focal depth for these purposes or (b) previously flattened nuclei are treated and examined (at any magnification). (2) RNA produces a remarkable effect if applied after protamine; within several seconds, the nuclei become filled with black dots in rectangular array. Discrete, curving, double rows of dots are often particularly prominent and can be traced for several microns. Present impression is that the dots represent protamine ribonucleate precipitated on the gyres of extended helices. If RNA precedes protamine, the effect is weak or non-existent, suggesting that protamine acts as a cationic bridge between DNA and RNA. The effect is equally striking in isolated nuclei from mature Drosophila (2n = 8) but we have not yet identified individual chromosomes.

d. Direction of Current Research: (1) Determining more precisely the relationship of the protamine-RNA effect to chromosome structure. Treatment of known chromosomes and Drosophila nuclei, as well as micromanipulation and other techniques, will be used.

(2) Treatment of brain nuclei. In post-mitotic cells, there is no teleological reason for genetic material to remain in the large linkage groups of chromosomes. A negative protamine-RNA effect would suggest subdivision of genetic material in these cells. Chromosome dissolution could even be a function of age, particularly in tissues with low mitotic indices.

(3) Treatment of material from rapidly growing tissues to determine whether the protamine-RNA effect is different in nuclei which have replicated their DNA before mitosis.

Many alternative experiments suggest themselves but the above seem to be of more immediate interest.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: J. A. Falzone, Jr.
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Binding of cations to proteins.

b. Principal Investigator: Wayne W. Everett (2/3 time)

Technical Assistance: Barbara Ann Randall (E.O.D. 6/8/59 - 2/3 time)

Objectives: To determine the effect of metal ions on the conformation and change of proteins. Specifically, (1) to distinguish between the effect of activating and non-activating metals on the shape of enzymes, e.g., arginase, and (2) to determine the effect of ion binding on the isomerization of bovine serum albumin.

Methods Employed: Solutions of the proteins in the absence of metal ions are compared with solutions containing metals in varying concentrations, using ultracentrifugation, electrophoresis, viscosity, and optical rotation.

c. Progress During Past Twelve Months: Ultracentrifugation studies with a sample of arginase obtained from Sigma Chemical Company have shown that the sedimentation constant is increased with increasing concentration of manganese ion. Electrophoresis indicates the presence of impurities, and it is desired to work out a scheme for the purification of amounts large enough for the required physical measurements. Recrystallization has proved impractical, but a method involving elution from a chromatographic column has been almost completely worked out.

The isomerization of bovine serum albumin has been studied electrophoretically at low pH, and the results from the literature duplicated. The addition of $MnSO_4$ produces a lowering in the mobilities of the isomerized molecules.

d. Direction of Current Research: First, the effect of different salts of manganese upon the electrophoretic mobilities of BSA will be investigated; subsequently other ions will be studied both by electrophoresis and ultracentrifugation.

Upon the completion of the purification of arginase; the ultracentrifugation experiments will be repeated with manganese and then extended to other metal ions.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Wayne W. Everett
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on relationships between development and aging: The normal histology and histochemistry of the various developmental stages of Drosophila from egg to fourteen day old adults.

b. Principal Investigator: Dietrich Bodenstein

Technical Assistance: Joanne Delp

c. Progress During Past Twelve Months: A complete developmental series consisting of about 60 different stages has been prepared. Part of this series has already been stained and is ready for study. We hope that these preparations will allow us to ascertain the histological changes associated with aging.

d. Direction of Current Research: Continuation of this project is uncertain due to my resignation.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Bodenstein, D.: Contributions to the problem of eye pigmentation in insects: studied by means of intergeneric organ transplantations in Diptera. Smithsonian misc. Coll., 137: 23-41, 1959.

Prepared by: Dietrich Bodenstein
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on the relationship between proliferation and differentiation of skeletal muscle myoblasts in vitro.

b. Principal Investigator: Irwin R. Konigsberg (80% time)

Technical Assistance: Marian Neufeld

Objectives: An evaluation of the postulate that growth (cell proliferation) and differentiation are mutually exclusive processes.

Methods Employed: Cell cultures of trypsinized embryonic skeletal muscle are maintained in monolayer culture in liquid media. Cell proliferation is measured by nuclear counts and spectrophotometric determinations of feulgen-DNA. Differentiation is assayed by the degree of multinuclearity, presence of cross-striated myofibrils and contraction of the multinuclear cells.

c. Progress During Past Twelve Months: The use of glycerol extraction has permitted the detection of cross-striated myofibrils in developing multinucleated muscle cells. Previous attempts, using conventional histological techniques had been negative. Qualitative observations strongly suggest that the amount of contractile protein is increasing with time and represents true increase in complexity. In this respect muscle cells differ from most cell types which under conditions similar to those employed here undergo loss of differentiative character ("de-differentiation"). The explanation of this apparent lack of conformity we believe to reside in the probable loss of proliferative capacity imposed when multinuclearity is reached.

Previous investigations with muscle cell cultures have led us to conclude that multinuclearity was a result of cell fusion. Histological and cytochemical evidence suggested that the terminus of the multinuclear cell was the preferential point of cell fusion. The sparsity of cross-striation in this area, even in well differentiated cells, strengthens this premise.

In attempting to establish cultures of small numbers of cells in which the quantitation of all types would be feasible, techniques have been perfected for cultivating aliquots of microliter quantities of cell suspensions under oil. Counts of syncytial nuclei and total nuclei indicate that the rate

of syncytia formation is a function of the starting population size. However, an upper limit of percentage of nuclei in syncytia is eventually reached and maintained. The mechanism of maintaining this limit is unknown. It probably does not involve the exhaustion of available myoblasts since it occurs even in the presence of an unchanged rate of proliferation of total nuclei. In the light of our present knowledge of multinuclear cell formation, this can only mean that nuclei are joining syncytia at the same rate that new nuclei are being produced. At present, an attempt to evaluate the contributions of cell number alone versus cell density is being made by varying the surface area over which the cells are dispersed. Preliminary results indicate the dependence is on cell density rather than cell number alone. This would indicate that "conditioning" of the medium plays a less significant role than does physical contact between cells.

An investigation of the frequency distribution of the quantity of feulgen-DNA complex per nucleus indicates a unimodal distribution around the diploid quantity in the nuclei of multinuclear cells. This is additional evidence for the post-mitotic nature of these nuclei. The distribution histogram of the mononucleated cells is, however, surprisingly irregular. The two expected peaks corresponding to diploid and tetraploid values are not as sharp as might be expected and several possible subsidiary peaks are suggested. The two possibilities that suggest themselves are: (1) Cell specific differences in DNA per nucleus (i.e., fibroblast nuclei differ from myoblast nuclei). (2) Changes in DNA per nucleus of cells multiplying in tissue culture (these changes would have to occur earlier than the well known aneuploid changes of cultured cells).

d. Direction of Current Research: The quantitation of microcultures will be continued in an attempt to simplify conditions still further. Specifically, we will try to establish conditions under which no cell proliferation occurs but in which viable, healthy cells can be maintained. Attempts to isolate and maintain single cells of known type will be continued.

Quantitation of feulgen-DNA will be continued. Measurements of feulgen-DNA distributions in mononucleated cells will be compared in the original cell suspension and after subsequent culturing.

Histological and histochemical investigation of developing multinuclear cells will be continued with particular emphasis on the contractile proteins and the cell terminus.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

The collaboration of Dr. B. L. Strehler has been had in the problem dealing with the quantitation of feulgen-DNA.

4. Publications and Awards:

1. Election to The Society for the Study of Growth and Development.

2. Election to the American Society of Zoologists.

Prepared by: I. R. Konigsberg
November 4, 1959

Serial No. NRI-49

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on oxidative phosphorylation.

b. Principal Investigators: D. R. Sanadi (25% time)
A. Fluharty (E.O.D. 10/1/59)

Other Investigators: None

Technical Assistance: J. A. Schwartz

Objectives: We are attempting to obtain evidence of whether a vicinal dithiol group is involved in oxidative phosphorylation.

Methods Employed: The effect of inhibitors like Cd^{++}, arsenite and γ-arsenophenyl butyrate which have a stronger affinity to dithiol groups than towards monothiols is being studied on oxidative phosphorylation and related partial reactions. It would be expected, also, that if a dithiol grouping is involved, only other dithiols (e.g., BAL) would reverse the uncoupling activity.

c. Progress During Past Twelve Months: Cd^{++} at levels below 10^{-4}M had no significant effect on the low ATPase activity of fresh mitochondria; nor did it change the strong ATPase activity elicited by the addition of 2,4-dinitrophenol.

γ-Arsenophenyl butyrate uncouples phosphorylation coupled to the oxidation of succinate, and the effect is reversed readily by dithiols and by much higher concentrations of certain monothiols.

d. Direction of Current Research: Further work on the effect of arsenite and arsenicals on the oxidation of other substrates is necessary to provide a basis for an acceptable generalization. The effect of the above inhibitors on sub-mitochondrial particulate systems will be studied.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: D. R. Sanadi
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on oxidative decarboxylation of α-ketoacids.

b. Principal Investigators: D. R. Sanadi (40% time)
R. L. Searls

Other Investigators: None

Technical Assistance: Patricia Knoll

Objective: Detailed study of the kinetics and mechanism of the thioctyl dehydrogenase component.

Methods Employed: Electrophoresis and ultracentrifuge studies to establish purity of the enzyme; kinetics of the reaction; effect of inhibitors.

c. Progress During Past Twelve Months: The α-ketoglutaric dehydrogenase complex which catalyzes several related reactions has been disrupted into smaller fragments by digestion with trypsin. Two distinct fractions have been obtained in a high degree of purity. One fraction is a flavoprotein which catalyzes the reversible dehydrogenation of reduced thioctamide or thioctate by DPN as well as the diaphorase reaction. All available evidence indicates that this enzyme is identical with Straub diaphorase whose natural substrate was not known.

The second fraction catalyzes the oxidative decarboxylation of α-ketoglutarate by artificial acceptors like 2,6-dichlorophenol indophenol. In the absence of electron acceptor, the ketoglutarate is decarboxylated to succinic semialdehyde.

d. Direction of Current Research: The mechanism of the flavoprotein reaction will be studied with special attention to the possible participation of unidentified cofactors or reactive sites indicated by certain anamolies in the spectral characteristics. The dye reducing fraction will be examined to give clues regarding the nature and role of the primary oxidative coenzyme and the mechanism of the cocarboxylase action.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Searls, R. L., and D. R. Sanadi: Dihydrothioctyl dehydrogenase--a flavoprotein. Proc. nat. Acad. Sci., Wash. 45: (5), 697-701, 1959.

Prepared by: D. R. Sanadi
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age changes in renal physiology.

b. Principal Investigators: J. Lowenstein (3/4 time)
M. J. Yiengst (1/2 time)
D. A. Faulstich (1/4 time - E.O.D. 7/1/59)
D. A. Cursler (1/1/59 - 6/30/59)

Other Investigators: N. W. Shock

Technical Assistance: Ramona Dorcas (Full time)
Margaret Sellmeyer (1/2 time)
Theresa Caryk (3/4 time - Maternity leave from 6/20 - 9/1/59)
L. Lee (1/2 time)

Objectives: To describe and elucidate the mechanisms of age changes in renal function.

Methods Employed: Determinations of renal concentrating ability are made by the Fishberg technique using the freezing point depression method as a measure of urine osmolarity. Creatinine clearances are determined by a modified alkaline picrate method using Lloyd's reagent to remove interfering non-creatinine chromogens.

The measurement of glomerular permeability utilizing dextrans of various molecular weights is being attempted. Methodology involving light scattering and viscosity measurements are being completed.

A method has been developed for estimation of glomerular clearance of hemoglobin based on the electrophoretic separation of free- and haptoglobin bound-hemoglobin. Standard methods are used for determining inulin and PAH clearances.

c. Progress During Past Twelve Months: The Fishberg-type dehydration test, on out-patient subjects from the longitudinal study, now includes a total of 130 tests. Since a sufficient number of subjects in the higher age groups have not been tested to date an agewise evaluation of renal concentrating ability has not been made for this group. However, preliminary observations of the data indicate a lack of age changes using this technique.

Endogenous creatinine clearances, on subjects from the longitudinal study, are being done on the above Fishberg urine samples. Two blood samples for plasma creatinine are collected just prior to and immediately following the period of dehydration. To date 130 subjects have been examined by this technique. Tests on more subjects as well as repeats on the same individual (just recently begun) will be necessary to properly evaluate the significance of an observed age regression. Simultaneous inulin and creatinine clearances are being done on subjects from the hemoglobin study to relate the clearance of true creatinine to inulin. Results of 10 such tests (5 periods per test) suggest a ratio of about 1.4 for $\frac{C_{Cr}}{C_I}$.

During the past 10 months attempts to isolate various dextran fractions from clinical dextran solutions have been attempted with only relatively good results. Dextran fractions with known average molecular weights have now been obtained from the Research Department of Commercial Solvents Corporation which will be used for standards. (Yiengst, Lowenstein and Faulstick).

Studies of glomerular clearance of hemoglobin have been carried out in 10 male subjects, aged 40 to 88, without evidence of renal disease. The plot of $UV_{HB}/100$ cc. glomerular filtrate against plasma concentration of "free" hemoglobin has been linear. The calculated ratio C_{HB}/C_{IN} has been .028 to .063. Renal "threshold" for free hemoglobin is 20-50 mg. %. There is evidence for small but consistent tubular reabsorption of free hemoglobin.

Urine osmolarity measurements were made on 24 hr. samples in a small group of older rats. On ad lib intake of food and water the majority of the animals showed a gradual decrease in concentrating ability between the ages of 18 and 24 months. Food intake and body weight remained essentially unchanged and reduced urine osmolarity was inversely related to water intake and urine volume.

Renal concentrating ability was followed during water deprivation for various time periods up to 3 days. All animals showed an increase in urine osmolarity in the second 24 hrs. of dehydration over the first 24 hr. period which in turn, was about 1/3 higher than the control level. All animals showed a return to control osmolarity levels within 24 hrs. after the end of water deprivation although several days were required for body weight to return to control levels. The maximal osmolarity attained during dehydration appears to be related to that animal's control value both of which decline with age.

d. Direction of Current Research: Work on the Fishberg type dehydration study will be continued until a sufficient number of older subjects from the longitudinal program have been examined. At such time, the data will be examined for aging changes as well as a comparison with chronic hospital patients.

Creatinine clearances will be continued on the out-patient group.

A longitudinal study, in which rats will be followed over an extended period of time, is planned. Urine osmolarity, creatinine clearance and proteinuria, both of degree and type (albumin, globulins, etc.) will be followed simultaneously at definite time intervals.

The standard dextran fractions are being utilized to calibrate equipment for future use of clinical dextran solutions in patients. Once the methodology is complete, changes in glomerular permeability with age as well as in various disease states will be investigated.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: J. Lowenstein
M. J. Yiengst
D. A. Faulstich
November 3, 1959

Serial No. NHI-52

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on relationships between development and aging: Nerve and muscle atrophy in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (1/4 time)

Technical Assistance: Joanne Delp (1/4 time)
Wilma Gabbay (1/4 time)

c. Progress During Past Twelve Months: For the histological aspect of this problem, fixation tests and nerve staining techniques have been conducted. Animals for this long-range project are being maintained.

d. Direction of Current Research: Continuation of this project is uncertain due to my resignation.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Dietrich Bodenstein
November 4, 1959

Serial No. NHI-53

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on relationships between development and aging: Humoral control of the accessory sex glands in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (1/4 time)

Other Investigator: Isabelle Sprague (Mt. Holyoke College - National Science Foundation)

Technical Assistance: Joanne Delp (1/4 time)
Wilma Gabbay (1/4 time)

c. Progress During Past Twelve Months: Transplantation and extirpation experiments were continued on this problem. The following results are now available:

(1) The juvenile hormone is present during the entire intermolt period of the nymphal roach. The allatum hormone titer seems to be somewhat higher at the end of the intermolt period.

(2) Young nymphal glands have the capacity to differentiate prematurely; i.e., the anlage of the gland in the fourth stage nymph is able to achieve adult characteristics if transplanted into the appropriate humoral environment. This has been elegantly demonstrated by splitting a single anlage into two halves and placing each half into a different age host; that is, into a different humoral environment.

(3) The state of maturity of the sex gland in intermediate hosts (i.e., animals intermediate between nymph and adult) is as follows. In slight intermediates the sex glands are almost nymphal in appearance. An increase towards adulthood of the external character is always correlated with a corresponding maturity of the sex glands.

(4) A comparison of the response of nymphal sex glands transplanted into a host undergoing a metamorphic molt and nymphal glands transplanted into an adult undergoing an experimentally induced molt was made. It was found that the metamorphic molt and the induced adult molt evoked the identical response in the juvenile sex glands; both molts induced adult characters. These results fit well our current ideas concerning the humoral milieu of last stage and adult animals.

d. Direction of Current Research: Continuation of this project is uncertain due to my resignation.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Dietrich Bodenstein
November 4, 1959

Serial No. NMI-54

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on relationship between development and aging: Studies on leg regeneration in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (1/4 time)

Technical Assistance: Joanne Delp (1/4 time)
Wilma Gabbay (1/4 time)

c. Progress During Past Twelve Months: (1) Experiments involving the isolation of parts of regenerates in vivo have given some interesting results. We have been able to produce triple legs experimentally. Such malformations have heretofore been found only in nature. In the light of these results we are able to gain insight into the causes of multiple organ formation in insects.

(2) The histochemistry of muscle formation in the regenerating roach leg is under investigation. The histological material is now prepared and ready for study. Details of the results are as yet not available.

d. Direction of Current Research: Continuation of this project is uncertain due to my resignation.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:
1. Bodenstein, D.: Le role des hormones dans la régéneration des organes des insectes. Scientia, 52: 1-6, 1959.

Prepared by: Dietrich Bodenstein
November 4, 1959

Serial No. NHI-55

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on relationships between development and aging: Humoral control of ovary development in the cockroach, Periplaneta americana, and in the mosquito, Aedes aegypti.

b. Principal Investigator: Dietrich Bodenstein (1/4 time)

Technical Assistance: Joanne Delp (1/4 time)
Wilma Gabbay (1/4 time)

c. Progress During Past Twelve Months: (1) The histcytology of the ovariole epithelium in nymphal and adult ovarioles is still under investigation. We have accumulated a rather large number of histological preparations for this problem.

(2) A whole series of ovary maturation stages of adult mosquitoes of different ages has been collected in order to investigate the histology and physiology of the corpora allata during egg maturation. Our original thought that there exists a correlation between the size of the allata and the state of ovarian development seems to be erroneous. There appears to be only a random size fluctuation of the corpora allata. This problem is still under investigation using more material, especially that collected from first and second pregnancies.

d. Direction of Current Research: Continuation of this project is uncertain due to my resignation.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Dietrich Bodenstein
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: The effects of aging on the developmental capacity of organ discs in the fruit-fly, Drosophila.

   b. Principal Investigator: Dietrich Bodenstein

      Technical Assistance: Wilma Gabbay

   c. Progress During Past Twelve Months: This project has been dropped due to unstable temperatures in the laboratory.

   d. Direction of Current Research: No further research is planned for this project.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Dietrich Bodenstein
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age changes in the chemical composition of various tissues of the rat.

b. Principal Investigator: M. J. Yiengst (1/4 time)

Other Investigators: C. H. Barrows, Jr.

Technical Assistance: Theresa Caryk (1/4 time)

Objectives: The objective of the investigation reported here is to determine (1) the effect of age on the serum proteins of the rat and (2) whether such age differences may be explained on the basis of serum protein synthesis.

Methods: Standard methods of chemical analysis were used for the determination of serum protein and albumin. These measurements were made on animals aged 3, 12 and 24 months. In addition, animals of these ages were subjected to 21 days of protein-free feeding followed by repletion with the standard diet for 3 and for 7 days.

c. Progress During Past Twelve Months: This work completes the study and includes the tests done on 3 and 12 month old animals. Albumin values showed no age changes in the control, depletion or repletion phases. All ages showed a significant drop during depletion with a return to control levels after 3 days of repletion. Globulin values of the 3 month animals were significantly lower than the two older age groups at the control level. By the seventh day of repletion both younger groups had raised their globulin levels to control values whereas the 24 month rats showed no gain over the 3-day repletion measurement.

d. Direction of Current Research: No further work is proposed for this project in the immediate future.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Reiff, T. R. and M. J. Yiengst: A rapid automatic semi-micro colloid osmometer. J. Lab. clin. Med. 53: (2), 291-298, 1959.

2. Yiengst, M. J., C. H. Barrows, Jr., and N. W. Shock: Age changes in the chemical composition of muscle and liver in the rat. J. Geront. 14: (4), 400-404, 1959.

3. Andrew, W., N. W. Shock, C. H. Barrows, Jr., and M. J. Yiengst: Correlation of age changes in histological and chemical characteristics in some tissues of the rat. J. Geront. 14: (4), 405-414, 1959.

Prepared by: M. J. Yiengst
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on the occurrence, isolation and properties of lipofuscin "age" pigments from human cardiac muscle.

b. Principal Investigators: A. S. Mildvan (60% time)
B. L. Strehler (20% time)

Other Investigator: D. D. Mark, Baltimore City Hospitals

Technical Assistance: M. V. Gee (35% time)

Objectives: Determination of the chemical structure, biochemical function (if any) and mechanism of formation of fluorescent particles that accumulate in the human myocardium with age.

Methods Employed: Quantitation of age pigment in myocardial sections was performed by the random shot technique previously described.

Isolation was done by tissue homogenization and differential centrifugation, purification by washing and differential centrifugation through discontinuous density gradients.

Elementary analysis is done commercially. Lipid, protein, and amino acid nitrogen were done by standard analytic procedures. Lipid fractionation is performed by chemical and chromatographic methods.

Enzymic studies were performed using standard spectrophotometric and polarographic assays.

Physical properties are studied with the Cary 14M spectrophotometer, the Perkin Elmer Infracord and the Aminco Bowman Spectrophotofluorometer.

c. Progress During Past Twelve Months: (1) The amount of age pigment in the hearts of individuals exposed to high energy radiation at the Hiroshima atomic bombing has been measured and was found not to differ significantly from the controls of the same age and race.

(2) Partial purification of age pigment in reasonably high yields (59-65% of calculated mass) has been achieved by differential centrifugation, repeated washings with sucrose solutions, electrolyte solutions and distilled water.

(3) The elementary analyses of two such preparations of these particles are:

	Sample 1	Sample 2
Carbon	58.19	55.05
Hydrogen	8.86	8.28
Oxygen	(20.98)*	21.43
Nitrogen	10.88	12.22
Sulfur	0.0	2.65
Phosphorus	1.09	.44
Iron	0.0	0.06

*Calculated by substraction.

(4) The percent lipid (28-46%) and percent non-lipid (72-54%) of these particles have been measured. We cannot ascertain, at present, whether the differences between these samples are due to inherent variation or to varying purity of the preparations.

(5) That the non-lipid fraction is largely protein is evidenced by:

(a) 15.8% nitrogen by weight.
(b) 75-85% of this nitrogen is ninhydrin positive after acid hydrolysis.
(c) Demonstration of no peaks other than the following in the H stretch region of the infra red spectrum, ($3250\ cm.^{-1}$; $3020\ cm.^{-1}$; $2900\ cm.^{-1}$; $2810\ cm.^{-1}$) which is compatible with protein.

(6) We have demonstrated that the fluorescence is mainly localized in the lipid fraction.

(7) The following mitochondrial enzyme systems have been sought and have not been detected in two fresh preparations of particles:

DPNH-cytochrome c reductase	No activity
DPNH-oxidase	No activity
Succinoxidase	No activity
Citric acid oxidase	No activity
Glutamic oxidase	Trace activity
Succinate-cytochrome c reductase	No activity

(8) Cathepsin activity (4.9-6.6 units*) has been detected in these particles (one preparation).

*(1 unit = 1 γ tyrosine/min. x ml. of suspension)

(9) The visible absorption spectrum of these pigment particles and of the lipid extract reveals no peaks; only end absorption.

(10) The infra red spectrum of the mixed lipid extract reveals peaks at the following positions:

3480	cm.$^{-1}$	1520-1540	cm.$^{-1}$	1086	cm.$^{-1}$
3220-3300	"	1450	"	1055-1060	"
2860-2900	"	1440	"	966- 969	"
2790-2830	"	1420-1430	"	926	"
1710-1725	"	1390-1395	"	873- 875	"
1640-1650	"	1370-1375	"	824- 826	"
1630	"	1220-1240	"	719- 720	"
1610-1620	"	1120-1125	"		

This data permits ruling out of certain structures but no unique positive identification as yet.

d. Direction of Current Research: Fractionation of lipid component is being attempted to determine what lipids are present and which of them are fluorescent.

Detailed enzymatic assays will be performed on fresh preparations, when available. Physical and chemical properties of the various fractions will be studied.

Biological experiments to induce or prevent formation of age pigment artificially are planned.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Strehler, B. L., D. D. Mark, A. S. Mildvan, and M. V. Gee: Rate and magnitude of age pigment accumulation in the human myocardium. J. Geront., 14: (4), 430-439, 1959.

Prepared by: A. S. Mildvan
November 4, 1959

4. Publications and Awards:

1. Reiff, T. R. and M. J. Yiengst: A rapid automatic semi-micro colloid osmometer. J. Lab. clin. Med. 53: (2), 291-298, 1959.

2. Yiengst, M. J., C. H. Barrows, Jr., and N. W. Shock: Age changes in the chemical composition of muscle and liver in the rat. J. Geront. 14: (4), 400-404, 1959.

3. Andrew, W., N. W. Shock, C. H. Barrows, Jr., and M. J. Yiengst: Correlation of age changes in histological and chemical characteristics in some tissues of the rat. J. Geront. 14: (4), 405-414, 1959.

Prepared by: M. J. Yiengst
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on the occurrence, isolation and properties of lipofuscin "age" pigments from human cardiac muscle.

b. Principal Investigators: A. S. Mildvan (60% time)
B. L. Strehler (20% time)

Other Investigator: D. D. Mark, Baltimore City Hospitals

Technical Assistance: M. V. Gee (35% time)

Objectives: Determination of the chemical structure, biochemical function (if any) and mechanism of formation of fluorescent particles that accumulate in the human myocardium with age.

Methods Employed: Quantitation of age pigment in myocardial sections was performed by the random shot technique previously described.

Isolation was done by tissue homogenization and differential centrifugation, purification by washing and differential centrifugation through discontinuous density gradients.

Elementary analysis is done commercially. Lipid, protein, and amino acid nitrogen were done by standard analytic procedures. Lipid fractionation is performed by chemical and chromatographic methods.

Enzymic studies were performed using chemical, spectrophotometric and polarographic assays.

Physical properties are studied with the Cary 14M spectrophotometer, the Perkin Elmer Infracord and the Aminco Bowman Spectrophotofluorometer.

c. Progress During Past Twelve Months: (1) The amount of age pigment in the hearts of individuals exposed to high energy radiation at the Hiroshima atomic bombing has been measured and was found not to differ significantly from the controls of the same age and race.

(2) Partial purification of age pigment in reasonably high yields (59-65% of calculated mass) has been achieved by differential centrifugation, repeated washings with sucrose solutions, electrolyte solutions and distilled water.

(3) The elementary analyses of two such preparations of these particles are:

	Sample 1	Sample 2
Carbon	58.19	55.05
Hydrogen	8.86	8.28
Oxygen	(20.98)*	21.43
Nitrogen	10.88	12.22
Sulfur	0.0	2.65
Phosphorus	1.09	.44
Iron	0.0	0.06

*Calculated by substraction.

(4) The percent lipid (28-46%) and percent non-lipid (72-54%) of these particles have been measured. We cannot ascertain, at present, whether the differences between these samples are due to inherent variation or to varying purity of the preparations.

(5) That the non-lipid fraction is largely protein is evidenced by:

(a) 15.8% nitrogen by weight.
(b) 75-85% of this nitrogen is ninhydrin positive after acid hydrolysis.
(c) Demonstration of no peaks other than the following in the H stretch region of the infra red spectrum. (3250 cm.$^{-1}$; 3020 cm.$^{-1}$; 2900 cm.$^{-1}$; 2810 cm.$^{-1}$) which is compatible with protein.

(6) We have demonstrated that the fluorescence is mainly localized in the lipid fraction.

(7) The following mitochondrial enzyme systems have been sought and have not been detected in two fresh preparations of particles:

DPNH-cytochrome c reductase	No activity
DPNH-oxidase	No activity
Succinoxidase	No activity
Citric acid oxidase	No activity
Glutamic oxidase	Trace activity
Succinate-cytochrome c reductase	No activity

(8) Cathepsin activity (4.9-6.6 units*) has been detected in these particles (one preparation).

*(1 unit = 1 γ tyrosine/min. x ml. of suspension)

(9) The visible absorption spectrum of these pigment particles and of the lipid extract reveals no peaks; only end absorption.

(10) The infra red spectrum of the mixed lipid extract reveals peaks at the following positions:

3480	$cm.^{-1}$	1520-1540	$cm.^{-1}$	1066	$cm.^{-1}$
3220-3300	"	1450	"	1055-1060	"
2860-2900	"	1440	"	966- 969	"
2790-2830	"	1420-1430	"	926	"
1710-1725	"	1390-1395	"	873- 875	"
1640-1650	"	1370-1375	"	824- 826	"
1630	"	1220-1240	"	719- 720	"
1610-1620	"	1120-1125	"		

This data permits ruling out of certain structures but no unique positive identification as yet.

d. Direction of Current Research: Fractionation of lipid component is being attempted to determine what lipids are present and which of them are fluorescent.

Detailed enzymatic assays will be performed on fresh preparations, when available. Physical and chemical properties of the various fractions will be studied.

Biological experiments to induce or prevent formation of age pigment artificially are planned.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Strehler, B. L., D. D. Mark, A. S. Mildvan, and M. V. Gee: Rate and magnitude of age pigment accumulation in the human myocardium. J. Geront., 14: (4), 430-439, 1959.

Prepared by: A. S. Mildvan
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on changes in localisation and/or concentration of molecular populations during aging.

b. Principal Investigators: B. L. Strehler (20% time)
I. R. Konigsberg (20% time)

Other Investigators: None

Technical Assistance: Malcolm Gee (35% time)

Objectives: General--These studies were undertaken in order to measure small amounts of materials in individual cells, their localisation and concentration, etc. The measurement of the absorption spectra of single age pigment particles *in situ* (sections) is one specific objective. A study of ploidy variation and other possible cytological effects of DNA change with age are projected.

Methods Employed: Spectrophotometry and histochemistry.

c. Progress During Past Twelve Months: Absorption spectra of age pigment *in situ* (microsections) and measurement of minute amounts of DNA made possible by construction of a recording microspectrophotometer.

d. Direction of Current Research: Measurement of ploidy variability in developing chick muscle cultures and of DNA variability/nucleus in young and old tissues.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: B. L. Strehler
November 4, 1959

Serial No. NMI-60

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on mathematical-physical expressions of population mortality.

b. Principal Investigators: B. L. Strehler (30% time)
A. S. Mildvan (40% time)

Other Investigators: None

Technical Assistance: M. Susanne Herman (25% time)

Objectives: To induce or deduce appropriate equations and assumptions to account for the shape of mortality curves.

Methods Employed: A theoretical and mathematical approach is used.

c. Progress During Past Twelve Months: The work has been essentially completed yielding a mathematical expression which makes correct predictions on a variety of physiologic and mortality data. See publication below.

d. Direction of Current Research: This work has been completed.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:
1. Strehler, B. L.: Origins and comparisons of the effects of time and high energy radiations on living systems. Quart. Rev. Biol., 34: (2), 117-142, 1959.

Prepared by: B. L. Strehler
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on the effects of temperature and other environmental factors on the aging process.

b. Principal Investigators: B. L. Strehler (15% time)

Other Investigators: None

Technical Assistance: M. Susanne Herman (75% time)

Objectives: To see what influence temperature and other factors have on the aging process in Drosophila.

Methods: Studies of the actuarial behavior of animals reared under controlled conditions and subjected to precise environmental stresses.

c. Progress During Past Twelve Months: It has been shown that Drosophila are not aged by short exposure to higher temperature (38.5°C./1 hr.). This indicates that aging in this organism is not due to a high activation energy process such as denaturation.

It has been shown that X-radiation doubles the longevity of D. melanogaster in doses up to 4500 R whereas D_2O in amounts from 20 to 40% cuts the lifespan in half. O_2 (100%) administration similarly shorten the life expectancy.

d. Direction of Current Research: Further studies of the aging of D. melanogaster by environmental factors is continuing.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: B. L. Strehler
November 4, 1959

Serial No. NHI-62

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Studies on the comparative physiology of senescence: Campanularia regression.

b. Principal Investigator: B. L. Strehler (15% of time)

Other Investigators: Matthew Pollack
Elizabeth Arnold

Technical Assistance: Malcolm Gee (30% of time)

Objectives: To determine source of Campanularia senescence.

Methods Employed: Manipulation of environment, electron microscopy, histochemistry, etc.

c. Progress During Past Twelve Months: Histological sections of Campanularia hydranths (young and old) were made and stained with Feulgen stain, Mallory's, H. and E., Pyronin and PAS reagents and examined for changes.

Electron microscopic sections were prepared and studied. Results of the above are inconclusive and will require further study.

Effect of X-radiation on senescence was studied as was effect of temperature on rate of aging. X-ray dosages up to 100,000 R did not appreciably shorten the lifespan of Campanularia. At low temperature (2.5°C.) the longevity was about 20 days as compared to 4-6 days at 19°C. Time lapse movies were taken of the regression process.

d. Direction of Current Research: More intensive histological examinations will be undertaken.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: B. L. Strehler
November 4, 1959

Serial No. NHI-63

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: The role of sulphydryl groups in muscle action.

b. Principal Investigators: J. J. Blum (40% time)

Technical Assistance: P. J. Buchanan

c. Progress During the Past Twelve Months: During the course of our study of the effect of parachloromercuribenzoate (PCMB) on the enzymatic activity of myosin A and myosin B, in which the SH groups on the protein play a key role, it was discovered that Zn^{++}, Cd^{++}, and Cu^{++} ions cause a pattern of acceleration and of inhibition of ATPase and of ITPase activity quite similar to that caused by PCMB. It has now been discovered that PCMB, Cu^{++}, Cd^{++}, and Zn^{++} interact with myosin in a time dependent fashion, the overall time course of the reaction being rather similar for all these substances, thus lending further support to the idea that myosin undergoes some conformation change when it interacts with its substrate, and that this hypothetical conformation change depends on the nature of the substituent at position 6 on the purine or pyrimidine ring of the nucleoside triphosphate. We have also demonstrated that the presence of ATP can prevent the interaction between PCMB and certain of the -SH groups on the myosin.

d. Direction of Current Research: We will try to quantitate the effect of ATP on those -SH groups which it protects by using radioactive N-Ethyl Maleimide to label the protein via its -SH groups. The kinetics of the interaction of Cu^{++}, Cd^{++}, and Zn^{++} with myosin A will be studied as a function of pH in an effort to assess the role of histidine groups in the enzymatic process. The interaction of these metals (and of PCMB) with myosin B will also be studied, with special attention to the superprecipitation phenomenon, where preliminary experiments have already shown that superprecipitation can be inhibited by certain concentrations of these metals provided that the myosin B is preincubated with the metal. We shall also seek to obtain direct physical evidence of conformation changes in this system, using viscosity, ultracentrifugation, and optical rotation.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: J. J. Blum
November 3, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Reaction time and electroencephalographic alpha frequency as functions of aging.

b. Principal Investigators: W. W. Surwillo (3/4 time)
M. D. Davidoff (1/5 time)

Technical Assistance: Sally Stram (1/8 time)
Jesse Yaffa (1/5 time until 8/31/57 when he retired)

Objectives: To investigate age decrement in simple reaction time with reference to age changes in the alpha frequency of the electroencephalogram. We hypothesize that the reaction-time differences between age groups can be accounted for, in part, by differences in the alpha frequencies observed between the two groups. We also hope to show that the frequently reported variability in simple reaction time, observed in the same individual over a group of trials, is a function of the alpha frequency present at the time of stimulation.

Methods Employed: As a consequence of the findings reported in last year's annual report, a number of changes in the experimental procedure described in that report have been instituted.

(1) An auditory stimulus has been substituted for the visual stimulus which was used in the original procedure. This increases considerably the amount of data which can be analyzed since with the subject's eyes closed a higher percentage of artifact-free recording is possible.

(2) The subject responds to the appearance of the auditory stimulus (a 250 cycle tone) by pressing a button with the thumb. This was substituted for the earlier procedure in which the subject responded by uttering a sound since we have reason to believe that, especially in some of the older subjects, considerable variability in the reaction times recorded was the result of transient changes in the respiratory system. In addition to recording the mechanical response consequent to pressing the button, we are also recording the muscle action potentials from the responding muscle. Since, even within the same subject, there appears to be considerable variability in the delay observed between the appearance of the muscle action potentials and the mechanical response, this measure may furnish us with an important variable in the evaluation of the role of alpha frequency in reaction time.

(3) In our last annual report, we noted that a portion of the variability observed in our reaction time measures appeared to be the result of changes in what we termed "central motive state". Since our second project is concerned with the determination of central motive state, we have added measures of central motive state to the present procedure. Hence, in addition to recording the electroencephalogram, reaction time, and muscle-action-potential response, we are recording simultaneously the amplitude of the spinal reflex at one second intervals and the instantaneous heart rate.

Analysis of the data will proceed according to the following scheme: Alpha waves in the period of time between stimulus and response are counted and the frequency of the alpha for each stimulus response pair is determined. Curves and correlations of reaction time vs. EEG frequency are obtained for each subject and for the group after the influence of the other variables on reaction time has been partialled out by appropriate procedures.

c. Progress During Past Twelve Months: Data collection could not be pursued for the first half of the year due to the disabling effect on our sensitive equipment of radio frequency interference. It required this time to diagnose and remedy this difficulty. Operations were resumed in July.

To date, 37 subjects of various ages have been tested. In addition, some pilot data has been obtained under conditions where we have attempted experimental manipulation of alpha frequency during the reaction time task by photic driving.

Analysis of these data has only been started.

d. Direction of Current Research: Testing will continue according to the above method. Emphasis will be placed in the coming period on analysis. Pending outcome of the analysis, plans are underway to determine the relation between alpha frequency and stimulus information in a choice reaction time task.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: W. W. Surwillo
November 2, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Choice reaction time as a function of aging.

b. Principal Investigators: Melvin Davidoff (1/5 time)
George Suci (1/3 time) (L.D. 8/31/59)

Other Investigators: None

Technical Assistance: Jesse Yaffa (2/5 time) (L.D. Sept. 1959)
Sally Stram (1/8 time)

Objectives: To investigate age decrement in reaction time with reference to stimulus complexity. In general terms, the hypothesis being tested is that age decrement increases as a function of increasing stimulus information.

Methods Employed: The subjects learn to name each of four symmetrically placed lights with one of four non-sense syllables. After achieving a certain learning criterion, the subject was to react to one light going off in each of a series of subsets of the four lights. The subsets consisted of combinations of 1, 2, 3, and all 4 lights. A series of 24 of each subset was presented. Reaction time to the light going off was measured by a 1/100 second timer which started with the presentation of the light going off and stopped when the subject uttered the appropriate non-sense syllable. The amount of information in bits was calculated for each subset of lights by finding $\log_2 x$, where $x = 1, 2, 3, 4$. The reaction times are averaged over the 24 presentations of each subset for each subject. Each subject produced, then, a relationship between information in bits and reaction time. These relations by inference from another study are supposedly linear, but this assumption needs to be tested. The prediction is that the older subjects produce linear regression but with a steeper slope than the younger; in other words, the older subjects take relatively longer to decide which light has come on when the possible number of alternatives increases.

The subjects used are subjects coming through the Branch's longitudinal studies who were college graduates and paid Johns Hopkins students.

c. Progress During Past Twelve Months: All our subjects have been run and the data collected and analyzed. Hypotheses were well substantiated. A paper reporting this study has been written.

One unanticipated result from the study was the fact that while the reaction times of the older subjects was longer all along the

line, they, on the average, learned the non-sense syllables faster than the younger subjects. We believe this to be due to imperfect matching between old and young subjects. This, however, does not add uncertainty to our reaction time results, since, if anything, this should contribute error in the conservative direction to the reaction time results.

d. Direction of Current Research: This project is completed unless some changes are indicated by the journal editors reviewing the report.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Melvin D. Davidoff
October 30, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: A study of central nervous system factors in aging deficit.

b. Principal Investigators: W. W. Surwillo (1/4 time)
Melvin D. Davidoff (1/10 time)

Technical Assistance: Sally Stram (1/4 time)

Objectives: The original aim of this project, as stated in the last annual report, was to investigate motor and sensory deficit in the framework of a study designed to provide data on central nervous system activity. Unfortunately, due to the practical circumstances of the continued lack of sufficient and adequate technical assistance (which was discussed in detail in the last annual report), we are now only concerned with the matter of motor deficit. Our present objective, therefore, is to determine the relation between motor deficit in aging and the activity level of the Brain Stem Reticular System.

Methods Employed: As a result of the lack of technical assistance, radical changes in the methods described in the previous annual report have been instituted.

(1) Pending a recently authorized accretion of personnel, measures of the galvanic phenomena of the skin as well as the control measures of skin temperature have been dropped.

(2) The project has, in terms of the collection of data, been combined with the project entitled "Reaction Time and Electroencephalographic Correlates as Functions of Aging". This procedure unfortunately precludes the possibility of any systematic investigation of the activity level of the Brain Stem Reticular System (BSRS) in sleep since the amount of subject time required to collect data for both projects during the same testing session is not available. Fortunately, however, a few of the more relaxed subjects do fall asleep for brief periods of time so we may have some data on this question.

The present method, then, is to record the amplitude of the spinal reflex (elicited by tapping the Achilles tendon) at one second intervals while the subject is occupied with the task of responding to the appearance of an auditory stimulus by pressing a button. Since the testing session is spread over a period of one and one-half hours, and the reaction times recorded prove to be quite variable, we have reason to believe that the subjects

display a wide range of central motive states throughout the session. Near the end of the testing session an attempt is made to raise this level by requesting the subjects to put forth additional effort in the attempt to produce shorter responses. The rationale behind the use of the spinal reflex as a measure of central motive state and the activity level of the BSRS was presented in the last annual report.

In addition to the spinal reflex, instantaneous heart rate is also recorded. Inclusion of this measure stems from some early pilot work carried out in conjunction with the determination of the activity level of the BSRS in sleep. Since (in order to study sleep as an experimental condition) we needed some indicator of the depth of sleep, we chose instantaneous heart rate as an indicator worth exploring. Simultaneous recording of the spinal reflex and instantaneous heart rate revealed the presence of a strong inverse relation between the two measures. Suspecting that the heart rate measure might assist in evaluating the relation of the spinal reflex to reaction time and motor deficit, this measure has been recorded as a companion to all recording of spinal reflex.

c. Progress During Past Twelve Months: Progress on this project was halted between November 1958 and July 1959 as a result of the same difficulty with the equipment referred to in Project No. NHI-98; i.e., the appearance of the radio frequency artifact in the recording equipment.

To date, 37 subjects of various ages have been tested. Analysis of these data has only been started.

d. Direction of Current Research: Testing will continue according to the above plan. Emphasis will be placed in the coming period on analysis of the data.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: W. W. Surwillo
November 2, 1959

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Serial No. NMI-67

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Care of patients housed on Ward B-2 (60 beds) who participate in studies performed in this unit. In addition, all males acceptable for admission to Baltimore City Hospitals Infirmary receive medical screening and treatment.

   b. Principal Investigators: Theodore Lundy (1/1/59 - 6/30/59)
   Dyrel Faulstick (7/1/59 - 12/31/59)
   (1/2 time)

   Other Investigator: Joseph A. Falzone

   Technical Assistance: Baltimore City Hospitals Clinical Laboratories

   c. Progress During Past Twelve Months: Assigned July 1, 1959 through December 31, 1959

   d. Direction of Current Research: Stated above.

2. Patient Days: Not applicable.

3. Collaborators: Baltimore City Hospitals staff and clinical facilities.

4. Publications and Awards: None.

Prepared by: Dyrel Faulstick
November 2, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age studies of cell particulates and fractions.

b. Principal Investigators: J. A. Falzone, Jr. (1/4 time)

Other Investigators: C. H. Barrows, Jr.
N. W. Shock

Technical Assistance: Wilbert Parson (3/4 time)

Objectives: This project is designed to measure age changes in the morphology, chemistry, and function of separable protoplasmic particles. The working hypothesis of these studies is that overall cell function may be well maintained until the moment of cell death by virtue of compensatory adjustments in the activities of its parts.

Methods Employed: Animals used will generally be the same as for Project No. 65; i.e., young adult and old rats aged 12 and 24 months, respectively. The basic technique is that of differential centrifugation of tissue homogenates at 0°-4°C. using an ordinary or ultracentrifuge depending upon particle size. Other techniques required by special problems are indicated below.

c. Progress During Past Twelve Months:

Nuclear Studies: Protein depletion has been reported to cause a 20% increase in mean DNA per nucleus (MDN) of rat liver. We could not confirm this. Controls: N = 8; MDN = 11.4 µg. Rats depleted 3 weeks: N = 8; MDN = 11.7 µg.

Mitochondrial Studies: Data from 23 rats derived from counts, optical density and protein nitrogen determinations on mitochondrial suspensions suggest that kidney mitochondria are swollen relative to those of liver. Measurements from published electron micrographs suggest that kidney mitochondria are longer than liver mitochondria *in situ*. Methods are being tested for a more extensive study of age differences in oxidative phosphorylation.

Miscellaneous: A simplified density gradient generator (no moving parts) has been constructed which gives results with liver nuclei equal to those of the more cumbersome mechanical device described earlier.

Attempts to isolate whole liver cells for metabolic studies have been partially successful. Very clean preparations have been obtained but yields do not exceed 30%. We have not used chelating agents.

Routine histological methods have been set up and are functioning well.

d. Direction of Current Research:
(1) Completion of mitochondrial study.
(2) Improvement of whole cell yield.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: J. A. Falzone, Jr.
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Thyroid function and age.

b. Principal Investigator: G. W. Gaffney (transferred to Bureau of State Services - 9/1/59)

Other Investigators: N. W. Shock
R. I. Gregerman
M. J. Yiengst

Technical Assistance: S. E. Crowder

c. Progress During Past Twelve Months: Data collected on age differences in (a) the rate of uptake of I^{131} by the thyroid gland, (b) level of protein bound iodine in the blood and (c) rate of disappearance of I^{131} tagged thyroxine from the blood have been statistically analyzed.

d. Direction of Current Research: Work has been completed on this project.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Baker, S. P., G. W. Gaffney, N. W. Shock, and M. Landowne: Physiological responses of five middle-aged and elderly men to repeated administration of thyroid stimulating hormone (thyrotropin; TSH). J. Geront., 14: (1), 37-47, 1959.

2. Gaffney, G. W., D. M. Watkin, and B. F. Chow: Vitamin B_{12} absorption: relationship between oral administration and urinary excretion of cobalt^{60}-labeled cyanocobalamin following a parenteral dose. J. Lab. clin. Med., 53: (4), 525-534, Apr. 1959.

Prepared by: N. W. Shock
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Verbal performance as a function of aging.

b. Principal Investigators: Melvin Davidoff (1/10 time)
George Suci (1/3 time) (L.D. 8/31/59)

Other Investigators: None

Technical Assistance: Sally Stram (1/4 time)
Jesse Yaffa (1/5 time) (L.D. Sept. 1959)

Objectives: The study of verbal skill has been somewhat neglected relative to other skills; e.g., motor. It has been our hope that this neglect would be modified and our belief that the study of verbal performance will lead to significant findings regarding age differences in a variety of psychological processes. Our intention has therefore been to study the relationships of selected parameters of verbal behavior to aging, and to eventually relate these parameters to other psychological processes such as communication between and with the aged. Two parameters of verbal behavior have been selected for preliminary study--encoding ability and meanings of age-relevant concepts.

(1) With respect to encoding, the following hypothesis will be tested: Errors and time of encoding increase as a function of age. This hypothesis is simply an extension of what has been found with other skills and performances. We wish to see if these findings generalize to verbal performance.

(2) With respect to meanings, the study will be descriptive; i.e., we will simply describe differences in concept meanings which exist to a significant degree between age groups.

Methods Employed: (1) This area of study has been discontinued due to lack of time during part of the year and then the resignation of Dr. Suci.

(2) The semantic differential will be employed to measure the meanings of certain concepts. Examples of possible concepts are DEATH, OLD MAN, ADOLESCENT, etc. These concepts were to be rated on seven-point scales defined by adjective-opposites; e.g., good-bad, active-passive, strong-weak. These scales were chosen on the basis of extensive research previously done at the University of Illinois. Preliminary testing in 1958 has lead to changes in the scales employed in the present study.

c. Progress During Past Twelve Months: (1) This project was discontinued.

(2) Most of the data for this part of the project has been collected. The major part of the analysis of the data was contracted out to be accomplished on the electronic computer maintained by the University of Illinois. This part of the analysis is completed. The remainder of the analysis and write-up of a report remains to be done.

d. Direction of Current Research: Analysis of the data is to be completed and the reports will then be written.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Melvin D. Davidoff
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Memory functions as related to age.

   b. Principal Investigators: Melvin D. Davidoff (2/5 time)
   George J. Suci (1/3 time) (L.D. 8/31/59)

   Other Investigators: None

   Technical Assistance: Sally Stram (1/4 time)
   Jesse Yaffa (1/5 time) (L.D. Sept. 1959)

Objectives: To study memory for different types of material and sense modalities as a function of aging. Two hypotheses are presently under investigation: (1) Aged subjects' short span memories are more susceptible to interference than younger subjects.

(2) The retention of verbal sequences is a function of sequence length, redundancy in the sequence and age.

Methods Employed: (1) The amount of interference as measured by anchoring effect on judgments of sizes of squares was to be observed for subjects of different ages. Subjects learned to judge the relative size of a set of five cardboard squares to a certain criterion of learning. The experimental group was then asked to judge the size of another set of five squares, each of which was larger than any of the squares in the first set. They were then asked again to judge the relative size of the first set of squares. The control group was not exposed to the "interfering" set. Instead, there was merely a conversational recess between the first and second presentation of the original set of cards, this recess being equivalent in time to the average time taken for second set presentation in the experimental group. The variable measured in this study is number of trials to the learning criterion. This study, involving 96 subjects, gave unequivocally positive results. The subjects studied, it should be noted here, were all people with less than a complete high school education.

(2) Word lists, ten, fifteen, twenty and twenty-five words in length, and of varying redundancy (such that each word occurs independently of the last in the list, two words are dependent but independent of the next pair, and so on through seven word dependency) were constructed. Each list was read to the subject at the rate of one word per second. The subject was asked to repeat as many words as he could remember. Scoring is in terms of the number of words recalled correctly. Analysis is in terms

of the interactions and primary effects of the variables of age, length and redundancy. Appropriate controls re possible effect of order of presentation of the word lists are incorporated in the study.

c. Progress During Past Twelve Months: (1) A "rough" and incomplete report of this study to date has been drawn up and is now due to be gone over and expanded for publication. At the same time, however, we have gone on to extend the area of this study along two lines. (a) Investigate the question of whether this interference difference with age in the judgment of card size will manifest itself in a sample of better educated people. (b) Whether this phenomenon will manifest itself in a quite different perceptual process. For test purposes, we have selected the estimation of time intervals. In regard to (a), the five card judgment task seemed too easy for this sample and we switched to six cards in the original set. The interference effect seems to be manifesting itself but not at all with the extreme clarity of the earlier study. However, we consider the present work to be preliminary and the study may yet have more changes made in it. In regard to (b), the results are so far not at all positive.

(2) Data collection in this study was completed this year. Scoring of the material and most of the work on analysis has been completed.

d. Direction of Current Research: (1) We will continue to collect data for a time along present lines. The situation will then be evaluated as to advisability for possible changes in the procedure. Changes would be most likely in stimulus magnitude and/or number. At the same time, we expect to have a manuscript covering the original study ready for publication before too long.

(2) Analysis is to be completed and a report prepared.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Melvin D. Davidoff
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age changes in cellular and tissue biochemistry.

b. Principal Investigator: C. H. Barrows, Jr. (3/4 time)

Other Investigators: N. W. Shock
J. A. Falzone, Jr.

Technical Assistance: Lois Roeder (3/4 time)
Frances Doran (L. D. 9/18/59) (3/4 time)
McKinley Brown (E.O.D. 9/17/59)

Objectives: The general purpose of this program is to examine various tissues of rats for changes associated with senescence in tissue and cellular metabolism. Specific problems investigated during this period have been:

(1) Enzymatic concentrations and age.
(2) Age differences in enzymatic activities during protein depletion and repletion.
(3) Studies on oxidative phosphorylation in mitochondria isolated from liver.

Methods Employed: (1) Various enzymatic activities as well as DNA and protein nitrogen of liver, kidney and heart of 1, 3.5, 12 and 24 month old rats (10 rats per age group) were determined by accepted standard procedures.

(2) Three additional groups (10 animals per group) of the 3.5, 12 and 24 month old rats were fed a protein-free diet for 21 days. One group at each age level was sacrificed (a) after the depletion period, (b) after three days of repletion (ad lib. feeding of our stock diet), and (c) after seven days of repletion. The tissues were analyzed as described above.

(3) In the studies on oxidative phosphorylation, two methods were employed. In the first, all components of the system were in the main compartment of the Warburg flask and the reaction was initiated by placing the vessel in the constant temperature bath. The rates for the first six minutes were therefore extrapolated from subsequent measurements. In the second method, glucose-hexokinase was placed in the sidearm and various times of equilibration were tested before the reaction was started by tipping the glucose-hexokinase into the main compartment.

c. Progress During Past Twelve Months: (1) Our attempt to classify individual enzymes into groups which follow similar age changes has not been completely successful. Furthermore, these data do not indicate that the same enzyme in different organs will behave in a similar manner at a given age. Finally, although changes were apparent between the ages of 1 and 3.5 months (growth changes), all but three comparisons did not vary by more than 10% between the ages of 3.5 and 24 months. The most notable exception was the increased catheptic activity of both liver and kidney in the aged (24 month old) as compared to the adult (12 month old) animal. This change may be interpreted as one associated with senescence.

(2) These data offer no evidence for the existence of an impaired protein synthesis in senescent rats and indicate only slight differences over the span of 3.5 to 24 months.

(3) Current studies on oxidative phosphorylation, employing the first described method, have shown (a) the rate of reaction is not linear with time, and (b) the rate of phosphorylation falls more rapidly than the rate of oxidation, resulting in a high P/O in the first six minutes. The high P/O may infer (a) that there is another pathway of phosphorylation which is heretofore unknown or (b) an inability to measure accurately the oxygen uptake during the early phase of the reaction. Varying a number of experimental conditions, such as the exclusion of substrate, glucose-hexokinase or oxygen, or the introduction of 2,4-dinitrophenol has failed to provide evidence that an unknown pathway is available. Moreover, using the second method and increasing the equilibration time up to 10 minutes prior to initiating the reaction has failed to indicate that the original observation was due to errors in oxygen uptake measurement. Studies are now in progress employing β-(OH) butyrate in which both the oxygen uptake and the acetoacetate formation are measured.

d. Direction of Current Research: (1) Studies by McCay showed that animals fed restricted diets have greater longevity than those fed diets which support optimum growth. It may be assumed that there are biochemical differences in the tissues of these two groups of animals which have resulted in the differences in longevity. Future experiments will include feeding animals various diets, which are believed to result in biochemical differences within the tissues, with the ultimate goal of determining the effectiveness of the diets in increasing longevity or maintaining youthful vigor in the senescent rat.

(2) Studies on oxidative phosphorylation of mitochondria isolated from liver will be continued until an adequate

method for the measurement of the rates and efficiency in animals of different ages can be obtained.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Falkone, J. A., Jr., C. H. Barrows, Jr., and N. W. Shock: Age and polyploidy of rat liver nuclei as measured by volume and DNA content. J. Geront., 14: (1), 2-8, 1959.

2. Viengst, M. J., C. H. Barrows, Jr., and N. W. Shock: Age changes in the chemical composition of muscle and liver in the rat. J. Geront., 14: (4), 400-404, 1959.

3. Andrew, W., N. W. Shock, C. H. Barrows, Jr., and M. J. Viengst: Correlation of age changes in histological and chemical characteristics in some tissues of the rat. J. Geront., 14: (4), 405-414, 1959.

4. Barrows, C. H., Jr., and B. F. Chow: Dietary proteins and synthesis of tissue proteins. Chap. 6, in: A. A. Albanese (Editor), Protein and Amino Acid Nutrition. Academic Press, New York, 1959, pp. 117-142.

5. Barrows, C. H., Jr., and B. F. Chow: Studies on enzymes in arterial tissue. Chap. 7, in: A. I. Lansing (Editor), The Arterial Wall; Aging, Structure and Chemistry. Williams & Wilkins Co., Baltimore, 1959, pp. 182-206.

6. Gregerman, R. I.: Adaptive enzyme responses in the senescent rat: tryptophan peroxidase and tyrosine transaminase. Amer. J. Physiol., 197: (1), 63-64, 1959.

Prepared by: C. H. Barrows, Jr.
November 4, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age changes in human performance.

b. Principal Investigators: A. H. Norris (1/4 time)
J. A. Falzone, Jr. (1/4 time)

Other Investigators: N. W. Shock
Felix Hügin

Technical Assistance: J. B. Melvin (2/3 time)
E. E. Howard
Edna Phillips (2/3 time)
M. F. Moody (2/3 time)
L. D. Ward

Objectives: This project is designed to study the effects of aging on (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations in performance observed in older people will be evaluated.

Methods Employed: Measured amounts of physical work are obtained in 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 cardiac output (by the dye method) are taken before, during and after standardized amounts of exercise. Each experiment involves analysis of 3-8 samples of expired air for standardization of automatic gas analyzers. Other studies include measurements of speed of nerve conduction, reflex delay time, and muscle action potentials. These phenomena are recorded on a six channel oscillograph or dual beam oscilloscope as the experiment demands.

c. Progress During Past Twelve Months: Measurements of reaction to touch of the foot and plantar flexor and superficial abdominal reflex times have been extended to larger numbers of subjects. Touch reaction times stimulated and recorded over the small muscles of the foot were increased significantly ($P = < .001$) with increase in age for subjects 20 through 92 years. Children 3-14 years old also showed significantly ($P = < .005$) increased touch reaction times. This measurement was made successfully in 111 of 128 subjects attempted.

Age differences were not demonstrated, however, for reflex times recorded from the small muscles of the foot, whether a brief scratch or a long scratch was used to stimulate the sole of the foot. Moreover, the long scratch results were characterized by much greater variability than the short scratch results. Both of these techniques gave adequate results in 65% of the subjects attempted. Short scratch stimulation of the abdomen provided reflex latencies which were shorter than similar latencies recorded from the foot and showed no age differences.

Exercise screening procedures have been initiated for subjects living outside the hospitals. In these tests muscle strength is compared with maximum cranking ability and high-level work output as has been done for hospital patients.

Studies of limb mechanics have been continued with the recent initiation of a series of tests designed to demonstrate the effects of loading the arm during rapid swinging.

d. Direction of Current Research: Measurements of foot reaction times and reflexes will be extended to subjects of a higher socio-economic level than have been tested and will be compared to nerve conduction velocities recorded in the same subjects.

An attempt will be made to find an index of mechanical efficiency for the "arm-wagging" exercise. Measurement of the effects of loading the arm will be continued.

The exercise screening and mechanical efficiency studies will be continued for the purpose of comparing subjects living outside the hospital with hospital inhabitants.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

Dr. Robert W. Ramsey, Medical College of Virginia, Richmond, Virginia

Dr. John Magladery, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

Dr. Robert D. Teasdall, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

4. Publications and Awards:

1. Norris, A. H. and N. W. Shock: Age changes in ventilatory and metabolic responses to submaximal exercise. In: 4th Congress of the International Association of Gerontology, Merano, Italy, July 14-19, 1957. Tito Mattioli, Fidenza, 1957, Vol. II, pp. 512-522.

Prepared by: A. H. Norris
November 7, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Age differences in body size and composition.

b. Principal Investigators: T. H. Lundy
A. H. Norris (1/4 time)

Other Investigators: N. W. Shock
M. J. Yiengst

Technical Assistance: C. J. Manaras

Objectives: This project is designed to describe age differences in body size and composition, to compare various size and composition measures made concurrently in individual subjects, and to examine the relationship of these age differences and comparisons to physiological responses.

Methods Employed: Height and weight data will be obtained by the usual anthropometric methods. The volume of the body will be measured by its displacement of helium in a closed chamber. The density of the body will be calculated from these measures after correction for total skeletal mass estimated from roentgenographic films of the humerus. Body fat will also be estimated from skinfold thickness and roentgenographic techniques. Estimates of lean body mass will include (1) basal metabolic rate determinations made with standard open circuit methods, (2) urinary excretion of creatinine, and (3) body water and fluid distribution determinations made from the distribution curves of injected antipyrine and sodium thiocyanate. This is a continuing program. These studies will be carried out in people of different ages and in the same people as they become older.

c. Progress During Past Twelve Months: Since January eighty additional subjects have had body composition studies, bringing the total of outpatient subject studies to 110 subjects, to date. In the initial 26 subjects in whom reliable estimates of body density were obtained, density values ranged from 0.991 to 1.064. When lean body mass was calculated from density and compared with lean body mass calculated from total body water (antipyrine distribution space), a correlation coefficient (r) of 0.828 was obtained. This may be compared to a correlation (r = 0.842) between lean body mass calculated from similarly determined densities and lean body mass calculated from total body water (tritium distribution space) reported by Behnke and Siri (1957) for a group containing

fewer older subjects than the present sample. Preliminary results indicate that peak obesity (percent body fat calculated from body water studies) occurs during the 5th decade in subjects living outside the hospital and in the 8th decade of life in subjects living in the hospital. However, both groups showed a decline in calculated lean body mass. The total amount of creatinine excreted in 16 hours was found to be correlated (r = 0.75) with calculated lean body mass.

We are continuing to introduce improvements in technique which are designed to increase reliability and day to day stability of the body volume determinations.

d. Direction of Current Research: The integrated measurement program which includes selected indices of body size and composition will be continued. Some techniques are provided through cooperation of investigators outside of the Public Health Service while others are standardized procedures used in this laboratory. Data are available for comparison with other physiological data which may be collected on subjects of these studies. Moreover, subjects whose size or composition varies widely from mean values will be selected for study, and experimental and therapeutic displacements of body composition may be attempted.

2. Patient Days: Not applicable

3. Collaborators: The Baltimore City Hospitals

Dr. Harald Schraer, The Bone Density Research and Evaluation Center, Pennsylvania State University, University Park, Pennsylvania

Dr. Stanley M. Garn, Fels Research Institute, Antioch College, Yellow Springs, Ohio

Dr. Saul P. Baker, Department of Medicine, Chicago Medical School, Chicago, Illinois

4. Publications and Awards: None

Prepared by: Theodore Lundy
November 2, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Pulmonary physiology as related to age.

b. Principal Investigator: Arthur H. Norris (1/4 time)

Other Investigators: Joseph A. Falzone, Jr.
David A. Cursler
Sister M. Stanislaus Huddleston
(July-September 1959)

Technical Assistance: John B. Melvin (1/3 time)
Mae F. Moody (1/3 time)

Objectives: This project is designed to describe age differences in pulmonary function. These studies involve measurements of the volumes of the lung compartments and functional capacities of the pulmonary system, including the mechanical aspects of bellows function and the responsiveness of the pulmonary system to experimental stimulation.

Methods Employed: Lung volumes are measured with standard methods including a helium washout technique which is used to estimate functional residual capacity. Lung volumes and functional capacities are compared with anthropometric and roentgenographic measurements of the chest. In addition, laboratory measurements of pulmonary function are compared with physiological responses to exercise and with clinical estimates of pulmonary and work performance limitations of older subjects.

This is a continuing program. Not only will subjects of different ages be compared, but individuals who are available will be measured at intervals of three to seven years.

c. Progress During Past Twelve Months: Since January 1959, subjects living outside of a hospital environment have been participating in these studies. When 83 of these subjects between 20 and 79 years were compared with subjects living in the old people's home of the hospital, they were found to have similar total lung capacities when this measure is expressed as a fraction of body size (TLC per square meter of body surface area). However, age group for age group they have smaller residual volumes and larger vital capacity and maximal breathing capacity than do hospital inhabitants. Indeed, the average maximal breathing capacity of the 70-79 year age group is similar to values previously reported for 40-50 year old subjects.

Lung areas have been measured from 17x24 inch posterior-anterior X-rays of the chest in 99 hospital subjects between 50 and 89 years of age. Both inspiratory and expiratory X-ray lung areas showed a significant ($P = < .001$) decrease with increase in age for both right lungs and left lungs.

Two estimates of the oxygen cost of ventilation have been compared for 13 subjects from 21 to 79 years of age. When ventilation was increased by having the subject breathe through a 1.5 L. dead space, interpretable records of oxygen uptake were obtained for 6 of the 13 subjects. When subjects voluntarily increased ventilation, interpretable records of oxygen uptake were obtained for 11 of the 13 subjects. Although the values of extra oxygen associated with the increased work of breathing divided by the extra air ventilated during the 5 minute test period varied widely (from 1.72 to 14.81 cc./L.), the voluntary overventilation technique proved more satisfactory in our hands.

d. Direction of Current Research: Estimates of the oxygen cost of lung ventilation will be continued in conjunction with exercise efficiency estimates in an attempt to provide better estimates of mechanical efficiency in old and young subjects. Comparisons of hospitalized vs. non-hospitalized subjects will be continued. Anthropometric and X-ray measurements will be compared with lung volume estimates made on the same subjects.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

Sister M. Stanislaus Huddleston, Assistant Professor of Biology, Marygrove College, Detroit, Michigan, was an American Physiological Society Research Fellow.

4. Publications and Awards:

1. Safar, P., L. Aguto-Escarraga, L. Drawdy, M. C. McMahon, A. H. Norris, and J. Redding: The resuscitation dilemma. Anesthesia and Analgesia, 38: (5), 394-405, 1959.

2. Safar, P., L. Aguto-Escarraga, L. Drawdy, M. C. McMahon, A. H. Norris, and J. Redding: Wiederbelebung II. Methoden der Mund-zu-Mund Beatmung. Der Anaesthesist, 8: 231-235, 1959.

Prepared by: A. H. Norris
November 7, 1959

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Longitudinal studies of human physiology and biochemistry.

b. Principal Investigators: N. W. Shock (Director)
A. H. Norris (Coordinator - 1/4 time)
J. A. Falzone, Jr. (Clinical Director - 1/4 time)

Other Investigators: M. D. Davidoff
M. J. Yiengst
G. J. Suci
W. W. Surwillo
Jerome Lowenstein
T. H. Lundy
A. S. Mildvan
Dyrol Faulstick
G. W. Gaffney
D. A. Cureler

Technical Assistance: Dora Goldblatt
Edna Phillips (1/3 time)
Llewellyn Perkins

Objectives: This project is designed to (1) facilitate repeated measurements of various physiological, biochemical and psychological variables in the same individuals as they grow older, (2) coordinate the utilization of the results of these measurements in establishing indices of aging and (3) compare the effects of socio-economic status on these results and the indices which may be derived therefrom.

Methods Employed: Participants are recruited by other participants. They agree to be available for a three day series of tests every 18 months for the rest of their lives (agreement in principle - there are no binding articles). A given series of tests will be repeated at a five year interval. Measurements of body size and composition, pulmonary function, kidney function, psychological function, neuromuscular function and cardiovascular function are made. Physical examinations and medical histories are performed with check lists to assure uniformity of recording. Reports of medical findings and pertinent test results are made to a participant's private physician only. Participants are treated as a group (name - Six S's which stands for Select Society of Seeking Scientists Saints and Sinners) and kept informed of the progress of the

total program and of test results for the group. Data are kept in restricted files and are summarized by calculating machine methods.

c. Progress During Past Twelve Months: The initial series of tests consisting of measurement of body composition and psychological, kidney, pulmonary and cardiovascular functions have been performed for over 100 subjects. A second series of tests has been initiated which consists of tests of kidney, neuromuscular, psychological and pulmonary function, as well as tests of response to exercise. We have demonstrated some differences between our subjects who live in the hospital and our participants who live outside institutions. The group who live at home have been found to be, on the average, 3 cm. taller than our hospital inhabitants while their average weight was similar to the average weight of subjects of the Baltimore Chronic Disease Survey (noninstitutionalized sample). The average weight of our hospital subjects was much lower than weights for either of these groups. Despite these marked differences in size, average ratios for total body water (antipyrine space) and extra cellular water (thiocyanate space) were similar when expressed as a fraction of body weight. Indeed, total lung capacity, which was larger for subjects living outside the hospital, was similar to that of hospital subjects when capacities of both groups were expressed as a fraction of body surface area. However, similarly expressed residual volume of the lungs was higher and vital capacity and maximal breathing capacity of the lungs were lower in hospital patients than in our subjects who live in their own homes. Basal heat production expressed as calories per square meter per hour was similar for the two groups of subjects.

d. Direction of Current Research: The present testing program will be continued. Results will be summarized and evaluated as they become available. In addition, methods of data handling are being examined to determine what will be appropriate and efficient for these studies.

2. Patient Days: Not applicable

3. Collaborators: The Baltimore City Hospitals

4. Publications and Awards:

1. Dack, S. (Moderator), M. Landowne, A. A. Luisada, J. H. Bland, and R. Harris (Panelists): Panel discussion on problems in the diagnosis and management of cardiovascular disease. J. Amer. Geriat. Soc., 7: (3), 221-239, 1959.

2. Shock, N. W.: Retrospect and prospect in the biological aspects of aging. Newsletter (Geront. Soc.), 6: (1), 3-5, 1959.

Prepared by: A. H. Norris
November 7, 1959

Serial No. NHI-77

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Structure of hemoproteins.

b. Principal Investigator: Gunther L. Eichhorn (1/3 time)

Other Investigator: Wayne W. Everett (1/3 time)

Technical Assistance: Albert J. Ochahr (1/2 time)
Mary Ann Stevan (2/3 time - E.O.D. 6/2/59)

Objectives:
(a) To elucidate the nature of the relationship between protein and prosthetic groups in such compounds as hemoglobin, cytochrome -C, etc.

(b) To evaluate the relationship between the structures of these compounds and their rotatory dispersion curves.

Methods Employed: Samples of various biologically important hemoproteins are prepared, and their absorption spectra and rotatory dispersion curves are determined. If the rotatory dispersion is anomalous in regions where the heme absorbs, the heme group must be an asymmetric center. It is then possible to subject the molecules to various chemical stresses, and to determine the effect upon the asymmetric center.

c. Progress During Past Twelve Months: The rotatory dispersion curve of catalase at pH 7 exhibits a Cotton effect, but at pH 11 no anomalous dispersion is observed. These experiments indicate that in the quarter molecules of catalase present at pH 11 the heme is symmetrically placed in the protein cavity, but that in the intact catalase molecules (which contain four hemes) present at pH 7 this symmetry is destroyed.

The heme has been removed from catalase, producing the apocatalase protein. This apoenzyme reacts with alkaline hemin to reform a substance which appears to be similar to the original catalase.

Further work on the rotatory dispersion of horse hemoglobin indicates that the splitting of the hemoglobin in acid and basic solutions occurs along two different axes. Optical rotation experiments with human hemoglobin in various modifications have given results similar to those previously reported for horse hemoglobin.

To obtain further insight into the relationship between the rotatory dispersion of hemoproteins and their structure, vitamin B_{12} was selected as a model for hemoproteins, because its structure is completely known, and because simple chemical changes in it are easily effected. Vitamin B_{12}, like the hemoproteins, exhibits a Cotton effect. The rotatory dispersion is not affected by the substitution of other ligands for the cyanide coordinated to the cobalt; it is, surprisingly, only slightly changed by reduction of the cobalt (which has a profound effect upon the spectrum). Only chemical changes which drastically change the shape of the vitamin B_{12} molecule have been found to result in significant changes in the rotation.

d. Direction of Current Research: The effects of chemical changes in hemoglobin and catalase will be pursued further, and extended to peroxidase and chlorophyll. Correlation with sedimentation behavior will be attempted in some cases.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Gunther L. Eichhorn
November 4, 1959

Serial No. NHI-78

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: Structure of nucleic acids.

b. Principal Investigator: Gunther L. Eichhorn (1/3 time)

Technical Assistance: Barbara Randall (E.O.D. 6/8/59 - 1/3 time)
Mary Ann Stevan (E.O.D. 6/2/59 - 1/6 time)

Objectives: (1) To find a method of determining terminal groups in polynucleotides; (2) To introduce a new technique for sequence studies in nucleic acids; and (3) To study the reaction of polynucleotides with the metal complexes of hydroxyaldehydes.

Methods Employed: Three of the four nucleotide bases of DNA and RNA have amino groups, which can be made to react with salicylaldehyde and metal ions to form complexes of widely varying stabilities. It is hoped that this reaction will distinguish between the four bases at terminal positions of a polynucleotide.

Varying quantities of metal ions and salicylaldehyde will be reacted with the nucleic acids; it is hoped that this reaction will create weak links in the polynucleotide chain; subsequent hydrolysis will then cleave the nucleic acid at specific points, in a manner resembling that of proteolytic enzymes.

Organic dialdehydes will be constructed with their aldehyde functions the correct distance apart for reaction with both amino groups of any given sequence in polynucleotides. Hydrolysis of the polynucleotides will then reveal the sequences that actually exist; correlation of the resulting information with enzymatic sequence studies should produce much longer nucleotide sequences than any that have been obtained so far.

c. Progress During Past Twelve Months: Cobalt complexes have been prepared of the reaction products of salicylaldehyde with adenine, cytosine, guanine, thymine, uracil, as well as with each of the corresponding nucleosides and the nucleotides. The reaction distinguishes between the bases, nucleosides, and nucleotides in a manner that should make it useful in qualitative and quantitative analytical schemes.

The reaction product with Nutritional Biochemical DNA resembles that with adenine.

Analogous reactions with nickel do not produce the differentiations noted with cobalt, but they do distinguish between adenine and the other nucleoside bases.

Techniques are being developed for the chromatography of the products from the cleaved polynucleotides.

d. Direction of Current Research: First, the chromatographic scheme for the separation of cleavage products will be completed. Their various polynucleotides will be subjected to the cobalt-salicylaldehyde reaction, under various conditions, to determine whether end groups can be made to react preferentially. The same compounds will be treated with nickel and salicylaldehyde, to determine if it is possible to utilize this reagent as a cleavage agent that is specific for linkages with adenine.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Gunther L. Eichhorn
November 4, 1959

Serial No. NHI-79

GERONTOLOGY BRANCH

Project Report

January 1959 - December 1959

1. a. Title: The function of metal ions in enzymatic reactions.

b. Principal Investigator: Gunther L. Eichhorn (1/3 time)

Technical Assistance: Albert J. Osbahr (1/2 time)
Mary Ann Stevan (E.O.D. 6/2/59 - 1/6 time)

Objectives: To arrive at an understanding of the mechanism by which metal ions participate in enzymatic reactions, and also to determine why different metal ions are required for different enzymatic processes.

Methods Employed: It is assumed that in any metal-catalyzed enzymatic process the metal ion must serve either (1) as an active site, by direct attachment to the substrate, or (2) it must react with the enzyme protein, and thus alter the secondary and tertiary structure of the latter, producing the active configuration. It is postulated that, whenever scheme (1) applies, the metal should be capable of bringing about a non-enzymatic reaction; failure of the non-enzymatic reaction, inversely, would favor scheme (2).

Specially selected enzymatic substrates are treated with metal ions under varying conditions, and the nature of the metal-substrate interactions detected by physical measurements, e.g., spectrophotometry. The interaction of the metals with the enzyme protein is studied by macromolecular techniques, such as ultracentrifugation.

c. Progress During Past Twelve Months: It has been found that various metal ions interact with the substrates of the enolase, aconitase, and aspartase enzymes to form similar complexes. These fall into two classes that can be described, in terms of the ligand field theory, as strong or weak field complexes. With some metal ions, e.g., chromium (III), both initial and product forms of the complex belong to the same class, whereas other metal ions, e.g., nickel (II), differentiate strongly between the initial and product forms.

Evaluation of the kinetics of the reaction of iron (II) with the aconitase substrates indicates that the aconitase reaction proceeds in the absence of enzymes but at tremendously lower rates.

Studies on the kinetics of the reaction between nickel ion, salicylaldehyde, and sarcosine revealed that no complexes are produced that are comparable to intermediates in the reaction with amino acids. The mechanism of Schiff base complex formation, such as occurs during transamination, thus does not involve a hydrated intermediate.

Experiments on the effect of metal ions on the shape of enzyme molecules, e.g., arginase, are discussed in a separate report.

d. Direction of Current Research: The studies on the reaction of metals with the enolase and aconitase substrates are being continued, and the effect of metals on enolase will be studied by ultracentrifugation and optical rotation techniques.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by: Gunther L. Eichhorn
November 4, 1959

Serial No. ZHI-80
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of Means for the Continuous Recording of pO_2 and pH in Flowing Blood.

Principal Investigator: Zena Taylor McCallum

Other Investigators: None

Technician: Carrie Scott

Cooperating Units: None

Man Years (calendar year 1959) Patient Days: None

Total:	.75
Professional:	.50
Other:	.25

Project Description:

Objectives - Methods Employed: The work over the past two years by Mrs. McCallum and Mrs. Scott on the adaptation of the Clark electrode for the continuous measurement of pO_2 in a stream of blood conducted across this electrode has been brought to the level of practical experimental use. It is now possible as a result of this development to continuously record the arterial and venous pO_2, arterial and venous pH, and the arteriovenous O_2 difference (Guyton) across a lower extremity before and during periods of exercise stimulated by electrical stimulation. In order to provide for the intra-experimental calibration of the pO_2 electrodes, a set of rotating disc oxygenators, brought into equilibrium with gases of known pO_2 and self contained in a constant temperature water bath, is now completed. It is thus possible not only to calibrate but to test the response time of both the arterial and venous pO_2 electrodes at any given time in the course of an experiment rather than to rely on the calibrations and response times previously obtained prior to the placement on the electrodes of the polyethylene membranes actually in use at that time.

This apparatus has been and will continue to be used in conjunction with both the exercise experiments and experiments on the isolated supported heart.

Proposed Course of Project: To continue refinement and reliability of determinations obtained with these instruments.

Serial No. NHI-81
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Regulation of the Ventricle's Contraction: the Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on Atrial and Ventricular Dynamics

Principal Investigator: Sarnoff, S. J., M.D.

Other Investigators: Mitchell, J. H., M.D.
Gilmore, J. P., M.S.
Linden, R. J., M.B., Ch.B., Ph.D.
Brockman, S. K., M.D.

Technicians: Perry, Frank
Furcell, Eugene
Whitted, Charles

Cooperating Units: None

Man Years (calendar year 1959)

Total:	1.30
Professional:	.75
Other:	.55

Project Description:

Objectives - Methods Employed: This project was a continuation and amplification of 1958 project #NHI-105. The hemodynamic observations made were as follows: a) continuous recording of pressure in the right and left atria, pulmonary artery, left ventricle and aorta; b) total aortic blood flow (cardiac output minus coronary flow); c) heart rate; d) changes in myocardial segment length. The dog with surgically induced heart block was in many instances used for the more explicit examination of the effect of autonomic stimulation on atrial function.

Major Findings: The experiments performed demonstrate

Part A, continued. (Title: The Regulation of the Ventricle's Contraction, etc.)

that the central nervous system has available direct efferent pathways to the heart over which it can, at any given heart rate, systematically regulate the ventricle's contraction by either of two means. First, it can control the atrial contraction over a wide range, augmenting the atrial contraction by sympathetic stimulation and diminishing it with vagal stimulation. The ventricle is thereby presented with more or less blood at the end of diastole, its end diastolic pressure and fiber length are modified, and a consequent alteration is made in the vigor of its contraction. This can transpire in the absence of any change in the contractile characteristics of the ventricle.

Secondly, the central nervous system, by way of cardiac sympathetic efferents, can directly cause the ventricle to contract more or less forcefully from whatever end diastolic pressure and fiber length has been obtained. The magnitude of the observed changes was noteworthy.

A more precise appreciation of the net effect of sympathetic impulses on the heart beating at any given rate is best appreciated by the following analysis. The more forceful ventricular contraction resulting from sympathetic stimulation produces more complete systolic emptying from any given end diastolic pressure or fiber length and, consequently, a lower diastolic impedance to ventricular inflow, i.e., the more complete systolic emptying results in a lower early and mid-diastolic ventricular pressure-length and pressure-volume curve. Finally, it is into the ventricle on this flatter part of its pressure-length curve, in which circumstance a small increase in pressure produces a large fiber length increase, that the more vigorous atrial systole propels blood and elevates ventricular end-diastolic pressure.

The net effect of efferent vagal impulses, at least with the intensities of vagal stimulation used in these experiments, is to diminish the vigor of atrial contraction; they did not in these experiments directly modify ventricular contractility.

The above described project has culminated in data which provide a formal means of broadening the basic Frank-Starling relationship and of integrating it with the activity of the central nervous system in relation to acutely induced changes. Two concise statements now appear to be appropriate for the heart operating at any given rate and in the absence of abnormal conditions such as hypoxia and acidosis.

Part A, continued. (Title: The Regulation of the Ventricle's Contraction, etc.)

1. If the effective catechol amine stimulus remains constant, the contraction of the ventricle varies with its end diastolic pressure and fiber length. If the end diastolic pressure and fiber length remain constant, the contraction of the ventricle varies with the effective catechol amine stimulus.

2. The central nervous system has available efferent neuronal pathways directly to the heart by means of which it can vary left ventricular end diastolic pressure and fiber length while keeping the effective catechol amine stimulus constant, means by which it can vary the effective catechol amine stimulus, or both.

These two statements comprise what we intend to call the "law of the innervated heart."

Proposed Course of Project: Project completed. Manuscript submitted for publication.

Part B included: No

Serial No. NHI-82
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Determination of Curve Relating Ventricular Diastolic Pressure and Myocardial Fiber Length

Principal Investigators: Linden, R. J., M.B., Ch.B., Ph.D.
Mitchell, J. H., M.D.

Other Investigators: None Technicians: Frank Perry

Cooperating Units: H-LTD

Man Years

Total: 0.4
Professional: 0.3
Other: 0.1

Project Description:

This project was an outgrowth and continuation of 1958 project #NHI-103. It was found possible to continuously and reliably record changes in the length of a selected length of left ventricular myocardium and to relate these changes to simultaneously occurring ventricular diastolic pressures.

Major Findings. By infusing blood and thus increasing ventricular diastolic pressure, the shape of the ventricular pressure-length curve could be determined. The natural frequency of the totally assembled heart-lever system was such that the segment length recorder also reliably followed changes in the length of the fibers of the ventricular myocardium consequent to atrial systole. These atrially induced changes in fiber length were found to be much greater than anticipated. Further, it was possible to better understand those circumstances under which the atrium makes a major contribution, namely, when the ventricle is on the flat part of its pressure-length curve.

Proposed Course: Project concluded.

Part B included: No

Serial No. NHI-83
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Regulation of the Ventricle's Contraction by the Carotid Sinus: Its Effect on Atrial and Ventricular Dynamics

Principal Investigator: Sarnoff, S. J., M.D.

Other Investigators: Mitchell, J. H., M.D.
Gilmore, J. P., M.S.
Brockman, S. K., M.D.
Linden, R. J., M.B., Ch.B., Ph.D.

Technicians: Perry, Frank
Purcell, Eugene
Whitted, Charles

Cooperating Units: None

Man Years (calendar year 1959) Patient Days: None

Total:	1.35
Professional:	.75
Other:	.60

Project Description:

Objectives - Methods Employed: The conventional view of the reflex function of the carotid sinus has been that it primarily influences heart rate, peripheral arteriolar resistance and venous distensibility. The techniques used were similar to those described in the project report title "The Regulation of the Ventricle's Contraction: The Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on Atrial and Ventricular Dynamics." In addition, however, techniques were used which allowed independent and controlled changes in carotid sinus pressure to be produced. Carotid sinus nerve stimulation was also used.

Major Findings:

The experiments performed demonstrate that the carotid sinus can increase the vigor of atrial contraction both by reflexly diminishing efferent vagal impulses and by augmenting the atrial contraction through sympathetic pathways and thus substantially modify ventricular filling. They also demonstrated the great increase

Part A, continued. (Title: The Regulation of the Ventricle's Contraction by the Carotid Sinus: Its Effect on Atrial and Ventricular Dynamics)

in ventricular stroke work and power that will be produced from whatever ventricular filling pressure is provided when the carotid pressure is lowered. These data establish support for the hypothesis that carotid sinus stimulation can markedly shift the ventricular function curve.

During the course of these experiments a new pattern of the baroceptor's functional role has been evolved which, even though based on a variety of observations and facts, seems to have an appealing unity and simplicity. This position states that a dominant physiologic responsibility of the carotid sinus in circulatory regulation is to augment or diminish the contraction of the ventricle. The basis for this position is as an integration of the data obtained in the course of this project with other previously available information gathered here and elsewhere. It is as follows:

1. Carotid hypotension diminishes venous distensibility. The net effect of such a change, if it alone occurs is an increased ventricular end diastolic pressure and fiber length and thus an augmented ventricular contraction. Splenic contraction would have the same effect.

2. Carotid hypotension augments and shortens the atrial contraction by means of the carotido-vago-atrial and the carotido-sympatho-atrial reflexes. The net effect of such an atrial augmentation, if it alone occurs, is an increased ventricular end diastolic pressure and fiber length and thus an augmented ventricular contraction.

3. The more complete systolic emptying consequent to carotid hypotension places the ventricle on a lower and flatter portion of its diastolic pressure-length curve. As a result there will be more filling and a greater fiber length elongation produced by any given atrial systole than if the more complete systolic emptying had not taken place.

4. Carotid hypotension directly augments the work produced by the ventricle from any given end diastolic pressure or fiber length.

5. Carotid hypotension augments ventricular power since it shortens the systolic time for any given amount of work produced, provides more rapid relaxation, and thus diminishes filling impedance. If this alone occurs, it provides for a longer interval of diastolic

Part A, continued. (Title: The Regulation of the Ventricle's Contraction by the Carotid Sinus: Its Effect on Atrial and Ventricular Dynamics)

filling than would otherwise occur and thus produces an augmented ventricular contraction, a factor which becomes especially important at high heart rates.

6. The catechol amines secreted by the adrenal medulla in response to a lowering of carotid sinus pressure would be expected to reenforce the effects enumerated under 1 through 5 above.

Since the carotid sinus, quite aside from its effect on heart rate and peripheral resistance, can reflexly modify both the filling and the performance characteristics of the ventricles over such wide ranges, it invites attention to its participation as one significant influence in the control of cardiac output in varying states.

Proposed Course of Project: Project completed. Manuscript submitted for publication.

Part B included: No

Serial No. NHI-86
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Role of the Carotid Sinus in Maintaining the Relative Bio-Chemical Normalcy of Active Tissue

Principal Investigators: Mitchell, J. H., M.D.
Remensnyder, J. P., M.D.

Other Investigators: S. J. Sarnoff, M.D.

Technicians: Scott, Carrie; Perry, Frank; and Edwards, Douglas

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None

Total:	1.0
Professional:	.40
Other:	.60

Project Description:

Objectives - Methods Employed: Having obtained what appears to be a reasonably comprehensive understanding of the manner in which neuronal impulses from the central nervous system can acutely modify the activity of the heart, and having demonstrated the profound effects that at least one of the important cardiovascular receptors can produce by the reflex activation of these efferent pathways, studies were initiated which are aimed at achieving a more complete understanding of the mechanisms involved in total circulatory regulation during varying states such as the change from rest to exercise. Some support was obtained in the experiments described above for the re-evaluated role of the carotid sinus in circulatory regulation. As a result of these experiments the baroceptor may now be likened to a voltage regulating element of a power supply which provides for an increased current flow and the maintenance of a constant voltage when one or more of the power consuming elements which it is supplying increases its utilization of current input. The observed data on the exercised lower extremities support this position. During rest, a lowering of the carotid pressure caused vasoconstriction which resulted in little change in lower extremity blood flow and little equilibrium change (although certain transients

Part A, continued. (Title: The Role of the Carotid Sinus in Maintaining the Relative Bio-Chemical Normalcy of active Tissue)

were of interest) in A-V O_2 and pO_2 in the face of the induced elevation of arterial pressure. Contrariwise, during exercise, when the blood flow to the exercised area had increased, the A-V O_2 difference had widened and the pO_2 had fallen, lowering of the carotid pressure at such times produced a marked increase in flow to the exercised area, a narrowing of the A-V O_2 difference, and an elevation of the pO_2.

Major Findings: The data obtained in this project invites attention to the role of the baroceptors in the regulation of cardiac output in varying states.

Proposed Course of Project: Examination of other afferents, especially vagal afferents and chemoreceptors which might exert a similar effect.

Part B included: No

Serial No. NHI-85
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Functional Sympathectomy during Muscular Activity

Principal Investigators: Remensnyder, J. P., M.D.
Mitchell, J. H., M.D.

Other Investigators: Sarnoff, S. J., M.D.

Technicians: Scott, Carrie; Perry, Frank; and Edwards, Douglas

Cooperating units: None

Man Years (calendar year 1959): Patient Days: None

Total:	1.0
Professional:	.40
Other:	.60

Project Description:

Objectives - Methods Employed: Fundamental to the basic hypothesis with which we are approaching the analysis of circulatory regulation has been the assumption that the fully integrated circulatory effort is really originated in the peripheral tissues of the body as first broadly suggested by Haldane. It was to be expected, therefore, that if any given tissue were to greatly augment its activity and thus its metabolic requirements, its vascular bed would have the ability to disregard, so to speak, those neural and humoral instructions to vary its tone and vascular resistance to which it would, in the absence of augmented activity, ordinarily be responsive. Since this assumption was the cornerstone of our overall hypothesis, it was desirable to ascertain whether it is experimentally demonstrable.

Two general types of experiment were employed. The first was one in which the total flow to both lower extremities was metered, before and during exercise, while simultaneously recording the arterio-venous O_2 difference, arterial and venous pO_2 and pH. Prior to exercise, three types of sympathetic stimulus were applied. These were a) the reflex increase in sympathetic tone consequent to lowering carotid sinus pressure; b) the emphatic sympathetic stimulation resulting

Part A, continued (Title: Functional Sympathectomy during Muscular Activity).

from stimulating the central cut end of the vagus nerve; and e) the injection of constricting doses of norepinephrine into the arterial line supplying the lower extremities. These were then repeated during a bout of exercise or, more properly, simulated exercise induced by electrical stimulation of the muscles of both lower extremities. The second type of experiment done was one in which the blood flow to each lower extremity was separately metered so that one extremity, the resting extremity, could act as the control while the opposite extremity was exercised.

Major Findings: The results of both types of experiments make it clear that the original assumption is correct, namely, that with augmented muscular activity, the vascular bed of the active area can disregard a sympathetic stimulus to which it would ordinarily be responsive. This might be termed the functional sympathectomy of activity.

Proposed Course of Project: Attempts to ascertain which aspect or aspects of the altered biochemistry of active areas provides the stimulus to vasodilation and confers independence from sympathetic stimulation.

Part B included: No

Serial No. NHL-86
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Comparison of Cardiovascular Hemodynamics during Atrial and Ventricular Stimulation

Principal Investigator: Sarnoff, S. J., M.D.

Other Investigators: Gilmore, J. P., M.S.
Mitchell, J. H., M.D.
Linden, R. J., M.B., Ch.B., Ph.D.
Brockman, S. K., M.D.

Technicians: Perry, Frank
Purcell, Eugene
Whitted, Charles

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None

Total:	1.35
Professional:	.75
Other:	.60

Project Description:

Objectives - Methods Employed: The objectives of this project were a) to ascertain whether, by causing the atrium to contract while the atrioventricular valve is closed, additional information could be obtained on the basis of which to further assess the importance of the atrial contribution to ventricular filling and b) to ascertain whether an abnormally propagated wave of depolarization influences the performance characteristics of the ventricle.

Major Findings: Both objectives were satisfactorily achieved. By closing the door, so to speak, on the atrium during atrial systole and thus depriving the ventricle of the filling pressure and fiber length it would otherwise have achieved, it was noted that the ventricular end diastolic pressure was significantly lower and the external work produced was thereby lessened. It was further observed that the amount of work produced by the ventricle from any given end diastolic pressure was lower during ventricular pacing than during atrial pacing. Analysis of high speed ventricular pulse contours produced results which are consonant with the explanation that there is a less rapid wave of depolarization, systole takes a longer period of time and the total ventricular

Part A, continued (Title: A Comparison of Cardiovascular Hemodynamics during Atrial and Ventricular Stimulation)

effort is appreciably less concerted and synchronous. Thus, when the first fibers are contracting, the flaccidity of those which are not as yet activated tends to impose the same hydraulic limitations as a ventricular aneurysm, e.g. diminish the effectiveness of the contraction. Similar considerations apply to the last contracting fibers.

Proposed Course of Project: Completed. Manuscript submitted for publication.

Part B included: No

Serial No. NHI-67
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Auto-Regulation of the Performance Characteristics of the Heart.

Principal Investigators: Mitchell, J. H., M.D.
Sarnoff, S. J., M.D.

Other Investigators: Gilmore, J. P., M.S.
Remensnyder, J. P., M.D.

Technicians: Scott, Carrie; Perry Frank

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None

Total:	.95
Professional:	.55
Other:	.40

Project Description:

Objectives - Methods Employed: Several apparently unrelated observations in the course of investigations in this laboratory over the past five years have given rise to a feeling of discomfort concerning the inadequacy with which we are able to comprehend certain specific aspects of the performance characteristics of the heart. First, it was observed that the ventricle of the isolated heart, beating at a constant rate and free of reflex or hormonal regulatory influences, would, over the entire range of stroke volumes examined, require no longer a time to put out any given stroke volume against a high aortic pressure than against a low one. In other studies, it was observed that if the work of the ventricle is increased solely by increasing aortic pressure, the ventricular function curve is much steeper than if the ventricular work is increased solely by increasing stroke volume. In still another study it was observed that when oxygen consumption is increased by increasing aortic pressure while holding stroke volume constant, there is a greater relative increase in coronary blood flow and a narrowing of the arteriovenous O_2 difference. Conversely, when O_2 consumption is increased by increasing stroke volume while holding mean aortic pressure constant, the increase in coronary blood flow is less marked and is, relatively speaking,

Part A. continued. (Title: Auto-Regulation of the Performance Characteristics of the Heart)

dominated by a widened arteriovenous O_2 difference. Put another way, coronary venous blood, presumably at least partially representative of conditions in the myocardium, showed less of a deviation from arterial blood when arterial pressure was elevated than when it was not as far as the O_2 content was concerned. It seemed not unlikely that, under these conditions, e.g., high aortic pressure, the coronary venous pO_2 and pH would also have deviated less from the arterial values.

Experiments were thus undertaken with more precise techniques of pressure recording and greater attention to the use of high speed pressure recording with increased resolution of diastolic ventricular phenomena. These made possible a clearer appreciation of the intimate changes produced by the action of catechol amines on the atrium and ventricle. It was then determined that raising aortic pressure by increasing the resistance to left ventricular ejection produced alterations in the pulse contours observed which were in every respect similar to those observed after the administration of norepinephrine. We were therefore led to the following considerations.

Major Findings: There exists a vast array of evidence suggesting that the responsiveness to catechol amine stimulation is a function of the biochemical environment of the stimulated effector organ. This gives rise to the hypothesis that an increased aortic pressure, by increasing coronary flow relative to O_2 utilization, so alters the biochemical environment of the myocardium as to render it more responsive to any given level of catechol amine and thus increase the *effective* catechol amine stimulus.

Proposed Course of Project: It will be the burden of future experiments to obtain at least partial evidence on the basis of which this hypothesis can be supported or, at least, re-examined.

Part B. included: No

Serial No. NHI-38
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Influence of Cardiac Sympathetic and Vagal Nerve Stimulation on the Relation between Left Ventricular Diastolic Pressure and Myocardial Segment Length

Principal Investigators: Mitchell, J. H., M.D., Linden, R. J., M.B., Ch.B., Ph.D, Sarnoff, S. J., M.D.

Other Investigators: None Technicians: Frank Perry

Cooperating Units: None

Man Years: Patient Days: None

Total: 0.7
Professional: 0.5
Other: 0.2

Project Description: Objectives: Methods Employed.

During the continuous and simultaneous recording of left ventricular diastolic pressures and changes in the length of a segment of left ventricular myocardium it was demonstrated that neither cardiac sympathetic nor vagal efferent nerve stimulation produces a change in ventricular extensibility. It was further shown that, at the heart rates studied, autonomic stimulation does not modify the end diastolic pressure-length curve. These data suggest that the augmented ventricular stroke work from any given end diastolic pressure is accomplished without an appreciable change in end diastolic fiber length.

Major Findings: Evidence was obtained which suggests that the abbreviation of diastole at high imposed heart rates or large stroke volumes leaves an inadequate time for ventricular relaxation to take place; under these circumstances sympathetic stimulation, by shortening systole and thereby lengthening diastole, permits the ventricle to remain on its "normal" pressure-length curve. This component of cardiac sympathetic efferent activity is peculiarly appropriate to the tachycardia that occurs with increased sympathetic outflow to the heart.

Part A. (continued)

Proposed course of project: Concluded. Manuscript submitted for publication.

Part B, included: No.

Serial No. NHY-89
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Submitted in conjunction with C.S. experiments (Norepinephrine and Epinephrine Estimation in Plasma).

Principal Investigator: S. J. Sarnoff, M.D.

Other Investigators: J. Cox
Mrs. Zena McCallum

Cooperating Units: None

Man Years (calendar year 1959)
Total: one-fourth
Professional: one-fourth
Other: one-fourth

Project Description:

Objectives: Methods Employed. The fluorometric method of Weil-Malherbe (EDA method) is being used for measurement of catechol amine (N & E) changes in coronary arterial and venous plasma in the cardiac sympathetic experiments. After considering other methods, the EDA method seemed best suited as it is sensitive and fairly reproducible. A number of difficulties were encountered in getting the method going but these for the most part seemed to have been ironed out by strictly following the procedure in detail.

In the process of improving and testing the technique, several small dogs were bled and aliquots of the plasma were analyzed for norepinephrine and epinephrine concentration. Usually during each run 10 aliquots were taken and half of them analyzed straight while known amounts of norepinephrine or epinephrine, or both, were added to the other half. From these determinations the per cent recovery was calculated.

Recovery of added norepinephrine and epinephrine seems to agree with results obtained by others using this method or slight modifications of it. The recovery of N averaged about 76% $\pm$ 20 and that of E was about 90% $\pm$ 15. Some difficulty was experienced in obtaining duplication in the control samples apparently due to very low concentrations ranges.

In addition to the data obtained above, during each intra-experimental run at least two recovery samples are included in

the analyses of the experimental samples.

Proposed Course of Project:

An attempt will be made to further establish greater precision and reproducibility especially in the very low concentration range and modifications will be made if necessary to improve the reliability and accuracy of the method in our hands.

Part B included: No

Serial No. NHI-90
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Reflex Elaboration of Catechol Amines by the Myocardium

Principal Investigators: J. P. Gilmore, J. H. Siegel, M.D.

Other Investigators: S. J. Sarnoff, M.D.

Technician: Joseph Miles

Cooperating Units: None

Man Years (calendar year 1959) Patient Days: None
Total: one-fourth
Professional: one-fourth
Other: one-fourth

Project Description:

Objectives; Methods Employed. To determine if the myocardial secretion of catechol amines, as indicated by changes in coronary sinus blood concentration, is modified by changes in carotid sinus blood pressure and if said changes are modified by sympathectomizing the heart (stellectomy). For this study, cardiac output is controlled via an extracorporeal perfusion pump, and coronary blood flow, left atrial pressure, and arterial pressure monitored continuously. Plasma concentrations of norepinephrine and epinephrine are determined by the method of Weil-Malherbe. Experiments to date show:

1. Electrical stimulation of the stellate ganglion increases significantly the concentration of norepinephrine in coronary sinus blood but has little effect on coronary blood epinephrine levels. It has also been found that upon cessation of stimulation there occurs an increase in coronary blood norepinephrine concentration above the levels observed during stimulation.

2. The norepinephrine concentration changes in coronary venous blood during carotid occlusion have been found to be variable, but yet appear to be modified by bilateral stellectomy. However, when the carotid occlusion is released, there occurs a great increase of norepinephrine in the coronary venous blood similar to what occurs when stellate stimulation is stopped.

Part A. (continued)

In these same animals, pressure-flow curves have been obtained before and during carotid occlusion.

Proposed Course of Project: To continue the study and further investigate the means by which catechol amines are secreted in and utilized by the myocardium; to extend observations on the effect of carotid pressure on the relation between pressure and flow and the extent, if any, of the change in this relation induced by changes of arterial pH.

Part B included: No

Serial No. NHI-91
1. Laboratory of Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of Stellate Stimulation on Arterial Pressure, Atrial Pressure and Renal Function

Principal Investigator: J. P. Gilmore

Other Investigators: S. J. Sarnoff, M.D.

Technician: Joseph Miles

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None

Total:	one-sixth
Professional:	one-sixth
Other:	one-sixth

Project Description:

Objectives; Methods Employed. Stimulation of isolated stellate ganglion in the dog.

Major Findings: Experiments to date have demonstrated:

1. When the isolated stellate ganglion of the dog is electrically stimulated there occurs an immediate diuresis which is independent of arterial pressure changes.

2. During stimulation the diuresis is accompanied by an increased inulin clearance with no change in urinary solute concentration.

3. Subsequent to vagotomy the extent of the diuresis produced by stellate stimulation is greatly diminished although the elevation of arterial pressure is at least the same or greater than that obtained before vagotomy. Inulin clearance also shows less of an increase after vagotomy during stellate stimulation.

4. The data suggest that the diuresis which occurs during stimulation of the stellate ganglion is the result of a reflex diminution of sympathetic efferent activity to the kidney which increases the glomerular filtration rate.

This interpretation is further suggested by the observation that sectioning the vagi which contain the aortic buffer nerves diminishes the extent of the changes in urine flow and glomerular filtration rate. The rapidity of the onset and cessation of the response further suggest that a neuronal rather than hormonal pathway is involved.

Proposed Course of Project:

To determine whether increasing sympathetic efferent activity as by carotid occlusion can modify the renal excretion of water and electrolytes and to determine whether the response is modified by vagotomy and/or stellectomy.

Part B included: No

Serial No. NHI-92
1. Lab. Cardiovascular Physiology
2. National Heart Institute
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Callicrein System

Principal Investigators: Webster, H. E., Ph.D.
Pierce, J. V., Ph.D.

Other Investigators: Sarnoff, L. C.
Emmart, E. W., Ph.D.

Technician: William Fisher

Cooperating Units: H LCNP
A LPT

Man Years

Patient Days: None

Total: 4-1/4
Professional: 2-1/4
Other: 2

Project Description:

Methods Employed: Methods for the detection of some of the various components of the callicrein-callidinogen callidin system have been developed:

(1) Callicrein is measured directly by the increase in femoral blood flow in the dog as previously described. However the response to plasma callicrein, as compared to the standard pancreatic callicrein, varies somewhat from one dog to another and at different times in the same dog even though the response of human pancreatic or urinary callicrein is similar to that of the standard callicrein;

(2) Callidinogen is measured indirectly on the guinea pig large intestine, by addition of excess callicrein, and measurement of the height of contraction due to the release of callidin. Human plasma callicrein does not release callidin as readily from human plasma as do the urinary and pancreatic callicreins. However it is much more effective in its release of callidin from guinea pig plasma. This effect does not appear to be related to differences in inhibitor concentrations to the callicreins and may be due to differences in their respective callidin inhibitor levels. It is possible

Part I (continued)

that the callidins produced by the various callikreins may differ from one another by biological or chemical differences have yet been found between the various callidins;

(3) Callidin is measured directly by its contraction of guinea pig large intestine. Comparisons of the callidins isolated from the various callikreins have not as yet been attempted;

(4) The plasma inhibitor (Callikrein Inhibitor I) towards pancreatic or urinary callikrein is a reversible inhibitor. At low levels of callikrein increasing levels of plasma fail to yield increasing inhibition of the callikrein. However, at high concentration of the inhibitor levels can be obtained by which the inhibition of the biological system in the dog;

(5) The irreversible inhibition of plasma callikrein (Callikrein Inhibitor II) can readily be measured at the level of plasma callikrein by the inhibition of the response to plasma callikrein as measured in the dog bioassay system so far tested (human, dog, rabbit, and guinea pig) contain greater concentrations of Callikrein Inhibitor II than Callikrein Inhibitor I;

(6) The callidin inhibitor level can be roughly estimated by measuring the rate of destruction of callidin following its liberation from callidinogen. A quantitative procedure for the measurement of this inhibitor has not been attempted.

Patient Material: Preliminary studies with the methods described above on patients with orthostatic hypotension or essential hypertension have furnished the following results. Hypotensive patients, as previously described, excrete low activities of urinary callikrein although the influence of age on the patients on the excretion of callikrein has not yet been properly controlled. These patients tend to have lower callikrein levels, lower plasma callikrein levels and, in the case of at least three, elevated decreases in plasma callikrein when upright as compared to the horizontal position. The potential plasma kallikrein Callikrein Inhibitor I was tested. Callikrein Inhibitor II was found to be somewhat lower in the hypotensive patients. Hypertensive patients, on the other hand, appear to vary in their excretion of urinary callikrein from low to normal to high normal. Their plasma callikrein level or higher than normal callidinogen levels have not been detected for their callikrein inhibitor levels. However, it has been noted that dogs following stellate stimulation

Part A. (continued)

a marked and erratic decrease in their excretion of urinary callicrein. This effect would appear to be neuronally mediated and modified by vagotomy.

Major Findings: Plasma callicrein can readily be activated from human plasma by the addition of 20% acetone v/v or by the addition of trypsin 1 mg./ml. Because plasma contains a proteinase in the euglobulin fraction called plasmin, it was of importance to definitely distinguish between plasmin and plasma callicrein. Although these two proteinases were similar in their inhibition with the various trypsin inhibitors, differences in the ratio of their inhibition could be shown, and there is little question that the proteinases are different. In addition, plasmin does not appear to be the proteinase responsible for the activation of callicreinogen in the presence of acetone.

Other factors in plasma which have not yet been definitely distinguished from plasma callicrein are the glass activated proteinase of Anderson and coworkers, Hageman's factor and the permeability factor of Milec and Wilhelm. Studies on comparison of these factors have been initiated and no differences have yet been found.

Chapman and Wolff (A.M.A. Arch. Internal Med. 103, 86-94, 1959) have reported the presence of a proteolytic enzyme similar to callicrein in the cerebrospinal fluid of patients with active disease of the central nervous system. An attempt has been made to detect this enzyme in patients with schizophrenia. However, all results to date have been negative. It is possible that these negative results may be due to lack of sensitivity of the bioassays which were employed, to incorrect storage of the cerebrospinal fluid or to differences between patients. It is planned to continue these studies in cooperation with Dr. Chapman and attempt to confirm his results.

Preliminary evidence has been obtained which suggests that human urinary callicrein is antigenic in the rabbit. This antibody will inhibit the biological action of urinary callicrein, but not that of pancreatic callicrein, and should provide a useful research tool. The antibody may be associated with the beta-globulins in rabbit serum and further studies are planned to elucidate this question.

Because plasma callicrein was inhibited *in vivo* with diisopropylfluorophosphate (DFP), a known inhibitor of esterolytic activity, it was thought possible that the callicreins might be capable of digesting synthetic esters. Tosyl L-arginine methyl ester (TAMe) has been found to be a substrate for the various

Part A. (continued)

callicreins. Crude urinary callicrein attacks this substrate at the same ratio of activity as does highly purified hog pancreatic callicrein (obtained from Dr. Moriya, Japan) and it is possible that urinary callicrein may be the main esterase excreted in the urine. Acetone activation has been found to activate at least two esterases, one inhibitable with soy bean trypsin inhibitor (SBI) and the other not. To date, correlation between SBI inhibitable proteinase and plasma callicrein has been consistent. It is possible that the SBI resistant proteinase may be the enzyme responsible for the liberation of callicrein from its callicreinogen (inactive precursor). Plasma callicrein would appear to be more active as a TAMe esterase than are either urinary or pancreatic callicrein and the ratio of inhibition of SBI to pancreatic trypsin inhibitor as determined in vitro is 1:40 as compared to an in vivo ratio of 1:10. It is possible, therefore, that acetone causes the activation of more than one proteinase inhibitable with SBI.

Major emphasis has been placed during the year on the purification of callidinogen. Methods have been devised for the purification of this substrate 83 times purer than normal plasma. This purified material still contains bradykininogen (the substrate for trypsin) and no differences have yet been found between these substrates, although the polypeptide(s) released by trypsin are either four times as active against the large intestine of guinea pigs or trypsin releases four times as many polypeptides. The substrate has been essentially freed of callicrein Inhibitor I and II and of plasma callicrein, but still retains detectable amounts of the callidin inhibitor.

Studies have continued on the purification of the various human callicreins. Urinary callicrein can be purified by adsorption on XE-64 and DEAE and the dialyzed and freeze-dried callicrein has a specific activity of 7 Frey Units (FU)/mg. Methods have also been developed for the extraction of pancreatic callicrein with dilute acetic acid and its purification by adsorption on DEAE. The activity of the final material was 4-8 FU/mg. Acetone activated plasma callicrein has been purified three-fold by its failure to adsorb on DEAE and its adsorption on carboxymethyl cellulose. Callicrein preparations of 0.33 FU/mg. have been obtained. Since the purest callicrein, prepared from hog pancreas, has a specific activity of 250-600 FU/mg., it is apparent that much greater purification can be expected in the future.

Proposed course of project: Continued investigation of the many varied components of the callicrein system.

Part B included: No

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: Studies on the Species Differences and Structures of Ribonucleases and Lysozymes.

Principal Investigators: C. B. Anfinsen, Trygve Tuve, Charles Sibley (Cornell University)

Other Investigators: Juanita Cooke, Technical

Man Years (Calendar year 1959): Patient days: None
Total:1-1/2
Professional: 1-1/2

Project Description:

Objective: To isolate and determine the structure of ribonucleases and lysozymes from a variety of biological sources. These studies have been undertaken since it seems a reasonable working hypothesis that the same general structure may occur in the active centers of all of the ribonucleases and lysozymes, but that variations in structural detail may exist in those parts of the structures which are not directly concerned with catalysis.

Methods employed: In a continuation of the previous work on the purification of pancreatic ribonucleases from sheep and pig tissue, ribonuclease has been isolated in moderately pure form from spinach leaves and from B. subtilis. Structural studies have not been done on the latter two enzymes, although studies have been made to determine the enzymatic specificities of these enzymes. These studies indicate a strong similarity to the pancreatic protein as regards temperature, stability, pH optimum, etc. Isolations have involved salt fractionation and column chromatography. Egg white lysozymes have been isolated from the egg whites of several dozen species of birds, mainly in the laboratory of Professor Charles Sibley at Cornell University, with whose collaboration this work is being carried out. Simple methods have been worked out for isolating pure lysozyme from egg white with a minimum of effort and a standard technique has been evolved for examining the general aspects of structure in sufficient detail to permit species comparisons. These comparisons involve the two-dimensional separation of peptides in trypsin digests of the reduced alkylating enzyme on paper sheets, using successive chromatography and electrophoresis.

Proposed Course of Research: It is proposed to continue these studies, particularly with the lysozymes, since they can be obtained in pure form from many hundreds of species. Professor Sibley has the active collaboration of many collaborators throughout the world, who are continuing to supply us with egg white material. The studies, in addition to furnishing informa-

tion on the common denominator of structure from species to species, should also permit an examination, at the chemical level, of the phenomena of speciation and hybridization.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Part B included Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

C. B. Anfinsen, Stig E.G. Aqvist, Juanita P. Cooke and Borje Jonsson, A Comparative Study of the Structures of Bovine and Ovine Pancreatic Ribonucleases, J. Biol. Chem. 234, 1118-1123 (1959).

Stig E.G. Aqvist and C.B. Anfinsen, The Isolation and Characterization of Some Ribonucleases from Sheep Pancreas, J. Biol. Chem. 234, 1112-1117 (1959).

Ray K. Brown, Barbara C. Tacey and C. B. Anfinsen, The reaction of Porcine and Ovine Ribonuclease with Antibody to Bovine Ribonuclease, in press.

C. B. Anfinsen, The Molecular Basis of Evolution, John Wiley & Sons, Inc. New York, N.Y. (1959).

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: The identification of the disulfide bridges in ribonuclease which are essential for activity; the chemical identification of ribonuclease in relation to activity.

Principal Investigators: C.B. Anfinsen, Edgar Haber, John Potts, A. Berger.

Other Investigators: None

Man Years (calendar year 1959): Patient days: None
Total: 1-1/2
Professional: 1-1/2
Other: --.

Project Description:

Objective: These studies are a continuation of our efforts to delineate the active center of ribonuclease. The relative importance of each of the four disulphide bridges in catalysis will be evaluated by the study of the kinetics of reduction of the disulphide bonds in relation to enzyme activity. The fully reduced protein, which has the form of an extended polypeptide chain with eight sulfhydryl groups, will be systematically degraded with proteolytic enzymes and other agents and subsequently refolded into an active form (see report by Frederick H. White, Jr.) to test for the essentiality of the parts removed by the degradative techniques.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

PART B included Yes No X

Serial No. NHY-95
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: Studies on the structure of lysozyme

Principal Investigators: Arnold M. Katz and Robert Canfield

Technical: Juanita Cooke

Man Years (Calendar year 1959) Patient Days: None
Total: 1
Professional: 1
Other: None

Project Description:

Objective: To devise column chromatographic techniques for the separation of the peptides in a tryptic digest of reduced, alkylated lysozyme.

Methods Employed: A new technique, for the precise separation of peptides has evolved through work in this department and elsewhere. Its applicability in the determination of the amino acid sequence of a protein enzyme of 15,000 molecular weight is being investigated as a model for future work. Crystalline lysozyme is reduced, alkylated and then digested with trypsin to give a reproducible mixture of peptides. This mixture is resolved into individual peptide spots by chromatography, followed by electrophoresis on large sheets of filter paper. The resulting pattern of peptides is called a "fingerprint" and is quite characteristic of this protein. All 18 of the theoretical number of peptides in the digests have been separated by paper chromatographic and electrophoretic techniques and analyzed for amino acid composition. The analytical values obtained, when summed together, are consistent with the theoretical expectations based on analysis of the intact enzyme. The column chromatographic methods also permit peptide separation and in large enough quantities for stepwise analysis of sequence as well as analyses for amino acid composition.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general. These studies on the structure of chicken egg-white lysozyme are of particular value since they will furnish a baseline for subsequent comparison of the structures of lysozymes isolated from numerous other species. (See report by Tuve, Sibley.)

Proposed Course of Research: The peptides separated by one or both of the techniques mentioned will be subjected to chemical study for the purpose of deducing the amino acid sequence of the peptide chain. Similar studies on egg-white lysozyme from other species will then permit a chemical comparison of the various lysozymes.

PART B included Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Katz, A.M., Dreyer, W.J. and Anfinsen, C.B., Peptide Separation by Two-Dimensional Chromatography and Electrophoresis (Fingerprinting) J. Biol. Chem. 234, 2897 (1959)

Katz, A.M., Amoz I. Chernoff, Structural Similarities Between Hemoglobins A and F, SCIENCE, in press.

B. In a continued study of the reduction of ribonuclease thioglycolic acid, mercaptoethanol, and cysteine were found to be equally effective as reagents for the reductive cleavage of protein disulfide bonds.

C. The mixed disulfide between thioglycolic acid and half-cystine residues (β-carboxy-β-aminoethyl carboxymethyl disulfide), which may form as an intermediate in the reduction of protein disulfide bonds, was observed to appear in significant quantities when shelf-aged thioglycolic acid was used as the reducing agent, but not with the highly purified reagent. The reason for this observation is not presently known

D. Comparative studies of native ribonuclease and of fully reduced, reoxidized ribonuclease have thus far revealed no significant differences in secondary and tertiary structure. This finding is of theoretical significance with regard to the mechanism of protein synthesis, suggesting that the formation of secondary and tertiary structure of a protein may be governed by information contained in the protein chain (e.g. amino acid sequence) rather than by the template from which the protein chain is formed.

E. The proteinases "subtilisin" and "nagarse", isolated from two different strains of B. subtilis, were found to be strikingly similar (but not identical) in their specificities toward ribonuclease.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical nature with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Investigation of the structural and functional relationships in ribonuclease will be continued. Further comparative studies of native, reduced, and reduced-reoxidized ribonucleases are planned. This work will be extended to other proteins to gain more insight into the mechanism of formation of secondary and tertiary structure.

PART B included Yes X

Individual Project Report
Calendar Year 1959

PART I

Project Title: Investigation of structural and functional relationships in ribonuclease

Principal Investigator: Frederick H. White Jr.

Other Investigators: None

Man Years (Calendar year 1959): Patient days: None
Total: 1
Professional: 1
Other: None

Project Description:

Objective: To investigate the structural and functional relationships of ribonuclease and other enzymes for the purpose of obtaining information on the mechanism of enzyme action.

Methods Employed: Previously elaborated methods of reduction and reoxidation of protein disulfide bonds have been used in a further investigation of the effects of these reactions on ribonuclease activity and structure. In studies on structural and functional relationships in this enzyme, proteolytic methods for the degradation of ribonuclease and its derivatives have been employed, followed by chromatographic and electrophoretic techniques for separating the resulting peptides and amino acids. Various methods of assay have been used for determination of ribonuclease activity.

Major Findings:

A. Kinetic studies on the inactivation of ribonuclease with either polythioglycolides (thiolesters derived from thioglycolic acid) or N-acetylhomocysteine thiolactone indicate a rapid loss of enzymatic activity which has been correlated with formation of sulfhydryl groups and the simultaneous disappearance of amino groups. These results demonstrate the essential nature of amino groups for enzymatic activity of ribonuclease.

Serial No. NHI-96

PHA--NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

F.H. White, Jr., Regeneration of Enzymatic Activity by Air-Oxidation of Reduced Ribonuclease, with Observations on the Effects of Thiolation During Reduction, J. Biol. Chem., in press.

C. B. Anfinsen and F.H. White, Jr., The Ribonucleases, The Enzymes, Vol. III, in press.

Serial No. NHI-97

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: Biochemistry of muscular contraction

Principal Investigators: W. Wayne Kielley
William F. Harrington

Other Investigator: Lisa Barnett (technical)

Man Years (Calendar Year 1959)
Total: 2 1/2
Professional: 1 1/2
Technical: 1

Project Description:

Objectives: It was observed some time ago that the ATPase activity of myosin, the protein unit of the contractile mechanism, exhibits a biphasic response to titration of the SH groups, with a 3-4 fold stimulation of ATPase activity occurring when approximately 1/2 of the SH groups are titrated, further titration leading eventually to complete inhibition. The question exists whether the two phases of this response are due to titration of specific groups or to statistical titration of the groups involved in each active center. It was planned to approach the problem using differential labeling of the groups with radioactive SH reagents followed by tryptic digestion and application of the "fingerprint" technique to identify the cysteine containing peptides. However, interpretation of the results would depend on whether the large myosin A molecule consists of a single or several peptide chains and if several, whether they are identical. Therefore, it was decided to approach this question first, using concentrated guanidine hydrochloride to disrrupt all of the secondary and tertiary structure, leaving only the primary covalent bonds of the peptide chain. Examination in the ultra centrifuge should then establish the sizes of the basic units. Myosin A is a rather large molecule (600,000 molecular weight) but offers a unique opportunity to establish some details of fine structure. It is a very asymmetric molecule (1600 Angstrom units long and 21Å in diameter) and the number of ways of arranging a single or several peptide chains in this shape are very restricted.

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Methods Employed: The molecular weight of native myosin has been determined using the approach to sedimentation equilibrium (Archibald) technic. These observations have demonstrated a concentration dependence of the apparent molecular weight that was not appreciated by other investigators applying this technic to myosin. It has been concluded that the true molecular weight is close to 619,000.

The same method has been applied to the determination of the substructure of myosin. For these studies the secondary, hydrogen bonded structure of myosin was destroyed by placing the protein in concentrated guanidine hydrochloride. In this medium the Archibald studies demonstrated that the native protein is dissociated into three units of approximately equal if not identical mass - 206,000. This conclusion was also supported by sedimentation, diffusion and viscosity studies. Consideration of the masses of the native protein and the subunit, the observed length and width and the optical rotatory and X-ray diffraction properties of the myosin molecule have led to the conclusion that the three polypeptide chains are each wound into an α-helix and the three helicies are twisted together in a rope form. In associated studies using the two-dimensional electrophoretic-chromatographics technics developed in this laboratory for separation of the peptides resulting from proteolytic digestion of proteins, it has been tentatively concluded that three polypeptide chains of myosin are chemically identical.

Significance to Heart Research: There is no immediate significance to heart research, though more extensive knowledge of the proteins involved in muscular contraction may be of eventual importance.

Proposed Course of Project: Current efforts are directed at a refinement of the technics used in establishing the chemical identity of the physical subunits of myosin. For this we have been using the highly sensitive and specific characteristics involved in labeling the sulfhydryl groups of the protein with a radioactive reagent. Since the 18 SH groups per chain react at different rates with the reagent and these functional groups are intimately involved in the reaction of myosin with adenosinetriphosphate, these technics offer an opportunity to determine which groups are directly involved in the enzymatically active site as well as some of the surrounding structure.

What appears to be the terminal peptide from the carboxyl end of the polypeptide chain (obtained by tryptic digestion) has also been isolated. Substantiation of these preliminary conclusions is in progress. This peptide will be of help in localizing the enzymatic center in the molecule as well as in answering the question of whether the 3 chains all have the same sense in the native myosin molecule.

Serial No. MHK-97

Present efforts are also directed at a determination of the mechanism of polymerization of myosin as it is found in the myofibril and the possible consequences of this polymerization reaction to the process of muscular contraction.

Part B included: Yes X

Serial No. NHI-97

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Kielley, W. W., and W. F. Harrington, A Model for the Myosin Molecule, Biochim. et Biophys. Acta (In press).

Serial No. MMY-08

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: Studies of the mechanism of genetic control of protein synthesis

Principal Investigators: William J. Dreyer
Michael J. Crumpton (Visiting scientist)
Elward Bynum
Christian B. Anfinsen

Other Investigators: Judith Wegman (technical) (June-August)

Cooperating Units: George Streisinger and Frank Mukai
Cold Spring Harbor, Long Island (Carnegie Inst. of Wash)

Man Years: (calendar year 1959)
Total: 3
Professional: 2
Technical: 1

Project Description:

Objectives: Recent genetic studies have made it clear that the genetic material, deoxyribonucleic acids (DNA), of living organisms exerts rigid control over the synthesis of proteins. It appears that a change in the chemical structure of DNA due to a mutation can result in a change in the structure of a protein synthesized under the control of the altered "gene". The structural analysis of such altered proteins should provide useful data relating to the translation of genetic information into protein structure. Ultimately, the knowledge gained should also be of considerable value in the study of structure and function of proteins and enzymes.

The genetic systems and the proteins currently being studied are those of the very simple "organisms", bacterial viruses.

Experimental:

1. Bacterial virus proteins -- Several proteins produced under the genetic control of the virus have been purified. Of these, the one to which we have devoted most attention recently is the bacterial virus lysozyme. Procedures have been developed which enable us to isolate the enzyme in pure form and in relatively high yield from a lysate of infected bacteria. The protein has been characterized by a variety of physical and chemical studies. It appears homogeneous in the ultracentrifuge, upon electrophoresis, and when subjected to column chromatography. Its optical rotatory properties suggest a high degree of coiling. Amino acid analysis reveals a similarity to egg white lysozyme in some respects. However, certain profound differences exist, such as the presence of a single cystine (or perhaps cysteine) in the viral enzyme. Various properties of the enzyme, such as pH optimum, thermal stability, and divalent cation stabilization have also been examined.

2. Development of methods for the study of protein structure -- Each advance in methodology seems to permit an improvement in both the quality and quantity of work that can be done in studies of protein structure. For this reason we have directed some of our efforts toward this end. Several minor but useful developments have been made in the past year:

a. The "fingerprint" method for the separation of peptides has been modified to permit rapid, preparative scale isolations with the same high degree of resolving power.

b. Quantitative amino acid analysis appears to be the rate limiting step in the study of primary structure of proteins. In order to minimize this restriction, a method used by Hanes, et al. has been simplified in several ways and now seems to be a satisfactory means of carrying out rather large numbers of quantitative amino acid analyses.

c. Theoretically, at least, the determination of the sequence in which a large number of peptides derived from a protein were originally arranged in that protein should become a relatively simple problem if one could produce exactly the "overlaps" needed -- without relying on chance. This end might be accomplished by using the proteolytic enzyme, trypsin. This enzyme hydrolyzes proteins at both lysine and arginine residues. Sela and Anfinsen have blocked lysine residues with carbobenzoxychloride thus permitting trypsin to act only on the arginine residues. Preliminary experiments aimed at preventing tryptic cleavage at arginine residues in a protein without affecting its action on lysine look rather promising. It is hoped that the use of both techniques on the bacteriophage lysozyme will enable us to produce (in two tryptic digests) the sort of overlaps needed in order to arrange the tryptic peptides in a unique sequence.

3. Genetic aspects of the problem -- In order to attempt a comparison between a genetic map and a "map" of changes in protein structure resulting from mutation, one, of course, needs both. The bacterial virus lysozyme appears to be particularly well suited to studies of protein structure. However, it was also necessary to demonstrate (a) that the enzyme is in fact produced under the genetic control of the bacterial virus (i.e., not the bacterium) and (b) that scoring of mutants is possible in cases where the enzyme is affected. In order to establish the first point, a peptide map comparison was made between enzymes from the closely related strains of bacterial viruses, T_2 and T_4. The peptide maps revealed small but distinct differences between the two, thus establishing the fact that the enzyme is produced under the genetic control of the virus.

The second requirement has been met, at least in a preliminary way by the development of methods permitting the detection of the enzyme surrounding each of the virus plaques formed on a petri plate. By the use of this technique we have isolated a number of potentially interesting types of mutants.

Drs. George Streisinger and Frank Mukai of the Cold Spring Harbor Genetics Laboratory are now collaborating with us on the genetic aspects of the problem. It is hoped that some valuable information on the genetics of the enzyme will come from that laboratory.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Experiments are continuing in an effort to gain information on the "genetic code" and the role it plays in controlling protein structure. Since the bacterial virus lysozyme appears to provide a most promising experimental system for these studies, work on the structure of this enzyme should be carried out. During the course of this work effort should be made to devise new, simpler, and more powerful methods for the study of protein structure.

An attempt must also be made to obtain bacterial virus mutants and/or strains which produce lysozymes with altered structures. Ultimately, it is by the determination of the nature of the changes produced in the enzyme as a result of mutation (particularly when induced by chemical mutagens with known mechanisms of action) that we hope to begin to gain an insight into the nature of the "genetic code".

PART B included: Yes X

Serial No. NHI-98

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Katz, A.M., W.J. Dreyer and C. B. Anfinsen, Peptide Separation by Two-Dimensional Chromatography and Electrophoresis, J. Biol. Chem. 234, 2897 (1959).

Serial No. NHI-99

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: The configurational properties of polymers of L-proline in solution

Principal Investigators: William F. Harrington
Itchak Steinberg, Weizmann Institute
Arieh Berger, Weizmann Institute
Michael Sela, Weizmann Institute
Ephraim Katchalski, Weizmann Institute

Cooperating Units: Weismann Institute of Science, Rehovot, Israel

Man Years (calendar Year 1959)
Total: 2
Professional: 2

Project Description:

Objectives: To gain information on the relationship between the proline residues of proteins and the specific configuration of polypeptide chains observed in the globular and fibrous proteins.

Methods: The configurational properties of long chains consisting of proline residues has been examined by studies of the hydrodynamic (sedimentation and viscosity) as well as optical rotatory characteristics of these polymers in various solvent systems. In addition, the kinetics of the interconversion of the two forms of poly-L-proline has been investigated.

Major Findings:

(1) Sedimentation and viscosity studies are consistent with the hypothesis that conversion of form I of poly-L-proline to Form II involves transformation of a right-handed helical structure to a left-handed helix.

(2) Solvent conditions have been found which favor reversal of left-handed helix to form the right-handed helical structure.

(3) The transformation in configuration has been shown to be dependent on cis-trans isomerizations at the peptide bonds of the polymers.

(4) The effect of neutral salt on the configuration of poly-L-proline has been studied and shown to involve a different mechanism than the organic solvent systems.

Significance to Heart Research: This work should help in clarifying the properties of certain proteins containing unusually high amounts of proline and hydroxyproline. Collagen and casein are examples of proteins whose structural properties appear to be primarily related to the proline residue.

Proposed Course of Project:

(1) Studies on collagens and gelatins from various sources with differing proline and hydroxyproline content will be continued.

(2) Similar studies on α and β casein will be initiated. These proteins have very high proline contents and preliminary investigation has shown that certain of their properties are closely similar to those observed for gelatin.

(3) Examination of simple model systems containing proline and hydroxyproline will be continued in collaboration with members of the Weizmann Institute, Israel.

PART B included No X

Serial No. MWR-100

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: Enzymatic studies on the gelatin → collagen fold transition

Principal Investigators: William F. Harrington
Peter H. von Hippel, Naval Medical Research Institu

Cooperating Units: Naval Medical Research Institute, Bethesda, Md.
(Dr. Peter H. von Hippel)

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2

Project Description:

Objectives: To examine the configurational changes in the polypeptide chains of gelatin during the gelation process. These studies should help in clarifying the structure of gelatin and collagen.

Major Findings:

(1) This work demonstrates the type of information which may be gained through the use of an enzyme to probe local changes in polypeptide chain configuration.

(2) The molecular configuration of ichthyocol gelatin has been examined at various temperatures using the proteolytic enzyme collagenase as a structural probe.

(3) On the basis of the studies above (and supporting optical rotation, viscosity and light scattering experiments on temperature and dilution effects) a model for the gelatin → collagen fold transition has been developed.

Significance to Heart Research: This work is aimed at a better understanding of the chemistry and function of collagen.

Serial No. MMT-100

Proposed Course of Project:

(1) Enzymatic studies on other collagen systems using trypsin and chymotrypsin will be initiated.

(2) Examination of configurational properties of various gelatins at low temperatures are contemplated.

Part B included Yes X

Serial No. NHI-100

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

von Hippel, P.H., and W. F. Harrington, Enzymatic Studies on the Gelatin → Collagen-fold Transition, Biochim. Biophys. Acta (in press).

Serial No. HMI-101
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: Use of Proteolytic Enzymes as Probes of the Secondary Structure of Fibrous Proteins.

Principal Investigators: William F. Harrington
Parker Small
Walter Englander

Man Years (Calendar Year 1959)
Total: 1

Project Description:

Objectives: It is well known that denatured or unfolded protein molecules are attacked much more readily by proteolytic enzymes than are their native counterparts. Recent developments suggest that many native protein molecules may be only partially folded and it seems likely, therefore, that the polypeptide chains in the amorphous regions of these protein molecules may be more rapidly cleaved than are the crystalline areas. Careful analysis of the kinetics of proteolysis should be of considerable value in elucidating the fine structure of these protein macromolecules.

Methods Employed: The kinetics of proteolysis of two fibrous proteins, fibrinogen and oxidized ribonuclease, have been investigated in the pH stat and in the polarimeter.

Major Findings:

(1) The kinetics are extremely sensitive to the presence of charged groups neighboring the sites of fissions as well as the polypeptide chain configuration in these regions.

(2) The hydrolysis of oxidized ribonuclease exhibits first order kinetics at pH values near neutrality, but the kinetics of splitting at alkaline pH regions are complex. This situation is independent of the configurational changes in the molecule.

(3) The kinetics of hydrolysis of fibrinogen are complex at all pH values examined. The reaction does not exhibit two discrete classes as was observed in the case of myosin.

Significance to Heart Research:

This work is aimed at a better understanding of the aspects of structure which make a protein fibrous in character.

Proposed Course of Project.

(1) Model synthetic polypeptide chains will be examined as a function of pH.

(2) The proteolysis of various gelatin molecules will be studied since the configurational transition in this molecule can be induced by temperature.

Part B included Yes No X

Serial No. NHI-102
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: Studies on the metabolism of triglycerides by adipose tissue and liver

Principal Investigator: Martin Rodbell

Man Years (calendar year 1959):
Total: 1
Professional: 1

Project Description:

Objectives: To study the conditions necessary for the removal of chylomicron triglycerides and their metabolism by adipose tissue and liver.

Methods Employed: Chylomicrons, labeled with C^{14} fatty acids, and artificial fat emulsions labeled with C-14 tripalmitin, were incubated with sections of rat adipose tissue. The radioactive lipids were isolated from the tissues after the incubation with the labeled substrates and were separated into free fatty acids and triglycerides. The specific activities and the quantity of these materials were determined by conventional techniques for titrating fatty acids and ester analyses. Sections of adipose tissue which had been incubated with artificial emulsions containing C-14 tripalmitin were similarly treated except that the triglyceride fraction was further fractionated by an alcohol-acetone precipitation at 5° into soluble and insoluble triglycerides. The insoluble fraction contained the C-14 tripalmitin whereas the soluble C-14 triglycerides represented those triglycerides formed from the hydrolysis and re-esterification of the fatty acids formed by the adipose tissue enzymes. Determination of the lipoprotein lipase content of the tissues were performed by established procedures.

Similar experiments were carried out with rat liver. In these experiments, the rat livers were perfused with rat blood containing synthetic fat emulsions containing C-14 tripalmitin. After determining the rate of disappearance of the fat from the blood, the livers were extracted for total lipid analyses. The radioactive lipids were subsequently fractionated into C-14 soluble triglycerides, C-14 tripalmitin, phospholipids, cholesterol, and cholesterol esters using silicic acid chromatography and the fractionation procedure employed for the separation of the soluble triglycerides from tripalmitin in the

adipose tissue experiments. The quantity and specific activities of these fractions were determined by conventional analytical techniques.

Major Findings:

1. (a) Rat chylomicrons and a synthetic fat emulsion were found to be taken up and metabolized to an equivalent extent by rat epididymal adipose tissue, suggesting that the chylomicron proteins (see project report, 1958) are not essential for fat uptake or metabolism by this tissue.

(b) Inhibition of lipoprotein lipase (the major site of this enzyme in the animal) did not substantially reduce the rate of uptake of triglycerides by adipose tissue, although there was a marked reduction in the production of fatty acids derived from chylomicron triglycerides or synthetic fat emulsions. These results suggest that the lipoprotein lipase is necessary for the metabolism of chylomicrons but not for their removal by adipose tissue.

(c) Chylomicron triglycerides and synthetic triglyceride emulsions were found to be taken up intact, prior to their metabolism, into a tissue compartment in which the triglycerides were no longer exchangeable with medium triglycerides and were inaccessible to removal by washing. The results suggest that the tissue compartment represents the rim of cytoplasm or cytoplasmic membrane surrounding the fat globule in the adipose cell.

(d) The effects of metabolic inhibitors such as cyanide and dinitrophenol and the nutritional requirements found to be necessary for maximum net incorporation of exogenous triglycerides suggest that the storage of chylomicron triglycerides in adipose tissue is mediated through the hydrolysis of the compartmentalized chylomicron triglycerides to fatty acids which are subsequently re-esterified to triglycerides.

2. (a) Similar to the adipose tissue, C-14 triglycerides were found to be taken up intact from the blood into the parenchymal cells of the rat liver. The blood triglycerides are found to be associated with the microsomes and the nucleus of the liver cells.

(b) Most of the blood C-14 triglycerides are converted to phospholipids, principally lecithin, and to triglycerides which have the composition of normal liver triglycerides.

(c) Tissues from diabetic rats remove blood triglycerides as rapidly as those from normal animals. The primary difference between the normal and diabetic livers is the fact that the liver pool size of endogenous triglycerides in the diabetic is at least three times that

found in the normal liver. In spite of this pool size, the specific activity of the liver phospholipids formed from the triglycerides are the same as in the normal liver. These results suggest that the newly entering triglycerides are spatially and metabolically separated from endogenous triglycerides.

(d) The results implicate the endoplasmic reticulum (microsomes) as the primary site for the absorption of exogenous fat by liver cells. It is proposed that the endoplasmic reticulum serves both as a channel for the passage of exogenous triglycerides into the cell as well as the site for the metabolism and the transformation of the exogenous triglycerides to other cellular lipids (lipoproteins) and the soluble lipoproteins eventually found in the blood.

Significance to Heart Research: Abnormal triglyceride metabolism is associated with many circulatory diseases. There is a tremendous void in our knowledge of the processes by which fat can be removed from the blood and metabolized in the body. It is hoped that studies of this type will provide a basic understanding of the normal and abnormal aspects of the cellular metabolism of lipids and their physiological role in cells.

Proposed Course of Project: (1) The studies of the relationship between the cellular components of the liver and adipose tissue responsible for the uptake and metabolism of fat will be continued with greater emphasis on the chemistry of the endoplasmic reticulum. (2) The enzymes responsible for the conversion of triglycerides to phospholipids will be studied with particular emphasis on the mechanism involved in this transformation.

Part B included: Yes X

Serial No. NHI-102

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards and Publications

Publications other than abstracts from this project:

Rodbell, M., The Removal and Metabolism of Chylomicrons by Adipose Tissue, J. Biol. Chem. (In press)

Serial No. NHI-103

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: Investigation of the physical and chemical properties of the glycoproteins of the yeast cell wall

Principal Investigator: Edward D. Korn in collaboration with D. H. Northcote, Cambridge University

Cooperating Units: Cambridge University, Cambridge, England

Man Years (calendar year 1959):
Total: 2/3
Professional: 2/3

Project Description:

Objectives: The structure, metabolism and function of conjugated proteins such as glycoproteins is a field of major interest in biochemistry. The cell wall presents a convenient system for the study of these problems. In particular, it was hoped to obtain homogeneous preparations of glycoproteins whose structure could be determined and which could be related to morphological entities in the yeast cell wall.

Methods and Major Findings: Yeast were broken by rapid vibration in the presence of small glass beads. The cell walls were then isolated by differential centrifugation, freeze-dried and stored over P_2O_5. The cell walls were examined for purity and structure by optical and electron microscopy. Cell walls were extracted with anhydrous ethylenediamine which dissolved approximately 50% of the material. The insoluble material was washed with methanol and ether and dried (Fraction C). The ethylenediamine was removed by evaporation and the residue suspended in water and dialyzed exhaustively. The insoluble material was collected and freeze-dried to give Fraction B (10% of original wall) and the supernatant solution freeze-dried to give Fraction A (35% of cell wall). Fraction A was largely one component by electrophoretic and ultracentrifugal analysis. It contained 12% protein and 2% glucosamine and 85% mannan (a mannose polymer). Its molecular weight by sedimentation diffusion measurements was 9.8×10^4. Fraction B contained 6% protein, 1% glucosamine and both mannan and glucan (a glucose polymer) made up the carbohydrate. Fraction C

again contained mannan and glucan in addition to 16% protein and 2% glucosamine. Of interest was the additional finding that classical preparations of "mannan" and "glucan" (always presumed to be pure polymers of mannose and glucose) actually contain 1.5 and 6% glucosamine respectively and a significant amount of residual protein (1.5 and 1%). The molecular weight of "mannan" was found to be 5.9×10^4. These studies were interpreted to indicate that the yeast cell wall is probably a complex polymer of mannan, glucan, and protein of which the materials isolated are partial degradation products. The protein and carbohydrate portions may be linked by the glucosamine. Chitin implicated to be present by earlier investigators does not seem to occur.

Significance to Heart Research: Conjugated proteins such as lipoproteins and glycoproteins are in high concentration in both plasma and cells. Significant work in this field has largely been impeded by a lack of adequate methods and basic understanding of the nature of the structures and their metabolism. To clarify some of these problems has been the prime goal of this research.

Proposed Course of Project: This project was undertaken at Cambridge University during a "Trainee" period and has now been discontinued.

Part B included: Yes X

Serial No. NHI-103

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Edward D. Korn and D.H. Northcote, Physical and Chemical Properties of Polysaccharides and Glycoproteins of the Yeast Cell Wall. Biochem. J. (accepted for publication)

Serial No. MHX-104

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A:

Project Title: Studies on heparin and lipoprotein lipase

Principal Investigator: Edward D. Korn

Other Investigator: Bertha Neal (technical)

Man Years (calendar year 1959)
Total: 2/3
Professional: 1/3
Technical: 1/3

Project Description:

Objectives: To study the structure and metabolism of heparin and related mucopolysaccharides, the relation of heparin to lipoprotein lipase and the role of lipoprotein lipase in fat metabolism.

Methods and Major Findings: This project had been discontinued in August 1958 when the principal investigator left for a year at Cambridge University. It was begun again in September 1959 with little results at this time. No report was submitted in December 1958 so what follows reports work done in 1958.

A new method for the isolation of small quantities of heparin in good yield from tissues was developed and applied to mouse mast cell tumors. The procedure involves digestion of the tissue with pancreatin, dialysis, and several precipitations of heparin from 1 M NaCl as the cetyltrimethyammonium complex. It was demonstrated that slices of mouse mast cell tumor could incorporate C-14 glucose and S-35 sulfate into heparin when incubated in vitro. It was further shown that soluble enzymes from mast cell tumors could catalyze the incorporation of S^{35}-sulfate into heparin. This system requires the addition of ATP and phosphoadenosyl phosphosulfate ("active sulfate", PAPS) was shown to be intermediate.

Significance to Heart Research: Heparin is not only a potent anticoagulant but also appears to be involved in lipoprotein metabolism. Lipoprotein lipase is an enzyme which probably plays a major role in lipid transport and metabolism.

Serial No. NHI-104

Proposed Course of Project: The major effort will be spent in an attempt to purify lipoprotein lipase in order to study its properties and, specifically, to determine directly if it is a mucoprotein which contains heparin or a similar mucopolysaccharide.

Part B included: Yes X

Serial No. NHY-104

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Meyer, K., Linker, A., Hoffman, P. and Korn, E.D., The Enzymatic Breakdown of Hyaluronic Acid and of Sulfated Mucopolysaccharides, Proc. of the International Symposium on Enzyme Chemistry, International Union of Biochemistry, 2, 132-134 (1958).

Korn, E.D., Preparation and Assay of Lipoprotein Lipase *in vivo* and *in vitro*. Methods of Biochemical Analysis, ed. D. Glick, Vol. VII, p. 145-192, Interscience Publishers, Inc., New York, 1959.

Korn, E.D., Observations on the Use of Cellulose Ion Exchangers for the Chromatographic Separation of Nucleotides, Biochim. et Biophys. Acta, 32, 554 (1959).

Korn, E.D., The Isolation of Heparin from Mouse Mast Cell Tumor, J. Biol. Chem., 234, 1325-1329 (1959).

Korn, E.D., The Synthesis of Heparin by Slices of Mouse Mast Cell Tumor , J. Biol. Chem., 234, 1321-1324 (1959).

Korn, E.D., The Enzymatic Sulfation of Heparin, J. Biol. Chem., 234, 1647-1650 (1959).

Serial No. NHI-105

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: Investigation of the ribonucleoprotein granules of rat liver ergastoplasm as sites for specific synthesis of cytoplasmic proteins

Principal Investigators: Richard W. Hendler
Elbert A. Peterson*, National Cancer Institute
Edward L. Kuff*, National Cancer Institute

*Separate reports on this project will be submitted by these investigators to their institute.

Cooperating Units: National Cancer Institute

Man Years (calendar year 1959) Total 1/2 (consisting of about 2 months work by each investigator)

Patient Days: None

Project Description:

Objectives: Current ideas in protein synthesis fix the ergastoplasm as the major cellular site for protein synthesis. The ergastoplasm consists of a lipid membrane, studded with ribonucleoprotein granules approximately 150 Å in diameter. It is believed that information for making particular proteins is contained in the configuration of the nucleic acids of these granules. If this is so, one should expect that the granules are heterogeneous. We are trying to fractionate these granules and determine if different granules do make different cytoplasmic proteins.

Methods and Major Findings: The proteins of the livers of rats were labeled in vivo by injection of radioactive amino acids. The radioactive soluble cytoplasmic proteins were characterized by their chromatographic behavior on columns of DEAE cellulose. The ribonucleoprotein granules were obtained by various treatments but predominently by treatment of the microsomes with deoxycholate and subsequent ultracentrifugation. Attempts to fractionate the granules on columns gave indications that this could be accomplished. Radioactive protein was released from the granules by treatment with versene or by

reincubation of the granules under controlled conditions. The proteins released by incubating the granules but not that released by versene treatment, behaved as normal soluble proteins upon chromatography on DEAE cellulose. The relative rate of accumulation of radioactive amino acid by all fractions of the liver has also been studied.

Significance to Heart Research: The dry weight of the body and its soft tissue structural material is mainly protein. The Heart Institute is concerned with understanding the normal function of heart tissue. The heart as a muscle is in a constant state of protein synthesis and degradation. Biochemical knowledge is based on the thesis that basic biochemical mechanisms are similar in the varied types of living tissue. The ability of a cell to function normally is based on the direction it receives. This direction must be in the form of chemical structures. It seems likely that many defects in tissues may be traced to defects in the processes determining aspects of specificity in the cell's metabolism.

Proposed Course of Project: The fractionation of RNP granules shall be continued with the objective of obtaining complete separation. Other methods than deoxycholate) will be looked for in order to obtain the granules. We shall try to improve the method of releasing labeled soluble protein from the granules. Attempts will be made to see if one class of granules will release only one class of normal soluble cytoplasmic protein.

PART B included: Yes No X

Serial No. NHI-106

1. Laboratory of Cellular Physiology
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: A chemical study of amino acid lipid complexes obtained from tissue

Principal Investigators: Richard W. Hendler
Evan C. Horning, LCNP, NHI
Marjorie G. Horning, LCNP, NHI

Cooperating Units: Laboratory of Chemistry of Natural Products, NHI

Project Description: Man Years (calendar year 1959) 1/4 in total

Objectives: To isolate and chemically characterize the amino acid lipid complexes obtained from tissues.

Methods and Major Findings: The partial fractionation of these complexes obtained from hen oviduct and E. coli has been achieved on columns of silicic acid. Large scale fractionations of material from hen oviduct have yielded milligram quantities of the complexes labeled with radioactive amino acids. The chromatographic behavior of some of this material has been compared on two different adsorbents in order to initiate studies of chemical character and in the hope of eventually improving fractionation.

Significance to Heart Research: The possible relation of lipid metabolism to the process of protein synthesis is being considered. Lipid metabolism is currently believed to exert a substantial influence in degenerative changes of the heart and blood vessels. If these amino acid lipid complexes do play an important role in the metabolism of tissues in general and the circulatory system in particular, a knowledge of their complete structure will be very important.

Proposed Course of Project: Attempts will be made to completely fractionate and chemically characterize amino acid lipid complexes from a variety of tissues.

Part B included: No X

Serial No. NHI-106 A

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Richard W. Hendler, Self-Absorption Correction for Carbon-14, Science, 130, 772-777 (1959).

Serial No. NHX-107

1. Laboratory of Cellular Physiology
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: A study of the possible role of amino acid-lipid complexes in the metabolism of E. coli

Principal Investigators: Richard W. Hendler
Richard M. Roberts, Carnegie Institute of Washington
Kenneth McQuillen, Carnegie Institute of Washington

Cooperating Units: Carnegie Institute of Washington, Washington, D.C.

Man Years (Calendar Year 1959)
Total 1/4 (in total)

Project Description:

Objectives: The possible role of lipids in the biosynthesis of proteins of the hen oviduct has been indicated by work with this tissue. A similar study in a completely different tissue capable of large scale protein synthesis was deemed desirable in order to study the generality of the indicated findings with the hen oviduct. The Carnegie group is considered to be the leading experts in the field of E. coli metabolism and a collaboration of this kind appears to be very suitable for the problem under study.

Methods and Major Findings: It was found that E. coli, in both the log and lag phases of growth could take up amino acids into chloroform soluble complexes. This material could be fractionated on columns of alumina-silica and silicic acid yielding patterns that were different from the ones obtained from hen oviduct. It was further found that the amino acid of the complex was in a very dynamic form capable of rapid incorporation and exit. As in the case of hen oviduct, this dynamic form was characteristic of lipid associated but not nucleic acid associated amino acid. Additional information with the E. coli strongly implicate these compounds as being involved in cell wall metabolism possibly concerned with the transport mechanism. Their role in protein synthesis per se is also a definite possibility. Preliminary experiments indicate a possible strict specificity of sites in the lipids for particular amino acids.

Significance to Heart Research: The possible relation of lipid metabolism to the process of protein synthesis is being considered. Lipid metabolism is currently believed to exert a substantial influence in degenerative changes of the heart and blood vessels. A general association of lipid and protein metabolism can be established only by studying several different tissues.

Proposed Course of Project: If time permits we will hope to pursue further aspects of amino acid-lipid involvements in the metabolism of E. coli.

Part B included: No X

Serial No. NHI-108

1. Laboratory of Cellular Physiology and Metabolism

2.

3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: A study of the mechanism of protein biosynthesis in the hen oviduct

Principal Investigator: Richard W. Hendler

Man Years (calendar year 1959)
Total: 0.59
Professional: 0.59

Project Description:

Objectives: The mechanism of protein biosynthesis is one of the most important areas in biochemistry about which the least is known. It is the purpose of this work to study the basic reactions of protein biosynthesis in a tissue highly specialized for performing this function, the oviduct of the laying hen.

Methods and Major Findings: The possible implication of lipid-type forms of the amino acids in protein synthesis was indicated from previous studies with the hen oviduct system. Further studies on the nature of this phenomenon as well as the character of the chemical entities involved were pursued. The effects on the lipid compounds of conditions unfavorable to protein synthesis were studied. Successful attempts at fractionation were achieved with countercurrent distribution and chromatography on columns of alumina-silica. Beginnings were made in the examination of individual lipid amino acid components from the standpoint of which amino acids are contained, which of the major lipid components are present and what is the relative stability of the amino acid lipid association. It was learned that the ability of amino acids to associate with lipids is general and not restricted to particular kinds. At present, it still appears possible that this is a phenomenon intimately related to the process of protein synthesis.

Significance to Heart Research: The dry weight of the body and its soft tissue structural material is mainly protein. The Heart Institute is concerned with understanding the normal function of heart tissue. The heart as a muscle is in a constant state of protein synthesis and degradation. Biochemical knowledge is based on the thesis that basic biochemical mechanisms are similar in the varied types of living tissue. By studying a tissue highly specialized with respect to the process of

protein synthesis (oviduct of the laying hen) it is hoped that knowledge of this vital phenomenon will be applicable to the tissues of man.

Proposed Course of Project: The isolation, purification, and characterization of the lipid-amino acid complex will be pursued. The possible role of these compounds in protein biosynthesis will be further studied.

Part B included: Yes X

Serial No. NHI-108

PHS--NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Richard W. Hendler, Passage of Radioactive Amino Acids through "Nonprotein" Fractions of Hen Oviduct during Incorporation into Protein, J. Biol. Chem., 234, 1466-1473 (1959).

Serial No NHI 109
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Triglyceride Mobilization and Deposition.

Principal Investigator: M. G. Korning
H. Maling

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):

Total: 2/3
Professional: 2/3
Other:

Project Description:

To study the effect of ethionine, ethanol and carbon tetrachloride on triglyceride metabolism and to determine the influence of adrenergic blocking agents on the metabolic processes involved in fatty acid and triglyceride mobilization.

Methods and Major Findings:

The administration of ethionine, ethanol and carbon tetrachloride to rats, in independent experiments, resulted in the accummulation of fat in the liver. Analysis of the total liver lipids by silicic acid chromatography showed a three to ten fold increase in the triglyceride fraction in the liver. Fatty acid composition data for the normal and fatty liver triglycerides and for the adipose tissue triglycerides are being obtained by gas chromatography. Preliminary results show that the fatty acid composition of the adipose tissue and the liver triglycerides in both the normal and fatty liver are very similar.

Part A. cont.

The immediate problem lies in determining whether the transport of these fatty acids occurs as NEFA (non-esterified fatty acids) or as triglycerides, or whether liver fatty acid synthesis is partially or wholly responsible for the observed effects. Evidence bearing on this point was obtained by experiments with C^{14}-acetate. The adipose fat in the rat was labeled by pretreating the animals with C^{14}-acetate in such a way that no radioactive acetate remained in the body pool when the chemical (ethionine, etc.) was administrated.

The radioactivity present in the triglycerides of the liver after treatment with ethionine and carbon tetrachloride was consistent with the theory of transport (acids or triglycerides) from adipose tissue. The experiments with ethanol appear to involve some synthesis of fatty acids as well as transport from adipose tissue. Ethanol thus becomes a special case which should be studied in greater detail.

It is not possible to state whether the triglycerides were transported as such from adipose tissue or were first hydrolyzed to free fatty acids in adipose tissue and then transported to the liver to be resynthesized into triglycerides. However the composition of the triglyceride fraction of the fatty liver is almost exactly that of the adipose tissue and this is compatible with the view that direct (triglyceride) transport occurs. An interesting point lies in the fact that the linoleic acid content of the liver triglycerides, in the fatty liver case, is the same as that in the adipose tissue triglycerides. Since the rat cannot synthesize this acid it is evident that the liver infiltration of this acid was at the expense of the depots, and that a transport and deposition phenomenon occurred.

The administration of adrenergic blocking agents (dibenamine, dibenzylene) to the animals 24-48 hours before ethionine treatment results in complete blocking of the transport effect. The response of the animal is naturally dependent on the method of pretreatment, but the current results indicate that when the concentration of dibenamine in adipose tissue reaches a maximum level, there is no release of fatty acids or triglycerides from the adipose tissue.

Part A con'd.

Proposed course of Research

The very large increase in liver triglycerides in these acute experiments is due to an increase in the rate of transport to the liver and probably also to a decreased catabolism of triglycerides by the liver.

It is possible that the lipase activity of the adipose tissue of the rats is influenced in opposite directions by treatment with ethionine, ethanol and carbon tetrachloride and by the adrenergic blocking agents. It is proposed to assay this activity in the variously treated animals to ascertain if such changes have occurred. If the catabolism of triglycerides is involved, there should also be changes in the liver handling of fats and these effects should be studied on an enzyme level.

Part B Included No X Yes

Serial No NHI 110
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Degradation of Cholesterol by Human Red Blood Cells.

Prinicpal Investogator: M. G. Horning
H. Danielsson
Prof. Sune Bergstrom

Other Investigator

Cooperating Units

Man Years (calendar Year 1959):

Total: 2/3
Professional: 1/3
Other:1/3

Project Description:

To study the in vitro degradation of cholesterol to bile acids.

Methods and Major Findings:

It was found that incubating human red blood cells with albumin suspensions of cholesterol-4-C^{14} resulted in the formation of several degradation products. Three classes of compounds were separated by reverse phase chromatography: acids, diols and triols. The acids formed did not include cholic acid or chenodeoxycholic acid, but one of the acids appeared to be identical with one formed by the liver mitochondrial system. It may be a di or trihydroxy coprostanic acid.

Part of the isotopic material in the triol peak was found to be identical with 3β, 5α, 6β-trihydroxy cholestane. One of the diols was identified as 7α-hydroxycholesterol. Apparently, 7β-hydroxy cholesterol is not formed, indicating that there is far less auto-oxidation in this system than in the liver mitochondria.

Part A cont'd

Methods and Major Findings:

Model compounds are being synthesized in order to determine by carrier dilution technique if 3α, 7α-dihydroxycholestane and 3 keto, 7α-hydroxycholestane are among the as yet unidentified products which have been isolated. These two compounds are probable intermediates in the conversion of cholesterol to bile acids. If the results are positive, it would be werthwhile to try to fractionate the blood cells for the enzymes involved in these reactions. The results so far certainly indicate that the blood may play a positive roll in the conversion of cholesterol to intermediates on the pathway to cholic and chemodeoxycholic acid.

Part B Included Yes X No

Serial No NHI 110

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B

Publications

1. On the oxidation of cholesterol by mouse liver mitochondria. Bile acids and Steroids 88
Henry Danielsson, Marjorie G. Horning. B.B.A. Vol. 34, 596 (1959).

Serial No NHI 111
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Metabolism of N-onium compounds and Methyl Donor Compounds in Anaerobic Microbes.

Principal Investigator: Hugh R. Hayward

Other Investigator:

Cooperating Units

Man Years (calendar Year 1959):

Total: 1 1/3
Professional: 1
Other: 1/3

Project Description:

To study the fermentation of choline as catalyzed by extracts of Vibrio cholinicus with regard to intermediates and reaction mechanisms, and to study the reactions involved in the esterification of inorganic phosphate coupled with this fermentation.

Methods and Major Findings:

(a) Further identification studies in the choline-fermenting organism revealed it to be a hitherto undescribed member of the genus Vibrio, and the name Vibrio cholinicus was assigned.

(b) Further study of the overall fermentation by sonic extracts has revealed the requirement of two heat labile fractions, one particulate and the other soluble after centrifugation for 90 minutes at 105,000 x g.

Part A. con't.

(c) Choline-dependent esterification of inorganic phosphate has been demonstrated in crude sonic extracts by using a glucose-hexokinase trap for the ATP synthesized.

(d) Studies of the effects of inhibitors on choline disappearance revealed marked inhibition by 2,4-dinitrophenol at concentrations known to uncouple phosphorylation from oxidation in aerobic systems; thus the possibility of electron transport-coupled phosphorylation exists in this anaerobic system.

(e) Preliminary studies on the possible role of the cytochrome of this organism revealed the choline-dependent appearance of the spectrum of the reduced form of the pigment in crude sonic extracts. These extracts also reduce sulfate, and by analogy to other sulfate-reducing bacteria the possibility exists that the cytochrome functions in the latter process.

(f) Some of the growth conditions for maximum production of the cytochrome have been established.

(g) Acetaldehyde has been shown to be an intermediate in the conversion of the 2-carbon moiety of choline to acetate and ethanol.

(h) The conversion of acetaldehyde to acetate and ethanol has been studied in crude sonic extracts and in partially purified protein preparations. The particulate fraction described above is not required. Absolute requirements for ADP, TPN, and a sulfhydryl compound have been demonstrated. In crude extracts a two to three-fold stimulation is observed on the addition of catalytic levels of a divalent cation, ferrous iron being the most effective of several examined. Similarly, the addition of catalytic levles of CoA markedly stimulates the overall dismutation, though no obligate requirement for it has yet been demonstrated.

Part A. cont'd.

(I) Some suggestive evidence has been obtained that betaine aldehyde may be an intermediate in the overall fermentation of choline, but conclusive demonstration of this point awaits clarification.

Proposed course of Research

Currently attempts are being made to fractionate and partially to purify the enzymes involved in the sequence of reactions from acetaldehyde to acetate so as to study these individual reactions. Attention will next be focused on the elucidation of the earlier intermediates in the fermentation with a view to localizing the site of phosphate esterification and studying the mechanism of this process. The possible site of action of the cytochrome will also be investigated. A series of anaerobic organisms isolated from soil on related substrates will be examined for cytochrome content.

Part B Included Yes X No

Serial NO. NHI 111

PHS -NIH
Individual Project Report
Calender Year 1959

Part B.

Publications:

1. Anaerobic Degradation of Choline: I Fermentation of Choline by an Anaerobic, Cytochrome-producing Bacterium, Vibrio cholinicus nov. spec., J. Bacteriology, in press.

2. Anaerobic Degradation of Choline II Preparation and Properties of Cell-free Extracts of Vibrio cholinicus, in ms.

Serial No NHI 112
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effects on Heart Muscle Triglycerides in Dogs after Large Doses of Catechol Amines and after Myocardial Infarctions

Principal Investigator: M. G. Horning
H. Maling

Other Investigator

Cooperating Units

Man Years (calendar Year 1959):

Total: 2/3
Professional: 2/3
Other:

Project Description:

Experimental partial heart failure (myocardial infarction) was induced by surgical means in dogs. The heart lipid changes were examined one to ten days after the infarction. A zone of fatty infiltration surrounded the infarcted area, while the remainder of the heart was normal. These lipid changes are being studied in detail.

It has been found that both triglyceride and phospholipid levels are affected in the infiltrated zone. The composition of the triglycerides is very much like that of the adipose tissue, which suggests that a transport and deposition phenomenon is involved. The disappearance of the triglycerides from the heart is currently being studied.

Large doses of catachol amines produce an effect which is related but not identical to that of the myocardial infarct experiments. A fatty infiltration of the heart tissue results, and this is chiefly triglyceride. Adrenergic blocking agents prevent this effect, but do not prevent the effect that occurs after infarction.

Part A cont'd

Methods and Major Findings:

The heart tissue lipid changes after catechol amine administration will be studied in detail and compared with those from the infarction experiments. An attempt will be made to study triglyceride utilization by the heart tissue.

Part B Included Yes _____ No X

Serial No. NIH 112
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigation of the Biosynthesis of Phenazine-1-carboxylic acid.

Principal Investigator: Mark Levitch

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):

Total:
Professional:
Other:

Project Description:

To define the biosynthetic pathway of phenazine-1-carboxylic acid in Pseudomonas aureofaciens Kluyver, using radioactive labeling and metabolic analogue inhibition studies.

Methods and Major Findings:

A. Radioactive labeling
1. Incorporation

A number of compounds have been tested for incorporation into phenazine-1-carboxylic acid in growing cultures, the most effective (highest specific activity) are acetate bicarbonate, alanine, serine, and methionine.

Part B Included Yes _____ No X

2. Chemical Degradation

In order to delineate the pattern of labeling of the phenazine compounds after incorporation of radioactive substrates, chemical degradation studies have been instituted. The phenazine-1-carboxylic acid has been converted to the α-amino compound in low yield. In order to obtain a more readily available starting material for ensuing reactions, chemical synthesis the α-methoxy and α-hydroxy derivatives has been accomplished.

B. Inhibition studies

II has been found that biosynthesis of phenazine-1-carboxylic acid is inhibited by relatively large concentrations (10 mg/1 ml.) of p-aminobenzoic acid. Reversal by low levels of aromatic amino acids has so far been unsuccessful. Complex medium has been purified to remove the major part of the aromatic amino acids for subsequent studies.

C. Cell-free preparations

Using previously grown cells synthesis has been obtained over short time intervals, accompanied by very slight increase in cellular material. Attempts to Obtain an active cell-free extract have so far been unsuccessful.

Proposed course of Research

Incorporation of radioactive substrates into phenazine compounds in resting cell, and eventually cell-free preparations are to be investigated; the PABA effect will be studied, especially the possibility of an accumulation of intermediates on the biosynthetic pathway. Additional inhibitor studies are planned.

Part B Included Yes ____ No X

Serial No. NHI 114
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calender Year 1959

Part A.

Project Title: (1) Isolation and Characterization of Compounds of Biological Interest.

Principal Investigator: Lin Tsai

Other Investigator:

Cooperating Units

Man Years (calender year 1959):

Total: .8
Professional: .5
Other: .3

Project Description:

The purpose of the research is to synthesize compounds for use as substrates in enzymatic studies and to attempt the isolation and characterization of biological intermediates.

Methods and Major Findings:

The structure of the bacterial degradation product of 1-ribityl-6,7-dimetyl-1,2,3,4-tetrahydro-2,4-diketo-quinoxaline, isolated by P. Z. Smyrniotis and E. R. Stadtman, was shown to be 6,7-dimethyl-2,3-quinoxalinediol by direct comparison with an authentic sample of this compound obtained by chemical synthesis. Other similar quinoxaline derivatives have been synthesized chemically and found to undergo degradation by the same microorganism.

Preliminary experiments directed toward the synthesis of a number of metabolic intermediates have been carried out. Their characterizations are as yet insufficient for definite assignment of structures.

Serial No. NHI 114

Part A. con't.

Proposed course of Research

Present studies will be continued.

Incidental Findings: None

Part B Included Yes _____ No X

Serial No. NHI 115
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Metabolism of Sulfonium Compounds

Principal Investigator: C. Wagner

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):

Total: .5
Professional: .3
Other: .2

Project Description:

To study the metabolic pathways by which sulfonium compounds are degraded by microorganisms.

Methods and Major Findings:

A motile, rod-shaped organism has been isolated by enrichment culture which is capable of growing anaerobically on dimethyl-β-propiothetin as the major source of carbon. The disappearance of the substrate is accompanied by the production of acetic and propionic acids as the major products.

Further work is being carried out in order to obtain a complete fermentation balance during the growth on this substrate.

Part B Included_____ Yes X No_____

Serial No NHI 115

PHS - NIH
Individual Project Report
Calendar Year 1959

Publications

1. The Metabolism of Glycogin in Tetrahymena pyriformis, C. Wagner and J. F. Hogg. (in press).

2. "The Conversion of Fat to Carbohydrate in Tetrahymena pyriformic", C. Wagner and J. F. Hogg, in press.

Serial NHI 116

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemistry of Differentiation in the Slime Mold.

Principal Investigator: Barbara E. Wright

Other Investigator

Cooperating Units

Man Years (calendar Year 1959)

Total: 2.3
Professional: 1
Other: 1.3

Project Description:

To analyze on a biochemical level various mechanisms contributing to the process of differentiation.

Methods and Major Findings:

(I) A collaboration with Dr. Erich Heftmann (NIAMD) and Mr. G. Liddel has resulted in the identification of the chemotactic hormone, acrasin, in D. discoideum: Δ^{22} stigmasten-3β-01. Based on the absence of activity in other fractions during the purification, as well as on rough estimates of recovery of acrasin, it would appear that this sterol is the major if not the only active compound present. Since acid hydrolysis was used during the purification, it is possible that the sterol exists in a bound form in vivo. Acrasin was identified by comparison to the authentic, synthetic material. Two derivatives of the natural and synthetic sterols were also prepared. Infrared spectra, melting points, rotations, and carbon and hydrogen analyses of the three natural compounds agreed well with the comparable synthetic materials.

Part B Included Yes X No

Part A con't.

(II) Studies on amino acid and protein turnover have been continued. It had been shown that extensive protein degradation occurs during development, and studies on S^{35} methionine uptake demonstrate that active protein synthesis also occurs (i.e., Turnover) throughout the differentiation process. Methionine protein is replaced by endogenous pool S^{35} methionine at the rate of about 7% per hour at preculmination. The rate of protein synthesis appears to decrease slightly during development. The relative rate of S^{35} methionine incorporation into ethanol soluble as compared to ethanol in soluble protein changes during differentiation.

The size of the endogenous "free" methionine pool is a function only of the stage of development, and is uninfluenced by exogenous methionine. However, exogenous S^{35} methionine can exchange with endogenous methionine, and the extent of this exchange (i.e., the specific radioactivity of pool methionine) is a linear function of the exogenous S^{35} methionine concentration.

Following a pulse of S^{35} methionine, the separation of various arbitrary classes of protein (by their ethanol solubility and by DEAE column chromatography) has revealed a striking heterogeneity in the specific radioactivity of these different protein classes. Evidence has been accumulated which indicates that the methionine molecules in the amino acid pool are "fixed" with respect to proteins into which they are incorporated. It appears that, on the average, pool methionine molecules which exchange readily with exogenous S^{35} methionine become incorporated into proteins of higher specific radioactivity; pool methionine molecules exchanging poorly with exogenous S^{35} methionine become incorporated preferentially into proteins of lower specific radioactivity.

Proposed course of Research

I. We are doing a specificity study of sterols related to acrasin. With some tritiated material, we will try to locate the part of the cell where acrasin acts, isolate

Part A con't

some bound derivative, etc. It might also be worthwhile to synthesize a water soluble derivative of acrasin, and to look into the possibility that it exists in soluble form in vivo.

II. All efforts on this project are concentrated purification of glucose 6 PO_4 dehydrogenase from the slime mold. One of the ends in view is to examine the rate of synthesis of a single protein at different stages of differentiation.

Part B Included Yes X No

Serial No NHI 116

Part B

1. Herman, E. C., and Wright, B. E. A 5'Nucleotidase activated by Ferrous Iron, JBC 234 122 (1959)

2. Wright, B. E., and Anderson, M. L., Biochemical Differentiation in the Slime Mold. Biochem. Biophysica Acta 31 310 (1959).

3. Wright, B. E. and Anderson, M. L., Amino acid Incorporation into protein in the Slime Mold Bacteriological Proc. (1959)

4. Wright and Anderson Protein and amino acid behavior during differentiation in the Slime Mold, Federation Proc. 18 (1959).

Serial No. NHI 117
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calender Year 1958

Part A.

Project Title: Isoprenoid Degradation in Aerobic Microorganisms.

Principal Investigator: Werner Seubert

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):
Total: 1.3
Professional: 1
Other: .3

Project Description:

Study of the mechanism of isoprenoid degradation.

Methods and Major Findings:

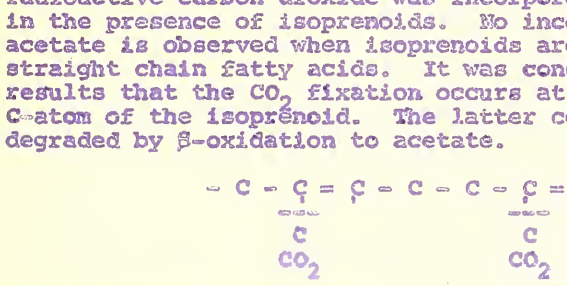
Classification of a citronellol degrading microorganism which had been isolated from soil. The identification studies revealed that the organism belongs to the genus Pseudomonas as a new species. The organism was designated Pseudomonas citronellolis.

In growth studies it was shown that the organism can utilize a variety of substrates. Among these are Ionine, which is a representative of the cyclic isoprenoids, and higher homologues of citronellol, like farnesol and farnesoic acid. The latter compounds have been included in the studies on the mechanism of isoprenoid degradation.

To obtain information about the pathway of citronellol degradation, growth filtrates have been analyzed for intermediates. It was possible to isolate an acid which was shown to be identical with synthetic citronellic acid by comparison of the infrared spectra.

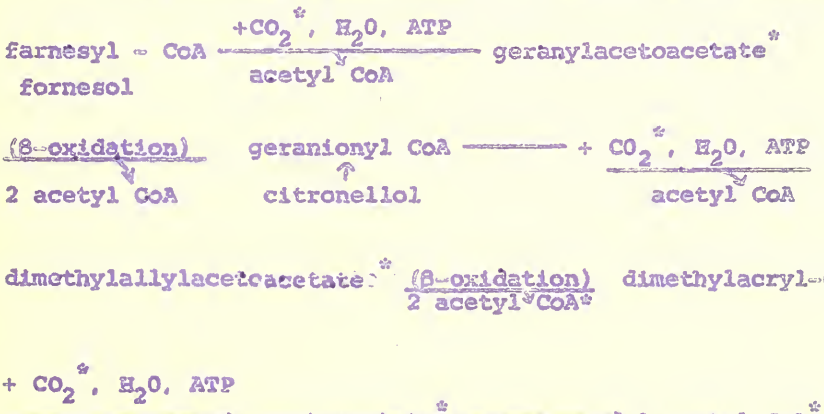
Part A. con't.

In resting cells which had been inhibited by arsenite, radioactive carbon dioxide was incorporated into acetate in the presence of isoprenoids. No incorporation into acetate is observed when isoprenoids are replaced by straight chain fatty acids. It was concluded from these results that the CO_2 fixation occurs at the branched C-atom of the isoprenoid. The latter could be further degraded by β-oxidation to acetate.



Mechanistically this would occur by a cyclic process analogous to the conversion of dimethylacryl CoA through acetoacetate to acetate. The higher homologues of dimethylacryl CoA in this case would be geraniony CoA and farnesyl CoA which contain 2 and 3 isoprenoid units respectively. The corresponding homologues of acetoacetate would be: dimethylallylacetoacetate and geranylacetoacetate.

This process is summarized by the following scheme;



The following experimental evidences favor this scheme.

Part A. con't

1) Incorporation of labeled Co_2^* into acetate in the presence of dimethylacryl CoA, geraniony1 CoA and farnesyl CoA in studies with enzyme extracts.

2) Incorporation of CO_2 into acetoacetic acid in the presence of dimethylacrylyl CoA.

3) Accumulation of carboxyl labeled β-ketoacids in the presence of labeled CO_2, arsenite and citronellic or farnesoic acid in studies with intact cells. In order to identify these β-ketoacids with the assumed intermediates acetoacetate, dimethylallylacetoacetate and geranylacetoacetate, the latter two compounds have been synthesized. However so far no satisfactory method for the chromatographic separation of these β-ketoacids could be developed.

Proposed course of Research

Further studies on the degradation of isoprenoids. Evidence for the conversion of geraniony1 CoA and farnesyl CoA to dimethylallylacetoacetate and geranyl-acetoacetate resp. according the postulated scheme.

Part B Included Yes X No

Serial No. NHI 117

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Publications:

1). A paper on the classification of the organism has been submitted to the J. of Bact.

Serial No. NHI 118
Laboratory of Cellular Physiol
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies in Heterocyclic Compound Metabolism: Alkaloid Biosynthesis

Principal Investigator: E. Kravitz

Other Investigator:

Cooperating Units

Man Years (calendar year 1958):

Total: 1.3
Professional: 1
Other: .3

Project Description:

This research project is aimed at studying the enzymic mechanism involved in the biosynthesis of heterocyclic compounds.

Methods and Major Findings:

Some studies on the biosynthesis of opium alkaloids were carried out with Papaver somniferum grown for us at the USDA Plant Industry Station in Beltsville, Md. The outlined experimental approach followed in these studies was to first, try to learn something of the rate of alkaloid biosynthesis and which alkaloids were formed during various phases of the development of the plant, then to attempt to locate the site, or sites of biosynthesis, and finally to attempt to solubilize by various means, the enzyme systems involved in their biosynthesis.

Methionine-Methyl C^{14} and β-C^{14} serine were vacuum infiltrated into plants of various ages. After one days incubation in water the plants were homogenized, and the

Part A. con't.

alkaloids extracted and separated by paper chromatography. Radioactive alkaloids were formed with plants of all ages. The major alkaloids containing isotope were papaverine and narcotine in 4 to 5 week old plants, thereafter, isotope was found in morphine and codeine as well (these are tentative identification of these compounds pending isolation of sufficient quantities for chemical analysis). The plants were then sectioned and experiments carried out measuring isotope incorporation into alkaloids in these different sections. It was found that root slices would incorporate methionine-Me-C^{14} into papaverine and narcotine (tentative identifications). The incorporation was not inhibited by CN^- but was decreased when acetate or glucose were added to the incubation vessels. A saturation curve effect was observed with increasing numbers of root slices indicating that something was limiting the incorporation, or an inhibitor of the synthesis was present in the roots.

All attempts to solubilize the enzyme system to date have been unsuccessful.

Along with the biological work, an ion exchange procedure has been devised for the separation of the major opium alkaloids. The procedure involves the use of Dowex-1-OH^- and Dowex-50-H^+ ion exchange resins and elutions from these resins with various H_2O-HCl-ethanol mixtures.

Proposed course of Research

The studies on the biosynthesis of the opium alkaloids will continue with root slices and it will be attempted to synthesize radioactive alkaloid from Tyrosine-U-C^{14} as well as the Methionine-Methyl-C^{14}. Attempts will then be made to separate the tyrosine incorporation from the methyl group incorporation by various inhibitors and thereby gain some insight into the overall biosynthetic process. In addition continual attempts will be made to solubilize the enzyme systems involved in these steps.

A second project is also underway to study the biosynthesis of alkaloids by cultures of Claviceps purpurea. Work has just begun on this project and the approach will be somewhat similar to that with the opium alkaloids. This project offers the distinct advantage that large quantities of the fungus can be grown for the biosynthetic studies.

Part B included Yes____ No X

Serial NHI 119

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: 1. Mechanism of enzymatic formation of threonine from O-phosphohomoserine.

2. Biosynthetic pathways of cystathionine and O-phosphohomoserine in Neurospora.

3. Role of lipoic acid in Butyribacterium rettgeri fermentation.

Principal Investigator: Martin Flavin
Charles Wittenberger

Other Investigator

Cooperating Units

Man Years (calendar Year 1959):

Total: 2.3
Professional: 1
Other: 1.3

Project Description: Methods and Major Findings

Threonine synthetase appears to be a single enzyme, which catalyzes an elimination of phosphate from O-phosphohomoserine coupled with a transposition of oxygen from γ to β position. In a continuation of studies of the mechanism of this novel enzymatic reaction, which has some features in common with early steps in squalene biosynthesis, the enzyme has been purified 500-fold from Neurospora, and some of its properties have been determined. The reaction requires added pyridoxal phosphate. During incubations in H_2O^{18} (in collaboration with Dr. Tetsuro Kono of the McCollum-Pratt Institute), O^{18} is incorporated into threonine but not into phosphate. O-phosphothreonine is not decomposed. Phosphate is therefore removed by elimination, rather than hydrolysis, with cleavage at a C-O bond. During incubations in 100% D_2O, two atoms of deuterium are incorporated into threonine, one in the α position. Incubation in H^3_2O results in the incorporation of very much smaller amounts of tritium, corresponding to the aquisition of 0.10 atoms of solvent hydrogen per mole threonine in the α position, and 0.025 atoms in the $\beta + \gamma$ positions. Residual, unreacted

Part A con'd.

phosphohomoserine acquires much larger amounts of tritium, which increase with time of incubation. A tentative mechanism has been proposed, involving intermediary formation of a Schiff base of vinylglycine and pyridoxal phosphate. A unique degree of discrimination against tritium is revealed. Tritium ions add to the β position of the vinylglycine intermediate at only 2 to 3% of the rate of proton addition.

It has so far been impossible to show cystathionine formation either from labeled cysteine or labeled aspartate, in homogenates and extracts of Neurospora. Labeled aspartate also does not label a pool ofphosphohomoserine.

Proposed course of Research

Projected studies of the threonine synthetase mechanism include: threonine degradation for separate isolation of β and γ hydrogens, phosphohomoserine degradation and chemical synthesis, reversibility studies, mechanism of binding of pyridoxal phosphate, and fluorimetric studies with pure enzyme. The pathway of phosphohomoserine formation remains to be determined, and may well be different from that in yeast and bacteria, since Neurospora lacks homoserine kinase. The enzymatic formation of cystathionine from homoserine, long implicated by nutritional and mutant studies, has never been demonstrated, and remains a puzzling problem. Assays are being developed for the purification of the corresponding cystathionine cleavage enzyme, which may well be a part of the condensing system in Neurospora.

The role of lipoic acid in the metabolism of Butyribacterium rettgeri, an anaerobe of intestinal origin, appears likely to be entirely different from any hitherto described for this vitamin. Lipoic acid is required for growth on lactate, but not on glucose or pyruvate. Anaerobic lactate fermentation by resting cells is sensitive to arsenite, but pyruvate fermentation, and the reduction of indophenol by lactate in dialyzed extracts, are not. Research will be directed toward determining the site and mechanism of action of lipoic acid in the anaerobic lactate fermentation.

Part B Included Yes X No

Serial No NHI 119

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Publications:

1. Mechanism of Enzymatic Formation of Threonine from O-Phosphohomoserine.
Martin Flavin and Clarence Slaughter,
Federation Proc. 18, 226 (1959).

2. Rapid and Specific Determination of Threonine.
Martin Flavin and Clarence Slaughter, Anal. Chem., in press.

3. Mechanism of Enzymatic Formation of Threonine from O-Phosphohomoserine.
Martin Flavin and Clarence Slaughter, Biochem. et Biophys. Acta., in press.

4. Threonine Synthetase.
Martin Flavin, "Methods in Enzymology," Article 127a, Vol. V. in press.

Serial No. NHT 120

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Energy-rich compound metabolism.
(2) Heterocyclic Compound Metabolism.

Principal Investigator: P. R. Vagelos
E. Kravitz
W. Sly
C. Wagner

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):

Total: 3.3
Professional 2.5
Other: .8

Project Description:

The purpose of all the problems carried on in general is to study the metabolism of various compounds at an enzyme level. The study of propionic acid metabolism in particular deals with the enzymatic synthesis of "energy-rich" compounds and their utilization in biosynthetic reactions. A knowledge of the details of fatty acid metabolism may lead to some observations that may be useful in the study of the clinical syndromes that are known to be associated with disorders of lipid metabolism. The study of heterocyclic compound metabolism deals in particular with the steps of synthesis and degradation of the alkaloids derived from poppy plants. The purpose of the latter work is to attempt to get some insight into the basic biochemistary of heterocyclic compounds in general.

Methods and Major Findings:

Propionate Metabolism: The study of propionate oxidation by cell-free extracts of Clostridium kluyveri has indicated

that malonyl coenzyme A is formed from propionyl-CoA. The enzyme which catalyzes the formation of malonyl CoA from malonyl semialdehyde CoA was partially purified by Dr. W. Sly. This purification however, will have to be extended in order to remove other interfering enzymatic reactions. Study of the further metabolism of malonyl-CoA indicated that it is not decarboxylated to acetyl-CoA and CO_2. An exchange reaction was discovered to occur between malonyl-CoA and $C^{14}O_2$. This reaction required acetyl-CoA plus boiled cell extract in addition to enzyme, malonyl-CoA, and $C^{14}O_2$. The factor in boiled-extract was found to be caproic acid, and it was further established that caproyl-CoA could replace the requirements for both boiled extract and acetyl-CoA. Also capable of stimulating the $C^{14}O_2$ exchange reaction were other saturated thiolesters, C-4 through C-12. (C-14 and above have not been tested). A chemical synthesis of β-keto thiolesters that do not have acid-labile pyrophosphate consituents has also been achieved.

Dr. C. Wagner has been working on another related problem, that is the metabolism of methylsulfonium compounds. He has isolated in pure culture an anaerobe that derives all its growth requirements from dimethylpropiothetin. It is felt that basic knowledge of the metabolism of sulfonium compounds in such an organism may reveal a mechanism whereby the organism derives energy for growth by neutralization of the positively charged sulfur.

Heterocylcic Compound Metabolism:

Dr. E. Kravitz has now shown that a number of C-14 labeled compounds are incorporated into alkaloids using homogenates of poppy plants. He has worked out a partial column chromatographic separation of the alkaloids produced by the plants. He has also begun studying ergot alkaloid production in a mold.

Direction of Current Research

The mechanism of the $C^{14}O_2$ exchange reaction and its possible relationship to fatty acid synthesis will be investigated. A careful study of the enzyme, malonyl

semialdehyde-CoA dehydrogenase, will be extended to animal tissues. The products of the reaction sequence in which dimethylpropiothetin is used for growth by the anaerobe will be studied. The alkaloid biosynthetic studies will be continued in both plants and molds using C-14 precursors and isolating the alkaloids produced. An attempt will be made to obtain cell-free extracts that produce alkaloids.

Part B Included Yes X No

Part B Serial No. NHI 120

Publications:

1. Propionic Acid Metabolism, International, Symposium on Enzyme Chemistry, Tokyo and Kyoto, Pan-Pacific Press, 1958, p. 86.

2. Propionic Acid Metabolism, I. The Purification and Properties of Acrylyl Coenzyme A Aminase J. Biol. Chem., 234, 490 (1959).

3. Propionic Acid Metabolism, II. Enzymatic Synthesis of Lactyl-Pantetheine, J. Biol. Chem. 234, 765 (1959).

4. Propionic Acid Metabolism, III. β-Hydroxypropionyl Coenzyme A and Malonyl Semialdehyde Coenzyme A, Intermediates in Propionate Oxidation by Clostridium kluyveri, J. Biol. Chem., 234, 2272 (1959).

5. Caproyl Coenzyme A Dependent Malonyl Coenzyme A Bicarbonate Exchange Reaction J. Am. Chem. Soc., 81, 4119 (1959).

6. Propionic Acid Metabolism, IV. Synthesis of Malonyl Coenzyme A, in preparation for J. Biol. Chem.

7. Acrylyl Coenzyme A aminase, in preparation for S. P. Colowick and N. O. Kaplan (Editors), "Methods in Enzymology," Vol. V, Academic Press, Inc., New York, N. Y.

Serial No. NHI 121
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A study of the metabolism of three-carbon compounds in the anaerobic microorganism Clostridium propionicum.

Principal Investigator: Howard Goldfine

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):

Total: 1.3
Professional: 1
Other: .3

Project Description:

To elucidate the metabolism of such three-carbon compounds as β-alanine, α-alanine and pyruvate in C. propionicum. Because these reactions, leading to the formation of acetate and propionate, appear to provide the organism with energy for growth, it is hoped that this study will lead to an understanding of the energetics of this organism.

2.
Methods and Major Findings:

The compound formed from labelled pyruvic acid was isolated and tentatively identified as γ-methyl, γ-hydroxy-glutamic acid (MHGA). However, it was found that the immediate precursor of this amino acid was γ-methyl, γ-hydroxy, α-ketoglutaric acid (MHKGA), a dimer of pyruvate which forms in solutions of pyruvate. Therefore, the enzymatic reaction observed was a transamination of MHKGA to MHGA. No enzymatic condensation of pyruvate was observed in extracts of C. propionicum. However, preliminary experiments with the fern, Adiantium pedatum, which contains large amounts of MHGA, indicate that MHGA is synthesized from labelled pyruvate in high yield.

Part A. con't Serial No NHI 121

The formation of propionic acid from β-alanine catalyzed by cell-free extracts of C. propionicum was studied. Evidence was obtained for the following enzymatic steps. β-alanine ⟶ β-hydroxypropionate ⟶ β-hydroxypropionyl CoA ⟶ acrylyl CoA ⟶ propionyl CoA ⟶ propionate. The formation of β-hydroxypropionate from β-alanine is believed to occur by a transamination of the amino group of β-alanine to pyruvate, hence to α-ketoglutarate, and the release of the amino group as free ammonia by the action of glutamic dehydrogenase.

However no evidence has been obtained for the formation of the other product of β-alanine transamination, malonyl semialdehyde

Part B Included Yes X No

Serial No. NHT 121

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Publications:

Propionic acid metabolism V. The conversion of β-alanine to propionic acid by cell-free extracts of Clostridium propionicum. In preparation.

Serial No NHI 122
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Enzymatic Mechanism of crotonyl-CoA Reduction by Diphosphopyridine Nucleotide

Principal Investigator: E. B. Brown, Jr.

Other Investigator:

Cooperating Units

Man Years (calendar year 1959):

Total: 1.3
Professional: 1
Other: .3

Project Description:

To study the process of phosphorylation coupled to electron transfer in a non-particulate, cell-free system;

Methods and Major Findings:

In the interval since the last annual report a number of experiments have been carried out which support the conclusion that the phosphorylation observed during the reduction of crotonyl-CoA to butyryl-CoA catalyzed by enzymes of C. kluyveri is produced by a process of dismutation rather than one of electron transfer. Attempts to reproduce exactly the experimental conditions of others who claim to have observed phosphorylation associated with electron transfer in this system have been unsuccessful. Consequently, we must conclude that reasonable doubt exists about the source of phosphorylation, and that other experimental approaches must be devised in order to settle the question.

Part B Included Yes X No

Part A cont'd

Project Title: 2. The Enzymatic Mechanism of crotonyl-CoA Reduction by Diphosphopyridine Nucleotide

Principal Investigator: E. B. Brown, Jr.

Project Description:

To separate, purify and characterize the ferrous iron-dependent nucleotidases in cell-free extracts of C. propionicum.

Methods and Major Findings:

Four separate ferrous iron-dependent nucleotidases were isolated and partially purified from cell-free extracts of C. propionicum. Two of these enzymes are mononucleotidases sharing a remarkable degree of heat resistance but differing in their sensitivity to versene (4×10^{-2}M); the other pair are heat-sensitive dinucleotidases separable on the basis of versene sensitivity. Successive action of a di- and mononucleotidase catalyze the irreversible decomposition of diphosphopyridine nucleotide to adenosine, nicotanamide mononucleotide and two equivalents of orthophosphate.

Part B Included Yes X No

Serial No NHI 122

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Publications:

1. A manuscript is being prepared for submission to the Journal of Biological Chemistry.

2. Two manuscripts have been prepared for submission to the Journal of Biological Chemistry.

   Nucleotidases of Clostridium propionicum I. Purification and Properties of Ferrous Iron-Dependent Mono- and Dinucleotidases by Elmer B. Brown, Jr. and Earl R. Stadtman.

   Nucleotidases of Clostridium propionicum II. Role of Electron Transport Systems in the Activation of a Diphosphopyridine Nucleotidase by Earl R. Stadtman, J. M. Earl and Elmer B. Brown, Jr.

Serial No. NMJ 123

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: 1. Studies of Ornithine Decomposition by *Clostridium lentoputrescens* M5

2. Studies of Lysine Fermentation by an unidentified *Clostridium* sp.

Principal Investigator: Vincent A. Tarantola

Other Investigator

Cooperating Units

Man Years (calendar Year 1959)

Total: 1.3
Professional: 1
Other: .3

Project Description

1. Ornithine decompositions (a) to establish the type of reaction involved in the initial deamination of ornithine; in particular, whether this reaction was oxidative or reductive in nature. (b) to determine whether the sequence of intermediates between ornithine and amino valeric acid were the same or differed from those reported in other microorganisms, e.g. *Neurospora*.

2. Lysine fermentation: To study the enzymatic decomposition of lysine by an anaerobe that utilizes this amino acid as its sole carbon source.

Methods and Major Findings:

1. Ornithine decomposition: The conversion of ornithine to proline and amino valeric acid has been found to occur in resting cells, dried cells and cell-free extracts prepared by alumina grinding and sonication.

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Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

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Individual Project Report
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Part A.

Project Title: Intermediary metabolism of amino acids with particular emphasis on those reactions involving overall reductive deaminations.

Principal Investigator: John K. Hardman

Other Investigator:

Cooperating Units:

Man Years (calendar year 1959):

Total: 1.3
Professional: 1
Other: .3

Project Description:

To obtain information concerning:

a) the intermediate steps involved whereby amino acids are converted to ammonia and the corresponding fatty acids and,

b) the anaerobic electron transfer processes involved, with special attention to a possibility of a phosphorylation mechanism.

Methods and Major Findings:

The metabolism of γ-aminobutyrate by an anaerobic bacterium, C. aminobutyricum, has been postulated to occur as follows: γ-aminobutyrate ⟶ succinic semialdehyde ⟶ γ-hydroxybutyrate ⟶ vinylacetate. Vinylacetate in equilibrium with crotonate undergoes a dismutation reaction giving rise to acetate and butyrate.

The reaction wherein succinic semialdehyde is reduced by DPNH to -hydroxybutyrate was studied in some detail. This enzyme, -hydroxybutyrate dehydrogenase, has been purified about 20-fold from crude extracts of

Part A con't.

this organism by means of protamine treatment, ammonium sulfate fractionation and chromatography on DEAE-cellulose resin. Extensive purification of this enzyme was difficult due to its instability under a variety of conditions and many techniques of enzyme purification were unsatisfactory. The properties of the purified enzyme have been investigated and it appears to be a zinc-sulfhydryl enzyme.

Proposed course of Research

Further investigation of the anaerobic intermediary metabolism of -aminobutyrate will be attempted; in particular, these studies will be directed toward:

a) determination of the activation step during aminobutyrate metabolism and
b) examination of reduction of vinylacetate to butyrate with respect to a possible phosphorylating mechanism.

Part B Included Yes X No

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Part B.

Publications:

1. Metabolism of ω-Amino Acids. I. Fermentation of δ-Aminobutyric Acid by Clostridium aminobutyricum, nov. spec. J. Bacteriol., John K. Hardman, T. C. Stadtman. In press.

2. β-Hydroxybutyrate Dehydrogenase, Methods in Enzymology, John K. Hardman, T. C. Stadtman, in press

3. Metabolism of ω-Amino Acids I. Fermentation of Δ-Aminovaleric Acid by Clostridium aminovalericum, nov. spec. J. Bacteriol. In press John K. Hardman, T. C. Stadtman.

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1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
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Part A.

Project Title: The Effects of Several Hormones on the Metabolism of Adipose Tissue Studied in Vitro.
(Partially reported here - see also report by Dr. Steinberg)
(Project started January, 1959 - not completed)

Principal Investigator: Martha Vaughan and Daniel Steinberg

Other Investigators: Ray Pittman, Helen Price

Cooperating Units: None

Man Years (calendar year 1959)

Total: 1.75
Professional: .75
Other: 1

Patient Days (calendar year 1959)

None

Project Description:

Objectives: To investigate the metabolism of adipose tissue in order to elucidate the mechanism by which this tissue regulates triglyceride synthesis and breakdown providing for storage of energy and its release in the form of fatty acids. To study the mechanism of action of the several hormones which apparently participate in the regulation of its metabolism.

Methods Employed: In vitro incubation of whole adipose tissue, homogenized tissue and functions of homogenates with measurements of the activity of certain enzymes and the amounts of several carbohydrate and lipid metabolites. Radioassay of C^{14} in various metabolic intermediates.

Major Findings: Some of the characteristics of the phosphorylase system in adipose tissue have been studied and the amounts of active phosphorylase determined after incubation of tissues under various conditions. It has been found that epinephrine, norepinephrine, glucagon and ACTH added in vitro all act to produce levels of active phosphorylase elevated above those of control tissues. This is analogous to the action of ACTH in adrenal tissue and to the effects of the other hormones in liver. The amounts of the respective hormones required to produce the effect in adipose tissue are of the same order of magnitude as those which are effective in other tissues.

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Along with their effects on phosphorylase activity all of these hormones increase the release of free fatty acids (FFA) from adipose tissue incubated in a medium containing bovine serum albumin and glucose. In addition, glucose uptake is enhanced in the presence of epinephrine or norepinephrine. As the amount of hormone present in the medium is decreased, however, the effect on glucose uptake becomes equivocal at a concentration (0.1 mg./ml.) of hormone where there are still distinctly significant effects on phosphorylase activity and on FFA. This appears to be true with ACTH also. The effects on phosphorylase and on FFA release are readily obtained at concentrations of the hormone (.02 - .04 U./ml.) that do not cause any consistent effect on glucose uptake.

Glucagon increases the level of active phosphorylase in adipose tissue when present in the medium at a concentration of 5 µgm/ml. Effects on glucose uptake and FFA release are variable with this amount of hormone. With large amounts of glucagon (20-100 µgm/ml) the effects on glucose uptake and FFA release are consistently observed. It is of interest that the maximal glucagon effect on FFA release obtained in these studies is considerably smaller than those obtained with the other hormones under similar conditions. The reasons for this quantitative difference in the effects of the different hormones is not apparent at present. In the presence of amounts of glucagon giving just barely significant effects on FFA release the increase glucose uptake is very striking. It is possible that a portion, or all of this effect is due to a minute amount of active insulin remaining over in the highly purified glucagon employed. (Adipose tissue is extremely sensitive to insulin.) Studies in progress with labeled glucose by providing a more sensitive measure of the uptake of glucose and some indication of its metabolic fate may enable us to decide (1) whether this effect is due to traces of insulin or to glucagon itself and (2) whether these several hormones do in fact affect glucose uptake when present at very low concentration or whether the effects on glucose metabolism and on the other parameters discussed above can be dissociated.

Sutherland and co-workers have shown that the effects of these hormones on phosphorylase are mediated by cyclic 3', 5' adenosine-monophosphoric acid, the accumulation of which is enhanced by these compounds. It is of interest then that no effect of this compound on phosphorylase activity or on glucose uptake by adipose tissue has been demonstrable. Its presence in the incubation medium leads to a decrease in FFA release and to depressed tissue

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levels of FFA. Although it has not been possible to demonstrate an effect of this nucleotide on the formation of active phosphorylase in fractions of adipose tissue homogenates preliminary experiments indicate that particulate fractions of homogenized adipose tissue when suitably fortified synthesize a compound possessing cyclic nucleotide activity when assayed in an enzyme system from liver.

Investigations of triglyceride synthesis and FFA release in adipose tissue homogenates are described in Dr. Steinberg's report.

Significance to Heart Research: This is a part of the basic research program of the National Heart Institute.

Proposed Course of Project: The interdependence and interrelationship of the various effects of these hormones in intact adipose tissue are under continued study. It is further planned to extend the studies of triglyceride synthesis and breakdown in adipose tissue homogenates as part of the over-all investigation of adipose tissue metabolism and its hormonal control.

Part B included - No.

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Individual Project Report
Calendar Year 1959

Part A.

Project Title: Synthesis and Degradation of Triglycerides in Adipose Tissue.
(Project started Sept. 1958 - not completed)

Principal Investigators: D. Steinberg, M. Vaughan, S. Margolis, A. Karmen.

Other Investigators: H. Price and R. Pittman

Cooperating Units: None

Man Years (calendar year 1959)
Total: 2.15
Professional: 1.15
Other: 1.

Patient Days (calendar year 1959)
None

Project Description:

Objectives: To study the mechanisms of synthesis and degradation of triglycerides in adipose tissue and to attempt to relate hormonal effects on fatty acid release to these mechanisms.

Methods Employed: In vitro incubation of epididymal fat pads and homogenates of fat pads, with measurement of fatty acid release, glucose uptake and oxidation, triglyceride synthesis from glucose and from fatty acids and other substrates, incorporation of radioactive glycerol into triglycerides.

Separation of radioactive lipids using silicic acid column chromatography, silicic acid paper chromatography, gas liquid chromatography and solvent partition.

Major Findings: Stimulation of fatty acid release from adipose tissue by glucagon added in vitro was demonstrated for the first time. It had been reported that glucagon given in vivo causes a fall in serum free fatty acid levels but this is probably explained by the hyperglycemia caused by glucagon. We have confirmed this drop immediately following intravenous glucagon but have shown that there is a rise in free fatty acids after the glucose level returns to normal. The further studies on the effects of glucagon, epinephrine and ACTH on adipose tissue are summarized in Dr. Vaughan's report entitled The Effects of Several Hormones on the Metabolism of Adipose Tissue Studied in Vitro.

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Synthesis of triglycerides in adipose tissue homogenates was demonstrated in a system dependent upon fortification with ATP and magnesium, coenzyme A, α-glycerophosphate, cysteine, sodium fluoride and buffer. C^{14}-palmitic acid incorporated into the neutral lipid fraction was shown to be present almost exclusively in triglycerides and diglycerides. Only traces of radioactivity appeared in the phospholipid and cholesterol ester fractions. The specific radioactivity of the diglyceride fraction was about 100 times that of the triglyceride fraction, implicating the diglyceride as an intermediate in the synthetic pathway. Glycerol could not be substituted for α-glycerophosphate. Fructose-1,6-diphosphate and dihydroxyacetone phosphate could replace α-glycerophosphate in this system.

This is the first report of triglyceride synthesis by adipose tissue homogenates. The system appears to be very similar to that reported by Weiss and Kennedy to be operative in liver homogenates. The probable reaction sequence is:

fatty acid + ATP + CoA → fatty acyl-CoA + AMP + PP (1)
2 fatty acyl-CoA + α-glycerophosphate → phosphatidic acid + 2 CoA (2)
phosphatidic acid → α,β-diglyceride + phosphate (3)
α,β-diglyceride + fatty acyl-CoA → triglyceride + CoA (4)

The one outstanding difference is that phosphatidic acid does not appear to accumulate in the adipose tissue homogenate whereas it is a major product in homogenates of liver. In the latter system magnesium ion inhibits the dephosphorylation of phosphatidic acid and since magnesium is required for fatty acid activation no single set of conditions is ideal for triglyceride synthesis. The conditions used here for adipose tissue appear to permit the reactions to go smoothly on to the triglyceride stage.

Parallel with these studies of triglyceride synthesis, the release of free fatty acids in homogenates has also been studied. It has been shown that, in the absence of fortification, there is a net increase in the free fatty acid pool. On the other hand, when all of the factors necessary for triglyceride synthesis are added, the fatty acid pool either remains reasonably constant or actually decreases. This indicates net synthesis of triglyceride.

Significance to Heart Research: Evidence obtained in this laboratory suggests that there may be an important connection between fatty acid mobilization and levels of lipoproteins in the serum. An understanding of the intimate mechanisms controlling fatty acid mobilization, in addition to the intrinsic value to basic science, may be helpful in elucidating homeostatic factors involved in the control of blood cholesterol levels.

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Proposed Course of Project: The characteristics of the homogenate system will be studied further in an attempt to demonstrate each of the postulated steps individually. The possible effects of hormones on triglyceride synthesis and on triglyceride degradation will be investigated. Studies have been started in collaboration with Dr. Korn to clarify the role of lipoprotein lipase in the fatty acid release demonstrated in homogenates.

Part B included - No.

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Part A.

Project Title: Ultraviolet absorption of fibrinogen.

Principal Investigator: Elemer Mihalyi

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1.0
Professional: 1.0
Other: 0

Patient Days (calendar year 1959)
None

Project Description:

Objectives: To study the status of tyrosine residues in fibrinogen.

Methods Employed: Ultraviolet spectroscopy, optical rotation, ultracentrifugation, chemical analysis.

Major Findings: To make possible the analysis of the UV spectrum of proteins and of spectrum changes caused by various agents, first a detailed study was performed of the ultraviolet absorption spectra of the aromatic amino acids. The effect of neighboring charges, hydrogen bonding agents, and polarizability of the solvent were investigated by differential spectrum techniques. Next the spectrum of fibrinogen was investigated. Fibrinogen contains appreciable amounts of tryptophane (19 per 10^5 gm) and tyrosine residues (30 per 10^5 gm). It was demonstrated that the absorption of tryptophane residues does not interfere with the spectrophotometric titration of the tyrosine residues. About 54% of the tyrosine residues has a normal dissociation with a pK of approximately 10.8, while the rest does not dissociate in the native molecule. It was demonstrated by concomitant solubility and optical rotation measurements that the dissociation of the second class of tyrosine residues and the denaturation of the protein proceed in parallel. Upon alkaline denaturation all of the tyrosine residues are titrated in a single class with a pK of about 10.8. Urea and guanidine denaturation, at neutral pH, causes the appearance of the differential spectrum characteristic for the perturbation of tryptophane

residues. There is no appreciable decrease in the height of the absorption curve. These data would suggest that the inability of some of the tyrosine residues to dissociate is not due to hydrogen bonding, but more likely to hydrophobic bondings. In view of the suggested central role of tyrosine residues in the polymerization process of fibrinogen, attempts were made to demonstrate changes in their status during the removal of the fibronopeptides and the following polymerization. No spectral changes of any sort were detected. This result contradicts the theory that tyrosine groups are liberated from hydrogen bonds by the removal of the fibronopeptides, which then become available for the subsequent polymerization step. However, the participation of tyrosine residues in the polymerization step cannot be excluded on this basis, because the number of residues involved is small, of the order of 3% of the total, and a relatively small change in the UV absorption of such a small fraction of the groups could not be detected with our present techniques.

Significance to Heart Research: No direct significance, however, the understanding of the mechanism of clotting of fibrinogen may help in understanding the causes of intravascular clotting.

Proposed Course of Project: Experimental work was largely completed, evaluation of the results and writing of the paper is in progress.

Part B included - No.

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Part A.

Project Title: Kinetic analysis of the pH changes associated with the fibrinogen-fibrin transformation.

Principal Investigator: Elemer Mihalyi

Other Investigators: Irwin H. Billick

Cooperating Units: None

Man Years (calendar year 1959)
Total 0.5
Professional 0.5
Other 0

Patient Days (calendar year 1959)
None

Project Description:

Objectives: Kinetic analysis of the pH change associated with the fibrinogen-fibrin transformation can give information on the mechanism of the attack of thrombin upon fibrinogen.

Methods Employed: Recording of pH, estimation of the peptides liberated and of the amount of polymerized fibrinogen during the clotting process.

Major Findings: At neutral pH the pH shift corresponds to a single first order reaction. At alkaline pH it can be analyzed in terms of two simultaneous first order reactions, one liberating, the other absorbing hydrogen ions. The reaction was run at different fibrinogen and thrombin concentrations and the usual enzyme kinetic analysis was performed yielding values for K_m and k_3 at different pH-s.

Significance to Heart Research: No direct significance, however, the understanding of the mechanism of clotting of fibrinogen may help in understanding the causes of intravascular clotting.

Proposed Course of Project: The experiments should be extended considerably in order to settle many controversies regarding the kinetics of the proteolytic phase of the fibrinogen-fibrin transformation. In special, correlation of the pH-shift with the splitting off of the peptides in one hand and the polymerization in the other hand is of great importance. The work will be completed probably in approximately 6 months.

Part B included - Yes.

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Part B: Honors, Awards, and Publications

Publications other than abstracts: None for this project.

NOTE: The following publications do not apply to this project, but they were published in 1959 and relate to project reported in 1958 entitled "Proteolytic Fragmentation of the Myosin Molecule" (Serial No. NIH 145), completed in October, 1958.

Mihalyi, E. and Harrington, W. F. Studies on the tryptic digestion of myosin. Biochim. Biophys. Acta. In press.

Harrington, W. F., von Hippel, Peter H., and Mihalyi, E. Proteolytic enzymes as probes of the secondary structure of fibrous proteins. Biochim. Biophys. Acta 32: 303, 1959.

Mihalyi, E. Conference on the chemistry of muscular contraction (Book review). Science 129: 1608, 1959.

Honors and Awards: None

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Part A.

Project Title: Metabolism of Free Fatty Acids.
(Started in 1955 - not yet completed)

Principal Investigator: Robert S. Gordon, Jr.

Other Investigator: Miss Amelia Cherkes

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1.25
Professional: .25
Other: 1.

Patient Days (calendar year 1959)
None

Project Description:

Objectives, and Methods Employed: In an attempt to elucidate the mechanism of production of free fatty acids, which have been shown in clinical studies to come from the periphery, the production of free fatty acid from isolated adipose tissue incubated in vitro has been studied. These investigations have been carried out using rats as experimental animals and there have been no significant clinical investigations during the calendar year 1959.

Patient Material: None

Major Findings: During 1958 it was shown that isolated rat adipose tissue would evolve free fatty acids when incubated in vitro. Further investigations of this system have clarified the effects of certain hormones and metabolic antagonists on the system. In an attempt to demonstrate an effect of heparin on the output of free fatty acids it was found that the major effect of heparin was to stimulate the release of lipoprotein lipase into the medium. The effect of various nutritional states, hormones and metabolic antagonists on the release of lipoprotein lipase was therefore also investigated.

Significance to Heart Research: The study of the metabolism of lipids is felt to be of importance in the ultimate understanding of atherosclerosis.

Proposed Course of Project: Further experiments will be undertaken along the lines already indicated.

Part B included - Yes.

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Part B:

Publications:

Cardon, P. V., Jr., and Gordon, R.S., Jr. Rapid increase of plasma unesterified fatty acids in man during fear. (Publication based on clinical investigations carried out primarily during calendar year 1957) J. Psychosomatic Research 4: 5-9, 1959.

Cherkes, A., and Gordon, R.S., Jr. The liberation of lipoprotein lipase by heparin from adipose tissue incubated *in vitro*. J. Lipid Research 1: 97-101, 1959.

Honors and Awards:

None.

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Part A.

Project Title: Study of the feasibility of altering milk fat to change its effect on blood cholesterol in man.

Principal Investigator: Donald S. Fredrickson

Other Investigators: None

Cooperating Units: (1) Dr. Joseph Shaw and Dr. S. Lakshmanan, University of Maryland Dairy Department
(2) Nutrition Department NIH

Man Years (calendar year 1959)
Total 0.1
Professional: 0.05
Other 0.05

Patient Days (calendar year 1959)
250

Project Description:

Objectives:

(1) To obtain butterfat containing a lower content of short chain fatty acids, and greater unsaturation (higher iodine number) by practical changes in dairy feed.

(2) Test the effect of normal and experimental butter fats on serum cholesterol in man.

Methods Employed:

(1) Using different feeds, Dr. Shaw and associates succeeded in obtaining two batches of butter fat from the same cows, one with iodine number of approximately 30 (regular feed), another of approximately 48 (about the highest obtainable with changes in feed alone).

(2) These fats were fed, as the sole source of fat, in liquid formula diets. Corn oil was fed before and after and between the two butter fat samples. Each fat was fed for three weeks. Blood lipids were measured three times weekly.

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Patient Material: Two normal control subjects.

Major Findings: The blood cholesterol level obtained with the two butter fats were identical and far higher than that obtained with corn oil.

Significance to Heart Research: This project proved fairly conclusively that variations in butter fat composition representing the extremes obtainable under practical dairy herd management are not sufficient to effect the tendency of butter fat to raise cholesterol in man. Successful alteration of this "undesirable feature" of butter fat would have very important implications in current dietary attitudes in prevention of atherosclerosis.

Proposed Course of Project: Project completed.

Part B included - No.

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PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the mechanisms of fat transport and metabolism. (Project started 1956 - not completed).

Principal Investigator: Donald S. Fredrickson

Other Investigators: Collaborating: Dr. Robert Gordon, Dr. John Stephenson (LTD-NHI), Mr. Arnold Jones (LTD-NHI), Dr. Eleazar Shafrir.
Technician: Mr. Katsuto Ono

Cooperating Units: None

Man Years (calendar year 1959)
Total: 2.0
Professional: 1.3
Other: 0.7

Patient Days (calendar year 1959)
10

Project Description:

Objectives: Activity limited this year to kinetic aspects of transport and metabolism of labeled free fatty acids (FFA) in plasma.

It previously having been established that FFA in plasma underwent rapid turnover, attention was mainly given to estimation of the net turnover of FFA and hence their contribution to meeting caloric demands under a variety of circumstances. This required a determination of (1) extent of recycling of FFA leaving plasma, (2) amount directly oxidized, (3) other fates of plasma FFA, and, above all, (4) the validity of the curves of plasma radioactivity several hours after FFA-C^{14} was administered.

Methods Employed:

(1) FFA-C^{14} was administered i.v. to humans, dogs and rats. Flux of labeled FFA carbon from plasma, in expired CO_2, and incorporation into plasma neutral lipids was measured. Rats were also sacrificed at 1-4 weeks after administration and the quantity, site and chemical nature of the residual FFA carbon determined.

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(2) The metabolic behavior and chemical nature of labeled FFA present in plasma 1-2 hours after administration was determined by cross-transfusion or chemical isolation and administration of this "late FFA-C^{14}" into other recipients. Paper and gas-phase chromatography (with cooperation of Dr. Arthur Karmen-LMD) also employed for identification.

(3) Data were subjected to kinetic analysis and the probabilistic theory of Dr. Stephenson used to obtain approximations of the probabilities for a given fate of an UFA molecule in plasma.

(4) In experiments with Dr. Eleazer Shafrir, the rates of turnover and oxidation of plasma FFA was measured in normal, adrenalectomized and hypophysectomized dogs before and after epinephrine.

Patient Material: Several normal control volunteers were trained for stationary bicycle exercise. FFA-C^{14} turnovers were measured while vigorously exercising to obtain a comparison of turnover with heavy metabolic demand.

Major Findings:

(1) Plasma FFA radioactivity at least 2 hours after administration of C^{14}-palmitate was found to be still in palmitate and subject to the normal rapid rate of removal (i.e. not abnormally bound in plasma). Thus recycling calculations from data using palmitate appear valid.

(2) "Late FFA radioactivity" after administration of highly unsaturated acids such as linoleic and γ-linolenic acid is not subject to rapid turnover and calculations based on data using these substrates are subject to enormous error. This residual FFA radioactivity is removed very slowly even after chemical isolation and re-administration. Since both linoleic and γ-linolenic acids are themselves removed rapidly, it is assumed new acids with unusual binding in plasma are formed. Their chemical nature is to be determined.

(3) Using labeled palmitate, the following rough approximations of the fate of an "ordinary" plasma FFA molecule are: (a) a probability of .2 - .35 that it will recycle at least once in plasma; (b) a probability of about .6 that it will be oxidized fairly directly without recycling; (c) since these probabilities do not add to one, some is unaccounted for. This last has been checked in rats and, indeed, 3-5 per cent of the labeled FFA carbon is still present after 4 weeks, over 90 per cent in lipid, mostly fatty acid, but some in sterols in skin.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rodbell, M., Fredrickson, D. S. and Ono, K. Metabolism of Chylomicron Proteins in the Dog. J. Biol. Chem. 234: 567-571, 1959.

Rodbell, M. and Fredrickson, D. S. The Nature of the Proteins Associated with Dog and Human Chylomicrons. J. Biol. Chem. 234: 562-566, 1959.

Fredrickson, D. S. and Gordon, R. S., Jr. Metabolism of Albumin-Bound Labeled Fatty Acids in Man. Proceedings of 4th Internatl. Conf. on Biochemical Problems of Lipids, Oxford, 1957.

Honors and Awards:

None.

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Individual Project Report
Calendar Year 1959

Part A.

Project Title: Primary Lipidoses (formerly titled Studies of Hyperlipidemic States in Humans, project begun in 1956 - a continuing study)

Principal Investigator: Donald S. Fredrickson (Studies on hypocholesterolemic agents in collaboration with Dr. Steinberg a part of this project)

Other Investigators: Mr. Katsuto Ono, technician

Cooperating Units: Pathology, NIAMD (Dr. Spicer)
Tissue culture, NIAID (Dr. Krooth)

Man Years (calendar year 1959)

Total: 0.5
Professional: 0.3
Other: 0.2

Patient Days (calendar year 1959)

410 (including OPD hours)

Project Description:

Objectives: The study of diseases characterized by "primary" accumulation of lipid in extracellular fluid (Essential Hyperlipidemia) or tissues. Emphasis is placed upon search for aberrant biochemical mechanisms in these conditions, but study is also directed toward classification, course, genetics, and treatment. Emphasis is upon hyperlipidemia but available tissue assays have been extended to permit study of patients representing diagnostic problems in sphingolipid metabolism.

Methods Employed: A weekly outpatient clinic is maintained at which referred patients are seen and followed. A pool of patients with hyperlipidemia is maintained for studies in this and related projects. Methods now available include cholesterol, phospholipid, glyceride, free fatty acid, cerebroside determination, complete fractionation of phospholipids, carotenoids and lipoproteins. Experimental drugs and diets are used in both in and out-patient studies. Histological study of tissue, including special histochemical preparations are made by Dr. Spicer (NIAMD). Dr. Krooth (NIMDB) working in Dr. Eagle's section, NIAID, cooperates in tissue culture studies.

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Patient Material:

1) In addition to follow-up study on about 35 old patients, blood samples were analyzed on about 50 new cases of essential-hyperlipidemia. Two pedigrees with familial hyperlipidemia were screened through three generations.

2) From the above, five cases were selected for in-patient study classified as essential familial hypercholesterolemia, essential familial hyperlipemia, hyperlipemia with diabetes, hyperlipemia with obesity, hyperlipemia with abdominal crises. An additional normal patient finished her second year on a corn oil formula.

3) Three cases of lipid storage disease were also studied; representing Gaucher's disease, a variant of Niemann-Pick disease, and unclassified reticuloendothelial disease.

Major Findings:

1) Insight into classification of hyperlipidemic syndromes, a step toward separation of biochemical determinants, has been increased. It was established that:

a. The clinical manifestations of hyperlipidemia apparently resulting from the same mutation can vary greatly within a sibship and from generation to generation.

b. There exist cases of severe hyperlipemia associated with obesity which can be "cured" by weight reduction and hospitalization and which have no apparent relation to (isocaloric) fat intake, either in amount or type fed.

A similar phenomenon has been observed in one patient with diabetes and severe hyperlipidemia. These findings imply that restriction of dietary fat may be of no importance in treating certain categories of hyperlipidemia.

c. The present chaotic classification has been found inadequate. A complete search of the literature has been made and a modified classification proposed. Review articles have been prepared on Essential Hyperlipidemia, Niemann-Pick, Gaucher's and Tay-Sachs diseases for a forthcoming book on inherited diseases.

d. Analytical techniques expanding the capacity of the laboratory to handle diagnostic problems involving complex lipids were put into operation.

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Significance to Heart Research: Most of the patients in the category under study have a significantly higher incidence of coronary artery disease presumably related to some abnormal aspects of lipid metabolism. They are encountered frequently by the average physician. Better methods of classification and treatment are urgently needed. Patients with tissue lipid storage represent problems in general lipid metabolism.

Proposed Course of Project: To continue as above. Bone marrow tissue cultures are being made on material from patients with lipid storage disease by Dr. Robert Krooth, NINDB. The aim is the development of in vitro systems for studying specific defects in fat metabolism.

Part B Included - Yes.

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PART B. Honors, Awards and Publications

Publications other than Abstracts relating to this project:

Fredrickson, D. S. Essential Hyperlipidemia. Chapter in Biochemical Basis of Inherited Diseases. McGraw-Hill, Publishers, New York. In Press.

Fredrickson, D. S. Infantile Amaurotic Family Idiocy. Chapter 17 of book: Biochemical Basis of Inherited Diseases by Stanbury, Wyngaarden and Fredrickson. McGraw-Hill, Publishers, New York. In Press.

Fredrickson, D. S. Niemann Pick's Disease. Chapter 18 of book: Biochemical Basis of Inherited Diseases by Stanbury, Wyngaarden and Fredrickson. McGraw-Hill, Publishers, New York. In Press.

Fredrickson, D. S. and Hoffman, A. Gaucher's Disease. Chapter 19 of book: Biochemical Basis of Inherited Diseases by Stanbury, Wyngaarden and Fredrickson. McGraw-Hill, Publishers, New York. In Press.

Honors and Awards relating to this project: None

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PART A.

Project Title: Pathogenesis of Atherosclerosis
(Started 1948 - not completed)

Principal Investigator: Joseph H. Bragdon, M. D.

Other Investigators: Alexander Michajlik (Rockefeller Fellow September, 1958 to September, 1959)
Philippe Laudat (NIH Fellow arrived October, 1959)
Carlos Schultz - technician
Carl Lauter and Edward Mougey (these techniciens, although under my supervision, spend most of their time running a general service laboratory in lipid chemistry).

Cooperating Units: None

Man Years (calendar year 1959)

Total: 3.0
Professional: 2.0
Other: 1.0

Patient Days (calendar year 1959)

None

Project Description:

Objectives: To study the pathogenesis of atherosclerosis through normal and abnormal lipid metabolism.

Methods Employed: Chemical and physical methods useful in analyzing lipids.

Major Findings: Preliminary findings indicate that the squirrel monkey responds to dietary fat in a manner similar to man. Microscopic atherosclerosis has been seen in one monkey after only 1 month of a coconut oil diet. Arrangements have been made to permit follow-up on a large scale.

It has been shown that the fatty acid composition of chylomicrons from human serum closely resembles that of the fat ingested. Exchange with tissue fatty acids occurs to a very slight extent, if at all.

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(Major Findings continued:)

It has been shown that intravenous heparin injection causes an obligatory oxidation of fat, even when large amounts of carbohydrate are available.

It has been shown in the rat that the feeding of carbohydrate markedly prolongs the emptying time of the stomach for fat. Carbohydrate by mouth or intravenously inhibits the absorption of fat from the small intestine.

Significence to Heart Research: Atherosclerosis causes coronary heart disease, which is the leading cause of death.

Proposed Course of Project: To continue along same lines.

Part B Included - Yes.

PHS-NIH
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Calendar Year 1959

PART B. Honors, Awards and Publications

Publications other than abstracts:

1. Bragdon, J. H. Chylomicrons and Lipid Transport. Annals of the New York Academy of Sciences 72: 845-850, 1959.

2. Bragdon, J. H. Editorial. J. Lipid Research 1: 2, 1959.

Honors and Awards relating to this project:

None

Serial No. NHI 13 A

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Interaction of carcinogenic hydrocarbons with serum lipoproteins. (Started 1958, completed March, 1959)

Principal Investigator: Joel Avigan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total 0.2
Professional 0.2
Other 0

Patient Days (calendar year 1959)
0

Project Description:

Objectives: To study both *in vivo* and *in vitro* the interaction of carcinogenic hydrocarbons with serum lipoproteins. To determine the role of these complexes in the transport of hydrocarbons in the organism.

Methods Employed: Labeled carcinogenic hydrocarbons were incorporated into serum *in vitro* by a method similar to that previously devised for incorporation of cholesterol (Project Report NHI-148 for 1958). The resulting solutions were ultracentrifugally fractionated and the amounts of label in each of the fractions determined.

Hydrocarbon solutions in lipoproteins were administered intravenously to animals and their disappearance rates from circulation were studied.

Uptake of labeled carcinogenic hydrocarbons from serum solutions by tissue slices was followed *in vitro*.

Major Findings: On incubation of whole serum with nonpolar hydrocarbons, the latter are bound predominantly by the various lipoprotein fractions. The compounds disappear at an extremely rapid rate from the circulation of rats when administered intravenously as a lipoprotein solution. Incubations of hydrocarbon solutions in serum with rat liver slices revealed the formation of a rapid equilibrium between the hydrocarbon in solution and that absorbed by the tissue. The amount taken up by the slices was proportional to the concentration in the medium.

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Significance to Heart Research: The interesting property of serum lipoproteins to complex various lipid substances may be important for their function in the organism.

Proposed Course of Project: Completed

Part B included - Yes.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Avigan, J. The interaction between carcinogenic hydrocarbons and serum lipoproteins. Cancer Research 19: 831-34, 1959.

Honors and Awards: None

Serial No. NHI 135

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Clinical Studies on Plasma Protein Metabolism (Started 1956 - not yet completed)

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: None

Cooperating Units: Dr. Thomas Waldmann, Metabolism Section, NCI

Man Years (Calendar year 1959)

Total: .75
Professional: .75
Other: 0

Patient Days (Calendar year 1959)

250

Project Description:

Objectives, Methods Employed and Patient Material: Certain patients formerly described as cases of "idiopathic hypoproteinemia" have been shown to have an accelerated catabolism of serum albumin (and presumably of other plasma proteins). Investigations with I^{131} labeled polyvinylpyrrolidone demonstrate the presence of an abnormal channel allowing transfer of plasma protein to the intestinal tract of these subjects. Similar transfer of plasma proteins has been found in certain cases of regional enteritis and ulcerative colitis. This abnormal permeability of the GI tract having been established, it is desirable to elucidate the nature of the lesion in the patients presenting with the clinical picture of idiopathic hypoproteinemia.

Major Findings:

(1) A significant number of new cases of "protein-losing gastroenteropathy" have been discovered in our own clinic and, in addition, the provision of I^{131}-PVP to qualified investigators in other institutions has made possible the discovery of further cases. The total number of patients recognized as having protein-losing gastroenteropathy is now almost 50.

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(2) A search for other related abnormalities in patients recognized as having protein-losing gastroenteropathy has shown the following features: in the majority of cases there is some measurable impairment of fat absorption. In a small but significant number of cases there is evidence of major disturbance of the lymphatic system (chylothorax, chylous ascites, or congenital lymphedema). A small but probably significant number of patients with transient disease have had eosinophilia.

(3) Biopsies of the intestinal mucosa have been obtained in six of these patients, as well as in three others who had had the transient form of protein-losing gastroenteropathy but who were not ill at the time the biopsy was obtained. The latter three cases yielded normal tissue but five of the former six showed the same lesion, all having dilated lymphatic vessels within the villi and mucosa of the small intestine.

(4) In March, 1959, it was possible for Dr. Gordon to carry out tests with I^{131} labeled PVP on patients in Bangkok suffering from Asiatic cholera. The results in these cases were uniformly normal, indicating that there was no loss of plasma protein into the intestine in this disease. This implies that the old concept of desquamation of the intestinal mucosa in cholera is erroneous.

Proposed Course of Project: It is anticipated that more patients with protein-losing gastroenteropathy will be discovered both here and in other parts of the world. Study of these cases may increase understanding of the nature of the lesion responsible for protein loss. It is anticipated that in coming months Drs. Gordon and Waldmann will prepare a major publication reviewing the experience to date.

Attempts to produce this protein-losing disorder in experimental animals by an attack on the lymphatic system will be carried out.

Significance to Heart Research: Investigation of hypoalbuminemia is of indirect importance to heart research, inasmuch as it will increase understanding of mechanisms of edema formation. Subsequent loss of plasma protein into gastrointestinal tract has been discovered in three cases of constrictive pericarditis and in one patient suffering from interatrial septal defect. It is possible that this process is of significance in the hypoproteinemia associated with certain cardiac conditions (especially constrictive pericarditis).

Part B included - Yes.

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Individual Project Report
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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Gordon, Robert S., Jr. Exudative Enteropathy: Abnormal Permeability of the Gastrointestinal Tract Demonstrable with Labelled Polyvinylpyrrolidone. The Lancet, Feb. 14, 1959, pp. 325-326.

Gordon, R. S., Jr., Barker, F. C., and Waldmann, T. Idiopathic Hypoalbuminemias: Clinical Staff Conference at the National Institutes of Health. Ann. Int. Med. 51: 553-576, 1959.

Gordon, R. S., Jr., Dasmoyavaja, A., and Benyajati, C. The Absence of Plasma Protein Excretion in Cholera. Submitted to Journal of Siamese Med. Sci. 6/9/59.

Honors and Awards: None

Laboratory of Cellular
Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of a Method for Counting Water Soluble Compounds in the Liquid Scintillation Counter. (Started April, 1958 - Completed)

Principal Investigator: Daniel Steinberg

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959)
Total .1
Professional: .1
Other 0

Patient Days (Calendar year 1959)
None

Project Description:

Objectives: To make it possible for the biochemist to obtain radioassay on polar compounds directly without the need of first converting them to an organic soluble form. Presently available methods for dealing with water soluble compounds are either limited to small quantities or involve time consuming conversion procedures.

Methods Employed: The basic principle is the use of a two-phase system - a solid phase consisting of finely divided fluorescent material and a liquid phase containing the compound to be assayed. The earlier studies utilized a plastic with diphenylstilbene in it (Pilot B); later it was found that crystals of anthracene and of diphenyloxazole were also suitable for the solid phase.

Major Findings: This technique has now been perfected so that it is a practical and general method for the radioassay of aqueous samples in the liquid scintillation spectrometer. Crystalline highly purified anthracene is the best and cheapest of a number of crystalline fluors that have been explored. The efficiencies obtained using 3 ml. of solution and 1 gm. of anthracene are as follows: tritium - 0.5%; C^{14} - 20%; Ca^{45} - 49%; I^{131} - 59%; P^{32} - 93%.

This method is now being used extensively in our own laboratory and in many laboratories across the country. It permits counting of CO_2 in alkali, proteins in dilute alkali, water soluble compounds in trichloroacetic acid supernatants and, in fact, any material soluble in water, dilute alkali or dilute acid. The reproducibility is excellent. An important advantage is the ability to recover the radioactive material unchanged after radioassay.

Significance to Heart Research: These studies represent a basic improvement in radioassay and the technique should be useful in many kinds of research, including research in the area of heart disease.

Proposed Course of Project: Completed.

Part B included: Yes.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Steinberg, D. Radioassay of aqueous solutions mixed with solid crystalline fluors. Nature 183: 1253-1254, 1959.

Honors and Awards relating to this project:

None.

Serial No. NHI 137

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epinephrine Induced Hyperlipidemia.
(Project started January 1, 1958 - not completed.)

Principal Investigator: Eleazar Shafrir

Other Investigators: Daniel Steinberg and Eugene Feigelson

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1.2
Professional: 1.0
Others: 0.2

Patient Days (calendar year 1959)
30

Project Description:

Objectives, Methods Employed, Patient Material, Major Findings:

As noted in last year's progress report, epinephrine induces a transient elevation of plasma free fatty acids (FFA) and a delayed elevation of plasma cholesterol and phospholipids occurring 24 hours after injection. These findings have now been confirmed by similar studies in rats.

It has been shown in dogs that adrenalectomy abolishes both the FFA and the cholesterol and phospholipid responses to epinephrine injection. Treatment with cortisone restores the ability of the dogs to respond to epinephrine in normal fashion.

Hypophysectomy likewise abolishes the FFA response to epinephrine and the cholesterol and phospholipid responses. The FFA response is restored by treatment with cortisone. The cholesterol and phospholipid responses are partially restored by ACTH treatment.

Clinical studies have been initiated in an attempt to extend these observations to man. Results obtained so far using 1 mg. total dose of epinephrine with or without cortisone treatment are equivocal.

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Significance to Heart Research: These observations suggest a hormonal basis for the hypercholesterolemia observed in patients under emotional stress. The hormonal control demonstrated here may be of significance in the homeostatic control mechanism regulating blood cholesterol levels.

The fact that both the FFA and the lipoprotein responses are abolished by removal of the adrenal or the pituitary gland suggests that there may be a causal relation between these two responses.

Proposed Course of Project: The clinical studies will be extended using larger doses of epinephrine in an attempt to duplicate more exactly the studies done in dogs.

Animal studies are being extended to include stress induced by cold, the effects of phloridzin and other factors known to influence adrenal activity and/or lipid mobilization.

Part B included - Yes.

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Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Shaffrir, E., Sussman, K. E. and Steinberg, D. The Nature of the Epinephrine-Induced Hyperlipidemia in Dogs and its Modification by Glucose. J. Lipid Research 1: 109-117, 1959.

Shaffrir, E. and Steinberg, D. The Essential Role of the Adrenal Cortex in the Response of Plasma Free Fatty Acids, Cholesterol and Phospholipids to Epinephrine Injection. J. Clin. Invest. In Press.

Honors and Awards relating to this project:

None.

Serial No. NHI 138
1. Laboratory of Cellular Physiology & Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Mechanism of Protein Synthesis. (Started August, 1956 - Completed Feb., 1959)

Principal Investigators: Martha Vaughan, Daniel Steinberg

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: .15
Professional: .15
Other: 0

Patient Days (calendar year 1959)
None

Project Description:

Objectives: To study the incorporation of certain unnatural amino acids into proteins as a means of learning something about the specificity of the biosynthetic mechanism and to examine tissue extracts for the presence of compounds which might be intermediates in protein biosynthesis.

Methods Employed: In vitro incubation of tissues. Fractionation, separation and purification of proteins, peptides and amino acids using solvent and salt fractionations, protein crystallization, column and paper chromatography, electrophoresis. Quantification of these materials using chemical and spectrophotometric methods. Radioassay of H^3 and C^{14}.

Major Findings: There were no new findings during the very last stages of the work on this project which was completed early in 1959.

Significance to Heart Research: This is a part of the basic research program of the Heart Institute.

Proposed Course of Project: Work on project discontinued at present.

Part B included - Yes.

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Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Vaughan, M. and Steinberg, D. The specificity of protein biosynthesis. Chapter for Volume XIV of Advances in Protein Chemistry. Academic Press, Inc., New York City. In press.

Steinberg, D., Vaughan, M., Sherman, F.G. and O'Dell, B.L. Acidic peptide conjugates in mammalian liver. Biochim. et Biophys. Acta. In press.

Vaughan, M. and Steinberg, D. Biosynthetic incorporation of fluorophenylalanine into crystalline proteins. Biochim. et Biophys. Acta. In press.

Honors and Awards: None.

Serial No. NHI 139

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Mechanism of Action of Dietary Fats in Relation to Serum Lipoproteins.
(Started July, 1957 - not completed)

Principal Investigators: Daniel Steinberg, Joel Avigan and Sven Lindstedt

Other Investigators: Hugh Vroman

Cooperating Units: None

Man Years (calendar year 1959)
Total: 0.9
Professional: 0.3
Other: 0.6

Patient Days (calendar year 1959)
350

Project Description:

Objectives: To determine the nature of the changes in lipoprotein and cholesterol metabolism effected by dietary fats and to explore the mechanisms of action involved.

Methods Employed: Clinical studies have been continued using liquid formula diets containing either coconut oil or an unsaturated vegetable oil, contributing 60% of the total caloric intake. Patients received 4-C^{14}-labeled cholesterol intravenously, given in the form of a complex with the patient's own serum lipoproteins (see 1958 annual report of Dr. Avigan for method). Serum samples are taken at intervals for determination of cholesterol level and cholesterol specific radioactivity. Complete fecal collections are made and the excretion of radioactivity in the form of sterol and of bile acids is determined.

With the collaboration of Dr. Sven Lindstedt, visiting scientist from Sweden, several patients were given tritium-labeled cholic acid at the same time as the C^{14}-cholesterol. Periodic bile samples were taken from the duodenum to permit determination of bile acid turnover.

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Patient Material, Major Findings: Cholesterol metabolism has been studied in several more patients fed formula diets containing saturated or unsaturated oils. The studies confirmed conclusions described in NHI-152, 1958, Nos. (1), (4), (5), (6). The hypocholesteremic effect of dietary unsaturated oils was not correlated with increased degradation and excretion of body cholesterol. Xanthomas removed on two occasions from a patient previously given labeled cholesterol contained the sterol in a predominantly esterified form. Its specific radioactivity was much lower than the specific radioactivity of serum cholesterol at the time of the removal. Results of the bile acid studies are not yet available.

Significance to Heart Research: Since the use of unsaturated fats in the diet offers great promise as an approach to hypercholesterolemia an understanding of the mechanisms involved is of obvious importance.

Proposed Course of Project: Further bile acid turnover studies are planned for comparison of normals with hypercholesterolemics and also for comparison of different types of hypercholesterolemics.

Part B included - No.

Serial No. NHI 1AO

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Distribution of radioactive cholesterol in the organism. (Started January, 1959 - completed July, 1959)

Principal Investigators: Daniel Steinberg, Joel Avigan, Hugh Vroman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)

Total: 0.4
Professional: 0.2
Other: 0.2

Patient Days (calendar year 1959)

None

Project Description:

Objectives: To study the rates of uptake and disappearance of isotopic cholesterol in various tissues in vivo and to determine the kinetic relationships involved.

Methods Employed: Rats and rabbits were given 4-C^{14}-cholesterol orally or intravenously and sacrificed after periods of time ranging from 6 hours to 7 weeks. The specific radioactivities of cholesterol in tissues and serum were determined.

Major Findings: The time relationships for disappearance of labeled cholesterol varied in the various organs. The specific activity in brain rose very slowly but its ratio to the specific activity in serum after 7 weeks was almost 2.0. The ratios for kidney, heart, lung and skeletal muscle increased faster than for brain and after 2 weeks all were larger than 1. The specific activities of cholesterol in liver and small intestine rapidly approached that of serum and remained close to it throughout the experimental period. The phenomena described have been satisfactorily explained on the basis of exchange of labeled cholesterol between serum and tissue pools and of a simultaneous first order disappearance of isotopic cholesterol from serum. The experimental tissue curves for the various tissues matched quite closely theoretical curves determined by assuming certain exchange rates between the tissue and serum pools. It is concluded that isotopic

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exchange processes may explain certain complex kinetic relationships observed when labeled cholesterol is administered. The results demonstrate the complex nature of the metabolism of C^{14}-cholesterol. They raise serious questions regarding the interpretation of studies based exclusively on the rate of disappearance of C^{14}-cholesterol from the serum compartment.

Significance to Heart Research: Evaluation of the limitations of isotopic labeling method for the study of cholesterol metabolism in patients and animals is of primary importance in view of its (too) frequent use.

Proposed Course of Research: None.

Part B included - No.

Serial No. NHI 1A1

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Inhibitors of Cholesterol Biosynthesis.

Principal Investigators: Daniel Steinberg, Joel Avigan, Hugh Vroman and Eugene Feigelson

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)

Total: 0.5
Professional: 0.3
Other: 0.2

Patient Days (calendar year 1959)

100

Project Description:

Objectives: To study the clinical effectiveness and mechanism of action of the serum cholesterol depressing agent: "MER-29". To isolate and characterize any metabolic intermediates accumulating as a result of administration of the drug.

Methods Employed: MER-29 is a powerful inhibitor of cholesterol biosynthesis from radioactive acetate. The radioactivity, however, accumulates in the non-saponifiable lipid fraction. Sterols were isolated from the livers of MER-29 treated rats. Separation of the various components by chromatography and other methods was attempted. Spectroscopic analysis and other physical determinations were carried out in the fractions obtained. In the in vitro studies rat liver slices were incubated in a paraffin oil solution of the drug.

Major Findings: On the basis of various determinations, it is concluded that the livers of MER-29 fed animals contain a substantial amount of an unknown sterol similar to, but not identical with, cholesterol. This sterol is also synthesized in vitro from labeled acetate, or mevalonate, when liver slices are incubated in the presence of "MER-29".

Clinical studies (250 mgm/day) were undertaken to determine whether response on a cholesterol-free diet might be more dramatic than results reported on regular diets. Results in 4 patients on formula diets show no greater response than has been observed by others on regular diets.

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Significance to Heart Research: In view of the possible usefulness of the drug in the management of hypercholesterolemia the elucidation of its mechanism of action is of considerable importance. The studies described may also yield information on the pathway of cholesterol biosynthesis.

Proposed Course of Research: Attempts to isolate and characterize the unknown sterol will be continued.

Part B included - No.

Serial No. NHI 142

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Biosynthesis of Serum Lipoproteins (Project started 7/1/57 - not completed)

Principal Investigators: Charles M. Redding, Daniel Steinberg, Joseph H. Bragdon, Eugene Feigelson

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)

Total: 0.7
Professional: 0.7
Other: None

Patient Days (calendar year 1959)

None

Project Description:

Objectives: To determine the site of synthesis of the serum lipoproteins and to study the factors regulating synthesis.

Methods Employed: Using C^{14} labeled amino acids we have been studying the synthesis of lipoproteins by rat liver slices and by the perfused rat liver. Lipoproteins have been isolated by the usual ultracentrifugal techniques and the identity of the isotopically labeled lipoproteins with serum lipoproteins has been studied by a method involving paper chromatography high voltage paper electrophoresis, and autoradiography.

Major Findings: The labeled lipoproteins synthesized by rat liver slices have now been definitely identified as α-lipoproteins indistinguishable from the circulating α-lipoprotein of the rat. This was shown by incubating with a complete mixture of highly radioactive amino acids, isolating the labeled lipoproteins from the medium, digesting these lipoproteins with trypsin and chymotrypsin, chromatographing the peptide mixture in two dimensions and making an autoradiograph of this chromatogram. It could be shown that the radioactive spots coincided with peptides on the chromatogram.

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The picture with respect to β-lipoprotein is less clear. Using the technique just described, it is found that some but not all of the radioactive spots coincide with peptide spots. It seems likely that the liver slice is producing β-lipoprotein but at the same time producing other lipoproteins.

It was shown that synthesis of the protein moiety of the lipoproteins proceeds at a normal rate despite changes in the rates of cholesterol synthesis. Feeding cholesterol, which inhibits cholesterol synthesis, or injecting Triton, which accelerates cholesterol synthesis, did not influence significantly the rate of lipoprotein protein synthesis. Liver slices taken from nephrotic rats appear to synthesize lipoprotein protein at a somewhat elevated rate.

Significance to Heart Research: The *in vitro* system devised with rat liver slices should provide a powerful tool for studying the synthesis of serum lipoproteins. From such a study we may learn about some of the physiological controls of blood lipid levels. Knowledge of such controls is of prime interest in understanding the pathogenesis and possible prevention of atherosclerosis.

Proposed Course of Research: This system will be used to try to determine whether deposition of fat in the liver leads to acceleration of lipoprotein synthesis. The preliminary findings in nephrotic rat liver will be further explored.

Part B included - No.

Serial No. HMY 1A3
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

NIH
Individual Project Report
Calendar Year 1959

PART A.

Project Title: Lipoprotein Metabolism in Nephrosis
(Project started September, 1955)

Principal Investigators: James H. Baxter and, in certain parts, Howard C. Goodman and James Allen. Claude Malmendier has recently started a related project.

Other Investigators: Robert Bowser (tech.)

Cooperating Units: None

Man Years (1959)
Total 2.5
Professional: 2.0
Other 0.5

Patient Days (1959)
120

Project Description:

Objectives: To characterize the lipoprotein abnormalities in nephrosis and to learn something of the pathologic mechanisms involved.

Methods Employed: Serum lipids and lipoproteins have been studied in patients and animals with nephrosis. Effects of steroid therapy, prolonged glucose infusions, and repeated infusions of albumin, dextran and other colloids have been investigated.
A comparison of the lipoprotein alterations caused by nephrosis and plasmaphoresis has been started, and Dr. Malmendier has begun a study of the metabolism of C^{14}-labeled unesterified fatty acid (UFA) and chylomicrons in nephrotic rats.

Major Findings: (a) The lipid and lipoprotein alterations in patients with nephrosis have been described in previous reports. Prolonged glucose infusions in 5 patients with nephrosis and in 5 control subjects caused some decrease in serum cholesterol and a decrease or little change in triglyceride. In 3 cases of nephrosis, however, the infusions caused a considerable increase in serum triglyceride and in lactescence and very low density lipoproteins. These results are described in J. Clin. Invest. (abstract) 38: 986, 1959.

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Infusions of 25 to 50 gm. of albumin per day for 1 to 4 weeks in patients with nephrosis in a majority of cases increased the concentration of serum albumin and caused the serum lipids and lipoproteins to change quantitatively and qualitatively toward normal. Similar changes in lipids occurred in 2 cases given infusions of dextran. Dr. James Allen gave nephrotic rats repeated injections of human albumin, dextran, P.V.P., and bovine gamma globulin. With all of these substances there was a decrease in lipids - particularly in triglyceride which was the lipid most markedly increased - far greater than could have resulted from changes in plasma volume (Clin. Res. Proc. 7: 278, 1959). These results suggest that hyperlipidemia in nephrosis may be in part a result of the loss of serum albumin and the consequent decrease in colloidal osmotic pressure of the serum. This portion of the project has been completed.

(b) In dogs with nephrosis, we have observed an increase in cholesterol and phospholipid - up to about twice normal levels, but no significant increase in triglyceride or lactescence. These lipid alterations are not as great as those seen in nephrotic patients, rats, and rabbits. The observations indicate that the rather small lipid alterations that have been produced in dogs by plasmaphoresis probably are not dissimilar to those which are produced by nephrosis. Effects of plasmaphoresis in dogs are being studied.

(c) Dr. Malmendier's early experiments suggest that there are some differences in tissue distribution of radioactivity and in rate of labeled CO_2 production after injections of labeled UFA in nephrotic rats compared with control rats.

Significance to Heart Research: It is hoped that information will be obtained concerning lipoprotein metabolism which will be applicable to various diseases involving lipid alterations.

Proposed Course of Project: The studies on plasmaphoresis, and those on metabolism of UFA and chylomicrons (by Dr. Malmendier) will be continued. Studies on the fatty acids in the lipoproteins of nephrotic animals on controlled diets are being considered.

PART B. Included - Yes.

Serial No. NHI 143

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Individual Project Report
Calendar Year 1959

PART B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Baxter, J. H., Goodman, H. C., and Havel, R.J. Lipid and lipoprotein alterations in nephrosis. J. Clin. Invest. Accepted for publication in March, 1960.

Honors and Awards: None

S[illegible] No. NHI 144
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Synthesis, Degradation and Interconversions of the Amaryllidaceae Alkaloids.

Principal Investigator: Henry M. Fales, Ph.D.

Other Investigators: None.

Cooperating Units: None.

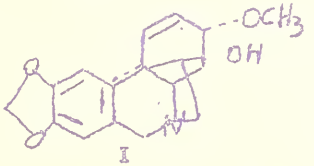
Man Years:
Total: 1
Professional: 1
Other:

Patient Days:
None

Project Description:

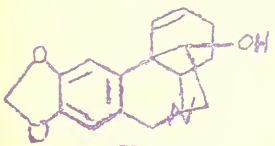
Progress During the Past Year:

The structure of haemanthamine (I) has been refined. The complete stereochemical and absolute configuration follows.

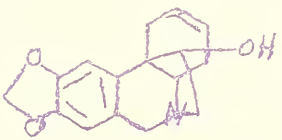


I

Crinamine is the methoxyl epimer of I and has same absolute configuration of the nucleus. Haemultin, obtained naturally and via degradation of I has been shown to consist of 2 isomers II and III.

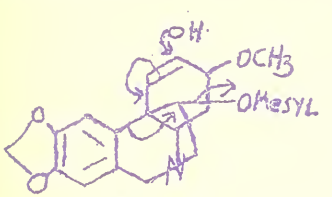


II

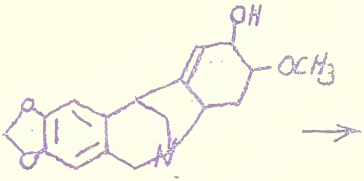


III

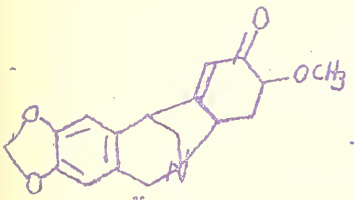
Compound I has been found to undergo an unusual rearrangement with $POCl_3$ or mesyl chloride in pyridine. The product, isohasmanthamine (IV) and montanine and coccinine have been degraded to a common product VI.



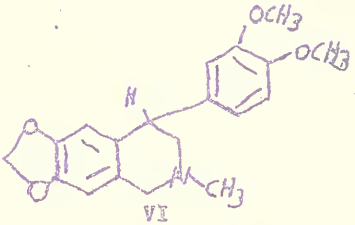
I



IV

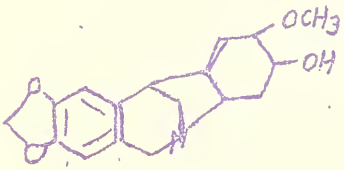


V



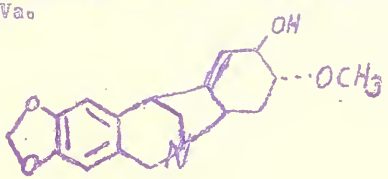
VI

The same rearrangement occurs with erinamine and II. The new ring system (5,11-methanomorphanthridine) so encountered has been simultaneously found to exist in the alkaloids montanine VII, coccinine and manthine; these alkaloids are discussed in a concurrent report.



VII

When methanol instead of water is used to decompose the reaction, manthine, the O-methyl ether of IV and montanine is obtained. Considering the probable reaction mechanism we can represent the absolute stereochemistry of isonatalensine as IVa.



IVa

In contrast with the other alkaloids of this family, there is no satisfactory biogenetic scheme for the direct formation of V. We are led to expect that a related rearrangement reaction also occurs in nature.

In all of the above alkaloids intramolecular hydrogen bonding from alcohols to ether groups or double bonds or aromatic rings has furnished important evidence for the stereochemistry of the molecules. This has been revealed by high dilution studies in non-polar media under conditions of high sensitivity and resolution with the Beckman IR-7 Grating Infrared Spectrophotometer.

Techniques for obtaining infrared spectra on very small quantities (5-50 μg.) of solid or liquid have been developed with the aid of the same apparatus.

Direction of Current Research:

Minor points concerning the chemistry of haemanthamine remain and the exact mechanism of its rearrangement will be sought. Sufficient quantities of the iso-alkaloids will be prepared for pharmacological testing. Biogenetic pathways for the alkaloids from radio-tyrosine are under investigation.

Part B included. Yes

Serial No. NHT 144
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

Wildman, W. C. and Fales, H.M., The Stereochemistry of Amaryllidaceae Alkaloids Derived from 5,10b-Ethanophenanthridine, J. Amer. Chem. Soc. 80, 6455 (1958)

Fales, H. M., Horn, D.H.S. and Wildman, W.C., Structure of Criwelline, Chemistry and Industry, 1415 (1959).

Fales, H.M. and Wildman, W.C., The Structures of Haemanthamine and Crinamine, J.Amer. Chem. Soc., 81, 0000 (1959).

Serial No. NMI-165
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Alkaloid Isolation and Structure Studies

Principal Investigator: Sidney M. Goodwin, Ph.D.

Other Investigators: None

Cooperating Units:

Man Years:
Total: .50
Professional: .50
Other: .00

Patient Days:
None

Project Description:

Progress During the Past Year:

The problem of the Lunasia alkaloids was concluded. Fourteen individual substances were isolated over the course of study of the heterocyclic bases of Lunasia amara Blanco of Philippine origin. Structural work was carried out by a combination of degradative, synthetic and instrumental studies. Following the determination of the structure of lunacrine, nuclear magnetic resonance methods were applied to several hydroxy substituted compounds, and in each case it was possible to determine the nature of the side-chain. Degradative procedures were used to correlate the instrumental data with proposed structures.

The experimental work relating to the isolation studies was summarized in a general paper outlining the nature of the entire problem, and the structural studies were summarized in separate papers. The "water-soluble" compounds were not investigated, since these are under study by Dr. J. R. Price, CSIRO, Australia.

Direction of Current Research:

No further work with these compounds is planned.

Part B included. Yes

Serial No. NHI-145
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

Goodwin, Sidney, Smith, A. F. and Horning, E. C., Alkaloids of Ochrosia elliptica Labill, J. Am. Chem. Soc., 81, 1903-8 (1959).

Goodwin, Sidney and Horning, E. C., Alkaloids of Lunasia amara Blanco. Structure of Lunacrine, J. Am. Chem. Soc., 81, 1908-1912 (1959).

Goodwin, Sidney, Shoolery, J. N. and Johnson, L. F., Nuclear Magnetic Resonance Spectra of Alkaloids. I. The Complete Structure of Lunacrine and Lunine, J. Am. Chem. Soc., 81, 3065 (1959).

Goodwin, Sidney, Shoolery, J. N. and Horning, E. C., Alkaloids of Lunasia amara Blanco. Hydroxylunacridine, J. Am. Chem. Soc., 81, 3736-38 (1959).

In Press:

Goodwin, Sidney, Smith, A. F., Velasquez, A. A. and Horning, E. C., Alkaloids of Lunasia amara Blanco. Isolation Studies, J. Am. Chem. Soc., 81, 0000 (1959).

Serial No. NHI-146
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cassine

Principal Investigator: E. J. Highet, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:
Total: 0.75
Professional: 0.75
Other: 0.00

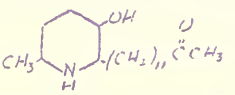
Patient Days:
None

Project Description:

Progress During the Past Year:

The extracts from several species of the genus Cassia have been examined. C. pereslteana has proven to be a superior source of cassine, yielding the crystalline hydrochloride by selective extraction of acid solutions by ethyl acetate.

Structural work on cassine has been extended to the point of providing the tentative structure for the alkaloid:



(1) Previous characterization had shown cassine to be a secondary amine, with a hydroxyl group, a methyl ketone, and an additional C-methyl and involving a single saturated ring system. The analytical result showing an N-methyl group, although readily reproducible, has been shown spurious, for the N-methyl derivative of cassine contains a single such group. The nuclear magnetic resonance spectrum substantiates these findings, and shows that the second C-methyl group occurs in the grouping: $CH_3CH<$

(2) The dehydrogenation product, shown by ultraviolet spectra to be a 3-hydroxypyridine, is shown by infrared and nuclear magnetic resonance spectra to be 2,6-dialkyl substituted, one of these groups being the second methyl group of cassine.

(3) Two-stage Hofmann degradation of N-methylcassine provides a small yield of a neutral material, and shows clearly that the nitrogen atom of cassine occurs within the ring system and bears no N-methyl group. Oxidation of this product, first by potassium permanganate and then by sodium hypoiodate, provides a dicarboxylic acid whose methyl ester was identified by gas chromatographic techniques as that of tetradecanedioic acid. This result requires that the hydroxyl group of cassine's 3-hydroxypiperidine system be on the side of the ring bearing the long alkyl chain, and provides the last piece of evidence required for the structure.

Direction of Current Research:

Further study of the Hofmann degradations is planned to adduce further evidence for the structure proposed. Synthetic procedures leading to this structure will be initiated.

Part B included. No.

Serial No. NHI-167

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Amaryllis Alkaloids

Principal Investigator: E. J. Highet, Ph.D.

Other Investigator: Helen Marie Walker

Cooperating Units: None

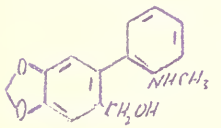
Man Years:
Total: 0.50
Professional: 0.25
Other: 0.25

Patient Days:
None

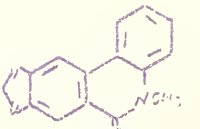
Project Description:

Progress During the Past Year:

Ismine. This alkaloid has been shown to have the structure I:



I

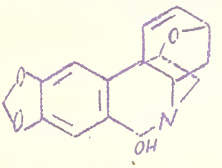


II

Analyses of the alkaloid and its picrate require the elemental composition of $C_{15}H_{15}NO_3$, with a single N-methyl group. The ultraviolet spectrum of the base hydrochloride suggests the existence of the 6-phenylpiperonyl system; that of the free base shows that the amino group occurs on the aromatic system. The infrared spectrum contains the characteristic peaks of the methylenedioxy aromatic system, and further shows the phenyl group to be o-substituted. Acetylation provides a neutral O,N-diacetate, demonstrable by the infrared spectrum. As anticipated of an aniline with a free para position, ismine couples with p-nitrobenzene diazonium chloride to give a red dye. Acid reflux of ismine, followed by ferricyanide oxidation in base provides the phenanthridone II, with a characteristic ultraviolet spectrum. Synthetic studies designed to produce ismine have been initiated, but are so far unsuccessful.

The occurrence of a compound of structure II among the Amaryllidaceae is striking. Since such a structure seems foreign to biogenetic schemes considered likely at present, ismine may represent a natural degradation product.

To provide further evidence for the existence of the aldehyde-ammonia group in haemanthidine, apo-haemanthidine methiodide, III, has been prepared, and shown to form a semicarbazone.



III

A simple ten-stage countercurrent distribution method for the isolation of galanthamine has been devised to allow the facile preparation of large quantities of this material.

Part B included. No.

Serial No. MWV-148
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Isolation and Characterization of Components of the Callicrein System from Urine, Pancreas, and Blood Plasma

Principal Investigator: Jack V. Pierce, Ph.D.

Other Investigator: Patricia A. Wagner (technical)

Cooperating Units: S. J. Sarnoff, L. C. Sarnoff, M. E. Webster, W. D. Fisher (Laboratory of Cardiovascular Physiology Serial No.)

Man Years
Total: 2
Professional: 1
Other: 1

Project Description:

Progress During the Past Year:

Introduction:

All evidence accumulated since our previous annual report further supports the assumption that we are in fact dealing with the callicrein system as defined by Werle and coworkers.

New procedures for the fractionation of human plasma have been devised toward the goal of isolating the components of the blood callicrein system. The chief innovation with respect to protein fractionation has been the extensive use of adsorbents in a batch manner at or near room temperature in place of fractional precipitation with salts and solvents. The adsorbents are DEAE (diethylaminoethyl) and CM (Carboxymethyl) celluloses and Amberlite XE-64, all of which are usually used chromatographically only.

Although DEAE and CM cellulose have been used chromatographically with some success, the adsorbent showing greater promise than these for the resolution of plasma and other proteins is hydroxyl apatite. With this adsorbent there is less tailing of the peaks (even with stepwise elution),

recoveries are generally better, and considerably high flow rates can be used without loss in resolution. One serious obstacle to its widespread use is the lack of a convenient and dependable method for preparing material with the desired adsorptive and other properties.

Methods for assaying the components of the callicrein system are described in the current annual report of Dr. M. E. Webster. Of especial interest is the use of the synthetic substrate, p-toluenesulfonyl-L-arginine methyl ester (TAMe), for the detection of callicrein as well as of other esterases present in human plasma which has been activated by various means.

Experimental:

1. Human Plasma Callidinogen. Five-fold diluted plasma was stirred 0.5 hr. with 40 mg. DEAE-C* per ml.≙** at pH 6.0 and 23° C. Elution of the adsorbate, recovered by filtration, with 0.1 M sodium phosphate buffer, pH 7.0, yielded a material which, after dialysis and lyophilization, contains about 10 per cent of the original dry weight and 100 per cent of the callidinogen activity. This step has been successfully applied twice to 2-liter lots of outdated human plasma.

Chromatography of such a preparation, though of lower potency than now available, on hydroxyl apatite and stepwise elution at pH 6.8 with sodium phosphate buffers of increasing ionic strength resolved the proteins present into about 12 peaks. Callidinogen activity was associated with one of the smaller ones. A 20-fold purification with 80 per cent recovery of activity was realized. Studies made by Dr. M. E. Webster revealed that this peak contains the plasma callidin inhibitor but is free of callicrein and callicrein inhibitors.

Although this preparation is only 83 times more pure than the original plasma, a purification of about 200-fold can now probably be achieved by the above two steps. It is hoped that a further increase in purity will result from rechromatography on hydroxyl apatite.

* DEAE cellulose prepared from Whatman Ashless Cellulose Powder by the method of Ellis and Simpson, J. Biol. Chem., 220, 939 (1956). This particular adsorbent had a capacity of 0.5 meq./g.

** Ml. ≙ means on the basis of a ml. of original plasma.

2. Human Plasma Callicrein. The callicreinogen in human plasma can be completely activated by treatment with acetone. Outdated human plasma at pH 7.5 was stirred at 25° C. with one-fourth volume of acetone and allowed to stand 4 hours. Dialysis, removal of the precipitate formed, and lyophilization of the supernatant yielded a material with about 2 Frey units (FU) per ml. This preparation (usually not dried but stored frozen at -20° C.) is made to five times the original plasma volume and

stirred 1 hr. with 50 mg. DEAE-C (0.8 meq./g.) per ml.- at pH 8.0 and 12°C. The filtrate was stirred immediately 1 hr. with the same amount of fresh DEAE-C, but now at pH 6.5 and 12°C. Elution of the adsorbate with 0.25 M sodium phosphate buffer, pH 6.8, gave a product with esterase activity for TAMe but with little callicrein activity. Finally, the second DEAE filtrate was stirred with 50 mg. CM-C (0.6 meq./g.) per ml.- at pH 6.0 and 12°C for 1 hr. The CM-C eluate (same phosphate buffer as above) was dialyzed and lyophilized to yield a white product with about 20 per cent of the starting TAMe activity and about 30 per cent of the starting callicrein activity. The results of these operations are shown in the table below:

Description of Fraction	$D_{280m\mu}$ per ml.-	TAMe Assay Units*/ml.-	Dog Assay FU/ml.-
Dialyzed and centrifuged acetone-treated O.D. human plasma	47.7	8.2	0.76
First DEAE filtrate, pH 8.0	12.9	4.2	.35
First DEAE eluate, pH 8.0	27.0	1.8	
Second DEAE filtrate, pH 6.5	7.7	2.1	.24
Second DEAE eluate, pH 6.5	4.8	1.3	.015
CM filtrate, pH 6.0	4.3	0.4	.015
CM eluate, pH 6.0	2.6	1.5	.25

*Arbitrary units

Preliminary experiments showed that plasma callicrein is strongly adsorbed to hydroxyl apatite on columns and can be eluted with about $\mu = 0.4$-0.5 sodium phosphate buffer, pH 6.8. Thus, the next step will be chromatography on hydroxyl apatite in an attempt at further purification.

3. Human Pancreatic Callicrein. Pooled human pancreas, obtained at autopsy and stored frozen, was thoroughly macerated and stirred with two volumes of 0.05 M acetic acid. After standing at 15°C for 5 hr., the mixture was filtered and the filtrate was adjusted from pH 4.5 to 6.0. The filtrate from 480 g. (wet weight) of pancreas was diluted to 4.8 l. and stirred 0.75 hr. with 12 g. of DEAE-SF** (0.34 meq./g.) at pH 7.1. Elution of the adsorbate with $\mu = 0.5$ sodium phosphate buffer, pH 6.0 dialysis and lyophilization of the eluate gave 0.86 g. of a light tan powder assaying 4-8 FU/mg. Further purification of this material will be undertaken as time permits.

** DEAE cellulose prepared from Solka Floc BW200.

4. Human Urinary Callicrein. Since the last report, 155 l. of male human urine have been processed by the KE-64 method. The combined 0.025-0.10 M sodium phosphate buffer, pH 6.0, eluates were stirred for 2 hr. with 22.5 g. of DEAE-SF[a] (0.34 meq./g.) at pH 7.0 and 15-20° C. The adsorbate was washed with water and eluted with 0.5 l. of 0.2 M sodium phosphate buffer -0.50 M sodium chloride, pH 7.0. Dialysis and lyophilization of the eluate gave 3.5 g. of a brown powder assaying 7 FU/mg.

Unlike hog and human pancreatic callicrein, human urinary callicrein was not precipitated by Rivanol (2-ethoxy-6,9-diaminoacridine lactate).

Chromatography of a small sample of the above preparation on hydroxyl apatite showed the adsorbed activity could be eluted with $\mu \simeq 0.10$-0.20 sodium phosphate buffer, pH 6.8. Recovery of callicrein activity was complete and a 3-fold purification was obtained.

5. Human Plasma Callicreinogen.- Hydroxyl apatite chromatography of the supernatant liquid from five-fold diluted O.D. human plasma gave 19 peaks by stepwise elution with 15 eluants ($\mu \simeq 0.01$-2.2 sodium phosphate buffer, pH 6.8). Attempts to locate the callicreinogen by both acetone and trypsin activation have so far failed, possibly due to the high concentration of salts in the combined and concentrated fractions. One more attempt will be made on these after dialysis.

Direction of Current Research

Further purification of the components of the callicrein system, particularly callidinogen, will be pursued.

Part B included. No.

Serial No. NHI-149
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Preparations, Purifications and Special Isolations

Principal Investigator: David L. Rogerson

Other Investigators: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. John C. Keresztesy and Henry E. Lutterlough, NIAMD. Large Scale Equipment.

Man Years:
Total: .40
Professional: .20
Other: .20

Patient Days:
None

Project Description:

Progress During the Past Year:

The 100 gallon fermentation unit has been utilized 42 times to grow a variety of microorganisms, particularly Escherichia coli. Due to improvements in the operating procedure and the mechanical systems of aeration, inoculation and antifoam control, these bacteria have been grown successfully with very few exceptions.

Approximately 50 gallons of hexane have been redistilled and polyethylene polymer, Chromosorb and particularly silicic acid have been prepared and graded in quantity for chromatographic uses. In addition, 300 pounds of inorganic salts have been purified by recrystallization.

Special isolations include the extraction of desired constituents from 30 liters of human blood plasma, 12 liters of fresh bovine blood, 60 pounds of potato starch, 5 pounds of bovine lung tissues and numerous small tissue samples from dogs.

Direction of Current Research:

To meet the increasing demands for use of the fermenter and to utilize its full potential, modifications to the unit will continue to be made so that a greater variety of bacteria may be grown successfully. Also,

the processing of animal tissues on both a small and large scale will be continued and new or improved isolation techniques will be developed as needed.

Part B included. No

Serial No. NHI-150
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Isolation of Alkaloids and Other Constituents from Plant Materials

Principal Investigator: David L. Rogerson

Other Investigators: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. B. G. Schubert, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md. Plant Identification and Procurements.
Dr. John C. Keresztesy and Henry E. Lutterlough, NIAMD and James H. Miles, NHI. Large Scale Equipment.

Man Years:
Total: .35
Professional: .10
Other: .25

Patient Days:
None

Project Description:

Progress During the Past Year:

Plant materials, mainly of the Amaryllidaceae, weighing in excess of 1100 pounds have been processed for alkaloids, and of the 26 plants processed, 15 were new observations. Also, desired constituents were isolated from an additional 537 pounds of plant materials.

Direction of Current Research:

Suitable isolation procedures will be used or developed for processing plant materials which are of chemical or pharmacological interest as determined through separate work.

Part B included. None.

Serial No. NHI-151
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Testing of Plant Materials for Alkaloids and Glycosides

Principal Investigator: David L. Rogerson

Other Investigators: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. B. G. Schubert, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md. Plant Identifications and Procurements.

Man Years:
Total .25
Professional: .10
Other: .15

Patient Days: None

Project Description:

Progress During the Past Year:

During the past year 69 plant materials have been received, 21 of which were supplementary to previously received materials. Included in the 48 new observations were 18 plant samples of the Amaryllidaceae, all of which gave positive alkaloid tests, and of the remaining 30 samples, 6 (20%) were found to contain one or more alkaloids.

In 30 of the 37 plant materials tested for glycosides, moderate to very noticeable activity or death in mice was observed. The samples were prepared for testing employing a modified extraction procedure of improved reproducibility.

Direction of Current Research:

Greater attention will be directed towards glycoside determinations using available plant materials and subsequent acquisitions which contain no alkaloids.

Part B included. No.

Serial No. NHI-152
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Lipids of Atheromatous Lesions, Plasma and Adipose Tissue from Patients with Atherosclerosis

Principal Investigators: C. C. Sweeley, Ph.D.
E. C. Horning, Ph.D.

Other Investigators: E. A. Moscatelli, Ph.D.
K. V. Anthony
K. Y. Cavitch

Cooperating Units: Baylor University and The Methodist Hospital, Houston, Texas.

Man Years:
Total: 1.0
Professional: 0.5
Other: 0.5

Patient Days
None.

Project Description:

Progress During the Past Year:

In order to study the lipids of aorta lesions and serum from patients with severe atherosclerosis considerable time was devoted to the standardization of difficult silicic acid chromatographic methods for the separation of various lipid classes. The present procedure involves a preliminary separation of total lipid from a tissue by solvent fractionation and subsequent chromatographic separation on silicic acid into neutral and polar lipids. These crude mixtures are separately chromatographed on silicic acid columns as follows: neutral lipids are fractionated with various hexane-benzene mixtures into hydrocarbons, cholesterol esters, triglycerides, and cholesterol; polar lipids are fractionated with various chloroform-methanol mixtures into non-choline phosphatides, inositol phosphatides, lecithins, sphingomyelins, and lysolecithins.

These analytical techniques yield class separations of the lipids on a relatively small scale. From the results is obtained the relative composition of each lipid class for the tissue studied.

The second phase of the analytical procedure involves the gas chromatographic analysis of the fatty acid mixture obtained from each lipid

class by hydrolytic or transesterification procedures and solvent extraction. Suitable techniques have been developed for preparing methyl esters of the fatty acids on a microscale. Analyses are obtained on two different polyester columns; the analyses include the saturated fatty acids from C_{10} to C_{24} and in addition include palmitoleic, oleic, linoleic, linolenic, eicosatrienoic, arachidonic and nervonic acids.

Direction of Current Research:

While considerable time has been expended to establish standard analytical procedures for the microanalysis of lipid mixtures, it has also been possible to apply these procedures in a preliminary sense to a study of the lipids of arterial lesions and serum from patients with atherosclerosis. From these experiments are obtained two types of data. First, the relative composition of each of the lipid classes is obtained and may be compared with corresponding data from other patients and from normals. In addition, comparisons of lesion lipids with those of serum may be made. Second, the precise composition of the fatty acid mixture within each of the lipid classes is determined. The various lipid classes within a given tissue may be compared in this sense and in addition an individual lipid class may be studied in a series of patients. These techniques provide a means of relating chemical changes to physiological changes in atherosclerosis.

Part B included: No.

Serial No. NH-153
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

FHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Micromethod for the Qualitative Analysis and Estimation of Sphingolipid Bases

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigator: E. A. Moscatelli, Ph.D.

Cooperating Units: None

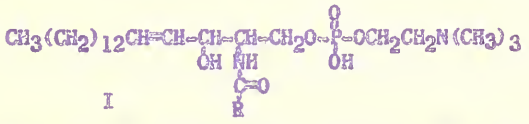
Man Years:
Total: 0.75
Professional: 0.75
Other: 0.00

Patient Days
None

Project Description:

Progress During the Past Year:

It has been possible to determine the total long-chain base content of a lipid sample (e.g., sphingomyelin, I) by several procedures. Although in some cases the methods provided adequate sensitivity it has not been possible, by these methods, to determine the relative concentrations of the individual sphingosine-like bases (dihydrosphingosine, phytosphingosine, etc.).



I

A method has been developed in this laboratory for the qualitative microanalysis and estimation of each of these sphingolipid bases present in a mixture. After acid hydrolysis of the lipid fraction the isolated mixture of bases is oxidized with sodium metaperiodate and the mixture of fatty aldehyde reaction products is analyzed by gas-liquid partition chromatography.

The method has been used to study, in a preliminary and orienting sense, the sphingolipids from a variety of animal tissues and plant sources. The presence of dihydrosphingosine has been detected in plant lipids for the first time. A new sphingosine-like base in human plasma has been shown to be associated with the sphingomyelin fraction of human plasma phosphatides. This unknown base, which has also been detected in bovine and dog plasma lipids, does not occur in bovine brain or spinal cord.

Direction of Current Research:

It will be possible to study the metabolic transformations of cerebrosides, glycolipids, and sphingomyelins in a new light, utilizing the gas chromatographic method for analysis of the long-chain base fractions as well as the fatty acids of these lipids.

Studies have been initiated to isolate the new long-chain base from human plasma in a pure form for elemental and spectral analysis and structural studies.

Part B included. Yes.

Serial No. NHI-153
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

Sweeley, C. C., A Gas Chromatographic Method for Sphingosine Assay, Biochim. et Biophys. Acta, 36, 268 (1959).

Sweeley, C. C. and E. A. Moscatelli, Qualitative Microanalysis and Estimation of Sphingolipid Bases, J. Lipid Research, 1, 40 (1959).

Serial No. NHI-154
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An Investigation of Stationary Phases for Gas-liquid Partition Chromatography

Principal Investigator: C. C. Sweeley, Ph.D.
E. C. Horning, Ph.D.

Other Investigator: None

Cooperating Units: None

Man Years:
Total: 0.25
Professional: 0.25
Other: 0.00

Patient Days
None.

Project Description:

Progress During the Past Year:

In the application of gas-liquid partition chromatography to the analysis of a mixture of high-boiling fatty acid methyl esters a severe limitation has been placed on the method by the problem of obtaining uniform coatings of polyester and other polar stationary phases on inert supports such as Celite and Chromosorb W. The most widely accepted method for preparing column packings has been relatively non-reproducible because of an inherent difficulty in obtaining uniform films of the stationary phase. The older method has been superceded by a procedure, developed in this laboratory, which involves a double-layered coating.

The inert support, usually a carefully screened diatomaceous earth, is first siliconized to give a non-polar, hydrophobic surface. A thin film of polyester is coated on top of the silicone layer by a solution technique. The process is simple and by its nature reproducibility of the technique and uniformity of the product are no longer a problem. Analytical separations on columns prepared by this method are uniformly good. There is some reason to believe that the column life may be somewhat enhanced.

Direction of Current Research:

A similar problem exists in the coating of films on long (100°-1000°) glass or metal capillaries for gas-liquid partition chromatography.

Preliminary experiments in this laboratory indicate the importance of the surface of the tube prior to coating, the polarity of the polymer used to produce a film, and even the nature of the solvent used to carry the polymer through the tube. Experiments have been designed to study these parameters. It is hopeful that for high resolution chromatography in the fatty acid field capillary columns will be realized with 200,000 to 500,000 theoretical plates (resolving power) capable of giving complete resolution of cis-trans and position isomers of unsaturated fatty acids such as oleic and linoleic acids.

Part B included. Yes.

Serial No. NHI-154
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

Horning, E. C., Moscatelli, E. A. and Sweeley, C. C., Polyester Liquid Phases in Gas-Liquid Chromatography, Chemistry and Industry, 751-752 (1959).

Serial No. NHI-155
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Structure of Amaryllidaceae Alkaloids.

Principal Investigator: W. C. Wildman, Ph.D.

Other Investigators: Y. Inubushi, Ph.D. (in part)
R. E. Lyle, Ph.D. (in part)
Patricia F. Highet (Technical)
Elizabeth A. Kiclar (Technical, in part)

Cooperating Units: None.

Man Years:
Total: 3.82
Professional: 2.32
Other: 1.50

Patient Days:
None

Project Description:

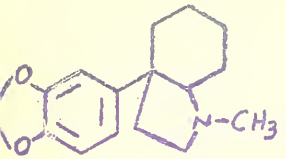
Progress During the Past Year

A. Isolation Studies.- The seeds of Crinum moorei J. D. Hook. have been found to contain the alkaloids lycorine, crinamidine, powelline, crinine and 1-acetyllycorine. 1-Acetyllycorine had not been isolated previously from natural sources. The bulbs of Nerine bowdenii W. Wats. have been shown to contain at least sixteen alkaloids, four of which, nerbowdine, bowdensine, 1-acetyllycorine and (+)-epicrinine, have not been reported to date.

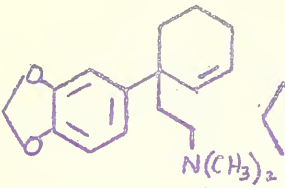
The various Crinum species called "Milk and Wine", C. zeylanicum, C. fribriatulum and C. erubescens, have been found to provide an abundant source for the rare alkaloid crinamine and a new alkaloid, 6-hydroxy crinamine.

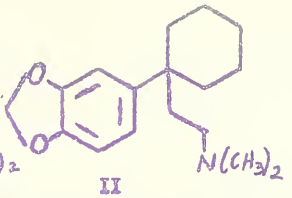
To provide sufficient quantities of the alkaloid galanthamine for pharmacological and possible clinical studies, a study is in progress to determine the most economical source. To date the cheapest source has been the King Alfred daffodil.

B. Structural Studies.- The C:D ring fusion of the alkaloids related to 5,10b-ethanophenanthridine has been shown to be cis by the Hofmann degradation of I to II. The structure of II was established by synthesis.



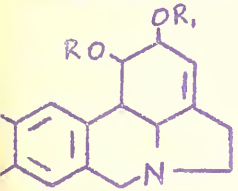
I



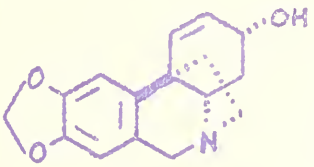


II

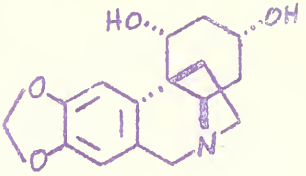
The structure of 1-acetyllycorine was established as III (R = Ac, R_1 = H) by acetylation to O,O-diacetyllycorine III (R,R_1 = Ac) and hydrolysis to lycorine (III, R,R_1 = H). (±)-Epierinine was shown



III



IV

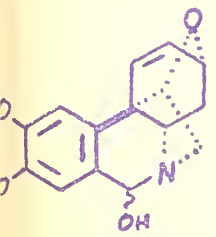


V

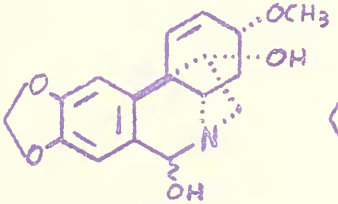
to possess structure IV by oxidation to (±)-oxocrinine (IV, = O instead of OH). This product was identical in all respects, except optical rotation, with (±)-oxocrinine, the structure of which was established earlier.

Nerbowdine has been shown to possess structure V by degradative and synthetic procedures. Bowdensine has been characterized and from preliminary experiments appears to be a hydroxy isomer or epimer of V.

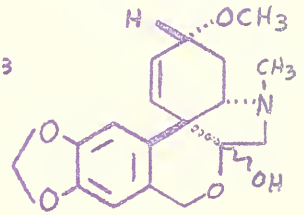
6-Hydroxy crinamine was proven to have structure VII by treatment with acid to afford apohaemanthamine (VI). Methylation of VII



VI



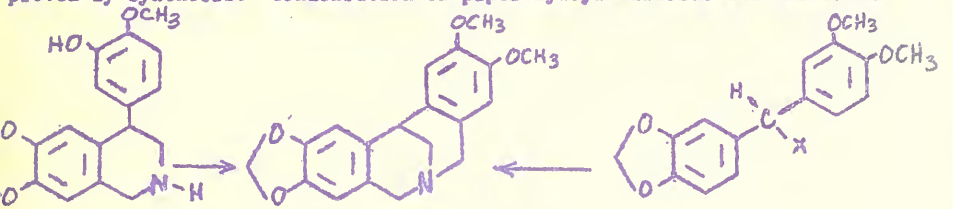
VII



VIII

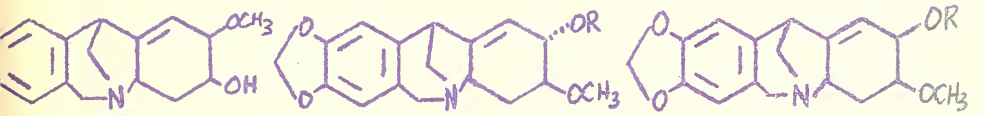
with methyl iodide and treatment of the product with cold dilute base gave criwelline (VIII, R = OH). The structure of criwelline was proven by O-methylation to O-methylcriwelline (VIII, R = CH_3), the methopicrate and methiodide of which were identical with that of the corresponding derivatives of O-methylisotazettine. The structure of O-methylisotazettine has been determined earlier.

Methylation of oxococcinine (IX) with formaldehyde and hydrochloric acid followed by diazomethane gave (X) the structure of which was proven by synthesis. Condensation of piperonyloyl chloride and veratrole



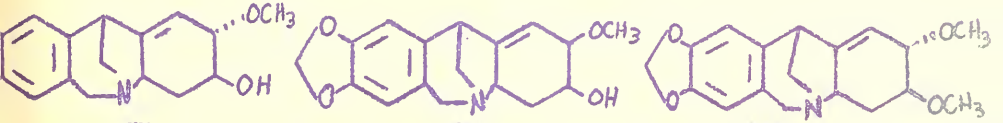
IX X XI

gave the expected ketone which was reduced with sodium borohydride to XI (X = OH). Successive treatment with thionyl chloride, copper cyanide and lithium aluminum hydride gave an amine (XI, X = CH_2NH_2) which was cyclized by formaldehyde and acid to X. From the now proven structure of IX, it became apparent that coccinine and montanine were isomers of XII. O-methylation of montanine was found to yield another alkaloid of the group,



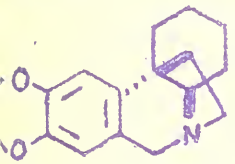
XII XIII XIV

manthine. Methylation of coccinine gave O-methylcoccinine. From the O-methylation of isohaemanthamine and epiisohaemanthamine, the structures of which had been established as XIII (R = H) and XIV (R = H) by this laboratory, there was obtained montanine and O-methylcoccinine, respectively. From this evidence, structures XV, XVI and XVII have been assigned to montanine, coccinine and manthine, respectively. This is the first report in the chemical literature of the existence of such a ring system.

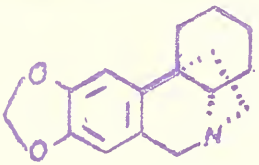


XV XVI XVII

The absolute configuration of the alkaloids derived from 5,10b-ethanophenanthridine has been presented. The alkaloids crinine, powelline, buphanisine, buphanidrine, buphanamine, crinamidine and undulatine are based on the (-)-crinane nucleus (XVIII). Vittatine, haemanthamine, haemanthidine, haemultine, crinamine, 6-hydroxycrinamine and (+)-epicrinine are based on the enantiomorphic (+)-crinane nucleus (XIX).



XVIII



XIX

Direction of Current Research:

The isolation and characterization of alkaloids and other physiologically active constituents of selected plant material will be continued. Preliminary studies on the biogenesis of selected alkaloids and the transformations of them by microbiological and mycological means will be started.

Part B included. Yes

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

Fales, H. M. and Wildman, W. C., Structure of Haemanthamine, Chemistry and Industry, 561 (1958).

Wildman, W. C. and Fales, H. M., The Stereochemistry of Amaryllidaceae Alkaloids Derived from 5,10b-Ethanophenanthridine, J. Amer. Chem. Soc., 80, 6465 (1958).

Fales, H. M., Horn, D. H. S. and Wildman, W. C., Structure of Criwelline, Chemistry and Industry, 1415 (1959).

Lyle, R. E., Kielar, E. A., Crowder, J. B. and Wildman, W. C., The Alkaloids of Nerine bowdenii W. Wats. and Crinum moorei J. D. Hook., J. Amer. Chem. Soc., 82, 0000 (1960).

Highet, P. F. and Wildman, W. C., The Hofmann Degradation of 3a-(3,4-Methylenedioxyphenyl)-1-Methyloctahydroindole, J. Org. Chem., 25, 0000 (1960).

Fales, H. M. and Wildman, W. C., The Structures of Haemanthamine and Crinamine, J. Amer. Chem. Soc., 81, 0000 (1959).

Serial No. NHI-156
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Long-Chain Esters; Amine Oxide Rearrangements

Principal Investigator: John C. Craig, Ph.D.

Other Investigators: None

Cooperating Units:

Man Years:
Total: .75
Professional: .75
Other: .00

Patient Days:
None

Project Description:

Progress During the Past Year:

(1) Formation of Long Chain Esters.- A study was carried out of the relationship between hemiacetals and esters for long chain compounds. This work was prompted by observations relating to the occurrence of plasmalogens and other substances which are clearly formed by reactions involving hemiacetal structures as intermediates. The formation of hemiacetals under a variety of conditions was studied, and ester formation was examined in a number of cases.

(2) Mechanism of Amine Oxide Rearrangements.- Several related amine oxide rearrangements were studied with a view to determining the requirements for the reaction, with particular regard to the mode of action of the catalyst. It was found that chelation of a specific type was necessary, and that the intermediate complex is most likely one involving an amino acid or hydroxy acid as well as the amine oxide in a chelate structure. The most satisfactory explanation for the course of the reaction involves a sequence of one-electron shifts in which there is a transient formation of iron IV. This leads to some interesting new aspects of the chemistry of iron complexes.

Direction of Current Research:

The amine oxide studies are being continued at the University of Sydney, Australia.

Part B included. No.

Serial No. NHI-157
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

A

Project Title: Mechanism of Action of New Type of Antidepressant Drug, Tofranil (Imipramine)

Principal Investigator: Dr. F. Sulser

Other Investigator: Mr. James Watts

Cooperating Units: Dr. F. Sulser on Fellowship from Swiss Academy of Medical Sciences until April 30, 1959, now on NIH Fellowship.

Man Years (calendar year 1959): Patient Days: None
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: Tofranil is structurally related to chlorpromazine, but acts as an antidepressant drug. It is not a monoamine oxidase inhibitor. Moreover, it does not produce excitation in normal man or animals, rather it elicits a slight sedation. Thus the drug has an action on the abnormal state which it does not show on the normal state. In this regard it is like aspirin which lowers temperature only when it is higher than normal.

The purpose of these studies is to ascertain the mechanism of Tofranil action in terms of brain neurohormones.

Methods Employed: Usual chemical and pharmacological procedures.

Major Findings: Tofranil in doses that do not affect alcohol action can block the ability of reserpine to potentiate alcohol. This action is maximal in about 3 hours and persists for several hours. Tofranil also blocks the potentiating action of reserpine on barbiturates.

In rats, Tofranil not only blocks the effect of reserpine in potentiating hypnotics, but shows a delayed action in preventing other central actions of reserpine including sedation, ptosis and miosis. Tofranil, however, does not interfere with the release of brain amines by reserpine.

Tofranil does not antagonize the central actions of chlorpromazine.

Chlorpromazine by itself potentiates the action of alcohol and barbiturates in mice. But in addition chlorpromazine can act like

Serial No. MHI-157

Tofranil having a delayed action in blocking the ability of reserpine to potentiate alcohol and barbiturates.

In dogs, chlorpromazine blocks reserpine-induced salivation. In addition preliminary results suggest that Tofranil may block the ability of reserpine to increase the activity of liver tryptophane peroxidase.

The working hypothesis that we now have is that phenothiazines and related compounds may have two actions - 1) central adrenergic blocking and 2) central serotonin blocking. Chlorpromazine exerts both effects with the anti-adrenergic action predominating. Tofranil exerts both actions with the anti-serotonin predominating. It is of considerable interest that Tofranil has a powerful anti-serotonin activity in vitro on smooth muscle.

Significance to the Program of the Institute: This project may be a good example of the principle that to screen drugs for chronic diseases, the abnormal state in animals should be used as a model.

Proposed Course of Project: 1) To ascertain whether Tofranil acts centrally by blocking the action of free serotonin. 2) To see whether our model screening test can be used to develop better Tofranil analogues.

rt B included: No

Serial No. NHI-158
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

t A

Project Title: Biochemical Mechanism of Action of Reserpine and Related Compounds
A. Evidence that Tranquilizing Action of Reserpine is Associated with Change in Brain Serotonin and not in Brain Norepinephrine

Principal Investigators: Dr. F. Barbara Orlans
Dr. Gertrude P. Quinn
Dr. F. Sulser
Dr. K.F. Finger

Other Investigators: None

Cooperating Units: Dr. Orlans received a Ciba Fellowship until September, 1959, now is a Visiting Scientist. Dr. F. Sulser, Fellowship from the Swiss Academy of Medical Sciences, until April 30, 1959, now on NIH Fellowship. Dr. Finger, Guest Worker from Chas. Pfizer and Co.

Man Years (calendar year 1959): Patient Days: None
Total: 2
Professional: 2
Other: 0

Project Description:

Objectives: The purpose of this study is to answer the crucial question whether action of reserpine is due to loss of brain norepinephrine or is associated with change in brain serotonin. This question has been complicated by discovery that reserpine releases brain serotonin and norepinephrine at the same rate, to same extent and for same period of time. Until the question is answered, it is difficult to determine the true role of brain serotonin.

Methods Employed: Spectrophotofluorimetric assay of serotonin and norepinephrine; classical pharmacologic procedures.

Major Findings: Other workers have convinced the scientific world that reserpine tranquilization is due to loss of brain norepinephrine, by reporting that reserpine and raunescine in rats, can produce sedation and release of brain norepinephrine without release of brain serotonin, and by reporting that certain tranquilizing benzoquinolizines elicit sedation in mice related to the extent of norepinephrine, but not of serotonin release in brain.

Our results show that reserpine and raunescine in rats liberates serotonin and norepinephrine to almost exactly the same degree; also

that is is not possible to link the central actions of the benzoquinolizines (Ro 4-1284 and Ro 4-1398) with either one of the amines.

However, we have found a reserpine analogue, dimethylaminobenzoyl methylreserpate (Su 5171) releases a considerable proportion of brain norepinephrine in rabbits without releasing significant amounts of brain serotonin and without eliciting sedation. Sedation is only seen with doses large enough to release 50% of brain serotonin.

The strongest evidence, with reserpine itself, that sedation is not due to loss of brain norepinephrine, is provided by experiments in which rats and rabbits subjected to stress are not sedated. The norepinephrine in the brains of these animals is depleted, but serotonin is lowered only slightly. (For details see report entitled "Stress Interaction with Reserpine).

These results show that the tranquilizing actions of Rauwolfia alkaloids are not related to change in norepinephrine but, as we originally suggested, to the change in brain serotonin.

Significance to the Program of the Institute: These studies indicate a role of serotonin in brain as a neurohormone of an integrative neuronal system that is antagonistic to an adrenergic (reticulo-activating) system.

Proposed Course of Project: The finding of compounds that selectively release brain amines permits, for the first time, experiments which give promise of showing in a conclusive fashion, the effects and perhaps the physiologic role of free serotonin and norepinephrine in brain.

Part B included: Yes

Serial No. NHI-158

Publications:

Brodie, B.B., Finger, K.F., Orlans, F.B., Quinn, G.P. and Sulser, F.: Evidence that tranquilizing action of reserpine is associated with change in brain serotonin and not in brain norepinephrine, in press.

Brodie, B.B., Spector, S. and Shore, P.A.: Interaction of drugs with norepinephrine in brain. Pharmacological Reviews 11, 548, 1959.

Brodie, B.B.: Chapter in book entitled "Pharmacologic Approach to the Study of the Mind", Charles C. Thomas, 1959.

Serial No. NHI-159
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies in Biochemical Behavior
I Survey of Pituitary Response to Various Chemical and Physical Stimuli

Principal Investigators: Mr. Roger P. Maickel
Dr. W. Robert Jondorf
Dr. Erik Westermann

Other Investigators: None

Cooperating Unit: None

Man Years: (Calendar Year 1959): Patient Days None
Total: 0.9
Professional: 0.6
Other: 0.3

Project Description:

Objectives: Unicellular organisms exhibit "behavior" by adapting their biochemical reactions to the stimuli of environmental changes. Although each individual cell in higher animals may possess some degree of automatism in adjusting to environmental change, the information from the outside world is usually transmitted through sensory organs to the brain. It is logical, therefore, to consider that biochemical adjustments to environment are mediated by the brain. Thus the brain may control not only psychological and physiological "behavior" but also biochemical "behavior". This control is presumably transmitted to peripheral enzymes and substrates by the hypothalamic pituitary secretion. These pituitary hormones are involved in a number of "axes" including the pituitary-adrenal, pituitary-thyroid, pituitary-gonad, etc. Thus the pituitary hormones may control enzymes directly or through hormones released from other endocrines.

The purpose of the present project is multifold:

1) The nature of and reason for the biochemical changes which occur in response to changes in environment.

2) The nature of the "irreversible" changes which may occur with exaggerated or persistent environmental changes and which may result in chronic diseases.

3) The mechanisms whereby the hypothalamus controls the various pituitary hormones.

4) The interaction of drugs with the hypothalamic-hypophysical-endocrine axis.

5) The extent to which the therapeutic effects of electroshock, insulin and metrazol shock and intravenous procaine are due to effects mediated through the pituitary.

Major Findings: Preliminary to studies of the control of biochemical behavior it was necessary to obtain information on the temporal relation between stimulation of the pituitary by various chemical and physiologic stimuli and the appearance of measurable responses.

Typical stimuli applied to rats were cold, intradermal formaldehyde, electroshock, ethionine, reserpine, ethanol, dibenamine, carbon tetrachloride, 3-methylcholanthrene, chlorpromazine, and Tofranil. The results are summarized as follows: (None of these responses occurred in hypophysectomized animals.)

1) Adrenal ascorbic was rapidly reduced and returned to normal in 4 to 6 hours. With persistent stimuli (cold and methylcholanthrene) the adrenal ascorbic acid level returned to a higher than normal level despite continuation of the stress. This finding, hitherto unreported, probably reflects increased ascorbic synthesis in the entire animal. In guinea pigs, which are unable to make ascorbic acid, the depletion was more complete and levels remained below normal during persistent stress.

2) Plasma corticosterone (main adrenal corticoid in rat) levels increased to a maximum in 1 to 4 hours and returned to normal shortly after. However, with persistent stress (cold and methylcholanthrene) the high levels were maintained for duration of stress.

3) Plasma non-esterified fatty acids usually increased to maximum in 1 to 4 hours and then returned to normal. The high levels were maintained by cold and perhaps by methylcholanthrene. Anomalous results were obtained with electroshock which lowered the fatty acids and Trofranil, which had a delayed response.

4) A chemical picture of "stress" was elicited by "anti-stress" compounds such as reserpine and chlorpromazine. These compounds produced effects in doses as low as 1 mg/kg. These results are surprising in view of present concept of stress.

5) The activity of tryptophane peroxidase (TPO) was increased by all stimuli except alcohol and electroshock which have not yet been studied.

6) Although many stimuli caused increased fatty acids (presumably coming from depot fat) only some of them resulted in deposition of liver triglyceride, e.g., cold, ethanol, ethionine, carbon tetrachloride increased liver fat whereas methylcholanthrene and dibenamine did not. This suggests something must also occur at tissue to cause fatty deposition.

Significance to the Program of the Institute: Pituitary control of body metabolism may be a key to understanding of some chronic diseases.

Proposed Course of Project:

1) Study of nature of the pituitary response to various stimuli by use of drugs which act on brain.

2) Outline biochemical behavior in terms of biochemical systems which change in response to stimuli.

3) Particular effect of various stimuli on amino acid synthesis and catabolism, neurohormone synthesis and metabolism, microsomal drug enzymes, TPNH oxidase.

Part B included: No

Serial No. NHI-160
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

art A

Project Title: Biochemical Mechanism of Action of Reserpine and Related Compounds.
B. Mechanism of Action of Cardiovascular Effects

Investigators: Dr. F. Barbara Orlans
Dr. K.F. Finger
Dr. Rossmary Cass

Other Investigators: None

Cooperating Units: Dr. Orlans, Ciba Fellow till September, 1959, now Visiting Scientist; Dr. Finger, Guest Worker from Chas. Pfiser and Co.; Dr. Cass, Riker Fellow.

Man Years (calendar year 1959): Patient Days: None
Total: 2
Professional: 2
Other: 0

Project Description:

Objectives: Interpretation of cardiovascular effects of reserpine in terms of changes in peripheral neurohormones.

Major Findings: Studies on peripheral action of reserpine reported last year have been completed. It has been shown unequivocally that reserpine lowers sympathetic tone, not by reducing central sympathetic activity (which in fact seems to be increased) but by producing chemical sympathectomy through loss of peripheral norepinephrine. The new drug Syrosingopine (Singoserp) is selective in that over a large dosage range it releases peripheral but not central amines. This compound thus can produce chemical sympathectomy (lowered blood pressure, heart rate, decreased response to carotid occlusion and stimulation of sympathetic ganglia etc.,) with minimal central sedation. This explains the value of this compound in hypertension since it is likely that it will have effects on doses which will not produce depression.

Serial No. NHI-160

In collaboration with Ciba Labs., it was found that the ester linkage of reserpine is not essential for its action and other linkages can be substituted. This has led to development of new series of reserpine analogues which selectively deplete peripheral norepinephrine. One to these is now in clinical trial.

Studies with Guanethidine (Su 5864), a Ciba compound, show that this drug may also affect blood pressure by producing chemical sympathectomy. It depletes heart norepinephrine, but not brain norepinephrine. It may be presumed to act by a different mechanism than reserpine since it does not release serotonin and acts much more slowly than reserpine.

Significance to Program of Institute: The discovery that drugs may selectively release peripheral norepinephrine, opens a new way of screening for hypotensive drugs.

Proposed Course of Project: The study of reserpine analogues without the ester sidechain will be continued. Studies will be carried out on the problem of whether Guanethidine may act by blocking synthesis of peripheral norepinephrine perhaps by interferring with conversion of dopamine to norepinephrine.

t B included: Yes

Serial No. NHI-160

Publications:

F.B. Hughes Orlans, K.F. Finger and B.B. Brodie: Biochemical and pharmacological actions of syrosingopine (Su 3118), a reserpine analogue which selectively releases peripheral norepinephrine. J. Pharmacology and Experimental Therapeutics, in press.

Honors and Awards: None

Serial No. NHI-161
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

A

Project Title: Biochemical Mechanism of Action of Reserpine
C. Stress Interaction with Reserpine

Principal Investigator: Dr. F. Sulser

Other Investigator: Mr. James Watts

Cooperating Units: Dr. F. Sulser on Fellowship from Swiss Academy of Medical Sciences until April 30, 1959, now on NIH Fellowship.

Man Years (calendar year 1959): Patient Days: None
Total: .8
Professional: .5
Other: .3

Project Description:

Objectives: To study the mechanism whereby "stress" prevents the action of reserpine and to use "stress" as a tool to elucidate role of brain amines.

Methods Employed: Standard pharmacologic and chemical procedures.

Major Findings: If rats and rabbits subjected to cold are given reserpine and kept in the cold, they are not sedated nor do they lose therighting reflex. Brain norepinephrine is depleted, but brain serotonin is almost normal. If animals are now brought to room temperature, the brain serotonin is slowly released and only when it is 50% of normal then sedation is evident.

In contrast to the effect on reserpine action, cold had not effect on chlorpromazine action. These results show that reserpine tranquilization is linked with change in brain serotonin.

Metrazol (IP) or formaldehyde (intradermal) also prevents reserpine sedation and release of brain serotonin suggesting that the phenomenon involves some substance in the pituitary. Hypophysectomized rats subjected to cold, are sedated and show the usual release of brain serotonin, indicating the probability that a pituitary substance is needed to prevent reserpine action.

Significance to the Program of the Institute: May indicate a relationship between the action of drugs and the emotional status of the organism.

Proposed Course of Project: 1) Identification of pituitary material which can prevent release of brain serotonin by reserpine. 2) By selectively releasing brain amines in the presence of monoamine oxidase inhibitors (to protect released amines) it should be possible to compare the central actions of norepinephrine and serotonin released at their actual sites of action; and in particular to show whether or not they may be hormones of antagonistic neuronal systems.

B included: No

Serial No. NHI-162
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

:t A

Project Title: Biochemical Mechanism of Action of Reserpine and Related Compounds. D. Effect of Reserpine on Storage of Catecholamines and Serotonin

Principal Investigators: Dr. S. Spector
Mr. R. Kuntzman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: .4
Professional: .4
Other: 0.0

Project Description:

Objectives: The tranquilizing effect of reserpine has been related to the release of serotonin in the brainwhile the hypotensive effect has been related to the release of norepinephrine at peripheral nerve endings. The present report describes the effect of reserpine on the serotonin and catecholamine binding sites in the brain.

Major Findings: Reserpine has been shown to impair the ability of brain to store serotonin. Since reserpine also depletes norepinephrine in the brain it was of interest to determine if reserpine impairs binding sites of catecholamines. The rise in brain dopamine after the administration of dopa (dihydroxyphenyl(alanine), to normal and reserpine treated rabbits indicates that reserpine impairs the ability of the brain to bind dopamine. Since dopamine is a catecholamine, it is used as a model for norepinephrine, and therefore the results imply that reserpine affects norepinephrine binding sites.

The decline in brain amines caused by reserpine can be prevented by pretreatment with monoamine oxidase inhibitors. In addition the sedation elicited by reserpine is reversed. Our interpretation has been that reserpine releases the amine, which however remain elevated but in a free form; the excitement is then due to the high level of free amines. An alternate explanation is that the monoamine oxidase inhibitors somehow prevents reserpine from releasing the amines. Using a

reversible monoamine oxidase inhibitor, harmaline, we have shown that in animals pretreated with the inhibitor and then given reserpine, brain amine levels are maintained only during the time that monoamine oxidase is inhibited. This strongly supports the view that monoamine oxidase inhibitors do not prevent the release of the amines, but maintain the brain amine levels by blocking the metabolism of the free amines; and the main pathway for norepinephrine metabolism is deamination by monoamine oxidase.

Significant to the Program of the Institute: These studies aid in understanding the action of drugs demonstrated to be useful in the treatment of hypertension. Furthermore, they may help suggest normal roles for serotonin, norepinephrine and dopamine.

Proposed Course of Project: Possible role of monoamine oxidase in controlling the size of the stored amine pools will be studied.

rt B included: No

Serial No. NHI-163
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

rt A

Project Title: Pharmacologic Mechanism of Reserpine Action in Brain

Principal Investigator: Dr. Fridolin Sulser

Other Investigator: Mr. James Watts

Cooperating Units: Dr. F. Sulser is on Fellowship from the Swiss Academy of Medical Sciences (until April 30, 1959), now on NIH Fellowship.

Man Years (calendar year 1959): Patient Days: None
Total: 0.5
Professional: 0.1
Other: 0.4

Project Description:

Objectives: Reserpine has been postulated to act centrally by stimulating through serotonin, a neuronal system (trophotropic) which integrates the parasympathetic system with somatomotor and psychic functions. This system seems to act in opposition to a system (ergotropic) which integrates the sympathetic system with somatomotor and psychic functions. This latter system is an adrenergic system which is antagonized by chlorpromazine.

If this conception is valid, then reserpine, in contrast to chlorpromazine, should increase the parasympathetic output from the central nervous system.

Methods Employed: Usual methods of classical pharmacology.

Major Findings: Our results show definitively that reserpine stimulates central parasympathetic areas, in contrast to chlorpromazine which inhibits centrals ympathetic areas. Thus, the two drugs must act on different (presumably opposing) neuronal systems.

In a previous report it was shown that reserpine produces miosis, lacrimation and enhanced light reflex by central parasympathetic stimulation. The present reports describe effects on salivation in unanesthetized dogs. Reserpine elicits profuse salivation from cannulated submaxillary gland. This effect is parasympathetic since it is blocked by atropine. It is central, for it is blocked by section of chorda tympani when acetylcholine and pilocarpine still produce salivation, and is prevented by ganglionic blocking agents (Ecolid). The salivation is also blocked by barbiturates, but it is not known whether this inhibition is central or peripheral.

Tetrabenazine, a synthetic benzoquinolizine, which releases brain amines, produces effects like those of reserpine.

Salivation, after reserpine, terminates in about 5 hours, though the animals are deeply sedated and the gland can still respond to cholinergic stimuli. The reason for the stopping of salivation is not known, but is probably significant in an understanding of reserpine interaction with neurohormones.

Chlorpromazine does not produce salivation, though it blocks the salivation elicited by reserpine (see Tofranil report).

Significance to the Program of the Institute: Knowledge of reserpine action will lead to an understanding of the role of norepinephrine and serotonin in normal brain function.

Proposed Course of Project: Further studies will be made to show that reserpine and chlorpromazine act on opposing integrative systems concerned in the control of behavior. Since "behavior" includes autonomic function, reserpine as well as chlorpromazine are valuable tools to study central control of cardiovascular functions.

lt B included: Yes

Publications

Brodie, B.B.: Effects of chlorpromazine, reserpine and monoamine oxidase inhibitors on cardiovascular control by interaction with central and peripheral neurohumoral agents. American Heart Jo. 58, 82, 1959.

Honors and Awards: None

Serial No. MHI-166
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

A

Project Title: Studies on the Physiological and Biochemical Effects of Monoamine Oxidase Inhibitors

Principal Investigators: Dr. Sydney Spector
Mr. Ronald Kuntzman

Other Investigator: Mrs. Carolynne Cirsch

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1.5
Professional: 1.2
Other: 0.3

Project Description:

Objectives: Monoamine oxidase inhibitors are assuming considerable importance in the treatment of depressed mental states, hypertension and angina pectoris. The present study is designed to gain some understanding of the biochemical mechanisms by which these drugs exert their action.

Major Findings: As discussed previously, the use of monoamine oxidase inhibitors has led to the finding that serotonin has a very rapid turnover in rabbit brain (half-life about 10-15 min.). However, norepinephrine has a much slower turnover (half-life several hours). In view of the presence of dopamine (3-hydroxytyramine) in brain, it was of interest to ascertain its turnover. The rate of increase in brain dopamine level after a rapid acting monoamine oxidase inhibitor JB 516) is similar to that of serotonin, about 50% in 15 min. The rapid increase in the dopamine content of the brain indicates that monoamine oxidase is important in the metabolism of this catecholamine.

Brain levels of serotonin and dopamine rise rapidly while norepinephrine increases slowly; however, the excitation elicited with the inhibitors is related with the increased brain levels of norepinephrine and not, with the levels of serotonin or dopamine. The above relationship was made with the following monoamine oxidase inhibitors: Iproniazid, phenylisopropylhydrazine (JB 516), phenylisobutylhydrazine (JB 835). However, JB 807 which is the isopropyl derivitive of JB 516 elevated the brain levels of serotonin and norepinephrine to the same extent as the other inhibitors and excitation was not observed.

The duration of action of these monoamine oxidase inhibitors varies with different species. In mice a single dose of this inhibitor causes complete inhibition of the enzyme for 18 hours, while in rabbits the enzyme remains inhibited for 48-72 hours. The rapid disappearance of monoamine oxidase inhibition in mice suggests that the enzyme may be rapidly formed in this species compared to the rabbit.

The benzyl analogue of serotonin (BAS) has been reported to be an inhibitor of monoamine oxidase. This compound causes hypotension and depression. However, our studies indicate that the levels of brain serotonin and norepinephrine are not altered by BAS and the inhibitory effect of BAS on monoamine oxidase is very weak.

Significance to the Program of the Institute: These studies increase our understanding of the mechanism of action of drugs which are useful in the treatment of various disease states. Furthermore, they may help suggest normal roles for serotonin, norepinephrine and dopamine, substances implicated in the function of homeostatic mechanisms in the body.

Proposed Course of Project: Monoamine oxidase inhibitors, other than the hydrazine type, will be investigated in order to establish their mechanism of action. Of specific interest is a potent monoamine oxidase inhibitor which has a triple bond, but lacks a hydrazine group.

B included: Yes

Serial No. NMI-164

Publications:

Spector, S., Shore, P.A. and Brodie, B.B.: Biochemical and Pharmacological Effects of the Monoamine Oxidase Inhibitors, Iproniazid, 1-phenyl-2-hydrazinopropane (JB 516), and 1-phenyl-3-hydrazinebutane (JB 835), J. Pharmacol. and Exp. Therap., in press.

Brodie, B.B., Spector, S., and Shore, P.A.: Interaction of Drugs with Norepinephrine in the Brain. Pharmacol. Rev. 11: 548-564, 1959.

Brodie, B.B., Spector, S. and Shore, P.A.: Interaction of Monoamine Oxidase Inhibitors with Physiological and Biochemical Mechanisms in Brain. Ann. N.Y. Acad. of Sci. 80: 609-616, 1959.

Honors and Awards: None

Serial No. NHI-165
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

: A

Project Title: Micromethods for the Determination of Norepinephrine and Serotonin

Principal Investigators: Dr. P.A. Shore
Mr. R. Kuntzman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: Methods for estimation of norepinephrine and serotonin in tissues have been developed in this laboratory. In order to estimate the levels of these amines in discrete areas of brain or in ganglia, it was necessary to modify these methods to increase their sensitivity by an order of magnitude.

Major Findings: The sensitivity of the chemical methods for norepinephrine and serotonin have been increased ten times. With these methods we can accurately measure as little as 0.040 µg of norepinephrine and .200 µg of serotonin.

Significance to the Program of the Institute: These methods are invaluable in studies of the role of norepinephrine and serotonin as neurohormones.

Proposed Course of Project: These methods will be used to investigate the role of norepinephrine and serotonin in nerves and ganglia. They will also be used to study the relationships between norepinephrine and serotonin in developing animals. In general, these methods will be used to study the two amines in areas where they are found in low concentrations of where only small amounts of tissue are available.

: B included: No

Serial No. NHI-166
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

A

Project Title: The Physiological Function of the Catecholamine in the Autonomic Ganglion

Principal Investigators: Dr. S. Spector
Mr. R. Kuntzman

Other Investigators: None

Cooperating Units: Dr. E. Costa, Galesburg State Research Hospital, Galesburg, Ill.

Man Years (calendar year 1959): Patient Days: None
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: The stimulation of the preganglionic fibers of the sympathetic nervous system results in the release of both acetylcholine and catecholamines from the ganglia. The ganglionic function of the catecholamine is not well understood. The present study is designed to gain some understanding of the physiological function of the catecholamine in the ganglia, and whether many drugs which are known to exert an effect through a ganglionic action may not be influencing the content of catecholamines in the ganglion.

Major Findings: The administration of reserpine to cats causes a 90% decrease in the total catecholamine content of the superior cervical ganglion.

The post ganglionic potential was recorded from the cervical sympathetic ganglion of the cat anesthetized by electro-coagulation of the reticular formation. Three hours after a dose of reserpine which will cause a severe loss of catecholamine content in the ganglion, the post ganglionic potentials are increased and the preganglionic threshold reduced. Six hours later the response from preganglionic stimulation is further increased.

Significance to the Program of the Institute: These studies may add to our knowledge of the autonomic nerve system and the control of the cardiovascular system.

Proposed Course of Project: 1) To determine whether drugs which may influence the ganglionic catecholamine content by either depleting the levels or elevating them, have an effect on nerve transmission.

2) Study the effects of reserpine on parasympathetic ganglia.

t B included: No

Serial No. NHI-167
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Project Title: The Variation in Norepinephrine and Serotonin Concentration in Fetal and Immature Rat Brain

Principal Investigator: Dr. Niilo Karki

Other Investigator: Mr. R. Kuntzman

Cooperating Units: Dr. Karki is on U.S. Grant-in-aid Fellowship from Finland.

Man Years (calendar year 1959): Patient Days: None
Total: 0.3
Professional: 0.3
Other: 0

Project Description:

Objectives: To examine the relative and absolute concentrations of norepinephrine and serotonin in rat brain before and after birth until maturity and relate these values to those found in the adult brain.

Methods Employed: Standard pharmacologic and chemical procedures.

Major Findings: The level of serotonin and norepinephrine in the brain of the fetus at an estimated 1-4 days before parturition were approximately 25-40 per cent and 14-20 per cent of the adult respectively; at this time the absolute concentration of serotonin was 0.16 μg/g and that of norepinephrine 0.086 μg/g. At two weeks the norepinephrine was 40% and the serotonin 70% of the concentration of these substances in the adult. As the age of the animal increased the amount of the two neurohormones increased progressively until at the age of six weeks reached 85-90 per cent of the values of 8-10 weeks old control animals.

Significance to the Program of the Institute: Norepinephrine and serotonin have been postulated to be neurohormones and it is therefore of importance to study the relationships of these substances, to one another, in the developing animals.

Serial No. NHI-167

Proposed Course of Project: Since serotonin and norepinephrine are found only in small amounts in developing rats, it is of interest to investigate the effect of drugs that act on the brain. To determine if the lower levels of these amines are related to enzyme concentrations, the enzymes involved in the synthesis and metabolism of these amines will be investigated. In addition, norepinephrine and serotonin will be studied in various species (insects, reptiles, etc.), to gain more information concerning their role as neurohormones.

Part B included: No

Serial No. NHI- 168
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Mechanism of Uptake of Catecholamines by Brain Tissue.

Principal Investigators: Dr. Cedric W. M. Wilson
Dr. Hans Bengler
Dr. Elwood O. Titus

Other Investigators: Anna W. Murray
Herbert E. Spiegel

Cooperating Units: None.

Man Years (calendar year 1959):
Total: 1.25
Professional: 1.0
Other: .25

Patient Days (calendar year 1959): None.

Project Description:

Objectives - The catecholamines have important effects on brain function. In recent years considerable evidence has appeared to suggest that these substances serve as neurohumoral mediators. It is therefore important to understand the mechanism by which these substances enter nervous tissue. Two aspects of this problem are being investigated.

1) The mechanism by which the catecholamines pass from the plasma into the central nervous system through the blood brain barrier.

2) The mechanism by which these amines enter slices of various brain tissues in vitro. It is hoped that these experiments will clarify the nature of receptor sites for neurohumoral mediators.

Methods Employed - Chromatographic and extraction procedures are used to determine the amounts and the specific activities of tritium labeled catecholamines and their metabolites in tissues that have been exposed to labeled drug.

Patient Material - None.

Major Findings - In agreement with other workers it has been observed that epinephrine from plasma is rapidly concentrated in the pituitary of the cat, but not in other parts of the brain. Concentrations in the anterior lobe are invariably higher than in the posterior. When plasma concentrations of the order of 0.002 micrograms of epinephrine per ml are maintained by infusion, concentrations several times higher appear in the hypophysis. The concentrating mechanism appears to become saturated at plasma levels of the order of 0.01 micrograms of epinephrine per ml.

In the cat reserpine destroys the ability of the pituitary to concentrate epinephrine. The same drug markedly reduces the amount of epinephrine appearing in other tissues of the body after intravenous infusion. The blood level which can be maintained by infusion of epinephrine at a constant rate is reduced to about one fifth of control levels in animals that have received 3.0 milligrams of reserpine per kilo. This effect is as yet unexplained.

Several tissues including cortex, hypothalamus, thalamus, rhinencephalon and pituitary, but not cerebellum, will concentrate epinephrine when incubated in vitro with serum containing approximately 0.002 micrograms of labeled epinephrine. Concentrations about twice those of serum are attained in one hour. The concentrating mechanism is saturated at serum levels of approximately 0.010 micrograms, and is destroyed in tissues from animals that have received reserpine.

There is preliminary evidence that the pineal gland accumulates higher concentrations of epinephrine than do other brain tissues and that the concentrating mechanism is insensitive to reserpine.

Preliminary experiments with norepinephrine indicate that this drug will enter hypothalamus slices against a concentration gradient. In contrast to tritiated epinephrine, which is still accumulating in hypothalamus after a one hour incubation, labeled norepinephrine reaches a constant concentration in this tissue within a very few minutes.

Significance to the Program of the Institute - There is at present considerable uncertainty about the nature of the receptor sites at which physiologically active amines such as serotonin, epinephrine and norepinephrine exert their effects. Binding to protein or to membrane phospholipids, concentration in subcellular particles as salts of adenosine triphosphate, and active transport into the receptor cell have all been suggested as the means by which these agents reach their targets. An understanding of the molecular events at the receptor site is prerequisite to an understanding of the mechanism of action of these substances.

Serial No. NMI-160

Proposed Course of Project - Studies of the uptake of epinephrine and norepinephrine into brain tissue slices will be continued in the hope of establishing:

1) Whether the entry of amines into nervous tissue occurs by active transport, by a diffusion process, by irreversible interaction with some tissue components or by a mixture of these mechanisms.

2) To what extent the mechanism or mechanisms of concentration are specific for optically active forms of the amines.

3) Whether passage of the amines through a membrane represents an exchange for cations or whether the biochemical systems responsible for the transport of cations are in any way involved in the functioning of receptor sites.

Part B included No

Serial No. NHI-169
1. Lab. Chem. Pharm.
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Development and Use of a Fluorometric Method for Assay of Histamine in the Body

Principal Investigators: Dr. P. A. Shore
Dr. A. Burkhalter
Mr. V. H. Cohn

Other Investigators: None

Cooperating Units: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives: Despite the wide-spread recognition of the potent effects of histamine on various organs, and the role this amine might play in certain pathologic processes, little is known of its normal function. Research activity in this area of investigation should be enhanced by a simple and sensitive assay technique for histamine in various tissues and body fluids.

Major Findings: Development of an extremely sensitive fluorometric technique for the estimation of histamine in tissues has been completed. The method is based on the condensation of histamine with o-phthalaldehyde, both non-fluorescent, to yield a condensation product which is highly fluorescent.

Application of the technique to analysis of various organs reveals that the method is very specific for histamine in organ tissues. Urine, which contains little free histamine and a high concentration of ammonia, has proved difficult to analyze, but we have developed a modification for the separation of the ammonia and histamine fluorophors.

Analysis of the brains of rabbits and dogs has revealed that there is a relatively uniform distribution of histamine in gross divisions such as the cerebrum, cerebellum and brain stem, although higher concentrations may be found in the pituitary gland. These findings indicate that the histamine in brain may be associated with non-nervous, perhaps vascular, tissue.

Significance to the Program of the Institute: Because of the extensive distribution of histamine in the body and the marked effects of this amine on the cardiovascular system, there is a need for more information of its distribution and metabolism.

Proposed Course of Project: We expect to complete development of a method for estimation of urinary histamine. This will be an aid in other metabolic studies. We also hope to carry out a study of histamine distribution in finer structures of the nervous system.

Part B included: Yes

Serial No. NHI-169

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Shore, P. A., Burkhalter, A., and Cohn, V. H. A Method for the fluorometric assay of histamine in tissues. J. Pharmacol. Expt. Therap. 127: 182, 1959.

Serial No. NHI-170
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Diamine Oxidase and Diamine Oxidase Inhibitors

Principal Investigators: Dr. P. A. Shore
Mr. V. H. Cohn

Other Investigators: None

Man Hours (Calendar Year 1959): Patient Days: None
Total: 3/4
Professional: 1/4
Other: 1/2

Project description:

Objectives: Many unanswered questions remain concerning the nature and function of diamine oxidase (DAO) which appears to play a role in the metabolism of histamine. Thus there is disagreement among investigators as to the identity of "histaminase" and DAO. There are also marked species and sex differences reported in histamine metabolism which might be associated with DAO activity. There is also a question of the ability of monoamine oxidase to metabolize histamine since the monoamine oxidase inhibitor, iproniazid, has been demonstrated to block histamine metabolism *in vitro*. This project was initiated in an attempt to learn more of the nature and function of this enzyme and of the compounds which inhibit its activity.

Major Findings: An improved technique, based on our fluorometric histamine assay, has been devised for the measurement of DAO activity in tissues. Studies carried out with various monoamine oxidase inhibitors have indicated that each of monoamine oxidase inhibitors containing a hydrazine moiety inhibits DAO as well as MAO. The action of a typical inhibitor, iproniazid, appears to be irreversible since inhibition of DAO in the rat persists after disappearance of iproniazid from the body.

One potent non-hydrazine monoamine oxidase inhibitor, phenylcyclopropylamine (SKF 385), has no DAO inhibitory activity *in vitro* or *in vivo*, while aminoguanidine blocks DAO activity completely without affecting MAO. Thus it can be demonstrated that monoamine oxidase is incapable of metabolizing histamine *in vitro*.

Comparison of the DAO activity in male and female rats indicates that the sex difference reported in the ability to metabolize histamine *in vivo* cannot be explained by a difference in DAO activity.

Significance to the Program of the Institute: These studies are relevant to investigations into the metabolism and function of histamine and possibly other amines which affect the cardiovascular system.

Proposed Course of Project: We plan to carry out studies to demonstrate the identify of "histaminase" and DAO and to study the properties of DAO. We also plan to investigate the reported influence of endocrine hormones upon the activity of DAO.

Part B included: No

Serial No. NHI-171
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Histamine Binding and Release

Principal Investigators: Dr. P. A. Shore
Dr. A. Burkhalter

Other Investigators: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 1/2
Professional: 1/2
Other: None

Project Description:

Objectives: It is known that reserpine and other active Rauwolfia alkaloids effect the release of serotonin and catecholamines from a number of tissues including brain, intestine, adrenal gland, and platelets. It has been reported that reserpine also releases histamine from rabbit platelets. We are investigating the influence of reserpine on histamine levels in various tissues, and the mechanism of release of histamine from rabbit platelets.

Major Findings: We have found that reserpine does not effect the release of histamine from any of the organs studied (brain, liver, intestine, heart, kidney), although release from rabbit platelets can be readily demonstrated in vivo. This indicates that histamine is bound in most tissues by a mechanism quite different than that involved in the binding of serotonin or the catecholamines. We have found that only reserpine and other Rauwolfia alkaloids which release serotonin can release platelet histamine in the rabbit. Administration of serotonin or its precursor, 5-hydroxytryptophan, also causes the release of platelet histamine. Thus there is the distinct possibility that the serotonin released by reserpine in turn releases platelet histamine.

Attempts to demonstrate the release of platelet histamine *in vitro* have led to the surprising finding that although platelet serotonin is readily released by reserpine *in vitro*, neither reserpine nor serotonin releases platelet histamine *in vitro*.

Significance to the Program of the Institute: This project is designed to elucidate the factors involved in the binding and release of histamine from certain tissues

Proposed Course of Project: We plan to examine the possible reasons for the difference in behavior of platelet histamine seen *in vivo* and *in vitro*. We also plan to extend the study of histamine binding and release to a study of mast cell histamine.

Part B. Included: No

Serial No. NHI-172
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Sex Difference in the Metabolism of Histamine in the Rat

Principal Investigators: Dr. P. A. Shore
Dr. K. J. Netter (Visiting Scientist from Hamburg)

Other Investigators: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 1/4
Professional: 1/4
Other: None

Project Description:

Objectives: During the past few years it has been found that there exist at least two pathways of histamine metabolism. One of these is oxidative deamination of the histamine molecule by the action of diamine oxidase (probably identical with "histaminase"). The other, more recently discovered route is by N methylation of the iminazol ring of histamine by an enzyme, iminazol N methyl transferase. The relative importance of these pathways appears to be dependent upon species, sex, and histamine levels.

A marked sex difference in rats has been described. Male rats are reported to metabolize histamine chiefly by N methylation while female rats appear to utilize chiefly diamine oxidase. We are attempting to discover the enzymatic basis for this sex difference.

Serial No. NHI-172

Major Findings: Although this project is a new one, it has already been found that no significant sex difference exists in the activity in vitro of diamine oxidase, N-methyl transferase, or methionine activating enzyme, the enzyme which makes S-adenosylmethionine needed in methylation.

Significance to the Program of the Institute: This project is designed to help elucidate physiological factors important in the metabolism of histamine.

Proposed Course of Project: Further investigations will be made to determine the basis of the reported sex difference. The possibility exists that the difference lies in a different rate of synthesis of histamine. Thus if females synthesize considerably more histamine than males, two pathways of metabolism might be necessary, while males utilize only one pathway (methylation).

Part B Included: No

Serial No. NHI-173

1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

PART A

Project Title: Studies in Biochemical Behavior
II. The Physiological and Biochemical Response of Rats to Reserpine as Mediated by the Pituitary

Principal Investigators: Dr. Erik Westermann
Mr. Roger P. Maickel

Other Investigators: None

Man Years: (Calendar Year 1959): Patient Days: None
Total: 0.6
Professional: 0.5
Other: 0.1

Project Description:

Objectives: Previous work has indicated that the pharmacological actions of reserpine are mediated through the release of biogenic amines in the nervous system. It has been suggested that the sedation produced by reserpine may be a result of the persistent release of serotonin in the brain. It has been reported that reserpine can inhibit the responsiveness of pituitary-adrenal axis to stressful stimuli. It is of interest, therefore, to study the possible interrelationship of the reserpine actions on the hypothalamus and the endocrine glands.

Major Findings: It was of considerable surprise to find that reserpine, an anti-stress compound, elicits a pituitary-adrenal response characterized by depletion of adrenal ascorbic acid an increased level of circulating adrenal cortico steroids. Liver tryptophane peroxidase is also increased. The response seems to require more than 50% depletion of brain serotonin and catecholamines. No effects were observed in hypophysectomized rats.

Serial No. MEI-173

Significance to the Program of the Institute: In connection with the current widespread use of reserpine in the treatment of hypertensive states and mental disorders, it is of obvious interest to examine the effects of this drug on endocrines and on biochemical behavior.

Proposed Course of Project:

1) To ascertain whether release of amines in brain is related to the release of pituitary hormones. Such a relationship might provide a lead on how the hypothalamus controls the pituitary output and therefore biochemical behavior. Since reserpine in large doses depletes amines, it may also deplete some pituitary hormones.

2) Studies on whether reserpine, despite its own effect on pituitary, can block the effects of "stress."

3) Effect of reserpine on posterior pituitary will be studied.

Part B included: No

Serial No. NHI-174
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NHI
Individual Project Report
Calendar Year 1959

PART A

Project Title: Studies in Biochemical Behavior
III. The Pituitary Response of Animals to Administration of Various Carcinogenic Compounds

Principal Investigators: Dr. W. Robert Jondorf
Mr. Roger P. Maickel

Other Investigators: None

Cooperating Unit: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 0.5
Professional: 0.4
Other: 0.1

Project Description:

Objectives: In mammals the stimulation of pituitary adrenal response by a variety of physical and chemical agents leads to certain predictable fluctuations in such measurable parameters as adrenal ascorbic acid level, plasma corticosterone level, plasma non-esterified fatty acid level, etc. It was found that 3-methylcholanthrene administration intraperitoneally, to rats, gave extraordinarily prolonged measurable responses up to 48 hours. Since 3-methylcholanthrene is a well-known carcinogen, it was of interest to study its mechanism; and in addition to see whether other carcinogens also induced unusually prolonged pituitary responses.

Major Findings: The typical symptoms of pituitary gland stimulation encountered in these experiments with 3-methylcholanthrene and other stressors were: 1) depletion of adrenal ascorbic acid; 2) increase of plasma corticosterone; 3) increase in plasma non-esterified fatty acid; 4) increase in activity of liver tryptophane peroxidase oxidase; 5) antagonism of reserpine sedation. The symptoms 2, 3 and 5 were still substantially in evidence 24 hours after administration of 3-methylcholanthrene and tended to return to normal only after 48 hours.

Studies with 3,4 benzpyrene suggested that this compound did not affect the variation of any of the parameters beyond 6 hours. 2-Aminofluorene, another carcinogenic agent, when administered to rats, did not differ very markedly from its non-carcinogenic isomer, 4-aminofluorene, in influencing pituitary output and the consequent variation in the measured parameters. The effects persisted for about 6 hours.

It seemed therefore that 3-methylcholanthrene was the exception rather than the rule, since maximal pituitary-adrenal response could be induced not only for exceptionally prolonged period of time, but with exceptionally small doses of material. In the comparative experiments chemical doses of 10 mg/rat (150 - 200 g wt) were administered but it was found that with 3-methylcholanthrene the pituitary response could be induced with as little as 1 γ/rat. With hypophysectomized rats none of the symptoms 1 - 5 could be induced with 3-methylcholanthrene.

Significance to the Program of the Institute: The pituitary gland has been called the leader of the endocrine orchestra. It coordinates biochemical activity in the body. 3-Methylcholanthrene has emerged as a tool for studying pituitary function with unusual precision and sensitivity.

Proposed Course of Project: Further studies with 3-methylcholanthrene at the enzymatic level are felt to be necessary, in attempts to correlate hormonal influences with enzyme activity.

Part B included: No

Serial No. MEI-175
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies in Biochemical Behavior
IV. The Effect of Various Pituitary Stimuli on Various Enzymes in the Body

Principal Investigator: Mr. Roger P. Maickel

Other Investigators: None

Cooperating Unit: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 0.2
Professional: 0.0
Other: 0.2

Project Description:

Objectives: Tryptophan peroxidase, an enzyme which converts tryptophan to formylkynurenine, is located in the soluble fraction of mammalian liver. It has been suggested that administration of substrate produces an increased enzyme level due to substrate induction. A study of the response of this enzyme to various stimuli was undertaken.

Methods Employed: Rats (normal, hypophysectomized, adrenalectomized) were exposed to various stimuli and the response of liver tryptophan peroxidase oxidase was determined by the standard in vitro assay.

Major Findings: The liver TPO activity can be increased b many stimuli. Exposure to cold, administration of intradermal formaldehyde, or of a wide variety of compounds such as reserpine, barbiturates, chlorpromazine, and Marsilid all resulted in stimulation of enzyme activity. None of these stimuli was effective in hypophysectomized animals, nor did intraperitoneal administration of large doses of tryptophan produce increased enzyme activity in animals without a pituitary. Administration of sub-narcotic doses of a series of barbiturates resulted in increased TPO activity with the amount of increase related to the

duration of time the barbiturate remained in the animal. Intraperitoneal administration of tryptophan at a dose of 500 mg/kg produced a significant stress response, as indicated by a decrease in adrenal ascorbic acid and an increase in plasma corticoids and NEFA

Significance to the Program of the Institute: This enzyme (TPO) is the first step in a comprehensive study of the relationships of biochemical mechanisms (as typified by enzymes) to the endocrine and neural systems responsible for their control. The possible ramifications of these relationships may lead to an understanding of differences between normal and pathologic tissues.

Proposed Course of Project: Other enzyme systems will be studied with respect to their response to stimulation of various endocrine systems. The relationship of the biochemical mechanisms of specific organs to the output of pituitary hormones will be investigated Finally, the possible role of the endocrine glands in various biochemical malfunctions will be explored.

Other enzyme systems involved in the metabolism of amino acids will be investigated. The serum enzymes which are increased in cardiac damage (acid and alkaline phosphatase, glutamic-pyruvic and glutaminc-oxalacetic transaminases, and lactic dehydrogenase) will be studied, since some of them have been reported to increase after cortisone treatment.

Part B included: No

duration of time the barbiturate remained in the animal. Intraperitoneal administration of tryptophan at a dose of 500 mg/kg produced a significant stress response, as indicated by a decrease in adrenal ascorbic acid and an increase in plasma corticoids and NEFA

Significance to the Program of the Institute: This enzyme (TPO) is the first step in a comprehensive study of the relationships of biochemical mechanisms (as typified by enzymes) to the endocrine and neural systems responsible for their control. The possible ramifications of these relationships may lead to an understanding of differences between normal and pathologic tissues.

Proposed Course of Project: Other enzyme systems will be studied with respect to their response to stimulation of various endocrine systems. The relationship of the biochemical mechanisms of specific organs to the output of pituitary hormones will be investigated Finally, the possible role of the endocrine glands in various biochemical malfunctions will be explored.

Other enzyme systems involved in the metabolism of amino acids will be investigated. The serum enzymes which are increased in cardiac damage (acid and alkaline phosphatase, glutamic-pyruvic and glutamine-oxalacetic transaminases, and lactic dehydrogenase) will be studied, since some of them have been reported to increase after cortisone treatment.

Part B included: No

Serial No. NHI- 176
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Induced Synthesis by Foreign Compounds of Liver Microsomal Enzymes.

Principal Investigators: Dr. Allan Conney
Dr. J. J. Burns

Other Investigators: Dr. I. A. Michaelson
Miss Ruth Gastel

Cooperating Units:

Man Years (calendar year 1959):
Total: .9
Professional: .4
Other: .5

Patient Days (Calendar year 1959): None

Project Description:

Objectives - To study the effects of polycyclic hydrocarbons and drugs on liver microsomal enzymes that metabolize drugs and certain normally occurring compounds.

Patient Material - None

Major Findings - Pretreatment of rats with several drugs such as phenobarbital, barbital, phenylbutazone, aminopyrine, or orphenadrine markedly increases the activity of liver microsomal enzymes that metabolize zoxazolamine, phenylbutazone, hexobarbital, aminopyrine and 3,4-benzpyrene. The increases in enzyme activity are paralleled by accelerated drug metabolism in the intact animal and by a shortened duration of drug action. For instance, the duration of hexobarbital narcosis and zoxazolamine paralysis was markedly shortened by pretreating rats with phenobarbital and other durgs that increased the activity of liver microsomal enzymes. The administration of certain drugs to rats increases the activity of the liver microsomes to metabolize the same or a closely related compound. Thus, the administration of phenylbutazone, aminopyrine, 3,4-benzpyrene or phenobarbital will respectively increase the ability of rat liver microsomes to metabolize phenylbutazone, aminopyrine, 3,4-benzpyrene or hexobarbital. The observation that pretreatment of animals with one barbiturate can speed up the metabolism and shorten the action of another barbiturate suggests that tolerance to this type of drug may result from an accelerated rate of metabolism of the barbiturate to pharmacologically inactive metabolites.

Serial No. NHI-176

Significance to the Program of the Institute - Studies on induced enzyme synthesis will give information on the general problem of factors which regulate the duration of drug action.

Proposed Course of Project - 1) To further study the stimulatory effect of hydrocarbons and drugs on various other drugs such as Miltown, Soma, Benadryl, Demerol, and salicylates. Parallel experiments will be carried out on the effect of drugs to shorten the duration of drug action.

2) To study the effect of drugs on the liver enzymes that metabolize various normally occurring compounds. Included among the enzymes that will be studied are glucose-6-phosphate dehydrogenase, Δ^4-3-ketosteroid reductase and L-gulonolactone oxidase.

3) To investigate the possibility that pretreatment of human subjects with drugs results in accelerated drug metabolism.

Part B included Yes

(Attachment I)

Serial No. NHI-176

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Conney, A.H. and Burns, J.J.: Stimulatory Effect of Foreign Compounds on Ascorbic Acid Biosynthesis and on Drug-Metabolizing Enzymes. Nature 184: 363-364, 1959.

Conney, A.H., Gillette, J.R., Inscoe, J.K., Trams, E.C. and Posner, H.S.: Induced Synthesis of Liver Microsomal Enzymes Which Metabolize Foreign Compounds. Science 130: 1478-1479, 1959.

Honors and Awards relating to this project:

None.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Effects of Thyroxin on Liver Microsomal Enzymes.

Principal Investigator: Dr. Allan Conney

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: .2
Professional: .2
Other: None

Patient Days (Calendar year 1959): None

Project Description:

Objectives - To study the effect of thyroxin on liver microsomal enzymes which metabolize drugs and certain normally occurring compounds.

Major Findings - Pretreatment of rats with thyroxin increases the activity of the liver microsomal enzyme system that metabolizes zoxazolamine. The increased enzyme activity is paralled in vivo by a shortened duration of zoxazolamine paralysis. In contrast to this, pretreatment of rats with thyroxin does not shorten the duration of hexobarbital narcosis.

Significance to the Program of the Institute - Studies on the effects of thyroxin on drug-metabolizing enzymes will provide information concerning factors that regulate the duration of drug.

Proposed Course of Project - 1) To further investigate the action of thyroxin and other hormones on liver microsomal enzymes.

2) To further study the shortened duration of drug action in animals that have been pretreated with thyroxin.

3) To compare the rate of drug metabolism in hyperthyroid, hypothyroid, and normal subjects.

Part B included No

Serial No. BMI-176
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Glucuronic Acid Pathway.

Principal Investigators: J. J. Burns
Allan H. Conney

Other Investigators: Carole Evans
Natalie Trousof
Anne Murray

Cooperating Units: New York University Research Service, Goldwater Memorial Hospital, New York.

Man Years (calendar year 1959):
Total: .16
Professional: .4
Other: .12

Patient Days (calendar year 1959): None.

Project Description:

Objectives - 1) To characterize the various reactions involved in the glucuronic acid pathway.

2) To study the effect of drugs on the glucuronic acid pathway.

3) To study the role of the glucuronic acid pathway in the metabolism of galactose.

Patient Material - None.

Major Findings - 1) Largely as a result of our studies on the biosynthesis of L-ascorbic acid, a new pathway of glucose metabolism has been uncovered which is shown on the attached chart. This pathway is of importance not only for the synthesis of L-ascorbic acid but it accounts for the origin of L-xylulose, the sugar excreted by patients with essential pentosurea. We have found that a variety of foreign compounds possessing complete unrelated chemical and pharmacological properties stimulate the metabolism of glucose through this pathway. These drugs include the hypnotics, Chloretone and barbital; the analgesics, aminopyrine and antipyrine; the muscle relaxants, orphenadrine and meprobamate; the antirheumatic agent, phenylbutazone; the antihistamine drug, Benadryl and the carcinogenic hydrocarbons, 3-methylcholanthrene 3,4-benzpyrene and 1,2,5,6-dibenzanthracene. The enhanced formation

of L-ascorbic acid through the glucuronic acid pathway may represent an adaptive response on the part of the body to foreign compounds. In this connection reports have recently appeared from Japan indicating that the administration of D-glucuronic acid reduces the toxicity of various foreign compounds in animals by an unknown mechanism. It is of considerable interest that those drugs which are potent in stimulating the synthesis of L-ascorbic acid such as phenobarbital, barbital, aminopyrine and the previously mentioned carcinogenic hydrocarbons, also increase the activity of liver microsomal enzymes which metabolize various foreign compounds (see report on Studies on the Induced Synthesis by Foreign Compounds of Liver Microsomal Enzymes). These results suggest a relationship between the stimulatory effect of foreign compounds on the glucuronic acid pathway and on the activity of drug metabolizing enzymes in liver microsomes.

2. Studies carried out during the past year have also shown the marked effect of various drugs in stimulating the metabolism of D-galactose through the glucuronic acid pathway. An effect of these drugs on galactose metabolism has also been demonstrated in vitro. Pretreatment of rats with drugs was observed to increase markedly the activity of the enzymes in the liver which convert D-galactose to D-glucuronic acid.

Significance of the Program to the Institute - Studies on the effects of drugs on the glucuronic acid pathway may give important information for carbohydrate metabolism in general and may point out new "detoxification" mechanisms in the body.

Proposed Course of Project - 1) Further studies will be carried out to determine the mechanisms by which drugs stimulate the metabolism of glucose or galactose through the glucuronic acid pathway. In particular, an attempt will be made to determine which enzymatic activities are increased in response to drugs. Studies will also be carried out to determine the structural features required for a drug to exert this affect on carbohydrate metabolism.

2) The possible physiological significance of this drug effect on the glucuronic acid pathway will be investigated. Experiments will be carried out to determine whether the administration of D-glucuronolactone and L-gulonolactone can reduce certain toxicity of drugs since these carbohydrates have been reported by others to have a protecting effect against certain drug toxicities.

3) Further experiments will be carried out to investigate the possible relationship between the stimulatory effect of drugs on ascorbic acid biosynthesis and their ability to increase the activity of drug metabolizing enzymes in liver microsomes.

4) Studies will be carried out on the development of enzymes of the glucuronic acid pathway. The fetal activity of enzymes involved in biosynthesis of ascorbic acid and of microsomal enzymes which metabolize foreign compounds will be determined. The activity of these enzymes as well as the animals response to drugs (as reflected by ascorbic acid excretion or by enhanced enzyme activity) will be measured as a function of age from fetus to adult. These studies may give information on the mechanism of development of enzymes involved in the biosynthesis of ascorbic acid.

Part B included Yes

Individual Project Report

Part B Honors, Awards and Publications

Publications arising from the observations from this project:

Eisenberg, F., Jr., Dayton, P.G., and Plaga, J.J. Studies on the Glucuronic Acid Pathway of Glucose Metabolism. J. Biol. Chem. 234, 250-253, 1959.

Ashwell, G., Kanfer, J. and Burns, J.J. Studies on the Enzymes of L-Xylulose Formation by Rabbit Kidney. J. Biol. Chem. 234, 472-475, 1959.

Burns, J.J., Kanfer, J. and Ashwell, G. Formation of D-Xylulose from L-Gulonic Acid in Rat Kidney. Biochim. Biophys. Acta 34, 468-469, 1959.

Papadopoulos, N.M. Incorporation of Glucuronolactone-C14 into Connective Tissue Polysaccharides. Proc. Soc. Exper. Biol. and Med. 100: 338-339, 1959.

Burns, J.J., Conney, A.H., Dayton, P.G., Evans, G., Martin, D.C. and Taller, D. Stimulatory Effect of Drugs in Rats on Ascorbic Acid Biosynthesis via the Glucuronic Acid Pathway. J. Pharm. Exptl. Therap. In press.

Honors and Awards relating to this project:

None

[illegible]

[illegible]
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Triglyceride Mobilization and Deposition

Principal Investigator: Dr. [illegible]

Other Investigators: Dr. William [illegible] Butler, Jr.

Cooperating Units: Dr. Marjorie [illegible], [illegible], NHI
Dr. Benjamin Highman, NIAMD

Man Years (calendar year 1959): Patient Days: None
Total: 1.3
Professional: 0.3
Other: 1.0

Project Description

Objectives: To study the mechanisms involved in the mobilization and deposition of neutral fat. To determine the role of epinephrine and norepinephrine in triglyceride mobilization and deposition.

Methods Employed: The triglyceride content of tissues is measured by a modification of the direct method of van Handel and Zilversmit (J. Lab. and Clin. Med., 50:152, 1957).

Major Findings: The triglyceride content of dog heart was elevated the day after an infusion of a large dose (0.85 mg/kg) of norepinephrine. This triglyceride deposition was prevented by the adrenergic blocking agent, phenoxybenzamine, [illegible] 1 hour before the infusion.

Triglyceride deposition in rat liver has been produced by carbon tetrachloride subcutaneously or orally, ethanol orally, [illegible] parenterally, and exposure to cold. [illegible] triglyceride deposition in the liver was partially prevented by the adrenergic blocking agents, phenoxybenzamine (Dibenzyline), [illegible] and [illegible]. We are now determining the [illegible] relationships between the degree of blocking of triglyceride deposition, the dose of adrenergic blocking agent, and the [illegible] between the administration of the adrenergic blocking agent and the [illegible] of the [illegible] which [illegible] triglyceride [illegible]. [illegible] of adrenergic blocking agents [illegible]. [illegible] sections of the liver [illegible] Dr. [illegible] and Dr. [illegible] [illegible] [illegible].

Ethanol did not induce triglyceride deposition in hypophysectomized rats. Triglyceride deposition in the liver after carbon tetrachloride and ethionine was less in hypophysectomized rats than in control rats.

In collaboration with Dr. Marjorie Horning, triglyceride deposition in the liver was induced by ethionine, ethanol, and carbon tetrachloride in rats after the incorporation of C^{14}-acetate into the triglycerides of liver and adipose tissue. The chemicals produced a marked increase in the total counts per minute of the liver triglyceride; this is most easily explained in terms of mobilization of labelled fat from adipose tissue. The experiments also indicate that ethanol stimulates the synthesis of triglyceride, thus causing an increase in the specific activity of adipose tissue and liver triglycerides.

Significance to the Program of the Institute: This project should increase our understanding of the processes involved in triglyceride mobilization and deposition. We may obtain some clues to the nature of hepatic cirrhosis and atherosclerosis.

Proposed Course of Project: We hope to clarify the role of the pituitary and the sympathetic nervous system in the mobilization and deposition of triglyceride.

Part B included: No

Serial No. NHI-180
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies in Biochemical Evolution. The Ontogenetic Development of Drug Enzymes.

Principal Investigators: Mr. Roger P. Maickel
Dr. W. Robert Jondorf

Other Investigators: None

Cooperating Unit: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: Studies with the tadpoles of terrestrial amphibia such as toads and some frogs and the juvenile aquatic stage of terrestrial salamanders showed that these immature animals lacked the liver enzymes for metabolizing foreign compounds. In contrast to this, the adult of the species did have the enzymes. These results suggested that a parallel course of "appearance" of the enzymes might be found in the development from aquatic to terrestrial life which occurs in the transition from fetal to postnatal life in mammals. Preliminary results in guinea pigs and mice indicate that the microsomal drug enzymes responsible for N-dealkylation, O-delakylation, sidechain oxidation, and glucuronide formation are not present in the fetus, but rather develop at about 4 to 10 days after birth. This is in agreement with the concept "ontogeny recapitulates phylogeny" since the fetal animal in its aquatic environment has no need for duplication of the enzymatic mechanisms available to it in the maternal livers. Transfer of lipid soluble substances across the placental membrane may thus be likened to similar transfer across the lipid membrane of the fish gill.

Serial No. MHI-180

Major Findings: Studies with chicken embryos show that these forms possess drug metabolizing enzymes in liver microsomes, which require TPNH and oxygen.

Relative Activity of Liver Microsomes

	Dealkylation of			
Age (days)	MMAP	N-Me-Aniline	Phenacetin	TPNH Oxidase
- 5	0.39	0.33	0.44	0.39
HATCHING				
+ 1	1.00	1.00	1.00	1.00
+ 7	0.88	0.52	0.62	0.34
+ 21	0.83	0.76	0.89	0.57

The possession of these enzymes by the chicken embryo may be related to its isolated milieu. It does not live in an environment similar to the tadpole or the mammalian fetus.

Significance to the Program of the Institute: These studies are important in understanding the development of enzymes phylogenetically and embryologically.

Proposed Course of Project: Studies to ascertain the part played by hormones in the development of the drug enzymes will be undertaken.

Part B included: No

Serial No. NHI-181
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies with Anticoagulants

Principal Investigator: Dr. Peter G. Dayton

Other Investigators: None

Cooperating Units: Drs. Murray Weiner and Theodore Chenkin, New York University Research Service, Goldwater Memorial Hospital, New York

Man Years (calendar year 1959):
Total: .25
Professional: .25
Other: None

Patient Days (calendar year 1959): None

Project Description:

Objectives - To study factors which affect the response to coumarin anticoagulants.

Methods Employed - Besides the usual coagulation tests, plasmas will be studied in the thromboelastograph (coagulograph) which measures clot firmness.

Patient Material - None

Major Findings - Barbiturates cause a decrease in coumarin anticoagulant levels in man, along with decreasing the hypoprothrombinemic activity of the anticoagulant. Starvation produces release of a heparinoid substance in guinea pigs.

Significance to the Program of the Institute - Wide variations in the clinical response to coumarin anticoagulants are a disturbing problem in the therapy of cardiovascular disease. Information obtained from this study may aid in the understanding of the mode of action of coumarin anticoagulants.

Proposed Course of Project - The observation that barbiturates antagonize the hypoprothrombinemic action of orally administered coumarin anticoagulants in man has been correlated with lower plasma levels of the anticoagulant. Further, when guinea pigs are pretreated with large doses of parenterally administered barbital, the hypoprothrombinemic effect of parenterally administered coumarin anticoagulant is completely antagonized. It is hoped that it will be possible to determine whether the effects observed in guinea pigs and man are acting through the same or different mechanisms

Part B included Yes

(Attachment 1)

Serial No. NHI- 181

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Chenkin, T., Dayton, P.G., Weisberg, L. and Weiner, M.: Effect of Starvation, Acenocoumarin and Vitamin K on the Coagulation Pattern of the Guinea Pig. Exper. Med. and Surgery 17: 219-224, 1959.

Honors and Awards relating to this project:

None

Serial No. NHI-182
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Lack of Blood-Brain Barrier in Certain Parts of the Brain

Principal Investigators: Dr. Elwood Titus
Dr. Cedric W.M. Wilson

Other Investigators: None

Cooperating Units: Dr. Wilson was a Eli Lilly Fellow

Man Years (calendar year 1959): Patient Days: None
Total: 0.7
Professional: 0.7
Other: 0.0

Project Description:

Objectives: To study the kinetics of entry of substances into those parts of brain reported to have a high affinity for administered dyes.

Methods Employed: Established chemical and dissection methods.

Major Findings: N-acetyl-4-aminoantipyrine (NAAP) and sulfoguanidine enter the pituitary, the intercolumar tubercle and the area postrema at about the same rates as they enter liver and muscle, and much more rapidly then they do other parts of the brain. NAAP enters both lobes of the pituitary at about the same rate.

Preliminary results indicate that radioactive sodium and radioactive epinephrine also enter the pituitary much more rapidly than the rest of the brain.

Significance to the Program of the Institute: May lead to a better understanding of interrelationship of brain and peripheral organs.

Proposed Course of Project: Studies will attempt to ascertain whether drugs can affect the brain (e.g., through the pituitary to produce "stress") without having to cross the blood-brain barrier.

Part B included: No

Serial No. NHI-183
1. Chemical Pharmacology
2. Enzyme Drug Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Mechanism of TPNH-dependent Enzyme Systems in Liver Microsomes

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. Jerome J. Kamm

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.9
Professional: 0.2
Other: 0.7

Project Description:

Objectives: To elucidate the mechanism of the microsomal enzyme systems requiring TPNH. Many drugs and other foreign compounds are oxidized by microsomal enzyme systems that require TPNH and oxygen. In addition, a number of normally occurring compounds, such as steroid hormones and cholesterol, are also formed by enzyme systems which require TPNH (or DPNH) and oxygen. The similarity in the requirements of these enzyme systems suggests their mechanisms may be similar. It is therefore extremely important to learn the mechanism of this type of oxidative process, first shown in this laboratory in studies of drug metabolism.

Methods Employed: Established Methods.

Major Findings: In previous work it was shown that TPNH is oxidized by liver microsomes to yield hydrogen peroxide even in the absence of a drug substrate. Although 4,4'-Diaminodiphenyl sulfide, does not affect the rate of oxidation of TPNH, but it causes a decrease in the formation of hydrogen peroxide equivalent to the amount of 4,4'-diaminodiphenyl sulfoxide formed. p-Ethoxyacetanilide similarly causes a decrease in formation of hydrogen peroxide equivalent to the formation of its metabolite, p-hydroxyacetanilide. These results are consistent with the following view. A "hydroxyl donor" is formed in microsomes from TPNH and oxygen. In absence of a drug substrate, a part of the "hydroxyl donor" participates in hydroxylation reactions that form normally occurring substances like lanosterol and cholesterol, and the rest of it breaks down to hydrogen peroxide. In the presence of a drug substrate, however, a part of the "hydroxyl donor" is used by a number of non-specific 'drug enzymes" to hydroxylate the foreign compounds. The concept may be formulated as follows:

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1. TPN + O_2 + "factor" ⟶ oxygen-factor complex ("hydroxyl donor") + TPN

In absence of drug.

2a. "Oxygen-factor complex + normal substrates ⟶ hydroxylation of normal substrates + "factor"

2b. "Oxygen-factor complex" ⟶ H_2O_2 + "factor"

In presence of drug

2c. "Oxygen-factor complex" + drug ⟶ oxidized drug + "factor"

Significance to the Program of the Institute: These studies provide a better understanding of the metabolism of many drugs.

Proposed Course of Project: Studies of the effects of other drug substrates on hydrogen peroxide formation will be undertaken. Studies will also be made to determine whether microsomal TPNH oxidase is a component of the drug enzyme systems.

Part B included: Yes

Publications:

Brodie, B.B., Gillette, J.R. and La Du, B.N.: Enzymatic metabolism of drugs and other foreign compounds. Annual Review of Biochemistry 27, 427, 1958.

Honors and Awards: None

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Metabolism of Sulfur Compounds

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. Jerome J. Kamm

Cooperating Unit: None

Man Years (calendar year 1959):

Total .4
Professional: .1
Other: .3

Patient Days (calendar year 1959):
None

Project Description:

Objectives: To study the metabolism of sulfur compounds.

Methods Employed: Previously described methods for 4,4'-diamino diphenyl sulfide and 4,4'-diaminodiphenyl sulfoxide.

Major Findings: TPNH-dependent enzyme systems in the liver microsomes of guinea pig (NIH strain) catalyze the oxidation of chlorpromazine and 4,4'diaminodiphenyl sulfide (DDS) to sulfoxides. Sulfoxidation accounts for about 90% of the DDS metabolism, but only about 50% of the chlorpromazin metabolism. Liver microsomes from the Hartley strain metabolize chlorpromaz at about 25% of the chlorpromazine metabolism by preparations from Hartley can be accounted for by sulfoxidation. These results indicate that chlorpromazine is metabolized not only by sulfoxidase but also by other enzyme system in liver microsomes, and that the relative activities of the enzyme systems metabolizing chlorpromazine differ from one strain to another.

Significance to the Program of the Institute: These studies provide a better understanding of the enzymatic metabolism of sulfur compounds

Proposed Course of Project: Studies on sulfur metabolism will be continued as a part of the project entitled "Mechanism of the TPNH-dependent Enzyme Systems in Liver Microsomes".

Part B included: No

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Enzymatic Oxidation of Nicotine

Principal Investigators: Dr. Howard B. Hucker
Dr. James R. Gillette

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1
Professional: 1
Other: 0

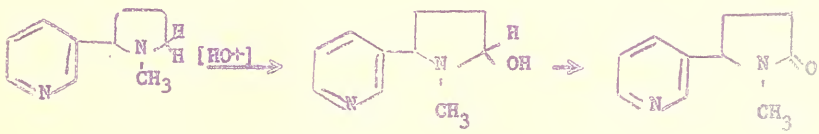
Project Description:

Objectives: To study the enzymatic systems(s) by which nicotine is metabolized in animal organisms.

Methods Employed: A differential solvent extraction method for the simultaneous determination of microgram quantities of nicotine and cotinine.

Major Findings: A major product of nicotine oxidation in liver microsomes was previously reported to be cotinine. It seemed probable that the reaction proceeds in two steps; the first is the hydroxylation of nicotine to a cyclized amino aldehyde (hydroxynicotine), and the second the oxidation (presumably by a dehydrogenase similar to aldehyde oxidase) to cotinine. After the addition of cyanide (to inhibit the dehydrogenase) an intermediate was isolated which appears to be identical with synthetically prepared hydroxynicotine.

Accordingly, the metabolism of nicotine is tentatively formulated as follows:



Nicotine Hydroxynicotine Cotinine

It is probable that hydroxynicotine is the precursor of many other urinary metabolites of nicotine and therefore is the key intermediate in the metabolism of this alkaloid. From the theoretical point of view, this compound is important because it supports the concept that the first step in oxidative dealkylation may be hydroxylation of an N-alkyl group.

Significance to the Program of the Institute: The present study is contributing specifically to an understanding of the metabolism of nicotine, a drug of considerable pharmacological importance. It is also of general interest since the metabolic pathway elucidated here very likely is important for the metabolism of various heterocyclic compounds of which many are useful drugs.

Proposed Course of Project: Further studies are planned on the hydroxy precursor of cotinine. Purification of this intermediate will be attempted in order to study its conversion to cotinine in more detail Development of a method for the acid derived from cotinine will also be undertaken so that formation of the acid can be measured. In addition, other heterocyclic compounds will be examined to determine whether they are metabolized by the same general pathway. Studies of other heterocyclic compounds may lead to an understanding of how heterocyclic ring systems aresplit in the body.

Part B included: yes

Serial No. NHI-185

Publications:

Hucker, H.B., Gillette, J.R., and Brodie, B.B.: Cotinine: An oxidation product of nicotine formed by rabbit liver. Nature, 183, 47 (1959).

Hucker, H.B., Gillette, J.R., and Brodie, B.B.: Enzymatic pathway for the formation of cotinine, a major metabolite of nicotine in rabbit liver. J. Pharmacol. Exp. Therap., submitted for publication.

Honors and Awards: None

Serial No. MHI-186
1. Chemical Pharmacology
2. Enzyme Drug Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Metabolism of Alcohols and Aldehydes

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mrs. Agnes Gaudette

Cooperating Units: Mrs. Gaudette is working under a fellowship from J.E. Seagrams and Sons

Man Years (calendar year 1959): Patient Days: None
Total: 1.2
Professional: 0.2
Other: 1.0

Project Description:

Objectives: 1.Duration of action of most drugs is dependent on their rate of metabolism. Accordingly, any variation in enzymatic activity among individuals will result in a variation in the time that a given dose of a drug will be active. By determining the rate of metabolism of alcohol in a large number of individuals, we hope to obtain some idea of the extent of variation in humans to metabolize drugs.

2.Drugs such as imipramine (Tofranil) have a pronounced effect on the action of alcohol in mice. It is of considerable importance to determine whether the effects are due to potentiation of the action of alcohol on the active sites in the central nervous system or to a decrease in the activity of alcohol dehydrogenase.

3.The normal function of alcohol dehydrogenase is not clear. It seems unlikely that sole normal function of this enzyme is to oxidize vitamin A alcohol to vitamin A aldehyde, the compound found by Wald to be important in the formation of rhodopsin. It is hoped that by studying alcohol dehydrogenase in animals of various phyla, we might learn of other possible normal functions of this enzyme.

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Methods Used: A method for measuring small concentrations (5.0 γ/ml) of alcohol in the blood has been developed. Alcohol in 0.01-0.5 ml of blood is isolated by a diffusion technique and is oxidized by yeast alcohol dehydrogenase. The DPNH formed during the enzymatic reaction is measured spectrophotometrically at 340 mμ.

Major Findings: The rates of metabolism of alcohol in four humans were studied after giving orally small doses (200-500 mg/kg). In two subjects the rates approached zero during the entire course of the reaction; in two other subjects the rates were zero order at the higher blood levels but changed to first order at concentration in blood of about 100 γ/ml. The data, however, were not precise enough to relate these observations to changes in Vmax or Km.

In preliminary studies, it was observed that imipramine (Tofranil), stimulates the rate of alcohol metabolism in mice. This was not expected since imipramine prolongs the sleeping time of mice given alcohol. It is concluded, therefore, that imipramine acts as a true potentiator of alcohol rather than as a prolonging agent.

Significance to the Program of the Institute: This study should provide better insight into mechanism of action of a number of drugs. Moreover, studying the rate of metabolism of alcohol in a large number of humans should give us an idea of the extent of variation in man's ability to metabolize drugs.

Proposed Course of Project: Since the calculation of Vmax and Km for alcohol dehydrogenase in vivo requires unusually precise data, the method for the estimation of alcohol in the blood must be improved. The effects of imipramine and other drugs on alcohol metabolism in vivo will also be studied.

The metabolism of a number of alcohols by alcohol dehydrogenase from animals of various phyla and the effect of inhibitors on this enzyme will be studied. From these data, we should learn whether the alcohol dehydrogenase of the lower animals is identical with that of mammals.

Part B included: No

Serial No. NHY-187
1. Chemical Pharmacology
2. Enzyme Drug Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Model Enzyme Systems in the Study of Drug Metabolism

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. James V. Dingell

Cooperating Unit: Lab. of Physical Biology, Dr. Edwin Becker

Man Years (calendar year 1959): Patient Days: None
Total 0.3
Professional: .1
Other: .2

Project Description:

Objectives: A number of foreign compounds are oxidized by enzyme systems localized in the microsomal fraction of mammalian liver. The mechanism of these reactions, however, is not clearly understood. The present studies were undertaken to determine possible mechanisms for the microsomal reactions.

Major Findings: Ceric sulfate oxidizes an equivalent amount of chlorpromazine to a red intermediate that decomposes to chlorpromazine sulfoxide. Although Dusinsky and Liskova have suggested that the red intermediate is a free radical, our E.M.R* studies indicate that the concentration of the free radical can account for only 1-10% of the red intermediate. Moreover, titration of chlorpromazine with ceric sulfate indicates that the reaction takes place by a two electron transfer rather than one. It was also found that the intermediate decomposes by a second order reaction, and that the rate of decomposition is decreased by a large excess of chlorpromazine. The mechanism of the oxidation of chlorpromazine by ceric sulfate is therefore not clear.

Significance to the Program of the Institute: Studies with model systems may provide an insight into possible mechanisms of drug metabolism.

Proposed Course of Project: Studies to determine the mechanism of the ceric sulfate system will be continued.

Part B included: No

* Electron Magnetic Resonance

Serial No. NHI-188
1. Chemical Pharmacology
2. Enzyme Drug Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Physiological Distribution and Metabolism of Imipramine (Tofranil), a Drug used in Treatment of Psychiatric Depression.

Principal Investigator: Dr. James R. Gillette

Other Investigators: Dr. Gertrude P. Quinn
Mr. James V. Dingell

Man Years (calendar year 1959): Patient Days: None
Total: 0.85
Professional: 0.35
Other: 0.5

Project Description:

Objectives: To study the plasma disappearance, tissue distribution, urinary metabolites and enzymes catalyzing the metabolism of imipramine.

Methods Employed: A sensitive method for the assay of imipramine has been developed. Imipramine is extracted from alkalinized aqueous solutions into heptane and returned to an aqueous phase of diluted HCl. An aliquot of the acid phase is made alkaline and the fluoresence activated at 290 mμ is measured at 410 mμ. Fluorescence is proportional to imipramine in concentrations of 0.4 to 10 γ/ml.

Major Findings: Fifteen minutes after 20 mg/kg of imipramine was administered intravenously into rabbits, the concentration in plasma was less than 1 γ/ml. During this time, the drug accumulated in tissues, especially in lung, kidney and spleen. Since only trace amounts were found in tissues after 24 hours, imipramine is rapidly metabolized.

Significance to the Program of the Institute: These studies should provide a better understanding of the mechanism of action of imipramine.

Proposed Course of Project: The major metabolites of imipramine will be isolated from urine and identified. The metabolism of the drug in tissue preparations will also be studied.

Part B included: No

Serial No. MHI-189
1. Chemical Pharmacology
2. Enzyme Drug Interaction
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Metabolism of 1-phenyl-2-hydrazinopropane (JB 516)

Principal Investigator: Dr. Howard B. Hucker

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.3
Professional: 0.3
Other: 0

Project Description:

Objectives: To study the physiological disposition of JB 516.

Methods Employed: A solvent extraction method for the determination of microgram quantities of JB 516.

Major Findings: JB 516 was shown to disappear from the plasma very rapidly after intravenous administration.

Significance to the Program of the Institute: The present study is contributing to the better understanding of the physiological disposition of JB 516, a drug currently of much clinical interest.

Proposed Course of Project: A method that will permit study of the distribution of JB 516 in the body is being developed.

Part B included: No

Serial No. NHI-190
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Metabolism of 6-Chloropurine (6-ClP)

Principal Investigators: Dr. Daniel Duggan
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.10
Professional: 1.10
Other: None

Patient Days (calendar year 1959): None

Project Description:

Objectives - The ultimate objective of this investigation is to elucidate the biochemical mechanisms by which 6-chloropurine, and possibly other related purine antimetabolites, effect specific inhibitions of anabolic processes. To this end, studies of the metabolism of 6-ClP have been extended to isolated tissue systems and intracellular fractions, and investigations of the metabolic effects of 6-ClP on various synthetic systems in vivo and in vitro have been undertaken.

Methods Employed - a) Catabolism of 6-chloropurine: All assays of starting drug and its metabolites have been accomplished by conventional radioautography of urine and tissue fractions following injections of 6-chloropurine-8-C^{14} of high specific activity. Metabolites were characterized by radiochromatography in admixture with authentic reference compounds prepared enzymically from hypoxanthine-8-C^{14} or 6-chloropurine-8-C^{14}.

b) Metabolic effects of 6-chloropurine: Available techniques were employed for the isolation and estimation of nucleic acid fractions (Tyner and Heidelberger 1952; Schmidt and Thannhauser 1945), total protein (Feigelson, 1959) and total lipid (Folsch, 1957); the effects of 6-ClP upon these three anabolic systems were studied by following the respective rates of incorporation of phosphorus-32, glycine-1-C^{14} and acetate-1-C^{14} by liquid-scintillation, and gas-flow counting techniques.

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Patient Materials: None

Major Findings: a) Metabolic fate of 6-ClP-C^{14}: In the whole rat, in liver slices, and in isolated nucleic, 6-ClP serves as a moderately effective precursor of the adenine and guanine of both RNA and DNA. Preliminary results suggesting a direct incorporation of 6-ClP as such into RNA have been shown to be an artifact, resulting from the acid hydrolysis of adenine during chromatography of the purine bases.

In the rat, isotope administered as 6-ClP-8-C^{14} is excreted in the urine only as starting drug and its oxidation product, 6-chlorouric acid, and as the normal end products of purine catabolism, allantoin, uric acid and urea. Quantitation of these components suggests an hydrolysis of the 6-chloro substituent in vivo to the extent of fifty per cent.

b) The metabolic effects of 6-chloropurine: The respective in vitro inhibitions of xanthine oxidase and uricase by 6-ClP and its oxidation product are operative to only a minor degree in the whole rat, as evidenced by only a slight accumulation of hypoxanthine and uric acid label in urine following administration of hypoxanthine-8-C^{14} and therapeutic dosages of 6-ClP.

The turnover of both RNA and DNA, as followed by the incorporation of inorganic Phosphorus-32, is inhibited by 6-ClP in vivo, and to a lesser degree, in liver slices and in isolated liver nuclei. Similar inhibition studies using carbon-14 labeled nucleic acid precursors indicated a greater degree of inhibition by 6-ClP, but these data apparently only reflect a dilution of labeled intermediate by the hypoxanthine formed in vivo by the hydrolysis of 6-ClP.

The incorporation of glycine-1-C^{14} into the total protein fraction of rat liver in vivo and in liver slices is inhibited by 6-ClP in dosages of 100 mg/kg.

The inhibition by 6-ClP of the incorporation of acetate-1-C^{14} label into lipid fractions of rat liver was found to be within the wide limits of variability of control animals, so these experiments were abandoned. In liver slices, a slight degree of inhibition (30-40%) is effected by 6-ClP at concentrations of 200 µg/ml of medium

Proposed Course of Project: Various aspects of the "fate" and inhibition studies described above will be repeated in more rapidly proliferating systems (microorganisms, ascites cells, solid animal tumors) to detect possible qualitative differences in the metabolism of 6-ClP, and quantitative differences in the metabolic effects of the drug. Should any such differences be evident, the same techniques will be applied to resistant strains.

Part B included Yes

(Attachment I)

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Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Duggan, D.E. and Titus, E.: 6-Chloropurine and 6-Chlorouric Acid as Substrates and Inhibitors of Purine-Oxidizing Enzymes. J. Biol. Chem. 234: 2100-2104, 1959.

In preparation:

Duggan, D.E. and Titus, E.: The Fate of 6-Chloropurine-8-C^{14} in the Rat. for J. Pharm. and Exptl. Thera.

Honors and Awards relating to this project: None.

Serial No. NHI-191
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Secretion of Substances into Bile

Principle Investigator: Dr. Lewis S. Schanker

Other Investigator: None

Cooperating Units: In collaboration with Dr. C. Adrian M. Hogben, Department of Physiology, The George Washington University School of Medicine, Washington, D.C.

Man Years (calendar year 1959): Patient Days: None
Total: 1/12
Professional: 1/12
Other: None

Project Description:

Objectives: To describe the means by which substances pass from the bloodstream into the bile.

Methods Employed: Anesthetized rats with ligated renal pedicles and cannulated bile ducts received an intravenous injection of a radioactive-labeled substance. Bile, plasma, liver and muscle were assayed for the isotope after various times.

Major Findings: Previous work on this project indicated that 3 lipid-insoluble substances enter the bile at rates roughly related to their molecular size. Thus, the steady state bile/plasma ratios are: inulin, 0.09; sucrose, 0.21; and mannitol, 1.16. The 3 compounds are distributed in the extracellular space of skeletal muscle (11 to 13% of the wet weight), but they appear to penetrate liver cells to varying degrees. For example, inulin and sucrose have liver spaces of about 24% and mannitol, which has a space of 72%, is apparently distributed in the total water of the liver.

The present report describes further studies on the hepatic distribution of mannitol, and determinations of the albumen space of liver.

D-mannitol-1, 6-C^{14} was administered intravenously to rats with ligated renal pedicles and cannulated bile ducts. The animals were killed at various times, and the liver and muscle assayed for the isotope.

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The mannitol space of muscle was about 10% after 10 minutes; it rose to 12% after 1 hour and remained at this value for the next 4-1/2 hours. In liver, the mannitol spacerose from a value of 64% at 10 minutes to a value of 73% at 2 hours; the value remained constant for the next 3-1/2 hours. Respiratory CO_2, collected from these animals, contained less than 1% of the injected radioactivity after 5-1/2 hours. Paper chromatograms of the bile and plasma revealed a single radioactive spot with the same R_f as mannitol. These results strongly suggest that mannitol is not metabolized by the rat, and that the unchanged molecule readily penetrates into liver cells. Further evidence that hepatic cells are permeable to mannitol was supplied by the observation that liver slices swell when suspended in an isotonic solution of the substance. For example, the slices increase in weight by 20% after 10 minutes of incubation in this medium.

Evidence that sucrose and inulin are able to penetrate liver cells would be available if a molecule larger than inulin were found to have a liver space smaller than that of inulin. The liver space of I^{131}-labeled human serum albumen was found to be 16%, a value considerably lower than that observed with inulin or sucrose.

Significance to the Program of the Institute: This study may lead to a better understanding of the permeability of hepatic cells and the means by which drugs and metabolites are secreted into bile.

Proposed Course of Project: (1) Further investigations of the permeability of liver cells. (2) Investigations of the biliary secretion of drugs and metabolites.

Part B included: No

Serial No. MHI-192
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Transfer of Substances from Cerebrospinal Fluid into Blood

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. John J. Jeffrey

Cooperating Units: None

Man Years (calendar year, 1959): Patient Days: None
Total: 1/3
Professional: 1/6
Other: 1/6

Project Description:

Objectives: To determine the routes by which solutes and fluid leave the cerebrospinal fluid compartment. To study the formation and fate of cerebrospinal fluid.

Methods Employed: An 18-gauge spinal needle was inserted into the cisterna magna of anesthetized dogs. After the removal of 2 ml of cerebrospinal fluid (CSF), 1 ml of saline solution containing inulin-carboxyl-C^{14}, sucrose-C^{14} or phenol red was injected through the needle, and this was followed by 1 ml of the previously removed CSF.

The needle was then plugged and left in place for the duration of the experiment. Urine was collected from an indwelling catheter at hourly intervals and the bladder washed well with water after each urine collection to insure a quantitative recovery of the urine sample. The urine and washes were assayed for the injected substance and, after 6 to 9 hours, a sample of CSF was removed for assay.

Major Findings: Inulin, sucrose and phenol red appear in the urine in significant amounts within 1 hour after their intracisternal injection, and they continue to be excreted throughout the experimental period of 6 to 9 hours. Terminal CSF concentrations and urinary excretion data suggest that inulin leaves the CSF compartment at the slowest rate, sucrose at a more rapid rate, and phenol red at the most rapid rate.

Before definite conclusions can be made from these data, it must be proven that there is no leakage of CSF around the indwelling cisternal needle. However, the observation that 3 lipid-insoluble compounds leave the CSF compartment at significantly different rates strongly suggests a route of exit that is closed by a barrier quite different from the barrier which impedes the entry of foreign organic compounds into the central nervous system.

Significance to the Program of the Institute: Knowledge of the distribution and fate of drugs injected intracisternally will help to clarify a large amount of pharmacologic data previously obtained by injecting drugs at this site. An understanding of the formation and fate of CSF and its components would have many valuable applications in the fields of pharmacology and therapeutics.

Proposed Course of the Project: (1) Refine the techniques of the present experiments to prevent the possible exit of CSF from its compartment through an unnatural route. (2) Study the exit from CSF of other substances. (3) Obtain data which will permit estimation of the volume and rate of formation or turnover of CSF.

Part B included: No

Serial No. NHI-193
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Penetration of Drugs into Erythrocytes

Principal Investigator: Dr. Lewis S. Schanker

Other Investigators: Mr. Panayotis A. Nafpliotis
Mr. John J. Jeffrey

Cooperating Units: None

Man Years (calendar year, 1959): Patient Days: None
Total: 1-1/6
Professional: 1/3
Other: 5/6

Project Description:

Objectives: To study the permeability characteristics of cells and to determine which properties of drugs govern their entry into these cells.

Methods Employed: Erythrocytes obtained from citrated human blood, which was previously stored at 5° C for 3 weeks, were suspended in Tyrode solutions (pH 7.4) containing various drugs. Twenty ml of the suspension, which contained a packed cell volume of 1 ml, was shaken in an incubator at 37° C in an atmosphere of air. After various times, the cells were isolated by centrifugation, washed twice with Tyrode solution, and assayed for the drug.

Major Findings: Highly lipoid-soluble drugs like aniline, procaine amide and salicylic acid enter erythrocytes so rapidly that the rates cannot be measured with the present technique. Three amines with relatively low lipoid-solubilities, serotonin, epinephrine and norepinephrine, penetrate the cells at slower rates. The rates of entry of these compounds are related to their lipoid-solubilities; thus, serotonin has the highest fat-solubility and enters the cells most rapidly; norepinephrine has the lowest fat-solubility and enters most slowly.

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Quaternary ammonium ions like Darstine and procaine amide ethobromide penetrate the cells at very slow rates in accord with their low lipoid-solubilities. In contrast, preliminary results with another class of lipoid-insoluble ions, the sulfonic acids, indicate that these substances penetrate the cells many times faster than do the quaternary ammonium ions.

The results suggest that the boundary of the red blood cell is lipoid in character -- it is readily penetrated by lipoid-soluble drugs, but penetrated with difficulty by compounds with low lipoid-solubilities. Preliminary observations suggest that the erythrocyte membrane is more permeable to organic anions than to organic cations.

Significance to the Program of the Institute: Studies of the factors which govern the penetration of drugs into living cells should increase our understanding of cell membranes in general.

Proposed Course of the Project: (1) Investigate the penetration of erythrocytes by other organic compounds. (2) Investigate the intracellular binding of drugs and intracellular pH. (3) Investigate the mechanisms involved in the distribution of molecules and ions across the cell membrane.

Part B included: No

Serial No. NHI-194
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Absorption of Glucose from the Colon

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. Panayotis A. Nafpliotis

Cooperating Unit: None

Man Years (calendar year 1959): Patient Days: None
Total: 1/6
Professional: 1/12
Other: 1/12

Project Description:

Objectives: To describe the absorption of glucose and other sugars from the colon.

Methods Employed: Saline solutions (pH 7.2) containing glucose-C^{14} were passed through the colon of the anesthetized rat at a rate of 0.2 ml per minute. The degree of absorption was estimated from the decrease in concentration of the perfusion fluid after a single passage through the colon.

Major Findings: The rate of glucose absorption in the colon appears to be much slower than that in the small intestine. At a concentration of 0.028 mM, the lowest concentration studied, the proportion absorbed is 70%. As the concentration of glucose is raised, the per cent absorbed decreases according to kinetics of the Michaelis-Menten type, and at concentrations greater than 5 mM, the per cent absorbed is too small to measure (less than 2%).

The transport of glucose across the colonic epithlium is depressed by other sugars. For example, the absorption of glucose from a 0.055 mM solution was depressed from a value of 19% to a value of 7% in the presence of 5 mM D-galactose. The per cent depression of glucose transport by 5 mmol per liter of various sugars was: D-galactose, 63%; D-mannose, 63%; L-sorbose, 32%; and D-fructose, 5%.

The results suggest that glucose is slowly absorbed from the colon by a specialized transport mechanism which can be saturated and which is competed for by certain other sugars.

Serial No. NHI-194

Significance to the Program of the Institute: Knowledge of the means by which substrates required by the cell cross cell boundaries should increase our understanding of cellular functions.

Proposed Course of Project: Determine whether glucose is transported across the colonic epithelium against a concentration gradient.

Part B included: No

Serial No. NHI-195
1. Chemical Pharmacology
2. Cellular Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Transport of Pyrimidines and Purines Across the Intestinal Epithelium

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. Dominick J. Tocco

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1-1/3
Professional: 1/3
Other: 1

Project Description:

Objectives: To describe the mechanisms by which naturally occurring pyrimidines and purines and structurally related compounds penetrate cell membranes.

Methods Employed: Intestinal absorption was investigated in the anesthetized rat. A saline solution (pH 7.2), containing a pyrimidine or purine, was continuously circulated through the small intestine, and the decrease in concentration measured after 1 hour. The transfer of these substances across the intestinal epithelium was also investigated in vitro using everted sacs of small intestine.

Major Findings: Previous work on this project indicated that the pyrimidine thymine is absorbed from the small intestine by two mechanisms: (1) passive diffusion; and (2) a specialized transport process. The specialized transport process appeared to be involved also in the absorption of uracil, since this pyrimidine competitively inhibits the transport of thymine.

The present report describes the intestinal absorption of uracil, the transport of uracil and thymine across the intestinal wall in vitro, and the effect of various substances on the transport of these pyrimidines.

Like thymine, uracil is absorbed by a combination of passive diffusion and specialized transport. The transport process, evident at low concentrations of the pyrimidine, becomes saturated as the concentration is raised; the kinetics of the process are of the Michaelis-Menten type. The rate of specialized transport of uracil is identical with that of thymine, suggesting that the two pyrimidines have the same affinity for the transport system. The passive absorption of uracil, evident at high concentrations, follows Fick's law of diffusion. The observation that the rate of passive transfer of uracil is slower than that of thymine is explained by the lipoid character of the intestinal epithelium, since uracil has a lipoid-solubility lower than that of thymine.

The specialized transport of thymine is depressed by the purine hypoxanthine, the pyrimidines uracil and cytosine, and the foreign compounds 6-azathymine and 6-azauracil, suggesting that the transport mechanism may be involved in the absorption of many purines, pyrimidines and related structures. The failure of D-glucose or L-histidine to depress the transport of thymine indicates that the transport process is different from those which transport a number of sugars and amino acids.

When solutions of uracil or thymine (0.02 mM) are placed on either side of the intestinal wall in vitro, the pyrimidine is transported, from the mucosal to the serosal side, against a concentration gradient. Serosal/mucosal concentration ratios of about 3 to 4 are attained on incubating the intestinal sacs for 1 hour at 37° C in an atmosphere of oxygen; when oxygen is replaced by nitrogen, the concentration gradient disappears.

When the concentration of the pyrimidine is raised from .02 to .05 mM, the gradient developed in 1 hour is significantly reduced; on raising the concentration to 0.5 mM, the gradient disappears. This progressive reduction in the concentration gradient is explained by the significant rates of passive transfer of uracil and thymine. Thus, raising the concentration of the pyrimidine results in saturation of the active transport process, while the rate of passive transfer is greatly increased.

Significance to the Program of the Institute: Knowledge of the mechanisms by which natural substrates traverse the cell boundary should increase our understanding of the functions of the cell and its components.

Proposed Course of Project: (1) Ascertain whether other species possess the pyrimidine transport mechanism. (2) Investigate the effect of metabolic inhibitors on the transport of pyrimidines. (3) Search for possible intermediates formed during the active transport of pyrimidines. (4) Investigate the permeation of other cells by pyrimidines and purines.

Part B included: Yes

Serial No. NHI-195

Publications:

Schanker, L.S. and Tocco, D.J. Active transport of some pyrimidines across the rat intestinal epithelium. J. Pharmacol. and Exper. Therap., in press, 1959

Honors and Awards: None

Serial No. NHI- 198
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemical Inhibition of Cholesterol Biosynthesis.

Principal Investigators: Dr. Herbert Wiese
Dr. Elliott Schiffmann
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.50
Professional: 0.50
Other: None

Patient Days (calendar year 1959): None

Project Description:

Objectives - The identification of several intermediates in the biosynthesis of cholesterol has made it possible to design antimetabolites which could inhibit the formation of cholesterol at specific points in the sequence of biosynthetic reactions. The objectives are: 1) To study the inhibitory properties of a number of compounds. 2) To determine the extent of suppression of sterol biosynthesis in vivo by compounds previously screened in vitro. 3) To study the metabolism of compounds which effectively inhibited cholesterol synthesis in vivo. 4) To study the mechanism of the inhibitory material at enzymatic levels.

Methods Employed - Isotopic cholesterol has been isolated from biological systems as the digitonide and the specific radioactivity of the product determined.

Some general synthetic procedures were used in the preparation of inhibitory compounds.

Major Findings - It has been found that Δ^3,3-methyl pentenoic acid and 3-hydroxy-3-methyl valeric acid inhibit the biosynthesis of cholesterol in rat liver homogenates. This effect is measured by the relative incorporation into cholesterol of isotopic mevalonic acid, an extremely efficient precursor of the sterol. The hydroxy acid is four times as effective as the olefin in this system.

Serial No. NHI-196

Significance to the Program of the Institute - One of the aims of this project is the development of a potent inhibitor of cholesterol biosynthesis. The effects of such a compound might be the suppression of cholesterol synthesis in vivo and the reduction of cholesterol deposition in arteriosclerotic plaques. Supniewski et al. have reported the clearing of experimentally induced plaques in pigeons by means of Δ^3,3-methyl-pentenoic acid.

Proposed Course of Project -

1. The preparation of several other compounds to be tested as inhibitors of cholesterol biosynthesis.

2. The testing in vivo of the compounds which effectively reduced cholesterol synthesis in vitro.

3. The determination of the enzymatic sites of action of the effective inhibitors.

Part B included No

Serial No. NHI-197
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Metabolism of Sterols

Principal Investigators: Dr. Elliott Schiffmann
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.00
Professional: 1.00
Other: None

Patient Days (calendar year 1959): None

Project Description:

Objectives - The intermediates in the biosynthesis of certain more polar steroids from sterols remain unknown. The biogenesis of the cardiotonic lactones is particularly obscure. Evidence from this laboratory indicates a role for cholesterol as a precursor of the cardiotonic lactones in the parotoid gland of the toad, Bufo marinus. It is conceivable that some of the phytosterols may serve a similar function in the formation of plant steroids of cardiotonic activity.

The aims of this work are: 1) To define the metabolic pathways between sterols and polar steroids, as exemplified in the biosynthesis of cardiac-active principles.

2) To investigate the effect of polar steroids upon certain biochemical processes.

Methods Employed - The cultivation of plant material has been carried out on a limited scale. Conventional chromatographic and isotope assay procedures. Synthetic organic chemical procedures.

Patient Material - None

Major Findings - 1) Although γ-sitosterol accounts for most of the sterol fraction in the parotoid gland of B. marinus, no conversion of γ-sitosterol-H^3 (prepared by Wilzbach

tritiation) to radioactive cardiac lactones could be demonstrated in this toad in vivo. Both labeled cholesterol, which is an efficient precursor of the lactones, and mevalonic acid-C^{14}, which is an efficient precursor of cholesterol, gave a radioactive sterol fraction in the gland when injected in vivo. It appears that β-sitosterol represents an end product of sterol metabolism in the gland.

2) Mevalonic acid-2-C^{14} was not incorporated into the toad lactones or into the convallatoxin of Convallaria majalis grown in a liquid medium containing the labeled precursor.

3) The data indicate that the biosynthesis of cardiac lactones may be analogous to origin of bile acids in that a rate limiting hydroxylation of the C_{27} sterol precedes the metabolic attack on the side chain. Synthesis of the postulated intermediates 21-hydroxy cholesterol and 14β,21-dihydroxycholesterol, both of which are unknown compounds, has been undertaken. Preliminary experiments indicate that reaction of a long chain alkyl triphenyl phosphonium halide with 21-hydroxy-20-keto steroids offer a practical synthesis of these compounds.

Significance to the Program of the Institute - The relationship of cholesterol to the biosynthesis of cardiac-active substances is still unexplored. The definition of such a relationship may enable one to understand the mode of action of polar steroids on an enzymatic level.

Proposed Course of Project - 1) The attempt to synthesize a hydroxylated sterol will be carried on, since such a compound is a probable intermediate in the biological formation of the lactones.

2) It is proposed to test the incorporation of label from isotopic leucine into the toad lactones since the latter amino acid is a precursor of isoprenoid metabolites.

3) The biosynthesis of convallotoxin from Lily of the Valley will be further investigated using tracer techniques.

Part B included No

Serial No. NHI-198
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Metabolism of Ascorbic Acid

Principal Investigators: Dr. J. J. Burns
Dr. Allan H. Conney
Dr. Peter G. Dayton

Other Investigators: Mr. Julian Kanfer
Miss Ruth Gastel
Miss Carole Evans
Miss Natalie Trousof

Cooperating Units: New York University Research Service, Goldwater Memorial Hospital, New York.

Man Years (calendar year 1959):
Total: 1.80
Professional: .65
Other: 1.15

Patient Days (calendar year 1959): None

Project Description:

Objectives - To determine the mechanisms required for the formation and for the degradation of L-ascorbic acid.

Patient Material- None.

Major Findings - 1) We have previously reported that ascorbic acid is synthesized in rats from glucose through D-glucuronic acid and L-gulonic acid. During the past year the following mechanism has been found:

D-glucose (or D-galactose) → uridinediphosphoglucose → uridine-

diphosphoglucuronic acid → D-glucuronic acid-1-PO_4 → D-glucuronic ac

The importance of this pathway in the formation of L-ascorbic acid has been established in experiments in vivo in which the conversion of glucose-1-C^{14} or galactose-1-C^{14} to D-glucuronic, L-gulonic and L-ascorbic acids was measured. Thus, the ascorbic acid formation may be added to other synthetic mechanisms in the body which require uridine nucleotides and these now include the synthesis of glucuronides, glycogen, amino sugars and mucopolysaccharides.

2) Further studies have been carried out on the enzyme system in rat kidney which decarboxylates L-ascorbic acid. The enzyme has been purified and 2,3-diketo-L-gulonic acid was shown to be the actual substrate in the reaction. L-Lyxonic and L-xylonic acids were identified as products of L-ascorbic acid metabolism. Similar enzyme systems have also been observed by us in guinea pig and rat liver.

3) All attempts to demonstrate the synthesis of L-ascorbic acid in microorganisms by the pathway demonstrated in animals have failed. It is possible that microorganisms either possess an entirely different pathway for synthesis or they lack the ability entirely to make the vitamin.

During the past year we have been able to adapt a certain strain of yeast to grow on L-ascorbic acid as its sole carbon source. This observation is of importance since it may furnish a useful approach for studying the enzymes involved in the metabolism of the vitamin.

Significance to the Program of the Institute - Ascorbic acid is necessary for the maintenance of normal connective tissue which is important for the integrity of the cardiovascular system.

Proposed Course of Project - 1) Further studies will be carried out on the enzymes required for the formation of D-glucuronic acid. In particular the system needed in the conversion of uridinediphosphoglucose to D-glucuronic acid through D-glucuronic acid-1-PO_4 will be characterized.

2) Further studies will be carried out on the enzymes involved in the breakdown of L-ascorbic acid in animals. The metabolism of lyxonic acid and xylonic acid in animals will be investigated. The results of such experiments will aid in evaluating the importance of these two pentonic acids in the overall metabolism of the vitamin.

3) Ascorbic acid has been reported previously to be cleaved to oxalate in animals. It has been suspected that the four carbon sugar acid L-threonic acid, may be the other product of this reaction but no definitive information has appeared on this point. Studies are now under way in an attempt to identify L-threonic acid as a product of the vitamin's metabolism. If this should be the case, the further metabolism of L-threonic acid will be studied in animals.

4) The observation that a strain of yeast can be adapted to grow on L-ascorbic acid furnishes a useful approach for studying the mechanism required in ascorbic acid metabolism. Attempts will be made to determine the end product of ascorbic acid in this yeast system and the specific enzymes involved will be characterized.

Part B included Yes

(Attachment I)

Serial No. NHI-198

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J.J.: Biosynthesis of L-Ascorbic Acid; Basic Defect in Scurvy. Am. J. Med. XXVI: 740-748, 1959.

Dayton, P.G., Eisenberg, F., Jr. and Burns, J.J.: Metabolism of C^{14}-Labeled Ascorbic, Dehydroascorbic and Diketogulonic Acids in Guinea Pigs. Arch. Biochem. and Biophys. 81: 111-117, 1959.

Kanfer, J., Burns, J.J. and Ashwell, G.: L-Ascorbic Acid Synthesis in a Soluble Enzyme System from Rat-Liver Microsomes. Biochim. Biophys. Acta 31: 556-558, 1959.

Burns, J.J., Fullmer, H.M. and Dayton, P.G.: Observations on Vitamin C Activity of D-Ascorbic Acid. Proc. Soc. Exper. Biol. and Med. 101: 46-49, 1959.

Burns, J.J., Trousof, N., Evans, C., Papadopoulos, N. and Agranoff, B.W.: Conversion of Myo-Inositol to D-Glucuronic Acid and L-Gulonic Acid in the Rat. Biochim. Biophys. Acta 33: 215-219, 1959.

Burns, J.J. and Ashwell, G.: L-Ascorbic Acid. Enzymes, Vol. II, in press.

Burns, J.J.: Vitamin C Activity of D-Ascorbic Acid. Proc. of the IV International Congress of Biochemistry, Vienna, 1958, in press.

Burns, J.J.: L-Ascorbic Acid. Chemical Pathways of Metabolism. Vol. II, in press.

Burns, J.J. and Conney, A.H.: Water-Soluble Vitamins, Part I (Ascorbic Acid, Nicotinic Acid, Vitamin B_6, Biotin, Inositol). Annual Review of Biochem., in press.

Evans, C., Conney, A.H., Trousof, N. and Burns, J.J.: Metabolism of D-Galactose to D-Glucuronic Acid, L-Gulonic Acid and L-Ascorbic Acid in Normal and Barbital-Treated Rats. Biochim. Biophys. Acta, in press.

Honors and Awards relating to this project:

None.

Serial No. MHI-199
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Distribution of Ascorbic Acid.

Principal Investigators: Dr. George R. Martin
Dr. J. J. Burns

Other Investigators: None

Cooperating Units: Fellowship from McNeil Laboratories, Philadelphia, Pennsylvania.

Man Years (calendar year 1959):
Total: 1.2
Professional: 1.2
Other: None

Patient Days (calendar year 1959): None.

Project Description:

Objectives - To investigate the factors which control the physiological disposition of L-ascorbic acid.

Patient Materials - None.

Major Findings - Following the intravenous administration of L-ascorbic acid-1-C^{14}, there is a marked difference in the rate at which the ascorbic acid in various tissues equilibrates with serum ascorbic acid. These rates are not related to the level of ascorbic acid normally present in a given tissue. Various studies have indicated that ascorbic acid is unable to penetrate cell barriers. The oxidized, unionized form of ascorbic acid, dehydroascorbic acid, readily penetrates cell barriers and is then reduced to ascorbic acid. Other in vivo studies indicate that circulating ascorbic acid is oxidized to dehydroascorbic acid by certain tissues such as the kidney and the intestine prior to the general distribution of the vitamin.

Significance to the Program of the Institute - L-Ascorbic acid is necessary for the maintenance and production of collagen present in the cardiovascular system.

Proposed Course of Project - 1) Nothing is known concerning the mechanism whereby ascorbic acid is absorbed by the intestine or reabsorbed by the kidney. Present studies suggest that the oxidation of ascorbic acid to dehydroascorbic acid would promote

the intracellular penetration of the vitamin and thus favor its absorption and retention. Studies will be carried out to determine the importance of this process in determining the retention of ascorbic acid and some of its analogues.

2) The presence of ascorbic acid in extremely high concentrations in certain tissues suggests that ascorbic acid exists in some sort of bound form. Studies will be carried out to characterize this bound ascorbic acid.

Part B included No

Serial No. NHI-200
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Isolation of Cardiotonic Substances from Mammalian Tissues.

Principal Investigators: Mr. Herbert Spiegel
Dr. Stephen Hajdu*
Dr. Elwood O. Titus

Other Investigators: None

Cooperating Units: *Laboratory of Kidney and Electrolyte Metabolism, NHI.

Man Years (calendar year 1959):
Total: 1.25
Professional: .50
Other: .75

Patient Days (calendar year 1959): None

Project Description:

Objectives - Earlier reports have discussed evidence for the existence in mammalian tissues of substances that can exert digitalis like effects. It is the purpose of this project to isolate and identify such substances in the expectation that a knowledge of their structure may clarify their role, if any, in the functioning of the cardiovascular system.

In addition to lysolecithin, which has previously been reported to account for part of the cardiotonic activity of mammalian tissue extracts, there appear to be a number of lipoidal substances as yet unidentified. The immediate objective of this project is the characterization of these substances.

Methods Employed - The cardiotonic activity of tissue extracts is assayed in the isolated frog ventricle according to a procedure developed by Dr. Stephen Hajdu. This method depends upon the ability of digitalis like substances to prevent the decrease in contractile force which normally results from lengthening the intervals between stimuli. It offers considerable advantages in sensitivity and specificity and may be used for quantitative determinations of cardiac steroids.

The fractionation of tissue extracts is carried out for the most part by conventional chemical means. For the separation of very closely related, very non-polar lipids, chromatography on silicic acid has been most useful.

Patient Material - None.

Major Findings - Several active factors, all acidic lipids of very low polarity have been obtained from extracts of mammalian tissue. These substances, which occur in amounts equivalent to 8 to 60 micrograms of strophanthidin per kilo of tissue, are listed:

1) Beef blood factor -- probably cis vaccenic acid.

2) Beef heart factor -- an acidic lipid but not a simple fatty acid. Infrared spectra suggest a lactonized -hydroxy fatty acid. May be a component of more complex lipids which are partly broken down during the isolation procedure.

3) Rabbit serum factor -- an acidic lipid but not a simple fatty acid. Infrared spectra suggest an unsaturated lactonized hydroxy acid or less probably a [illegible]. Occurs as a component of more complex phospholipids and appears as an artefact of isolation. Interesting because of its high activity which is approximately equivalent to that of digitoxigenin on a molar basis.

4) Rabbit heart factor -- [illegible] (5).

5) Rabbit red cell factor -- Factors 4 and 5 are readily distinguishable from the preceding substances by chromatography. They are less well characterized. They have the same chromatographic properties and may be identical with each other and with a chromatographically similar lipid from the standard rabbit diet which has high cardiotonic activity. Variability in dietary intake would explain the considerable variation in yields of these substances.

6) Toxic factor from rabbit [illegible] -- An acidic lipid is contracture of the frog heart in a manner [illegible] to the saponin. Chemical data [illegible] spectrum [illegible] absorption. Probably an unsaturated fatty acid.

Significance to the Program of the Institute - There is no evidence that these pharmacologically active simple lipids play a role in maintaining ventricular contractility. Their physiologic role remains obscure. It is interesting that similar structural features, i.e. cis 11,12 unsaturation or lactonization with a hydroxyl group, appear to confer both cardiotonic activity and the ability to cause contraction of smooth muscle upon otherwise inactive fatty acids.

The effects on contractility of the frog ventricle probably reflect the influence of these lipids on passage of ions through membranes to the contractile proteins. These studies may help to clarify the role of lipids in membrane function.

Proposed Course of Project - Efforts to complete the characterization of the more active factors will continue.

Part II [illegible] No.

Serial No. NHI-201
1. Chemical Pharmacology
2. Organic Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of the Properties of Phospholipase D.

Principal Investigators: Dr. Herbert Weiss
Dr. Elwood O. Titus

Other Investigator: None.

Cooperating Units: None.

Man Years (calendar year 1959):
Total: 1.00
Professional: 1.00
Other: None

Patient Days (calendar year 1959): None.

Project Description:

Objectives - Phospholipase D is an enzyme that removes choline from lecithin and ethanolamine from cephalin. It has been found thus far only in plants but is presumed to be present in mammalian tissues, particularly since phosphatidic acids, which are products of its activity, have been recently found in normal mammalian tissues.

The purposes of this project are. purification of the enzyme, identification of the cofactors required for its activity, a search for its presence in animal tissues, and a study of its significance in the metabolism of phospholipids.

Methods Employed - Conventional methods of enzyme isolation were employed. Enzyme activity was measured by assay of liberated choline as the enneaiodide. Phospholipids were chromatographed on silicic acid.

Patient Material - None.

Major Findings - Highly purified fractions of phospholipase D prepared by ammonium sulfate fractionation require both calcium and one or more lipid activators found in soybean phospholipids. An activator isolated by solvent fractionation and repeated chromatography has been identified as phosphatidyl inositol.

Serial No. NHI-201

Kinetic studies of the effect of phosphatidyl inositol on lecithin hydrolysis indicate that a micelle containing approximately 3 molecules of activator to 1 of lecithin is the preferred substrate. Quantities of activator in excess of this ratio have little effect, and in high concentrations the inositide becomes inhibitory.

Either the electrical charge or some structural peculiarity of the hydrophilic inositide micelle must be important in the interaction with the enzyme, since solubilization of lecithin by various detergents is far less effective in enhancing its enzymatic hydrolysis than is the addition of inositide.

Significance to the Program of the Institute - Lack of recognition of the peculiar cofactor requirements of phospholipase D may have prevented its identification in animal tissue. It is possible that this enzyme may be of importance in the metabolism of mammalian phospholipids.

Proposed Course of Project - Further studies of the mechanism of activation by phosphatidyl inositol will be carried out. A search for the enzyme and a study of its role in mammalian systems will be undertaken.

Part B included No

Serial No. NHI-202
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Effects of Coronary Occlusion in Dogs Treated with Reserpine or Phenoxybenzamine

Principal Investigator: Dr. Harriet M. Maling

Other Investigators: Mr. Victor H. Cohn, Jr.
Mrs. Alice Williams
Mr. Duffy E. McBrayer

Cooperating Unit: Dr. Benjamin Highman, WIAMD

Man Years (calendar year 1959)
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days: None

Project Description:

Objectives: To determine the effects of coronary occlusion in dogs with hearts depleted of norepinephrine by reserpine and in dogs pretreated with an adrenergic blocking agent. These experiments were planned to test the concept of Harris and Richins (1955) that the ventricular tachycardia resulting from myocardial infarction in dogs is due, at least in part, to epinephrine and norepinephrine, which are liberated from the necrotic myocardium and which may act upon the functional cells bordering the infarct.

Methods Employed: Myocardial infarction was produced by the two-stage occlusion procedure of Harris (Circulation 1: 1318, 1950). Spontaneous ectopic activity and ventricular sensitivity were measured as in a previous study (Maling and Moran, Circulation Research 5: 409, 1957).

Serial No. MHI-202

Major Findings: The usual spontaneous ectopic activity and prolonged ventricular hypersensitivity occurred after two-stage coronary occlusion in dogs pretreated with reserpine or the adrenergic blocking agent, phenoxybenzamine. There was no significant difference in the elevations in serum enzyme levels and in either the gross or histological appearance of the infarcts in treated and non-treated dogs. It is unlikely that release of norepinephrine from the infarcted area during necrosis has a significant role either in the usual development of spontaneous arrhythmias and myocardial hypersensitivity or in the deposition of neutral fat around the infarct.

Significance to the Program of the Institute: Our findings indicate that the catecholamines in the myocardium do not contribute significantly to the spontaneous ectopic activity and the cardiac hypersensitivity after coronary artery occlusion. Depletion of the catecholamines by reserpine does not prevent the triglyceride deposition in the border.

Proposed Course of Project: This project has been finished.

Part B Included: Yes

Serial No. NHI- 202

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Maling, Harriet M., Cohn, Victor H., Jr. and Highman, Benjamin: The effects of coronary occlusion in dogs treated with reserpine and in dogs treated with phenoxybenzamine. J. Pharmacol. & Exper. Therap. 127: 229-235, 1959.

Honors and Awards: None

Serial No. NHI-203
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Some Similar Effects after Large Doses of Catecholamines and Myocardial Infarction in Dogs

Principal Investigator: Dr. Harriet M. Waling

Other Investigators: Mrs. Martha A. Williamson
Mr. William H. Butler, Jr.
Mr. Duffy E. McBrayer

Cooperating Units: Dr. Benjamin Highman, NIAMD
Dr. Marjorie Morning, LCPM, NHI

Man Years (calendar year 1959): Patient days: none
Total: 0.4
Professional: 0.2
Other: 0.2

Project Description:

Objectives: To compare in dogs some similar effects after large doses of catecholamines and myocardial infarction. These effects include triglyceride deposition in the heart, ventricular hyperexcitivity, and elevated serum enzyme levels.

Methods Employed: Myocardial infarction was produced by the two-stage occlusion procedure of Harris (Circulation 1: 1318, 1950). Serum glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase were measured by the method of Reitman and Frankel (Am. J. Clin. Path. 28: 36, 1957). Serum lactic dehydrogenase was measured by a procedure outlined in a bulletin distributed by the Sigma Chemical Company. The heart muscle was carefully trimmed to remove the coronary arteries and the epicardium, which contains many fat cells. The triglyceride content of the heart muscle was measured by a modification of the direct colorimetric method of van Handel and Zilversmit (J. Lab. & Clin. Med. 50: 152, 1957).

Serial No. NHI-203

Major Findings: In dogs, both myocardial infarction and intravenous infusion of large doses of catecholamines produce sustained ventricular hypersensitivity, as indicated by exaggerated ectopic responses to small doses of norepinephrine. This hypersensitivity is associated with myocardial fatty changes. The triglyceride content of the heart is elevated the day after an infusion of a large amount of norepinephrine. Both myocardial infarction and large doses of catecholamines cause marked elevations in serum glutamic-oxalacetic and glutamine-pyruvic transaminases, lactic dehydrogenase and alkaline phosphatase. The adrenergic blocking agent, phenoxybenzamine, prevents the triglyceride deposition in the heart, the prolonged ventricular hypersensitivity, and the elevations in serum transaminases and lactic dehydrogenase after large doses of catecholamines; it does not prevent these changes, however, after coronary occlusion.

Significance to the Program of the Institute: This project has increased our knowledge of the changes after myocardial infarction and large doses of catecholamines

Proposed Course of Project: In collaboration with Dr. Marjorie Horning, a more complete analysis will be made of the lipid composition of normal heart and hearts from dogs killed 1 day after infusions of large doses of norepinephrine. A study is also in progress of the lipid composition of infarcted and non-infarcted heart and the border of infarcts at varying times after coronary occlusion.

Part B Included: Yes

Serial No. NHI-203

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Highman, B., Maling, H. M. and Thompson, E. C. Serum transaminase and alkaline phosphatase levels after large doses of norepinephrine and epinephrine in dogs. Am. J. Physiol. 196: 436, 1959.

Maling, H. M. and Highman, B. High altitude tolerance of normal dogs and dogs with myocardial infarcts. Am. J. Physiol. 196: 507, 1959.

Maling, H. M., Cohn, V., Jr. and Highman, B. The effects of coronary occlusion in dogs treated with reserpine and in dogs treated with phenoxybenzamine. J. Pharmacol. & Exper. Therap. 127: 229, 1959.

Maling, H. M., Highman, B. and Thompson, E. C. Some similar effects after large doses of catecholamines and myocardial infarction in dogs. Manuscript submitted to the American Journal of Cardiology.

Honors and Awards: Participation in the symposium on "The Catecholamines in Cardiovascular Pathology," which was held at the University of Vermont, College of Medicine, August 23-26, 1959.

Serial No. NHI-204
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Pharmacology of JB 516 and Other Inhibitors of Monoamine Oxidase

Principal Investigators: Dr. Harriet M. Maling
Dr. Sydney Spector

Other Investigators: Mrs. Martha A. Williams
Mr. Duffy E. McBrayer

Cooperating Unit: Dr. Benjamin Highman, NIAMD

Man Years (calendar year 1959) Patient Days: None
Total: 1.0
Professional: 0.4
Other: 0.6

Project Description:

Objectives: To study the pharmacological actions of JB 516 and other monoamine oxidase inhibitors. To correlate the changes in behavior and neurological symptoms (see Annual Report for 1958) with pathologic findings and levels of serotonin and norepinephrine in various parts of the central nervous system.

Methods Employed: Observations were made on unanesthetized dogs, cats, rabbits and squirrel monkeys. Monoamine oxidase inhibitors were administered daily, subcutaneously, orally or intravenously, usually 5 days per week, for periods up to 35 weeks. Records were kept of changes in behavior and personality, neurological symptoms, serum enzyme levels, rectal temperature, and hemoglobin and hematocrit values. Movies were made of some animals.

The monoamine oxidase inhibitors under study include iproniazid, racemic JB 516, dextro-JB 516, levo-JB 516, JB 835, Ro 5-0700, Ro 5-0831/1, SKF 835 A, Nialamid, Nardil and A-17767. Control drugs include amphetamine, d-amphetamine and isoniazid.

Arterial blood pressure and lead II electrocardiograms were recorded in each dog before it was killed for chemical analyses of tissues and pathologic studies; records were obtained of resting values and the responses to tilting and injections of drugs.

Major Findings:

Changes in behavior and neurological symptoms: On small doses of iproniazid (5 mg/kg) or JB 516 (0.1 to 0.2 mg/kg), dogs were alert, happy, noisy and active. They ate greedily. With large doses of iproniazid (15 to 30 mg/kg) and JB 516 (2 to 4 mg/kg), dogs soon became depressed. Dogs receiving iproniazid became markedly anemic, with hemoglobin values as low as 5 to 7 grams. They developed reticulocytosis and Heinz bodies were seen in some red blood cells. Dogs receiving 25 to 30 mg/kg iproniazid usually died within 2 weeks.

The most marked neurological symptoms were seen in dogs receiving JB 516, JB 835 and Nardil (1 dog after 4 doses of 10 mg/kg). All dogs which received at least 3 doses of 4 mg/kg JB 516 developed marked neurological symptoms. Regardless of dose (0.5 to 4.0 mg/kg/ subcutaneously), almost all dogs which received a cumulative dose greater than 40 mg/kg developed marked neurological symptoms Neurological findings included unsteadiness during standing, sinking of the hind legs, rigidity of the hind legs, extensor spasms of the front legs, ataxia, tremor at rest, uncontrollable jerking of the head, nystagmus, hoarse barking, loss of weight and lowered rectal temperature.

Two cats died 4 and 6 days after the fifth dose of 4 mg/kg JB 516. Both cats showed an extreme lowering of rectal temperature, inability to stand and walk, general irritability, and salivation Before receiving the drug, both cats were healthy, affectionate and frequently purred. On 2 mg/kg JB 516, cats died or were killed terminally after 16 to 22 doses. The most prominent early symptoms were an impaired righting, shown by an inability to land on the feet when dropped, salivation, and a fall in rectal temperature. Experiments are now in progress with cats on lower doses.

JB 516 is well-tolerated for long periods by rabbits. On doses of 2, 3, and 4 mg/kg, rabbits became alert and more active. They remained healthy and gained weight. The only rabbit on 10 mg/kg daily is depressed.

One squirrel monkey is receiving 10 mg/kg JB 516 daily, 5 times a week. It is planned to continue the experiment for 12 weeks. At the end of 6 weeks, the monkey is healthy and much tamer.

Levels of serotonin and norepinephrine. In cats and dogs, the prolonged administration of various monoamine oxidase inhibitors caused marked increases in the concentration of serotonin in various parts of the central nervous system. The highest levels of serotonin were found in the midbrain, hypothalamus, thallmus, pyriform lobe, olfactory tract and spinal cord. The levels of norepinephrine were not appreciably changed.

Pathologic Studies: Examinations have been made of the brains of 47 dogs on prolonged subcutaneous administration of JB 516 in doses from 0.1 to 4.0 mg/kg. No definite lesions in the brain were observed in dogs which received cumulative doses less than 40 mg/kg. Fourteen dogs received cumulative doses between 40 and 72 mg/kg. Degenerative lesions were observed in the inferior olivary nucleus in 11 dogs and in the pyriform lobe, which includes the amygdaloid nucleus, in 7 dogs. Five dogs had lesions in both the inferior olivary nucleus and the pyriform lobe. A marked lesion in the inferior olivary nucleus was observed in the only dog on 0.5 mg/kg JB 516 which received a cumulative dose greater than 40 mg/kg; this dog was killed after a cumulative dose of 41.5 mg/kg.

Four dogs received JB 835, 4 mg/kg subcutaneously, 5 days per week, for 3 1/2 to 5 weeks; cumulative doses were 72 to 100 mg/kg. Lesions were observed in the inferior olivary nucleus in all 4 dogs. Two of the four dogs also had lesions in the pyriform lobe.

Lesions in the olivary nucleus and pyriform lobe, comparable to those noted after JB 516 and JB 835, have not been seen in the brains of 8 dogs which received cumulative doses of 590 to 1410 mg/kg iproniazid; daily doses were 5 to 20 mg/kg. Such dogs often showed marked hemosiderosis of the viscera, indicating excessive hemolysis.

Pathologic studies in dogs are incomplete with the other inhibitors. Pathologic studies on cats, rabbits and monkeys are incomplete.

Significance to the Program of the Institute: The toxicity of JB 516 in animals is important since this drug has been given a clinical trial in the Institute.

Proposed Course of Project: The pharmacological, chemical and pathologic studies will be completed as soon as possible. It is planned to submit a manuscript to the Journal of Pharmacology and Experimental Therapeutics.

Part B Included: No

Serial No. NHI-205
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on New Drugs for Arthritis and Gout.

Principal Investigators: Dr. J. J. Burns
Dr. Peter G. Dayton
Dr. Allan Conney

Other Investigators: Dr. L. Sicam
Miss Dolores Taller
Mr. Miguel Landrau

Cooperating Units: New York University Research Service at Goldwater Memorial Hospital, Department of Medicine, Mount Sinai Hospital, New York and Geigy Laboratories, Basel, Switzerland.

Man Years (calendar year 1959):
Total: .65
Professional: .65
Other: None

Patient Days (calendar year 1959): None

Project Description:

Objectives - 1. Phenylbutazone, a synthetic pyrazolone derivative, has found considerable use in the treatment of various arthritic diseases. Although phenylbutazone is a potent antirheumatic agent, its usefulness is limited by such side effects as edema, gastrointestinal hemorrhage, skin reactions and occasionally agranulocytosis. A simple non-steroidal molecule with the potent antirheumatic effects of phenylbutazone, but lacking its undesirable side effects, would be of paramount importance to the therapy of rheumatoid arthritis, rheumatic fever, gout and related musculoskeletal disorders. A collaborative search for such a drug has been undertaken with Geigy Pharmaceuticals. Promising compounds which have been screened for anti-inflammatory effect in animals will be tested in patients with active arthritis.

2. Studies are being carried out also with drugs both in the phenylbutazone series and in the zoxazolamine series to find new uricosuric agents for the treatment of chronic gout. Promising compounds are synthesized for us either by Geigy Pharmaceuticals or McNeil Laboratories and their activities are tested in gouty subjects.

Major Findings - I. Largely as a result of our studies four new drugs for the treatment of arthritis and gout have been found.

1. Oxyphenbutazone - This drug was originally shown by us to be a para-hydroxy metabolite of phenylbutazone in man. Oxyphenbutazone has the potent antirheumatic effect of phenylbutazone in acute arthritis and gout. The drug has been studied extensively in many clinics, and it appears to lack some of the undesirable side effects of phenylbutazone. For this reason it will probably be introduced in the near future into medical practice.

2. Sulfinpyrazone (Anturan) - This compound was also shown in this laboratory to be a metabolite in man of a phenylbutazone derivative. Subsequently it was found to be one of the most potent uricosuric drugs yet described, and this observation has been confirmed by many other workers. Recently sulfinpyrazone has been introduced into medical practice for the treatment of chronic gout.

3. Zoxazolamine (Flexin) - Studies in this laboratory indicated that Flexin, a muscular relaxant drug, has extremely potent uricosuric activity. This was a rather surprising observation since Flexin has an entirely different chemical structure than any other drug which enhances the urinary excretion of uric acid. In view of its marked uricosuric activity, Flexin is now being used clinically in the treatment of chronic gout.

4. Chlorzoxazone (Paraflex) - In the course of our metabolic studies with Flexin, it was found that the drug was converted in the body to a hydroxyl derivative. This metabolite (Paraflex) possesses potent muscular relaxant properties, but no uricosuric activity. Paraflex has been introduced as a new drug for the treatment of muscle spasm.

II. Studies carried out by us with 70 different analogues of phenylbutazone have fairly well established the structural features required in the molecule for the various pharmacological activities of the drug. This information is of considerable importance in finding still more useful drugs in the phenylbutazone series for the treatment of arthritis and gout. Parallel studies have also been carried out with various derivatives and metabolites of zoxazolamine (Flexin) and it is now possible to make predictions concerning the structural features required in this chemical series for uricosuric and muscular relaxant activity.

Proposed Course of Project - 1. Further studies will be carried out to establish the relationship between chemical structures and pharmacological effect in the phenylbutazone series. Six new analogues are now under study which have structural changes in the molecule not hitherto investigated. The possibility that introduction of cyclic groups into the wide chain may enhance antirheumatic activity will be explored.

2. During the past year information has been obtained on two new drugs in the phenylbutazone series which may be valuable in the treatment of chronic gout. One of these drugs, a para-methylsulfone derivative, has been found to exert prolonged uricosuric activity in man. This observation is correlated with its relatively long half-life in the body (about 24 hours). Another drug, keto oxyphenbutazone, has been shown in studies carried out in collaboration with Dr. J. E. Seegmiller of the N.I.A.M.D. to possess prolonged and potent uricosuric activity. Since there is considerable need for a long acting uricosuric drug in the treatment of gout, it is planned to evaluate further these two drugs in chronic gout. In particular, their effectiveness will be compared with sulfinpyrazone and zoxazolamine. It should be noted that a disadvantage of the latter two agents is their rapid rate of metabolism in man which necessitates frequent medication to maintain a desired uricosuric effect.

3. A major route of metabolism for sulfinpyrazone in man has been shown to involve hydroxylation in one of its benzene rings. This metabolite has been isolated from urine and identified. Experiments are now under way to elucidate the further metabolism of this sulfinpyrazone.

4. A recent report indicated that an N-acetyl derivative of zoxazolamine has potent uricosuric activity. This compound could possibly be an active metabolite of zoxazolamine. A supply of this compound has been made available to us and we intend to investigate this point.

Significance to the Program of the Institute - A non-toxic potent antirheumatic drug would be of considerable value in the treatment of rheumatic fever. Studies on how drugs effect uric acid excretion increase our general knowledge on the mechanisms by which various naturally occurring compounds are excreted by the kidney.

Part B included Yes

(Attachment I)

Serial No. NHI-205

PHS-NIH
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Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Yu, T.F., Burns, J.J., Dayton, P.G., Gutman, A.B. and Brodie, B.B.: A p-Nitro Analogue of Phenylbutazone Possessing Potent Antirheumatic, Sodium Retaining and Uricosuric Properties. J. Pharma. Exptl. Thera. 126: 185-189, 1959.

Conney, A.H. and Burns, J.J.: Physiological Disposition and Metabolic Fate of Chlorzoxazone (Parafiex) in Man. J. Pharma. Exptl. Thera., in press.

Conney, A.H., Trousof, N. and Burns, J.J.: The Metabolic Fate of Zoxazolamine (Flexin) in Man. J. Pharma. Exptl. Thera., in press.

Honors and Awards relating to this project:

None.

Serial No. NHI-206
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Antiarrhythmic Drugs

Principal Investigator: Dr. Harriet M. Maling

Other Investigator: Mrs. Martha A. Williams

Cooperating Unit: None

Man Years (calendar year, 1959)
Total: 0.1
Professional: 0.05
Other: 0.05

Patient Days: None

Project Description:

Objectives: To test drugs for antiarrhythmic activity against the spontaneous ectopic activity which is conspicuous in unanesthetized dogs the day after ligation of the anterior descending coronary artery. To study other pharmacological actions of antiarrhythmic drugs.

Methods Employed: The anterior descending coronary artery of dogs is ligated by the two-stage occlusion procedure of Harris (Circulation 1: 1318, 1950). Electrocardiograms and arterial pressure are recorded the day after occlusion when spontaneous ectopic activity is most marked. The drugs being tested are injected intravenously in appropriate doses over a period of one minute. Observations are continued for a period of at least one hour after the drug.

Major Findings: The following compounds have been tested for antiarrhythmic activity during the past year: reserpine, WIN 5494-B and Atarax. Reserpine did not show antiarrhythmic activity. Atarax has been tested in only 1 dog; it showed moderate antiarrhythmic activity. WIN 5494-B definitely slowed the ectopic rate in 3 dogs, but did not convert an ectopic rhythm to a sinus rhythm.

Significance to the Program of the Institute: Testing selected drugs for antiarrhythmic activity in dogs may lead to the discovery of a clinically-useful antiarrhythmic drug.

Proposed Course of Project: We hope to complete our studies on an Abbott series of barbiturate derivatives which possess antiarrhythmic activity. Some of these drugs were tested during 1958.

Serial No. NHI-206

Additional experiments should be done with Atarax.

Part B included: No

Serial No. MH-207
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Metabolism of Amines

Principal Investigator: Sidney M. Hess

Other Investigators: C arles A. Chidsey

Cooperating Units: Non

Man Years:
Total: 2.0
Professional: 2.0
Other:

Patient Days:

Project Description:

A sensitive and specific method of analysis has been developed for a number of monoamine oxidase inhibitors. Among those were methods developed for harmaline, harmine, and tetrahydroharmine. Harmaline was administered and the physiologic distribution determined. The degree of monoamine oxidase inhibition with respect to time after administration was also determined. The drug was shown to be effective when absorbed from the gastro-intestinal tract but it required about a 10 fold larger dose by mouth to produce the same effect as were produced by an i.p. injection. Patient studies showed the drug disappeared very quickly from the blood stream after oral administration. Preliminary studies on relatively low levels of the drug administered intramuscularly also showed a rapid disappearance.

A sensitive (to 0.7 μg/gm tissue) and specific method for the determination of tryptamine in tissues was developed. The method involves cyclization with formaldehyde followed by dehydrogenation to form the highly fluorescent product norharman. Using this method tryptamine was demonstrated in the tissues of rats, guinea pigs, dogs and other animals after treatment with a monoamine oxidase inhibitor followed by tryptophan. The highest level in the brain (2.6 γ/g) was found in a dog, the highest level in the liver (5.5 γ/g) was found in the rat. Levels in normal rat brain approached the limits of sensitivity of the method and at present there is no good evidence that tryptamine occurs in normal brain tissue in the rat or guinea pig.

Central excitation was noted in those rats treated with monoamine oxidase inhibitors and tryptophan. To determine if this central action was caused by tryptamine, or serotonin, which is derived from tryptophan,

a series of experiments was completed using a number of monoamine oxidase inhibitors under varying conditions. Rats pretreated with a monoamine oxidase inhibitor showed a marked increase in both serotonin and tryptamine levels in brain. The livers of these animals showed no significant increase in serotonin and a barely significant level of tryptamine at the dosage of tryptophan used. No direct relationship was found between brain levels of serotonin or tryptamine and central excitement. Excited rats were produced whose brain levels of the two amines could be varied to be higher or lower than the levels in other rats which were not excited. The effectiveness of the monoamine oxidase inhibitors in producing the amines was not related necessarily to the ability of the inhibitor to produce excitement.

A technique for perfusing rat liver in situ was adapted for the study of metabolic changes of drugs or natural constituents of blood. A marked increase in serotonin found in the perfused liver was shown to be an artifact resulting from the trapping of serotonin present originally in the platelets. A marked stimulation of the tryptophan peroxidase system after adding tryptophan to the perfusate was clearly demonstrated. This technique promises to be a powerful tool for uncovering metabolic products too toxic or fleeting or present in quantities too small to be found by injecting the parent compound under investigation into the normal animal.

Metabolism of amines via aromatic hydroxylation is also of interest, and the hydroxylating system of rat and rabbit liver microsomes and supernate are being investigated. Efforts are under way to elucidate the mechanism and to purify the enzyme(s) responsible for forming the various hydroxylated amines which are products of phenylalanine, o-tyramine and m-tyramine.

Part B included: Yes

Serial No. NHI-207

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Hess, S.M., and Udenfriend, S. A Fluorometric Procedure for the Measurement of Tryptamine in Tissues. J. Pharm. and Exptl. Therap. 127: 175-177, 1959.

2. Hess, S.M., Redfield, B.G., and Udenfriend, S. The Effect of Monoamine Oxidase Inhibitors and Tryptophan on the Tryptamine Content of Animal Tissues and Urine. J. Pharm. and Exptl. Therap. 127: 178-181, 1959.

Serial No. NHI-208
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Amino Acid Decarboxylases

Principal Investigator: Herbert Weissbach

Other Investigators: None

Cooperating Units:
Dr. B. Witkop, Laboratory of Chemistry, NIAMD
Mr. W. Lovenberg, United Fruit Company and Section of Experimental Therap., NHI

Man Years:
Total: 0.15
Professional: 0.15
Other:

Patient Days:

Project Description:

Although normally only small amounts of amines are excreted in the urine, recent work in vivo employing monoamine oxidase (MAO) inhibitors has uncovered the presence of many of these compounds, heretofore suspected but never identified. Levels of other amines which are normally excreted could be markedly elevated after MAO inhibition. It thus became obvious that the decarboxylation of a series of amino acids was a normal process in the body and experiments were designed to study this process in more detail. 5-Hydroxytryptophan (5HTP) decarboxylase had been studied previously and experiments were begun on the further purification of this enzyme. The preparation from guinea pig kidney has now been shown to decarboxylate not only 5HTP, but also tryptophan, DOPA, tyrosine, phenylalanine and histidine. The old concept that there are specific decarboxylases for the individual amino acids in animal tissues is now being questioned since this one preparation has such a wide activity. Other amino acids (aliphatic) will be tested to see if such compounds as ethanolamine, γ-aminobutyric acid, and some diamines are formed from the corresponding amino acids by this preparation.

Alpha-methyl DOPA has been shown to inhibit this decarboxylase preparation with DOPA as substrate competitively. The affinity of various substrates for the enzyme has been determined and it will be of interest to see whether all of these substrates are inhibited by α-methyl DOPA; the degree of inhibition being determined by the affinity of the various substrates for the enzyme.

Purification of this preparation will continue and other tissues will be examined for similar non-specific decarboxylase activity. Other unnatural amino acids will be tested either as substrates or inhibitors of this enzyme.

In examining the major pathway of tryptophan degradation leading to the formation of nicotinic acid, it can be seen that two amino acids are normal intermediates; kynurenine and hydroxykynurenine. It had been suggested previously that these compounds might be decarboxylated, to the corresponding amines, and then converted by MAO to derivatives which could condense to form 4-hydroxyquinoline and 4,8-dihydroxyquinoline respectively. In initial studies these amines (kynuramine and hydroxykynuramine) were shown to be excellent substrates of MAO; the development of a simple assay for this enzyme resulted (see above). However, attempts thus far have failed to show any appreciable decarboxylation of kynurenine, and studies are now in progress to see whether hydroxykynurenine is decarboxylated by crude tissue extracts and microorganisms.

Part B included: No

Serial No. NHI-209
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Uptake of Serotonin by Platelets

Principal Investigator: Herbert Weissbach

Other Investigators: Betty G. Redfield

Cooperating Units:
Dr. T.O. Titus, Laboratory of Chemical Pharmacology, NHI

Man Years:
Total: 0.5
Professional: 0.5
Other:

Patient Days:

Project Description:

Although other workers have studied this phenomena, and available evidence indicates that this process is one of active transport, very little is known concerning the mechanism. Most of the previous studies employed platelets suspended in plasma, although it had been shown that uptake occurs in a saline medium. The present studies were designed to determine what is required for maximal uptake of serotonin by platelets in saline media, and whether the active transport system could be isolated and studied independently of other processes.

Marked differences were found in the uptake of serotonin at various pH values. At pH 7 and above, serotonin uptake by the platelets was dependent on the serotonin concentration (saline containing both K^+ and $PO_4^{\equiv}$), while below pH 7 the uptake of serotonin was nearly maximal at low levels of exogenous serotonin (less than 15 µg/ml). It thus appeared that at the higher pH values diffusion was accounting for a large proportion of the serotonin uptake, while at the low pH values uptake occurred mainly through an active process. It was therefore decided to study the uptake of serotonin at pH 5.7, where the data indicated that there was very little diffusion occurring. At this pH some serotonin uptake proceeded in a saline medium, although it could be doubled by the addition of small amounts of both K^+ and $PO_4^{\equiv}$. K^+ alone resulted in a 65% stimulation while $PO_4^{\equiv}$ gave a 40% stimulation. Some evidence that a pumping mechanism for K^+ was essential for serotonin uptake was indicated by the findings that the cardiac glycosides, which are known to inhibit the active transport of K^+ in other systems, inhibited the increased uptake of serotonin by the platelets in the presence of K^+. At higher pH values where diffusion accounted

for a large portion of the serotonin uptake the cardiac glycosides had no effect, and K^+ did not stimulate serotonin uptake. The platelets are known to have a fairly active anaerobic metabolism, but no stimulation of uptake was obtained with various glycolytic intermediates. However, fluoride was found to be a potent inhibitor of serotonin uptake and this is indicative of a requirement for glycolysis.

Most other amines were not taken up by platelets under these conditions, although a number of amino acids were. However, the amino acid uptake at pH 5.7 was not dependent on K^+ or $PO_4^{\equiv}$ and was not appreciably inhibited by fluoride.

The exact roles of K^+ and $PO_4^{\equiv}$ are not clear. Their importance in preserving the active transport system for serotonin was shown conclusively in experiments in which the leakage of serotonin from the platelets was measured over a period of 3 hours. Without any additions, the rate of serotonin leakage was much faster than it was in the presence of K^+, $PO_4^{\equiv}$, or both. Fluoride also stimulated the rate at which serotonin leaked from the platelets, indicating an energy requirement to keep the serotonin in the cell.

More studies are planned concerning the uptake of serotonin at higher pH values. Lactic acid formation at pH 5.7 will also be determined in the presence and absence of K^+ and $PO_4^{\equiv}$ to determine any effects that these ions may have on the rate of glycolysis, and on the relationship between glycolysis and serotonin uptake. Although reserpine is known to inhibit serotonin uptake markedly, only a slight inhibition (30-40%) was observed at pH 5.7. This will be studied to determine if something in plasma is needed for maximal reserpine inhibition.

Part B included: No

1. Lab. of Clinical
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Monoamine Oxidase (MAO)

Principal Investigator: Herbert Weissbach

Other Investigators: Thomas E. Smith

Cooperating Units:
Dr. R. Crout, Section of Experimental Therap., NHI

Man Years:
Total: 0.66
Professional: 0.16
Other: 0.5

Patient Days:

Project Description:

The major route of metabolism of the various amines derived from aromatic amino acids is via oxidative deamination. Although a soluble MAO preparation was previously purified some 10-20 fold, little is known about the mechanism of this reaction, or of the requirements of the

A rapid assay for MAO has been developed based on the action of this enzyme on kynuramine. This compound is a good substrate for MAO, and has a characteristic absorption peak at 360 mμ. The product formed after the action of MAO on kynuramine is 4-hydroxyquinoline, which no longer absorbs at 360 mμ but at 315 and 329 mμ. Thus, by measuring the decrease in absorption at 360 mμ one has a simple assay for MAO which should be useful in enzyme purification or screening studies. This work was submitted for publication.

Purification will be attempted using a soluble preparation from beef liver. It is suspected that this is a flavin enzyme and specific flavin anti-metabolites will be tried in vivo to see if they affect the enzyme activity.

With a purified preparation other amines will be tested to see if they are even weak substrates for MAO. Some of the compounds to be tried are ethanolamine, γ-aminobutyric acid, thyroxamine, β-alanine, and amines derived from the other aliphatic amino acids.

In the conversion of amines to their corresponding acids the two enzymes involved are MAO and aldehyde dehydrogenase. Preliminary studies have been done on further purification, tissue distribution and substrate specificity of the dehydrogenase. It is hoped to learn more about this enzyme and eventually to carry out further studies *in vivo* to determine what role this enzyme plays in the metabolism of various aldehydes formed in animals.

Part B included: Yes

Serial No. NHI-210

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Weissbach, H. The Metabolism of 5-Hydroxyindole Compounds. Symposium on Tryptophan Metabolism, American Chemical Society meeting, Division of Medicinal Chemistry, Atlantic City, N.J., Sept. 14, 1959, pp. 13-27.

Serial No. NHI-211
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Histochemical Studies on Monoamine Oxidase

Principal Investigator: Herbert Weissbach

Other Investigators: Betty G. Redfield

Cooperating Units:
Dr. G. Glenner, Laboratory of Pathology and Histochemistry, NIAMD
Dr. B. Witkop, Laboratory of Chemistry, NIAMD

Man Years:
Total: 0.5
Professional: 0.5
Other:

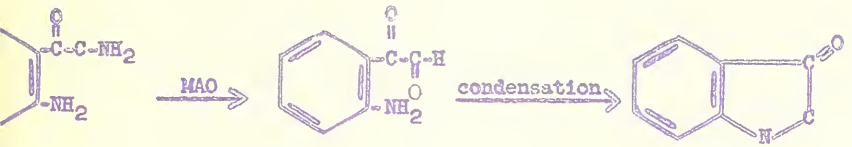
Patient Days:

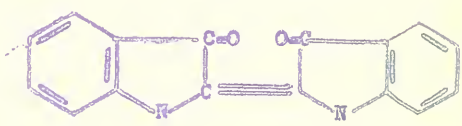
Project Description:

Earlier studies have shown that during the oxidation of tryptamine by monoamine oxidase (MAO) a concurrent reduction of the dye, iodo-nitro tetrazolium chloride (INT), took place. However, the data indicated that reduction of the INT was not due to a direct transport of electrons resulting from the action of MAO, but was dependent on the formation of the corresponding aldehyde, indole acetaldehyde. Thus, INT reduction was either due to spontaneous action of the aldehyde on the tetrazole, or resulted from the further oxidation of the aldehyde. With a simple method of preparing indole acetaldehyde it was possible to check these alternatives. Test tube experiments showed that the aldehyde spontaneously reduces INT, without the need of a tissue preparation. During this chemical reaction no indoleacetic acid was formed. In the presence of tissue the aldehyde was readily converted to pigment, which is also seen when tissues are incubated with tryptamine. It is possible that a pigment precursor is produced in the chemical oxidation of the aldehyde with INT.

A homologue of kynuramine, containing one carbon atom less, has been prepared and tested as a substrate for MAO since the product formed should yield indigo after condensation and thereby produce local staining in situ. However, the reaction has not proceeded at a rate comparable to kynuramine and no evidence of indigo formation has been observed in homogenates.

Serial No. NHI-211





Indigo Blue

Part B included: Yes

Serial No. MHI-211

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Glenner, G.G., Weissbach, H., and Redfield, B.G. The Histochemical Demonstration of Enzymatic Activity by a Nonenzymatic Redox Reaction Reduction of Tetrazolium Salts by Indolyl-3-Acetaldehyde. J. Histochem. and Cytochem. In Press.

Serial No. NHI-212
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Alternate Routes of Indoleamine Metabolism

Principal Investigator: Herbert Weissbach and Sidney Udenfriend

Other Investigators: Betty G. Redfield

Cooperating Units:
Mr. W. Lovenberg, United Fruit Company and Section of Experimental Therap., NHI

Man Years:
Total: 0.5
Professional: 0.5
Other:

Patient Days:

Project Description:

Previous studies here and in other laboratories have shown that 60-70% of administered serotonin is metabolized by monoamine oxidase (MAO), with a small percentage being converted to N-acetyl serotonin and serotonin-O-glucuronide. Studies were initiated to determine what affect MAO inhibitors, in vivo, would have on these alternate routes of serotonin metabolism, and whether previously unknown metabolic routes could be uncovered if MAO is inhibited.

In mice the major metabolite, resulting from the action of enzymes other than MAO is the O-glucuronide of serotonin. Normally 30% of an administered dose of serotonin can be recovered as the glucuronide, while after MAO inhibition as much as 80% can be recovered as the glucuronide. Two dimensional chromatography has shown the presence of 4 metabolites that always occur after serotonin administration and 2 which are occasionally seen. The metabolites which have been tentatively identified are 5-hydroxyindoleacetic acid, serotonin glucuronide, the glucuronide of 5-hydroxyindoleacetic acid, and n-acetyl serotonin. Another metabolite may be the sulfate conjugate of 5-hydroxyindoleacetic acid.

Administered tryptamine can be recovered almost quantitatively as indoleacetic acid, whether or not MAO is inhibited, although the rate of indoleacetic acid formation is much slower in the inhibited animals.

Part B included: Yes

Serial No. NHI-212

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Weissbach, H., King, W., Sjoerdsma, A., and Udenfriend, S. Formation of Indole-3-Acetic Acid and Tryptamine in Animals. A Method for Estimation of Indole-3-Acetic Acid in Tissues. J. Biol. Chem., 234: 81-86 (1959).

2. Udenfriend, S., Creveling, C.R., Posner, H., Redfield, B.G., Daly, J., and Witkop, B. On the Inability of Tryptamine to Serve as a Precursor of Serotonin. Arch. Biochem. and Biophys., 83: 501-507, 1959.

3. Udenfriend, S. Psychochemistry. Symposium on "A Pharmacologic Approach to the Study of the Mind", San Francisco, Calif., Jan. 1959. In Press.

4. Udenfriend, S. Biochemistry of Serotonin and Other Indoleamines, in Vitamins and Hormones, vol. 17, New York, N.Y., Academic Press, Inc. In Press.

Serial No. NHI-213
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Amines in Human Urine and Their Biogenesis

Principal Investigator: John B. Jepson

Other Investigators: None

Cooperating Units:
Dr. J. Oates, Dr. A. Sjoerdsma, and Miss P. Zaltzman, Section of Experimental Therap., NHI

Man Years:
Total: 1.0
Professional: 1.0
Other:

Patient Days:

Project Description:

The presence of tryptamine in urine and the demonstration of its formation by tryptophan decarboxylation prompted an investigation of the metabolism of the amine. It was known from Japanese reports that tryptamine was hydroxylated by liver microsomes. However, careful study has indicated that the sole product is 6-hydroxytryptamine and not the 7-hydroxy derivative or serotonin, as had been reported by the Japanese investigators. Rabbit and rat liver microsomes readily formed 6-hydroxytryptamine in the presence of tryptamine and TPNH. Apparently microsomes hydroxylate a large number of hydroxyindoles in the 6 position. Indoleacetic acid (IAA) was found to be an excellent substrate. As for in vivo studies, IAA was shown to yield appreciable amounts of 6-hydroxy IAA upon oral administration to man. Conversion of skatole to 6-hydroxy skatole was reported by Horning and Dalgliesh and administered dimethyl tryptamine was shown to yield 6-hydroxy dimethyltryptamine by Zara. These conversions were also found to be catalyzed by liver microsomes. However, it was not possible to demonstrate any 6-hydroxytryptamine in urine of animals or patients given tryptamine, even after inhibition of monoamine oxidase. Pharmacologically, 6-hydroxytryptamine is a much weaker agent than serotonin.

A procedure for the identification of amines in human urine was developed. This involves preliminary chromatography on a cation exchange column, elution, evaporation, desalting and two dimensional chromatography on paper. When chromatograms from normal urines were sprayed with ninhydrin 25-30 spots appeared. Thus far only a few have been identified,

since they react with phenol reagents. In addition to the tyramine and the O-methyl catecholamines normal urine contains ortho-tyramine and meta-tyramine. The tyramines are not formed through bacterial action and are not dietary in origin. Their biogenesis and significance is under investigation. The excretion of most of these amines is markedly increased following administration of monoamine oxidase inhibitors.

Of great interest was the finding of large amounts of phenylethylamine in the urines of two phenylketonurics on monoamine oxidase inhibitors. The amounts excreted were from 2 to 5 mg/day compared to $<$100 µg for normals. Phenylalanine decarboxylation occurs in brain and phenylethylamine is pharmacologically active (amphetamine like). This represents the first demonstration of the formation of abnormal amounts of a pharmacologically active agent in this mental disorder.

Studies on the identification of the other urinary amines are continuing.

Part B included: Yes

Serial No. NHI-213

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Sjoerdsma, A., Lovenberg, W., Oates, J., Crout, R., and Udenfriend, S. Alterations in the Pattern of Amine Excretion in Man Produced by a Monoamine Oxidase Inhibitor. Science, 130: 225, 1959.

2. Sjoerdsma, A., Oates, J., Zaltzman, P., and Udenfriend, S. Identification and Assay of Urinary Tryptamine: Application as an Index of Monoamine Oxidase Inhibition in Man. J. Pharmacol. and Exptl. Therap., 126: 217-222, 1959.

3. Jepson, J.B., Lovenberg, W., Zaltzman, P., Oates, J., Sjoerdsma, A., and Udenfriend, S. Amine Metabolism, Studied in Normal and Phenylketonuric Humans by Monoamine Oxidase Inhibition. Biochem. J. In Press.

Serial No. NHI-214
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Uptake of α-Amino Acids by Mammalian Cells

Principal Investigator: Sidney Udenfriend

Other Investigators: None

Cooperating Units:
Drs. G. Guroff, P. Greengard, and M. Chirigos, Geigy Chemical Corp.
Drs. J. Oates and A. Sjoerdsma, Section of Experimental Therap., NHI

Man Years:
Total: 0.33
Professional: 0.33
Other:

Patient Days:

Project Description:

The manner in which nutrients penetrate into cells is currently attracting great interest among biochemists and physiologists. Our interest in amino acid metabolism has made it desirable to study their penetration into various cells of the body.

Interesting information has been obtained on the uptake of tyrosine into the central nervous system. This amino acid was administered to rats (100 mg. per animal intraperitoneally) and its level in the blood was found to rise rapidly to 10 times normal values maintaining almost constant values for a period of up to two hours. At selected intervals during this period individual animals were killed and brain and plasma levels were determined with a procedure specific for tyrosine. (It should be pointed out that little or no metabolism of L-tyrosine takes place in brain during the intervals used). It has been possible in this way to demonstrate that 1) tyrosine uptake into brain is as rapid as into other tissues, 2) the endogenous ratio of brain tyrosine to plasma tyrosine (Br/Pl) is about 1.6; when the levels are increased over ten fold the endogenous ratio of Br/Pl is reestablished. 3) The entry of L-tyrosine is much more rapid than that of the D-isomer and the final value of Br/Pl for D-tyrosine is 1/5 - 1/10 that of the L-isomer. 4) The penetration of non-amino acid congeners of tyrosine (tyramine and p-hydroxyphenylacetic acid) is much less than that of the amino acid and no relationship exists between lipid solubility and penetration. 5) Amino acids such as tryptophan, fluorophenylalanine, leucine, valine, and isoleucine inhibited uptake of tyrosine into brain. In fact, with tryptophan at blood levels 5 times that of tyrosine, uptake was completely inhibited for a period of one hour. All these findings are consistent

with a catalytic process for penetration of L-tyrosine from the blood into the central nervous system.

When similar invivo studies were carried out with leg muscle we were surprised to find little evidence of a catalytic process for penetration.

In view of the in vivo findings on the penetration of tyrosine and its structural relatives into various tissues, the investigation of in vitro systems has begun, in order to permit the measurement of uptake at constant external concentration and under a great variety of conditions and free of hormonal influences. The "intact" diaphragm preparation of Kipnis and Cori offers several unique advantages for permeability studies. Among these are ease and speed of preparation, reproducibility of slice, and absence of cut cells in the slice itself. The preparation has been shown to retain its cellular characteristics during incubation and recent work has indicated that excised diaphragm remains metabolically active for several hours through utilization of endogenous sources.

Interestingly enough tyrosine does not appear to be taken up by means of so-called active mechanisms in the diaphragm. Low temperature coefficient (Q_{10} 1.3-1.5), lack of demonstrable pH dependence, distribution ratios less than unity, and failure to demonstrate competitive inhibition indicate a lack of enzymatic participation. It would appear that a diffusion mechanism can account for all the observations thus far except for a somewhat slower uptake of D-tyrosine. The failure of the diaphragm to lose tyrosine when suspended in buffer gave rise to speculation about the state of endogenous tyrosine. Preliminary isotope studies have indicated that uptake of tyrosine does not lead to equilibration with the endogenous pool of "free" amino acid.

The in vitro study will be extended to a consideration of other tissues with immediate plans for brain and spleen. The latter tissue is of interest due to the finding of a large concentration and an active uptake of L-tyrosine by this tissue in vivo. Another objective of this program is to determine the effect of structural modification of amino acids on their uptake into tissues. It is hoped that the study will yield results of interest both biochemically and pharmacologically.

It was shown that little or no conversion of D-tyrosine to L-tyrosine occurs in rats in vivo. An initial experiment indicates that the same is true for D-tryptophan.

An attempt was made to determine whether the high levels of phenylalanine in the blood of a phenylketonuric patient influences the rate of uptake of tyrosine into spinal fluid. The findings indicate that even if this were true lumbar puncture is not the way to approach this problem.

Part B included: Yes

Serial No. NHI-214

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Chirigos, M.A., and Udenfriend, S. A Simple Fluorometric Procedure for Determining Salicylic Acid in Biological Tissues. J. Lab. Clin. Med., 54: 769-772, 1959.

Serial No. NHI-215
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Physiologically Active Amines in Forms of Life Other than Mammals

Principal Investigator: Sidney Udenfriend and Herbert Weissbach

Other Investigators: None

Cooperating Units:
Mr. W. Lovenberg, United Fruit Company and Section of Experimental Therap., NHI
Dr. E.S. Kline, Walter Reed Army Medical Center

Man Years: Patient Days:
Total: 0.5
Professional: 0.5
Other:

Project Description:

Following the demonstration of serotonin and catecholamines in bananas it became of interest to determine how widespread was the distribution of physiological amines among edible plants. It was shown that serotonin, tryptamine, tyramine, dopamine, and norepinephrine are found in many ordinary fruits and vegetables such as plums, potatoes and oranges. However, the banana is still the richest source of these amines. This part of the project is essentially finished.

Another interesting finding relates to hydra. The "stinging organ" (nematocysts) of hydra is known to produce a potent toxin which rapidly kills the various organisms that the hydra utilizes for food. It was shown by Dr. Kline that this material is a protein, and an inhibitor of succinoxidase. Lower animal forms are known to contain 5-hydroxyindoles, and it was of interest to see if hydra also contained 5-hydroxyindoles. Large amounts of hydroxyindole material were found localized in the nematocysts and were found to be released with the toxin. The purified toxin, however, did not contain any 5-hydroxyindole material. The hydroxyindole has been shown to be basic and is very likely either serotonin or one of its N-methylated derivatives. The latter point has not been ascertained.

Part B included: Yes

Serial No. NHI-215

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Udenfriend, S., Lovenberg, W., and Sjoerdsma, A. Physiologically Active Amines in Common Fruits and Vegetables. Arch. Biochem. and Biophys. In Press.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Epinephrine, Norepinephrine and Related Compounds

Principal Investigator: Sidney Udenfriend

Other Investigators: Cyrus R. Creveling

Cooperating Units:
Drs. B. Witkop and M. Ozaki, Laboratory of Chemistry, NIAMD
Dr. R. Crout, Section of Experimental Therap., NHI

Man Years:
Total: 1.33
Professional: 0.33
Other: 1.0

Patient Days:

Project Description:

Studies on the biogenesis of norepinephrine have been temporarily discontinued to permit investigation of some of the degradative steps.

Norepinephrine in vitro can be either oxidatively deaminated or methylated. Both in animals and man, parenterally administered epinephrine and norepinephrine are largely metabolized through the action of O-methyl transferase. A simple nonisotopic procedure for measuring both the rates of metabolism of norepinephrine and of the appearance of the deamination and methylation products has shown that methylation is indeed a major route of metabolism of norepinephrine in the intact mouse. Several compounds have been found which markedly reduce the formation of normetanephrine without prolonging the chemical half-life of norepinephrine proportionately. These compounds are all methyl acceptors themselves and include pyrogallol, catechol, and glycocyamine. The first compound is the most active and has permitted further investigations on the relationship between monoamine oxidase (MAO) and catechol-methylpherase as pathways for metabolism of norepinephrine in various tissues. Using inhibitors of MAO and methylation it was shown that only the former can elevate levels of norepinephrine in rat brain and heart. Secondly, norepinephrine was administered intravenously to rats pretreated with either MAO inhibitors or methylation inhibitors. In both cases the amounts of norepinephrine in the heart were markedly elevated over untreated controls. However, blood levels were only elevated when methylation inhibitors were used. All these findings indicate that the major route of norepinephrine metabolism in sympathetically innervated organs (heart, brain, etc.) is oxidation catalyzed by MAO. Methylation disposes of that portion of catecholamines which passes into the blood and then through the liver. It is most likely then that MAO inhibitors do influence sympathetic activity in vivo. As for methylation inhibitors these studies suggest that they act mainly by preventing detoxication in the liver.

Part B included: Yes

Serial No. NHI-216

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Sjoerdsma, A., Leeper, L.C., Terry, L.L., and Udenfriend, S. Studies on the Biogenesis and Metabolism of Norepinephrine in Patients with Pheochromocytoma. J. Clin. Invest., 38: 31-38, 1959.

2. Senoh, S., Witkop, B., Creveling, C.R., and Udenfriend, S. 2,4,5-Trihydroxyphenethylamine, A New Metabolite of 3,4-Dihydroxyphenethylamine. J. Amer. Chem. Soc., 81: 1768, 1959.

3. Senoh, S., Witkop, B., Creveling, C.R., and Udenfriend, S. Oxidation Mechanisms of Catecholamines and the Biogenesis of Noradrenaline. Proc. of the IV Internat'l. Congress of Biochemistry, Vienna, Sept. 1-6, 1958, vol. 13, Colloquium on Oxygenating Enzymes.

4. Udenfriend, S., Creveling, C.R., Ozaki, M., Daly, J., and Witkop, B. Inhibitors of Norepinephrine Metabolism in vivo. Arch. Biochem. and Biophys., 84: 249-251, 1959.

5. Udenfriend, S. Survey of Chemical and Physical Methods for Measuring Catecholamines. Symposium on Catecholamines (Supplement to Pharmacol. Revs.), 11: 252-255, 1959.

6. Udenfriend, S., and Creveling, C.R. Localization of Dopamine-β-Oxidase in Brain. J. Neurochem. In Press.

7. Senoh, S., Creveling, C.R., Udenfriend, S., and Witkop, B. Chemical, Enzymatic and Metabolic Studies on the Mechanism of Oxidation of Dopamine. J. Amer. Chem. Soc. In Press.

Serial No. NHI-217
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biosynthesis of Choline

Principal Investigator: Kenneth D. Gibson

Other Investigators: Jean D. Wilson and John J. Pisano

Cooperating Units: None

Man Years:
Total: 1.7
Professional: 1.7
Other:

Patient Days:

Project Description:

Although much is known about the enzymic degradation of the humoral agent acetyl choline we know little about its overall synthesis except what has been obtained from experiments in intact animals.

Previous investigations in vivo have shown that choline is synthesized by methylation of ethanolamine. It is generally believed that two of the methyl groups are derived from the one carbon fragment pool, while the third is transferred from methionine. Rat liver slices were found to incorporate C^{14} from uniformly labelled ethanolamine and methyl-labelled methionine into phospholipid choline, in confirmation of previous work. Liver slices also incorporated C^{14} from uniformly labelled serine into phospholipid choline. The incorporation from methyl-C^{14} methionine into phospholipid choline also occurred in liver homogenates; it was stimulated by the addition of a mixture of cofactors. Incorporation has also been shown from C^{14} methionine into added phosphorylcholine; the latter was isolated by hydrolysis to choline and crystallization as the reinechate. The incorporation into phosphorylcholine was catalyzed to a small extent by rat liver particles and by the soluble portion of rat liver. However, particles and soluble protein together acted synergistically.

Current research is directed towards analyzing the above reaction and determining what part it plays in the biosynthesis of choline, and investigation of the intermediates in choline biosynthesis from ethanolamine and serine.

Part B included: No

Serial No. NHI-218
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Collagen Turnover

Principal Investigator: Sven Lindstedt

Other Investigators: Beverly Peterkofsky

Cooperating Units:
Dr. D. Prockop, Section of Experimental Therap., NHI

Man Years:
Total: 1.33
Professional: 0.33
Other: 1.0

Patient Days:

Project Description:

In the past year we have developed a simple method for the quantitative measurement of hydroxyproline in urine and other biological materials. Like several previous methods this one involves the oxidation of hydroxyproline to pyrrole and then a color reaction with Ehrlich's reagent. Earlier methods, however, were limited by a) materials other than pyrrole which interfered with the color reaction b) materials in urine and tissue preparations which drastically affect the yield of pyrrole in the oxidation step. We feel we have insured specificity of the color reaction by extracting the pyrrole into toluene before the color reaction. Since the first oxidation product of hydroxyproline is not soluble in toluene under alkaline conditions, we can remove any interfering substances which might be soluble in toluene by extracting the aqueous phase with toluene before the final formation of pyrrole. In addition we have stabilized the oxidation step by oxidizing hydroxyproline in the presence of a large excess of alanine. The alanine acts as a kind of "oxidation buffer," and overcomes the variable effects which solutes other than hydroxyproline may have on the oxidation potential.

By modifying our quantitative method for hydroxyproline we have developed a simple technique for determining the specific activity of carbon-14 or tritium labelled hydroxyproline in urine and tissues. As described above, the hydroxyproline is oxidized to pyrrole and the pyrrole extracted into toluene. An aliquot of the toluene is reacted with Ehrlich's reagent to determine the amount of pyrrole present and the remainder is mixed with a phosphor and counted directly in a liquid scintillation counter. The technique makes it possible for the first time to determine the specific activity of a large number of hydroxyproline samples simultaneously, and it is sensitive enough to follow urinary hydroxyproline specific activity for over three months after a small injection of proline-C^{14} into a rat.

We have applied our technique for hydroxyproline specific activity to following urinary hydroxyproline-C^{14} in the rat after a single injection of proline-C^{14}. The excretion curve for hydroxyproline-C^{14} demonstrates at least three distinct components, and each of these probably represent a separate compartment of body hydroxyproline. The first component of the excretion curve has a rapid turnover, and is probably accounted for by the small amount of free hydroxyproline present in urine. Since nearly all the hydroxyproline of the body is found in collagen, the origin of this fraction is uncertain, but it probably represents a small amount of direct hydroxylation of proline. The second component has a half-life of 5 to 10 days, and probably represents a fraction of body collagen which turns over at a very rapid rate. Preliminary experiments indicate that the second component is not found in the excretion curves of old animals, and rats of the same weight but different ages appear to have varying amounts of this component. The third component of the excretion curve has a half-life of 100 to 300 days, and probably reflects the turnover of the relatively inert deposits which form the bulk of body collagen.

The simple quantitative method for hydroxyproline makes it possible to study the effects of various experimental conditions on the total excretion of hydroxyproline. We plan to measure urinary hydroxyproline in conditions which drastically affect the amount of body collagen, such as carrageenin tumors, serum sickness, lathyrism. We will attempt to further identify the three pools of hydroxyproline revealed by the urinary excretion patterns of hydroxyproline-C^{14} referred to above.

Using our technique for urinary hydroxyproline specific activity we will study the effect of various experimental conditions on the excretion pattern of hydroxyproline-C^{14}.

Experiments on the enzymatic conversion of proline to hydroxyproline are now underway (see separate report).

We plan to undertake a study of collagenase, the enzyme which destroys collagen. The study would be directed toward isolating the enzyme from mammalian tissues and studying its properties.

Part B Included: No

Serial No. NHI-219
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biosynthesis of γ-Guanidinobutyric Acid

Principal Investigator: John Pisano

Other Investigators: David Abraham

Cooperating Units: None

Man Years:
Total: 0.7
Professional: 0.2
Other: 0.5

Patient Days:

Project Description:

In the previous report the biosynthesis of γ-guanidinobutyric acid from γ-aminobutyric acid and arginine was established. Transamidination was shown for the first time to occur in brain but the activity of this tissue was only 1/40 the activity of kidney or pancreas. While the evidence for transamidination in brain involving glycine was convincing, transamidination with γ-aminobutyric acid was more difficult to demonstrate in this tissue because this amino acid was only 1/10 as active as glycine.

Using purified transamidinase from pig kidney, it has been shown that β-guanidinopropionic acid and δ-guanidinovaleric acid are synthesized from β-alanine and δ-aminovaleric acid. The two guanidino acids have recently been reported to occur in rat liver and urine and human urine.

Present research is directed toward the purification of brain transamidinase in order to have enough enzyme to consistently demonstrate transamidination involving γ-aminobutyric acid. This goal now appears to be certain of attainment and the project will be completed within a few months.

Part B included: No

Serial No. MHI-220
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on a Bound Form of γ-Aminobutyric Acid in Brain

Principal Investigator: Jean D. Wilson and John J. Pisano

Other Investigators: None

Cooperating Units:
Dr. L.A. Cohen, Laboratory of Chemistry, NIAMD

Man Years:
Total: 0.7
Professional: 0.7
Other:

Patient Days:

Project Description:

Coenzyme A was isolated from beef brain in order to determine whether the previously described homopantothenic acid of brain might substitute in part for the β-alanine moiety. While coenzyme A isolated from brain contained no γ-aminobutyric acid a fraction was found in the course of this isolation which upon hydrolysis did yield γ-amino-butyric acid. Attempts have been made to assay this compound in various tissues and to characterize its chemical structure. While not present in appreciable quantities in beef liver or dog liver, kidney, muscle, or spleen, it has been found in the brain of dog, pig, and beef at levels from 300-9000 μg/kilo. As much as 600 micromoles of the material has been partially purified on charcoal and Dowex 50 columns and by paper electrophoresis. It is a peptide which contains histidine and γ-amino-butyric acid in what appears to be a one to one ratio. Although small amounts of other amino acids were present in this preparation it is currently believed that the peptide is the γ-aminobutyric acid analogue of carnosine. The compound was synthesized and thus far appears to be the same as the isolated material.

Current research is directed toward ultimate purification and positive identification of this compound and toward developing a simple method of analysis so that an evaluation of its physiological properties can be undertaken. Pharmacological activity of the isolated product and synthetic analogues (if any are made) will be investigated.

Part B included: Yes

Serial No. NHI-220

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Pisano, J.J., Wilson, J.D., and Udenfriend, S. γ-Aminobutyric Acid as a Possible Precursor of Other Physiological Substances. Symposium on Inhibition in the Nervous System and γ-Aminobutyric Acid, Los Angeles, California, May 1959. In Press.

Serial No. NHI-221
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Reports
Calendar Year 1959

Part A.

Project Title: Chemical Determination of the Metabolites of Epinephrine and Norepinephrine

Principal Investigator: John J. Pisano

Other Investigators: David Abraham

Cooperating Units: None

Man Years:
Total: 0.7
Professional: 0.2
Other: 0.5

Patient Days:

Project Description:

With the discovery that m-O-methyl-epinephrine, m-O-methylnorepinephrine and 3-methoxy-4-hydroxymandelic acid are the major metabolic end products of the catecholamines, the chemical determination of these compounds in urine should give an index of the overall catecholamine production in health and disease.

A simple method has been developed for the analysis of combined m-O-methylepinephrine and m-O-methylnorepinephrine in urine. The method involves the adsorption of the urinary metabolites on Amberlite CG-50, followed by elution and conversion of the metabolites to vanillin which is assayed spectrophotometrically.

The method has been applied to the diagnosis of pheochromocytoma and provides for the first time a simple test for the chemical diagnosis of this disease. Normal values are 0.6 ± 0.3 mg/day and in 8 patients with pheochromocytoma the values have averaged 25 mg/day, and ranged between 3 and 113 mg/day.

A method is also under development for the determination of 3-methoxy-4-hydroxymandelic acid, the oxidation product of the methyl adrenalines. This compound which is found in urine in larger amounts than the methylated amines, also reacts with periodate to form vanillin but the reaction is inhibited by certain urinary compounds.

Current research deals with an evaluation of solvent extraction and ion-exchange procedures as a means of purification of 3-methoxy-4-hydroxy-mandelic acid from urine prior to reaction with periodate to form vanillin. This work should be completed within this calendar year.

Part B included: Yes

Serial No. NMI-221

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Pisano, J.J. A Simple Analysis for Normetanephrine and Metanephrine in Urine. Clin. Chim. Acta. In Press.

Serial No. NHI-222
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biogenesis and Metabolism of Hydroxyproline

Principal Investigator: Chozo Mitoma

Other Investigators: Thomas E. Smith and Frances M. DaCosta

Cooperating Units: None

Man Years:
Total: 1.83
Professional: 0.33
Other: 1.5

Patient Days:

Project Description:

It is known that ascorbic acid deficiency affects collagen synthesis. However, the mechanism(s) whereby it is affected is somewhat obscure and current speculations span the spectrum from hydroxylation of proline and lysine to organization of the collagen molecule. Experiments in this laboratory during the last year have established that normal amounts of hydroxyproline are formed in the scorbutic guinea pig when collagen synthesis is markedly decreased. This would rule out ascorbic acid as having a direct role in hydroxyproline formation.

Ketoproline is known to occur as part of the actinomycin molecule in certain types of actinomyces. Studies have been conducted to show that the normally occurring ketoproline is probably of the L configuration. It has also been shown that those cultures which can form the ketoproline containing actinomycins convert ketoproline to hydroxyproline.

The enzyme responsible for the conversion of ketoproline to hydroxyproline has been purified about 30 fold from the supernatant fraction of rabbit kidney. Of a wide variety of substrates tested, the enzyme appears relatively specific for ketoproline.

Further attempts will be made to establish the configuration of ketoproline as found in actinomycins.

The enzyme involved in the conversion of ketoproline to hydroxyproline will be further purified and studied.

Part B included: Yes

Serial No. NHI-222

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Publications

1. Mitoma, C., Smith, T.E., DaCosta, F.M., Udenfriend, S., Patchett, A.A., and Witkop, B. Studies on 4-Keto-L-Proline. Science, 129: 95-96, 1959.

2. Mitoma, C., Smith, T.E., Friedberg, F., and Rayford, C. Incorporation of Hydroxyproline into Tissue Proteins by Chick Embryos. J. Biol. Chem., 234: 78-80, 1959.

3. Mitoma, C., Smith, T.E., Davidson, J.D., Udenfriend, S., DaCosta, F.M., and Sjoerdsma, A. Improvements in Methods for Measuring Hydroxyproline: Application to Human Urine. J. Lab. Clin. Med., 53: 970-976, 1959.

4. Mitoma, C., and Smith, T.E. Studies on the Role of Ascorbic Acid in Collagen Synthesis. J. Biol. Chem. In Press.

Serial No. NHI-223
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Oxidative Pathways of γ-Aminobutyric Acid (GABA) Metabolism

Principal Investigator: Chozo Mitoma

Other Investigators: Sven Lindstedt and John J. Pisano

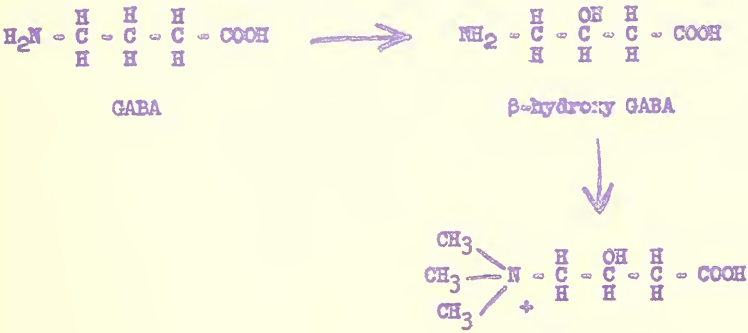
Cooperating Units: None

Man Years:
Total: 0.86
Professional: 0.86
Other:

Patient Days:

Project Description:

The presence of GABA in brain has excited interest in studies concerning its metabolism. The following pathway of GABA metabolism is suggested by the presence of carnitine in tissues:



GABA

β-hydroxy GABA

carnitine

The isolation of β-hydroxy GABA from brain has been reported as well as a demonstration of its enzymic formation from GABA. However, using C^{14} labelled GABA and with added β-hydroxy GABA as a trap, we have been unable to verify this. Furthermore, using C^{14} labelled β-hydroxy GABA and isotope dilution procedures it has been impossible even to detect this amino acid in brain or any tissue. Dr. Tallan of the Rockefeller Institute, utilizing β-hydroxy GABA which we sent him as a standard, was also unable to demonstrate this amino acid in brain.

When C^{14} labelled GABA, β-hydroxy GABA and glutamic acid were administered to rats the carnitine isolated from urine and tissues was found to contain no radioactivity. It would appear, therefore, that this compound arises in a manner other than the one shown above.

Further studies on carnitine formation and metabolism will be continued by Dr. Lindstedt on his return to Sweden.

Part B included: No

Serial No. NHI-224
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Metabolism of Hydroxylysine

Principal Investigator: Sven Lindstedt and Chozo Mitoma

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.65
Professional: 0.66
Other:

Patient Days:

Project Description:

Like hydroxyproline, hydroxylysine is also a bodily substituent which is found only in collagen. In previous studies it was shown that adapted Achromobacter metabolized hydroxyproline extensively. This same organism was also found to metabolize hydroxylysine and the product was identified as 5-hydroxypipecolic acid. With lysine as substrate little pipecolic acid was found since it was further metabolized to adipic acid. Using the bacteria it was possible to make C^{14} labelled hydroxypipecolic acid and to study its metabolism in animal tissues. It was found that unfortified mitochondria readily convert 5-hydroxypipecolic acid to 5-hydroxyadipic acid. The latter reaction has not been demonstrated in animal tissues before. The metabolism of the collagen substituent will be investigated further and attempts will be made to find these metabolites in urine.

Part B included: No

3 Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Renal Concentrating Mechanism

Principal Investigator: George A. Bray
(under supervision of Dr. Robert W. Berliner)

Other Investigators: None

Man Years: Patient Days: None

Total:	1
Professional:	1
Other:	0

Project Description -

Objectives: The project has been concerned with studying the problem of renal concentration using various methods, among which have been included radioautography, freezing and thawing of kidney slices, clearance studies and use of isotopes.

Progress during the past year: This has been in several fields which will be considered separately.

1. The ability of nephrotic rats to concentrate urine has been studied. A group of rats were examined for their maximal concentrating ability before and after the administration of rabbit anti-rat kidney serum. The induction of the nephrotic syndrome was shown to diminish the maximal urine concentration.
2. Radioautographs were used in several ways:
   a. C-14 urea, C-14 sucrose were given to concentrating rats and their kidneys removed and autographs made. The resolution was inadequate to allow interpretation.
   b. H-3 Diamox. Attempts to localize carbonic anhydrase with tritium labelled Diamox have thus far proved unsuccessful.
   c. Cl 36 localization in the stomach was studied in sections of frog stomach. Initial radioautographs showed suggestive localization in the region of the parietal cells, but subsequent attempts have been unsuccessful.
3. Isotopic reflux in the collecting ducts; an attempt was made to study the rate of diffusion of H-3 water and C-14 urea into the renal parenchyma from a solution of isotope injected into the renal pelvis under pressure after occluding the renal blood flow. The variation from one experiment to the next was too great to permit interpretation.
4. Medullary blood flow; the possibility of estimating renal medullary blood flow by measuring the appearance of H-4 water in the urine was investigated. The problem of recirculation of isotope and a rising blood level of tracer made interpretation of the results impossible.
5. Measurement of thawing of rat kidney slices; a modified approach to the measurement of the osmotic pressure in kidney slices as introduced

Part B included: No.

by H. Wirz has been used to study the renal concentrating ability. The following qualitative observations have been made. (Some quantitative observations have also been made, but more work is needed to adequately calibrate the temperature recording system.)

a. In all slices of the inner medulla, regardless of the distance from the papilla, the collecting ducts are seen to be the last elements to thaw, regardless of the rate at which the bath they are in is warmed up.
b. In one set of experiments with a water loaded rat, the loop of Henle was seen to thaw before the cortex, while collecting ducts and "distal tubules" melted later (i.e., had a lower osmotic pressure)
c. In sections of the outer medulla, the vascular bundles are the last elements to thaw, but at the time all of the elements in the inner medulla have thawed there are still numerous tubules scattered throughout the cortex down to the junction of the outer medulla and the cortex.

6. The observation that ureteral obstruction leads to a decreased urine concentration has been investigated in collaboration with Dr. John Jacmike.

Direction of Current Research:

1. The problem of the thawing of kidney slices is being pursued with the idea of obtaining quantitative values for the osmotic pressure of the tubules when they thaw.
2. The problem of ureteral obstruction still needs further work to elucidate the mechanism by which this occurs.
3. Tritiated Diamox is being resynthesized to study the binding of Diamox to carbonic anhydrase in association with Dr. Robert W. Berliner.
4. Plans are in progress to study the effect of lactate and other such substances on the renal excretion of uric acid.

Incidental findings of significance: None.

Serial No. MMY-226
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Determination of the true chloride content of tissues.

Principal Investigator: Ernest Cotlove

Other Investigators: None

Man Years:
Total: 1/2
Professional: 1/2
Other: 0

Patient Days: None

Project Description -

Objectives: Chloride is known to be the predominant anion present in extracellular fluid, but its concentration in cell fluid has been very uncertain. Reliable information on intracellular chloride would be important in understanding the behavior of electrolytes in the body. The difficulties in determining intracellular chloride have been two-fold: (1) the lack of a reliable criterion for evaluating various analytic methods for total tissue chloride which have yielded widely disparate results; and (2) the lack of a reliable method for estimating extracellular volume, and thereby the fractions of total tissue chloride which are extracellular and intracellular.

Progress during the past year:

The isotope dilution method of determining the true chloride of tissues, as described in the previous annual report, has been employed in the analysis of additional animal tissues, and has been used as a standard of reference for evaluation of simplified methods of analysis of tissues for chloride. The development of reliable, simplified, non-isotopic methods is necessary to enable other investigators to measure true chloride under a wide variety of experimental conditions. The isotope dilution method requires a relatively large amount of tissue for each analysis, requires specialized equipment and experience, and is extremely limited in the number of samples that can be analyzed at one time.

Part B Included: No.

Although the simplified analytic methods described in the last report appeared to be adequate, further experience showed a disturbing, erratic variability in the results, and failed to confirm initial results suggesting protein binding of chloride with apparent release by phosphate ion. The variability in results was traced to two factors, both related to the use of very small aliquots of tissue, as would be necessary under many experimental conditions: (1) inhomogeneity of these small aliquots of dried, pulverized tissue, especially in the relative amounts of connective tissue fragments (which have high Cl content); and (2) variable absorption (1 to 6% of dry weight) of water vapor due to the exposure of most of the surface of these small, extremely hygroscopic samples in a paper cup when weighing, even when this was accomplished rapidly. The variable and erroneous results arising from these factors could be avoided by: (1) finer pulverization of dried tissue, sieving through a 350 micron mesh, and careful mixing before sampling; and (2) weighing of aliquots into glass tubes used for extraction or digestion, with reweighing after further drying at 105° C. (tubes being glass-stoppered when out of oven or desiccator).

With these precautions in handling small tissue samples, the results of two simplified methods were found to correspond consistently and favorably with the isotope dilution procedure, namely: (1) extraction of dry tissue powder with 0.75 N nitric acid; and (2) digestion of tissue in hot dilute sodium hydroxide, protein precipitation with zinc hydroxide, and oxidation of interfering sulfhydryl groups with alkaline perborate, followed by titration with the automatic chloride titrator previously described. With these methods, the usual procedure requires only about 5 microequivalents of Cl in a tissue sample, yielding results reproducible within about 1%; but even smaller amounts, down to 0.3 microequivalent can be analyzed, though with decreasing precision.

The results of these two simplified methods of Cl analysis of dried tissues (and of serum by direct dilution), and those of the isotopic method, compare almost exactly (within about 1%), using the specific activity values through the oxidation-reduction stage of purification.

Incidental findings of significance:

In the isotope dilution analysis, there is a nearly uniform reduction in specific activity of 1-2% during the last stage of purification; reaction of concentrated sulfuric acid with dried alkaline chloride, liberating HCl gas which diffuses into dilute sodium hydroxide solution and is trapped as NaCl. The reduction in specific activity occurred both with tissue samples and NaCl standards (analytical grade reagent). Experimental results indicate that this finding cannot be explained by: (1) contamination with non-radioactive chloride due to reagents used in the diffusion step; (2) chemical impurity in the NaCl standard; (3) radiochemical impurity in the Cl-36 used; or (4) consistent analytic error in radioassay or in titration, in so far as this could be tested. The finding has eluded explanation, and the possible significance is uncertain.

Direction of current research:

Studies will be undertaken on the second phase of this project, through the use of C-14 labeled inulin and sucrose to measure extracellular space in rat tissue, which when combined with measuremants of total, true chloride of tissues would enable estimation of intracellular chloride.

Serial No. MKI-227
1. Kidney and Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Localization of Chloride Transport in the Gastric Mucosa.

Principal Investigator: Ernest Cotlove

Other Investigator: C. Adrian M. Hogben, Professor of Physiology and Chairman of Department of Physiology, George Washington University School of Medicine.

Man Years:
Total: 1/2
Professional: 1/2
Other: 0

Patient Days: None

Project Description -

Objectives: The secretion of hydrochloric acid by the stomach has been a subject of considerable interest for many years. Recent studies have shown that the isolated frog gastric epithelium actively transports chloride ions from nutrient to luminal solutions against an opposing electrochemical gradient. A comprehensive formulation of the mechanism of chloride secretion is still to be established, but certain specific features of chloride transport have been defined.

Chloride ion movement across the gastric mucosa has been shown to occur by active transport, by exchange diffusion, and by passive diffusion. In an earlier annual report, it was shown that chloride movement was at least five times faster across the luminal than the nutrient membrane of the epithelial cell, suggesting the localization of active chloride transport at one of these cell surfaces. This hypothesis was examined in the present study by measuring the effect on chloride movement of metabolic inhibition by 2,4 dinitrophenol, which reversibly inhibits active transport and exchange diffusion and has relatively little effect on passive chloride diffusion or total mucosal conductivity.

Progress during the past year:

The procedure described previously was employed, in which radioactive Cl-36 is placed into the nutrient bathing solution of one half of the isolated bull frog gastric mucosa and into the luminal solution of the other half, with measurement in the steady state of the two transmucosal fluxes of chloride, and of the specific activities of the

Part B included: No.

solutions of origin and the mucosal cells. From these measurements are derived the four values of cell influx and outflux at opposite cell surfaces.

In a series of control mucosae, when only the luminal solution contained Cl-36, the cell specific activity reached a high steady-state value; on the other hand, when only the nutrient solution was tagged, the cell specific activity was very low. The chloride turnover at the luminal membrane was over ten times faster than at the nutrient membrane, confirmation of earlier results. Under metabolic inhibition by 2,4-dinitrophenol (DNP), at concentrations of 6×10^{-5} M or 10^{-4} M in the nutrient solution, the cell specific activity results were reversed; the ratio of influx at luminal to influx at nutrient surfaces was altered from the control value of 16 to values of 0.3 and 0.2, respectively. The cellular fluxes at the nutrient membrane showed little change, but the fluxes at the luminal membrane showed a decrease of almost fifty-fold. The spontaneous potential and the active transport of chloride were abolished, and the transmucosal fluxes in both directions were reduced.

The inhibition of chloride flux by DNP is thus limited to the luminal cell membrane, reducing this flux much below that at the nutrient membrane, while the active transport of chloride is stopped and exchange diffusion is largely abolished. In the normal mucosa, therefore, the site of exchange diffusion must be largely or entirely at the luminal cell membrane. The inference is strong that the active transport of chloride is also at this membrane. In the DNP inhibited mucosa, the small luminal flux may still include a residual component of exchange diffusion, so that an even smaller value would be attributable to passive diffusion (permeation of ionic chloride). However, even if all the DNP inhibited flux at the luminal membrane is ionic chloride, it is still much lower than the nutrient flux, presumably also ionic. It would be advantageous for a chloride pump to be located at the luminal membrane with its relatively low permeability to ionic chloride.

Incidental findings of significance: None.

Direction of Current Research:

Preliminary studies, conducted in collaboration with Dr. George Bray of this Laboratory, have employed radioautography in an attempt to obtain direct confirmation of the inferred difference in cell fluxes and to localize the site of rapid chloride turnover and of active transport according to cell type: parietal cell or surface epithelial cell. Initial findings have been suggestively confirmatory, and more studies are planned.

Serial No. NHI-228
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on a mammalian cardiotonic protein system.

Principal Investigators: Stephen Hajdu
Edward Leonard

Other Investigators: None

Man Years: Patient Days: 120
Total: 2
Professional: 2
Other: 0

Project Description -

Objectives:

To collect information relevant to the physiological significance of the mammalian plasma cardiotonic protein system described in previous reports.

Progress during the past year:

1. Development of a quantitative assay for the components of the system. The system is comprised of 3 components, now called cardioglobulin A, B and C. Cardioglobulin B is the component which binds to the frog heart, cardioglobulin C contains the strongly bound calcium needed for activity, and the significance of A remains unknown except that it is needed for the activity of the system. Since any one of the factors could be limiting in determining the activity of the system, methods were developed for measurement of the concentration of each of the components. The general approach in measuring the concentration of one component was to determine the minimum amount of plasma required for activity in the presence of an excess of the other two components. This required:

a. Grading of the biological activity of the system. Since the system in sufficient concentration causes contracture, contracture of a given intensity was chosen as the assay end point, and a frog unit of any component was defined as follows: one frog unit of a component is that quantity required to cause an end-point contracture under standard assay conditions. The concentration of any component in terms of its biological activity is thus expressed in frog units (f.u.)/ml.

Part B included: Yes.

b. A stable source of cardioglobulin B. The fraction containing cardioglobulin B was prepared by sodium sulfate precipitation of human plasma and was rendered inactive with respect to the other components by dialyses against glycerol and against phosphate buffer at room temperature. Biological activity was maintained unchanged for periods as long as seven weeks by storage in dry ice.

c. A stable source of cardioglobulins A and C for assay of cardioglobulin B. Fresh plasma from male Sprague Dawley rats was used for this purpose since it contains negligible amounts of cardioglobulin B, but high concentrations of the other two components.

d. Unchanging sensitivity of the frog heart to the system. Hearts of frogs maintained under standard storage conditions at 12 degrees C for five weeks before use were uniform in their sensitivity to the system at least within any one season. The possibility of seasonal variations is under study.

II. Assay Results.

1. Relative ratios of the concentrations of the three components in human plasma. Approximate mean concentrations, in frog units/ml. are: cardioglobulin A, 5; B, 10; C, 2.5. Since the concentration of cardioglobulin A has always been observed to be well in excess of cardioglobulin C, determination of the minimum amount of plasma required for contracture of a heart in the presence of excess cardioglobulin B is always a measure of cardioglobulin C concentration.

2. Comparison of cardioglobulin C concentrations in patient groups. The results, expressed as the mean concentration in f.u./ml. are:

12 normals: 2.7, sigma .82.
8 hypertensives: 5.7, sigma 1.0.
8 aortic stenosis: 4.2, sigma 1.2.
14 patients with various diseases excluding above: 3.3, sigma 1.6.

The patient groups with hypertension and with aortic stenosis had significantly higher concentrations of cardioglobulin C than did the normals (hypertension vs. normal, p less than .001; aortic stenosis vs. normal, p less than .008).

3. Effect of lowering blood pressure: Administration of JB-516 to a patient with essential hypertension resulted in a lowering of blood pressure and a decrease in plasma concentration of cardioglobulin C. When the drug was stopped, blood pressure returned to normal levels and cardioglobulin C also increased.

4. Concentration of cardioglobulin C in congestive heart failure. Plasmas from 26 patients in congestive heart failure have been assayed. Very low concentrations of cardioglobulin C have been found in a number of plasmas from a group of 15 patients in whom no definite cause for the

failure had been determined. Thus 8 plasmas from this group had concentrations lower than the lowest value obtained in normal controls.

5. Cardioglobulin C in surgical patients. Plasma from 5 patients subjected to 30-45 minutes of extracorporeal circulation were assayed. When compared to the value found immediately after mixing patient and pump blood, the concentration of cardioglobulin C 3-5 hours after pumping was markedly diminished to 1/2 - 1/5 of the control. Values 24 hours after the operation were back to normal in 4 patients. In the fifth, who went into vascular collapse about 3 hours after operation and died despite vasopressor agents 27 hours postoperatively, the cardioglobulin C concentration at 24 hours was still about 1/5 the control. No significant change in cardioglobulin C was observed in two surgical patients not subjected to extracorporeal circulation (one mitral and tricuspid valvulotomy, one seven hour inguinal dissection for cancer).

Direction of current research:

Certain of the above observations provide circumstantial evidence suggesting that the system under study has something to do with cardiac function. Thus, cardioglobulin C concentration was increased in both hypertension and aortic stenosis, two conditions which appear to have little in common except for increased isometric tension developed by the left ventricle in systole. It was decreased in a patient with hypertension when systolic tension was decreased secondary to blood pressure lowering with JB-516. It could be postulated that this system is cardiotonic for the mammalian heart, is increased in compensatory fashion in the conditions mentioned, and if decreased by some pathological cause (idiopathic in the case of certain cases of congestive failure, iatrogenic in the case of the pump-oxygenator) might lead to myocardial insufficiency. This possibility will be examined further by (1) attempting to establish some meaningful correlations in cases of congestive heart failure with diminished cardioglobulin C; and by (2) observing the effects of destroying the cardioglobulin system in experimental animals.

Incidental findings of significance:

Cardioglobulin C concentrations have been found to be low in 4 out of a group of 6 patients with disseminated lupus erythematosus.

Part B.

Publications:

1) A serum protein system affecting contractility of the frog heart present in increased amounts in patients with essential hypertension. Circulation Research, 6:740, 1958

2) The Cellular Basis of Cardiac Glycoside Action. Pharmacological Reviews, 11:173, 1959

Serial No. NHI-289
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Active sodium transport and its metabolic substrate in human red cell ghosts.

Principal Investigator: Joseph F. Hoffman

Other Investigators: None

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description -

Objectives: The previous year's report summarizes the general aims of this research and discusses the advantages of employing the ghost system to meet these ends. It will be noted that this report deals with Na outflux whereas the former was concerned with K influx. The characteristics of the Na ghost system with respect to active and passive transport and exchange diffusion will be summarized in addition to experiments in which ATP is shown to be the specific and immediate substrate for the active component of Na transport.

Progress during the past year: In these studies the outflux of Na^{24}, from pre-labelled ghosts is measured under various conditions. Prelabelling is accomplished by hypotonic hemolysis of intact cells in the presence of tracer Na^{24}. The resultant ghosts when subsequently washed are found to retain 10-20% of the initial Na^{24}. Separate experiments indicate that this trapped amount resides in the interior of ghosts comprising only a small portion of the total ghost population. The characteristics of the outflux of this residual Na^{24} indicates that the ghost system is similar to intact red cells in this regard. Three components of the outflux in ghosts can be elaborated; active transport, passive transport and exchange diffusion. The active transport of Na, necessarily driven by metabolism, requires the presence of K in the extracellular phase and is blocked by strophanthidin. The dependence of the pump flux of Na on the external K concentration is quantitatively the same in ghosts as in intact cells. But the kinetics of Na outflux in ghosts is different from that of intact cells. The loss from ghosts follows a single rather than a double exponential. Presumably the heterogeneity referred to before accounts for this: that is, the intact cell population is also heterogeneous. This explanation differs from the generally accepted idea that the intact cell has two compartments of Na but only one of K.

Part B included: No.

The ghost system described above was then put to assay metabolic substrates for their possible role in activating and sustaining the Na pump mechanism. (Compounds normally impermeable to intact cells, e.g. phosphorylated intermediates, can be incorporated inside the ghost during the time of hemolysis, along with Na^{24}.) The results show that ATP alone is the basic energyzer of the pump and that any reaction that will generate ATP will run the pump. Thus, ADP alone or PEP will likewise s stimulate the pump (2ADP=1ATP + 1AMP; PEP + ADP = ATP + pyruvic acid). However, both of these reactions are about half as effective as ATP in stimulating the pump. In reactions involving PEP or the utilization of inosine, ADP, as a phosphate acceptor, has to be present in catalytic amounts if the pump is to be activated. Inosine, without ADP, is metabolized (to lactate) and also generates ITP. However, ITP will not stimulate Na outflux; this is so whether ITP is incorporated by itself at hemolysis or synthesized as a result of glycolysis. But under these same conditions (no ADP) adenosine will stimulate the pump. When ADP is present, both inosine and adenosine will stimulate the pump to approximately the same extent. There must be a pathway to make ATP from adenosine and ITP from inosine but it is found that only ATP is active. Thus the pump is specific for the purine base, adenine. Inhibitors such as iodoacetate and arsenate do not affect the pump when it is driven by the utilization of ATP. Competition for substrate can, however, be demonstrated. It has been shown by others and corroborated in the present study that glucose is not utilized by ghost systems, presumably because of the loss of hexokinase during their preparation. However, hexokinase (molecular weight=10^5) can be incorporated at hemolysis along with ATP and Na^{24}. Na outflux is stimulated, as before, by virtue of the ATP. But now the addition of glucose completely inhibits Na outflux. The reason for this is that the reaction, ATP + glucose + (hexokinase)$\longrightarrow$Glucose-1-PO_4 + ADP, completely utilizes the ATP and thus leaves no ATP for the pump to catabolize.

These experiments strongly imply that the pump itself is or has, as an intimate component in its structure, an ATPase. The evidence at present does not permit a more detailed molecular description. In support of this conclusion it should be mentioned that R. L. Post (Vanderbilt University) has isolated, in vitro, an ATPase from red blood cells that is activated by K^+ and Na^+ in precisely the same fashion as Na outflux is activated in both intact cells and their ghosts as above described. The activation by the cations is blocked by strophanthidin. In addition, ATP specificity is also found for the enzyme.

Direction of current research: We are currently determining the efficiency of the pump, i.e. the number of Na ions moved for each high-energy phosphate used.

Incidental findings of significance: None.

Serial No. NHI-230
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A study of the concentrating and diluting processes in the mammalian kidney.

Principal Investigator: John R. Jacnike

Other Investigators: Robert W. Berliner

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description:

Objectives: An elaboration of certain mechanisms operative in the concentration and dilution of the urine.

Progress during the past year:

1. A study of distal renal tubular functions by a modified stop flow technique has been completed. Experiments were conducted during water, NaCl, Na_2SO_4, and mannitol diuresis. Elimination of the renal pelvic dead space, the collection and analysis of small specimens, and isotopic labelling of distal convolution fluid provided relatively accurate localization and correlation of the movements of water and electrolytes in the distal tubular system. Previous observations indicating that permeability to water in the distal convoluted tubule is vasopressin dependent were confirmed by the present technique. The data also reveal that NaCl reabsorption occurs in the same portion of the tubule. The association of NaCl reabsorption and water permeability, in the area of the distal convolution, will serve to greatly reduce the volume of tubular fluid delivered to the collecting ducts per unit of time. As a result, the ability of the kidney to elaborate a highly concentrated urine is enhanced, by virtue of the reduced rate of solute free water abstraction (Tc_{H_2O}) necessary for the attainment of any given urine osmolality. In the absence of vasopressin (water diuresis), the distal convolution appears to be highly impermeable to water. On the other hand, as previously concluded by other workers, it has been shown that water diffuses out of the collecting duct in the absence of vasopressin. In addition to these observations related to urinary concentration, the stop flow data suggest that NaCl reabsorption occurs in the

Part B included: Yes.

collecting duct, as well as the distal convolution. Similarly, potassium secretion appears to occur in the collecting duct. These data also indicate that conclusions by previous workers, derived from stop flow studies, indicating a distal tubular site of potassium reabsorption were erroneous. This study has been written up and submitted for publication (1).

2. The effect of transient unilateral urinary obstruction on urine concentrating ability has been studied. Dehydrated dogs elaborating a highly concentrated urine were prepared by bilateral ureteral catheterization, through flank incisions. One kidney was obstructed for 5 minutes by intrapelvic injection of isotonic NaCl or the dog's own urine, at a pressure of 100-110 mm Hg. The contralateral kidney was used as a control. This procedure results in a reduced urine concentration on the obstructed side, which usually gradually approaches the control side, but remains depressed below control up to 2 to 3 hours after obstruction. Electrolyte excretory rates are not usually affected. Analysis of treated vs. control kidneys reveals a higher water content and lower Na and urea content (as well as concentration in tissue water) on the obstructed side. The mechanism underlying this phenomenon remains unexplained, and further studies are planned (see below). Incidental to these experiments, several studies on the time relationship between onset of vasopressin action and increase in urine osmolality were performed. In brief, urine osmolality rose rapidly during the first 20 minutes after Pitressin injection (during water diuresis), to levels in the range of 400-500 mOsm. Thereafter a much slower, but usually progressive increase in osmolality occurred over the next 60 minutes. The latter observation suggests that factors other than vasopressin activity which enhance urinary concentration (such as the accumulation of urea in the kidney medulla) may require a considerable period of time to produce their maximal effect. Thus, some correlation with the relatively prolonged concentrating defect following ureteral obstruction is suggested.

3. The effect of vasopressin on permeability of the collecting duct to urea has been studied. Dogs are anesthetized and both ureters catheterized. Urea is infused and a water diuresis established. When a steady state has been achieved and maintained for 30-60 minutes, one kidney is rapidly removed, and medullary slices analyzed for urea. The dog is then given Pitressin, urea infusion is continued, and mannitol is given to maintain a high rate of urine flow. The above procedure is then repeated when a steady state is attained. By this means we have compared the urea concentration ratio of the medullary tissue to that of the urine in the presence and absence of vasopressin. Thus far, this ratio is consistently higher in the presence of vasopressin, suggesting that the latter increases permeability to urea diffusion in the collecting duct epithelium.

Direction of current research:

The following studies are at present underway:

1. Further experiments as described in (3) above, investigating in particular the manner in which other variables such as the state

of hydration or urine urea concentration may affect the concentration ratio between tissue and urine.

2. As an extension of the studies outlined in (2) above, the instantaneous effect of ureteral obstruction on the tissue water, sodium and urea content is being studied further. The effect of simultaneous clamping of the renal artery is to be more clearly defined. In addition, in view of the increased water content observed in the medulla of obstructed kidneys, obstruction with a non-aqueous liquid (mineral oil) will be studied.

3. Some preliminary experiments concerned with the concentrating defect associated with K depletion are underway. Specifically, the response to osmotic loading with mannitol, as opposed to hypertonic NaCl, before and after K depletion is to be determined. It is proposed that any differential response to these agents present during K depletion (and absent in the normal state) would provide evidence for some cause other than, or in addition to, reduced permeability as an explanation for the concentrating defect in this state.

Incidental findings of significance: None.

Part B.

Publications.

(1) Jaenike, J.R. and Berliner, R.W. A study of distal tubular functions by a modified stop flow technique. J. Clin. Invest. (to be published).

Serial No. NHI-231
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the function of single nephrons.

Principal Investigator: Thomas J. Kennedy, Jr.

Other Investigators: None

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description:

Objectives:

1. To establish the precise loci along the course of the nephron at which specific functional processes occur.
2. To separate clearly and describe in as simple terms as possible the details and steps in contiguous processes under scrutiny.
3. To finally integrate each process into an interrelated complex oriented toward some total homeostatic goal.

Progress during the past year:

Progress during the last year has been extremely slow. On the methodological side, serious difficulties have been encountered in the measurement of chloride concentration in small samples. The nature of the difficulties included accuracy and speed. The accuracy problem has yielded to practice. Speed was impaired by virtue of certain characteristics of condensers utilized ("soakage", "hysteresis"). As a result of this property, the type of condensor utilized was difficult to discharge rapidly and completely. If the operator waited for the discharge process to be completed, the delay so encountered (10-20 minutes) limited the number of determinations which could be performed. If the condensor was incompletely discharged, then the residual charge contributed to the apparent value of subsequent analytical result, introducing intolerable errors. The difficulties described were quantitatively relatively unimportant so long as samples contained small amounts of chloride, and the nature and extent of the problem was really unrecognized until it became necessary to measure larger amounts of this ion. At this writing, the method for chloride determination seems to be rapid and accurate and (hopefully) trouble free.

Part B included: No.

A second difficulty has been in the acquisition of the technical skill to carry thru to obtaining puncture fluids on elements in the hamster papilla. This problem, too, seems, hopefully, on the way to liquidation.

A final annoying problem has involved the handling of samples. Particularly troublesome is the manipulation of small samples of blood, the prevention of clotting, the separation of serum from clotted blood, etc. On this score, things do not presently look very hopeful. A number of ideas have been entertained, all of which look promising but have so far invariably failed to solve the problem. The end result of our inability to regularly prevent clotting is that a significant fraction of samples obtained are not analyzeable.

To the extent that observations have been made, the following should be noted. Gross effects on the hematocrit of the papillary vasa recta are associated with osmotic diuresis. The visually estimated hematocrit falls to low values as urine flow increases, and, to the extent that the hematocrit in the samples is representative of that in the vessels, it seems to fall from about 40% to less than 10%. There is also a progressive fall in the chloride concentration in the vasa recta plasma from extremely high values observed on a few occasions in antidiuresis toward the concentration of systemic plasma.

Direction of current research:

1. To continue to attempt to elucidate the relationship of chloride in vasa recta, collecting duct urine, loop of Henle, and systemic blood during a variety of physiological states in the animal.
2. To extend these observations to the proximal and distal tubular urine.
3. To incorporate inulin determinations into the observations.
4. To use the unpunctured kidney throughout as an index of gross renal function.

Incidental findings of significance: None.

Serial No. MHI-232
1. Kidney and Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of ammonium salts on electrolyte excretion in the chicken.

Principal Investigator: Jack Orloff

Other Investigators: Luis Brenes
Laurence Earley
Melvin Kahn

Man Years: Patient Days: None
Total: 1 1/12
Professional: 1 1/12
Other: 0

Project Description -

Objectives: It had been previously observed in this laboratory that infusion of the ammonium salts of chloride, sulfate or nitrate into the leg vein of a chicken produces natriuresis and chloruresis on the ipsilateral side. This is generally associated with a decrease in the rates of excretion of sodium and chloride on the contralateral side. This phenomenon is being reinvestigated in order to obtain information concerning the mechanism of the diuretic response.

Progress during the past year: The observations noted above have been confirmed. The effect appears to be related to the administration of the ammonium ion since similar results have not been observed following injection of equimolar amounts of the corresponding sodium salts. Ammonium excretion generally rises following infusion of the ammonium salts from a control value of approximately 5 micro-equivalents per minute to 25-30 micro-equivalents per minute. The rate of excretion rises on the contralateral side as well but does not attain the same maximal rate. Systemic acidosis does not produce a diuresis since the leg vein infusion of HCl was without significant effect on Na^+ or Cl^- excretion. Differences in ammonium excretion on the two sides at comparable urine pH's indicate that the ammonium enters the urine from the tubule cells. However an associated relationship between ammonium excretion and urine pH such that more ammonium appeared in the more acid urines is consistent with the view that accumulation occurs by non-ionic diffusion.

Part B included: No.

Analogous results, in this instance bilateral, were obtained when the same salts were administered into the wing vein. The rate of infusion necessary to augment sodium excretion exceeded by a factor of two that required in the leg vein studies. Thus 200 micro-equivalents per minute of ammonium chloride did not elicit a natriuresis whereas 400 did. Results following injection of ammonium acetate either into the wing or leg vein were variable.

Preliminary studies indicate that the distribution of the ammonium ion between cells and extracellular fluid may be of significance with respect to the urinary changes. There is suggestive evidence that alkalinization of the extracellular fluid diminishes the response to an infusion of ammonium salt independent of the effect upon the urine pH.

Direction of current research: The effect of variations in extracellular, and theoretically of intracellular pH, on the response to equivalent amounts of ammonium salts are being examined. The comparative effects on electrolyte excretion of amino acids which augment ammonia excretion but do not (in preliminary studies) effect a diuresis are to be examined.

Incidental findings of significance: None.

Serial No. MHI-233
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The effect of chlorothiazide on the renal concentrating and diluting mechanisms.

Principal Investigator: Jack Orloff

Other Investigators: Laurence Earley
Luis Bremes
Melvin Kahn

Man Years:
Total: 1 1/12
Professional: 1 1/12
Other: 0

Patient Days: 60

Project Description -

Objectives: To determine the effect of chlorothiazide on urinary concentration in the absence of antidiuretic hormone in order to define its usefulness in the management of pitressin resistant diabetes insipidus.

Progress during the past year: One patient with nephrogenic diabetes insipidus is being studied. Chlorothiazide resulted in a prompt reduction in urine volume and an increase in urine concentration. The "improved" concentrating ability was most striking during the first three days of therapy, and was less striking when potassium supplement was added to the diet. Urine osmolality never exceeded that of plasma.

Direction of current research: Patient studies are continuing to determine the roles of filtration rate and sodium and potassium balance in this response, since it is known that the drug reduces filtration rate in normal man and may also induce significant potassium depletion. Studies in dogs are also planned to determine the effects of chlorothiazide on urine concentration during ADH administration and solute diuresis. It is hoped that such data will be helpful in determining the mechanism through which chlorothiazide affects urinary concentration (increased membrane permeability to water and/or diminished freeing of water at site of dilution and/or increased concentration of the medullary interstitium).

Incidental findings of significance: None.

Part B included: No.

Serial No. NHI-234
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of electrolyte metabolism in renal cortical slices.

Principal Investigator: Jack Orloff

Other Investigator: Maurice B. Burg

Man Years:
Total: 10/12
Professional: 10/12
Other: 0

Patient Days: None

Project Description -

Objectives: A further examination of the effect of various agents on Na^+ and K^+ metabolism, the flux of K^{42}, and the transport of PAH in renal cortical slices. Earlier studies in which the experimental method was discussed in detail were reported in the 1958 annual report. This report deals with a number of observations which were incomplete at the time of the initial summary.

Progress during the past year: It was reported by us that the cardiac aglycone strophanthidin, interferes with the accumulation of para-amino-hippurate (PAH) by rabbit renal cortical slices; and further that the drug decreases the K^+ content of kidney slices by inhibiting the active influx of K^+ from surrounding medium. Both of these effects can either be reversed or prevented by incubation of the slice in a medium containing a high concentration of K^+. In view of this it was suggested that the aglycone effect on PAH uptake may not be a primary effect of the drug on anion transport, but rather may be secondary to the associated interference with K^+ transport. This was established by comparing the PAH accumulation of two groups of slices; 1) a group in which the K^+ content was diminished to a varying degree by leaching the slices in low K^+ media in the absence of strophanthidin and 2) a group in which the K^+ content was lowered to a varying degree by incubation in medium of normal K^+ content containing the aglycone. The depression in PAH accumulation was similar in the two groups and was a function of the calculated intracellular K concentration. The reversal of the strophanthidin effect by a high concentration of K^+ may be simply explained on the basis of the forced response of an incompletely inhibited mechanism to "load". In other terms it is probable that the hypothetical pump by which K^+ is pumped into cells in exchange for extruded Na^+, although not acting at full efficiency in the presence of the aglycone, is still responsive to

Part B included: Yes

high concentrations of K^+, such that under these circumstances K^+ accumulation and those processes somehow dependent on the intracellular K^+ content will tend to approach their normal rates.

It is of interest in this light that the natriuretic effect of the aglycone in the intact chicken (discussed last year and considered to be due to interference with Na^+ and K^+ transport by renal tubular cells) is partially reversed by the concomitant administration of KCl.

Adrenal salt-active steroids have been reported to interfere with the inhibitory effect of the digitalis group on electrolyte transport. This has not been confirmed in these studies. Thus:

1. The effect of strophanthidin on the Na^+ and K^+ content and PAH accumulation of rabbit renal cortical slices was not prevented by incubation in the presence of large doses of a variety of potent adrenal steroids, including aldosterone.

2. In order to exclude the effect of endogenous hormone on the results, similar studies were performed on slices obtained from kidneys of adrenalectomized dogs to which exogenous hormone had not been administered for one week prior to sacrifice. The results were similar to those obtained in normal rabbit slices and further did not differ if medullary as well as cortical slices were used.

A study of the kinetics of K^{42} transport in kidney slices was extended during the last year. The method initially described by us in 1958 in which the uptake and washout of K^{42} can be observed repeatedly in single slices of cortex in a well scintillation counter has proved to be entirely satisfactory. The data obtained from a single slice is reproducible and the slice can be maintained in the steady state for as long as 200 minutes. Using this technique the following additional results have been obtained.

1. All of the K^+ in the slice is completely exchangeable with a half-time of approximately 15 minutes at 25° C.

2. Strophanthidin (10^{-5} M) reversibly reduces the influx of K^+ into the slice without affecting the efflux.

3. Strophanthidin (10^{-4} M) diminishes active influx of K^+ and increases K^+ efflux. These results are non-specific and are also observed with 2-4 DNP, salyrgan and anoxia. It has been concluded that the effect of 10^{-5} strophanthidin represents the "physiological" action of the drug, whereas the changes induced by anoxia, etc. and 10^{-4} strophanthidin in which passive efflux is also enhanced may represent an additional non-specific change in membrane permeability.

4. Although changes in medium pH affected K^{42} flux this cannot be interpreted at present.

Direction of current research:

1. Examination of the effect of medium K^+ on K^{42} flux. Preliminary studies are consistent with the presence of exchange diffusion of K^+.

2. Further examination of the pH effect on potassium flux.

3. Effect of strophanthidin on tissue swelling, either of the cortical slice or of an isolated membrane. Although interference with Na^+ and K^+ exchange by other agents generally results in tissue swelling, this is not certain for strophanthidin and may indicate a different mode of action of the drug on the linked exchange system.

Serial No. NHI-235
1. Kidney and Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The osmotic properties of artificial membranes and the erythrocyte membrane.

Principal Investigator: Victor M. Sidel

Other Investigator: None

Man Years:
Total: 1/2
Professional: 1/2
Other: 0

Patient Days: None

Project Description -

Objectives:

1) to explore the transfer of water, electrolytes and non-electrolytes in model membrane systems with particular reference to the theories of "bulk flow" of water through certain membranes under an osmotic gradient.

2) to explore the movement of water and certain non-electrolytes through the red blood cell membrane with particular reference to the theories of "leaky membranes" as derived from the viewpoint of irreversible thermodynamics.

Progress during the past year: (started work 7/1/59)

1. The rate of movement of water through air under osmotic and diffusion gradients has been measured. A bell jar was used to define the air "membrane" and small Petri dishes were used to define the aqueous compartments. Net water movement under an osmotic gradient was determined from the change in volume of distilled water and 5.0 molal NaCl solutions after 24 and 72 hours of equilibration. The rate of water movement in the carefully defined experimental system was 6.3×10^{-2} gm/hr. Diffusion of water was measured using tritiated water as a tracer for equilibration periods of 17 and 68 hours. The facilities of the Radiation Safety Laboratory were made available to us for these experiments. The rate of unidirectional diffusion of water in the same experimental system, corrected for isotope effect, was 0.32 ml/hr. The unidirectional diffusion flow was thus found to be five times the net osmotic flow, a result predicted for this system

Part B included: No.

by classical osmotic and vapor pressure depression theory. This result rules out "bulk flow" of water through the air membrane.

2. A model system utilizing a butanol "membrane" separating two aqueous compartments was set up using equipment devised by Dr. Joseph Hoffman and was used to measure the transfer of water and electrolytes (NaCl and KCl) under osmotic and diffusion gradients. Experiments with the model thus far give no evidence of "solvent drag" through the butanol membrane and therefore no evidence of "bulk flow" of water through the membrane. The partition coefficients of Na^+ and K^+ in butanol-water and isobutanol-water systems were measured as a part of these experiments. The partition coefficients were found to vary markedly with the salt concentration.

3. A "rapid-flow" system of the Hartridge-Roughton type with Millipore filters to separate erythrocytes from experimental media, previously used by Dr. Tosteson of this laboratory, was reactivated in an attempt to measure the rate of flow of water across the RBC membrane under an osmotic gradient. Dr. Hubert Ryan conducted many of these experiments. Several difficulties, particularly local hemolysis during filtration through the Millipore filters, has thus far prevented our obtaining consistent results for water flow.

Direction of current research:

1. Upon the return of Dr. Hoffman studies on the butanol membrane model will be resumed, with the use of Cephalin to enhance the transfer of electrolytes through the butanol. It is hoped to confirm and extend previous experiments showing the ability of this system to differentiate between Na^+ and K^+ in transport.

2. Efforts will be continued to make the rapid-flow system operate without hemolysis and to measure the rate of water movement across the RBC membrane. Changes in extracellular osmolality, Na concentration, and I^{131}-albumin will be used as indicators. The movement of labelled urea and its osmotic effects as tests of the "leaky membrane" hypotheses will also be studied.

Incidental findings of significance: None.

Serial No. MHI-236
1. Kidney & Electrolyte Metabolism
2. Experimental Cardiovascular Disease
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Physiology of Congestive Heart Failure

Principal Investigator: James O. Davis

Other Investigators: Nicholas A. Yankopoulos
Carlos E. Ayers
Charles C.J. Carpenter

Man Years:
Total: 2½
Professional: 2½
Other: 0

Patient Days: None

Project Description:

Objectives:

Area I. To determine the mechanism of increased aldosterone secretion in secondary hyperaldosteronism.
Area II. To define the biochemical defect in the failing myocardium.

Progress During the Year:

Area I. Mechanism of Aldosterone Secretion in Secondary Hyperaldosteronism.

Project I

1a. Title: The role of the anterior pituitary in control of aldosterone secretion in experimental secondary hyperaldosteronism.
1b. Investigators: James O. Davis, Nicholas A. Yankopoulos, Ferol Lieberman, John Holman and Robert C. Bahn.
1c. Progress During the Year: Project is complete and paper has been sent to the Journal of Clinical Investigation.

The effect of removal of the anterior pituitary on aldosterone secretion in dogs with hyperaldosteronism secondary to caval constriction was reported previously (The Physiologist 1:15, 1958); a 76-97% fall in adrenal vein aldosterone output occurred within 2 hours following hypophysectomy. In the present study, ACTH was administered following hypophysectomy of 2 dogs with caval constriction and aldosterone secretion remained at the high control level. To inhibit secretion of ACTH, large doses of

cortisone were administered to 7 dogs with experimental ascites. Aldosterone and corticosterone production was significantly lower than in dogs with chronic ascites without cortisone therapy and in one of the 7 animals aldosterone output was depressed to normal. Subsequent hypophysectomy of 5 of these 6 dogs resulted in a further decline in aldosterone and corticosterone output. Attempts to produce hypersecretion of aldosterone in simple hypophysectomized dogs failed: 1) adrenal vein aldosterone output was significantly lower than normal in 10 simple hypophysectomized dogs during maintenance on a low Na diet; 2) in 2 simple hypophysectomized dogs, acute thoracic caval constriction failed to produce an increase in adrenal vein aldosterone output. In a previous study, this stimulus produced hypersecretion of aldosterone consistently in normal dogs. It is concluded that the anterior pituitary and, specifically, ACTH plays an important role in the increased production of aldosterone in experimental secondary hyperaldosteronism.

Project II

2a. Title: Studies of aldosterone and corticosterone secretion in unanesthetized dogs.
2b. Investigators: James O. Davis, Carlos Ayers and Charles Carpenter.
2c. Progress During the Year: This project is almost complete.

Studies have been conducted in 5 normal dogs, 5 dogs with thoracic caval constriction and in 3 dogs with thoracic caval constriction following hypophysectomy. In 2 animals, observations were made first on normal dogs, then following caval constriction and, finally, following hypophysectomy. The technique consists of collection of adrenal vein blood from a chronic indwelling catheter.

In unstressed conscious normal dogs, the secretion of aldosterone and compound B was lower than in anesthetized dogs following adrenal vein cannulation; corticosterone secretion was influenced considerably more than aldosterone. The differences in steroid secretion between anesthetized animals subjected to surgery and unanesthetized dogs is probably attributable to increased ACTH output by the former group.

In unstressed conscious dogs with caval constriction, corticosterone secretion was very low (0.5 ug./min.) while aldosterone secretion was at essentially the same hight level as in anesthetized stressed animals with caval constriction. Hypophysectomy decreased both aldosterone and corticosterone secretion. These findings indicate a normal rate of ACTH secretion in experimental secondary hyperaldosteronism. It appears, therefore, that the role of ACTH is to support steroidogenesis by the adrenal cortex at a very high level rather than the initiation of increased aldosterone secretion.

Project III

3a. Title: Attempts to locate nervous receptors for control of aldosterone secretion in the central arterial tree.
3b. Investigators: James O. Davis, Charles Carpenter and Carlos Ayers.
3c. Progress During the Year:

The effects of chronic denervation of the cervical common carotid

artery, the carotid sinus and the cervical portions of the external and internal carotids have been studied in 2 dogs; no changes in electrolyte or aldosterone excretion were detected. Subsequently, the thoracic inferior vena cava was constricted. Increased aldosterone excretion, Na retention and ascites formation resulted. Both dogs were sacrificed for adrenal vein blood studies. Markedly increased rates of aldosterone secretion were observed in both dogs. When the 2 dogs were considered as a group, the rate of aldosterone secretion (.41 ug./min.) was significantly higher than the usual high rate of aldosterone output (.135 ug./min.) for dogs with no alteration other than caval constriction. The rate of aldosterone secretion in normal dogs studied under similar circumstances was .024 ug./min.

The effects of combined aortic arch and cervical carotid artery denervation have been studied in one dog. Following cervical carotid artery denervation as described above and caval constriction, the typical response with hyperaldosteronism resulted. The aorta was then denervated; aldosterone excretion in urine fell markedly but Na retention continued.

The possible role of abdominal mesenteric receptors has been studied by constricting the coeliac axis, superior mesenteric and inferior mesenteric arteries. The effects on aldosterone secretion have been observed in 2 dogs. In the first dog, a very small (8 mm. Hg) increase in systemic arterial pressure occurred following arterial constriction and no effect on aldosterone secretion resulted. In the second animal, a striking reflex increase in arterial pressure (60-70 mm. Hg) occurred and aldosterone secretion tripled.

These findings in all 3 regions (cervical, thoracic and abdominal) of the central arterial tree suggest the possibility of receptors which regulate aldosterone secretion.

Project IV

4a. Title: Effects of mid-brain transection and of pinealectomy on aldosterone secretion in normal dogs and in dogs with experimental secondary hyperaldosteronism.
4b. Investigators: James O. Davis, Evelyn Anderson, William K. Sponce, Hildegard Wilson, William Gay, Carlos Ayers and Charles Carpenter.
4c. Progress During the Year:

The rates of aldosterone and corticosterone secretion did not appear to be altered following mid-brain transection in 4 normal dogs. Also, subsequent bleeding increased aldosterone secretion in these animals. In dogs with caval constriction subjected to mid-brain transection, the response in aldosterone secretion varied. In some animals aldosterone output decreased while in others aldosterone output remained at the high level characteristic of dogs with thoracic caval constriction. The decrease in aldosterone output may represent a fall secondary to a drop in venous pressure rather than the interruption of apparent neural pathways. In one dog with caval constriction in which aldosterone output fell following mid-brain transection, subsequent bleeding stimulated aldosterone secretion. The study is not complete but available data suggest that interruption of

nervous pathways at the mid-brain level does not prevent a high rate of aldosterone secretion.

Pinealectomy of normal dogs was without effect on urinary aldosterone and electrolyte excretion. Subsequent caval constriction resulted in typical hyperaldosteronism.

Project V.

5a. Title: Effects of hypophysectomy and subsequent hemorrhage compared with decapitation followed by hemorrhage on aldosterone secretion.
5b. Investigators: James O. Davis, Charles Carpenter and Carlos Ayers.
5c. Progress During the Year:

The purpose of this study is twofold. First, to determine if hemorrhage will stimulate aldosterone secretion in the absence of anterior pituitary hormones and, secondly, to determine if the aldosterone stimulating hormone is secreted by the brain.

The first part of the study is complete. Hypophysectomy of normal dogs resulted in a 70-80% fall in aldosterone output. Subsequent bleeding was followed by a small but significant increase in aldosterone secretion.

The plan for the second part of the study is to decapitate the animal and wait 2 hrs. for ACTH to disappear and then bleed. If the aldosterone stimulating hormone is secreted by the brain, aldosterone secretion should remain unchanged after bleeding. On the other hand, if aldosterone secretion increases in response to bleeding, the data will indicate another source (other than the head) for the aldosterone stimulating hormone. Only one experiment has been performed for the second part of the study and this was unsuccessful.

Project VI

6a. Title: Effects of chronic thoracic caval constriction and of hypophysectomy on the biological half life of aldosterone and hydrocortisone in the dog.
6b. Investigators: Carlos Ayers, James O. Davis and Charles Carpenter.
6c. Progress During the Year:

The effects of chronic caval constriction on the metabolism of aldosterone are being studied to evaluate the possible role of a congested liver in contributing to the high blood level of aldosterone in congestive heart failure. Tritiated aldosterone is injected intravenously and the rate of disappearance of authentic aldosterone is studied. Since aldosterone is metabolized very rapidly, it is necessary to study the disappearance of H^3-aldosterone which is isolated from each plasma sample before determining the radioactivity present.

Several normal dogs have been studied but only one dog has been studied after caval constriction and, similarly, only one animal has been observed after hypophysectomy. The $T_{\frac{1}{2}}$ of aldosterone and hydrocorti-

sone for normal dogs is 15-30 min. Available data following caval constriction and hypophysectomy are not adequate to allow any definite conclusions.

Project VII

7a. Title: Attempts to isolate the aldosterone stimulating hormone from peripheral plasma of dogs with experimental secondary hyperaldosteronism.
7b. Investigators: James O. Davis, Elwood Titus, Carlos Ayers and Charles Carpenter.
7c. Progress during past year:

Attempts have been made to obtain an active extract of peripheral plasma by several different extraction procedures designed to yield various large groups of compounds such as steroids and proteins. Extracts have been assayed in simple hypophysectomized dogs. In our initial studies on this project, we obtained an extract consisting primarily of large polypeptides and proteins which gave 2 positive assays but we have been unable to reproduce these results. We are currently slightly altering our original extraction procedure in an effort to obtain activity.

Project VIII

8a. Title: Effects of hypertension produced by buffer nerve section on aldosterone secretion.
8b. Investigators: Charles Carpenter, James O. Davis and Carlos Ayers.
8c. Progress During past year:

This project was begun only recently. In 2 dogs, hypertension was produced by excising the carotid sinus bilaterally, by complete left cervical vagotomy and partial right cervical vagotomy. The mean arterial blood pressure in both dogs is approximately 200 mm. Hg. It is planned to obtain electrolyte balance studies and urine for aldosterone measurements. Finally, the animals will be sacrificed and adrenal vein blood aldosterone studies made.

Project IX

9a. Title: Sodium retention and ascites formation with only one kidney transplanted to the neck.
9b. Investigators: James O. Davis, John Holman, Carlos Ayers and Charles Carpenter.
9c. Progress During the past year:

Only one animal has been studied. The left kidney and adrenal were transplanted to the neck and the remaining kidney was removed. Control electrolyte balance data were obtained in the presence of only one kidney. The thoracic inferior vena cava was constricted. Sodium retention and ascites formation occurred and the changes were indistinguishable from those observed following caval constriction of normal dogs. No measurements of aldosterone have been completed. It is planned to remove the right adrenal.

Area II. The biochemical defect in the failing myocardium.

Project I

1a. Title: Chemical characterization of cardiac myosin from normal dogs and from dogs with experimental cardiac failure.
1b. Investigators: James O. Davis, William R. Carroll, Mary Trapasso and Nicholas A. Yankopoulos.
1c. Progress during year:

This project is complete and paper is nearly ready to go to press. The essential findings were presented in the last annual report.

Project II

2a. Title: Electrolyte and water content of the myocardium and the adrenal cortex in normal dogs, dogs with caval constriction and dogs with experimental cardiac failure.
2b. Investigators: Nicholas A. Yankopoulos, James O. Davis, and Ernest Cotlove.
2c. Progress during past year: The project is complete and the manuscript is being prepared for publication.

Project III

3a. Title: The water and electrolyte content and the contractile proteins of cardiac muscle from hypophysectomized dogs.
3b. Investigators. James O. Davis, Nicholas A. Yankopoulos and William Gay.
3c. Progress during year.

This project is complete and the data have been partially analyzed. It is planned to complete the analysis of the data and to write the paper during 1960.

Direction of Current Research

Area I.

It is planned to concentrate on locating peripheral receptors for control of aldosterone secretion and in evaluating the possibility of an afferent nervous limb. Studies will be made to determine the source from which the aldosterone stimulating hormone is secreted and to determine its chemical nature.

Area II.

No studies are planned in this area.

Incidental findings of Significance: None.

Part B.

Publications:

1. Davis, James O., Bernard Kliman, Nicholas A. Yankopoulos, and Ralph E. Peterson: Increased aldosterone secretion following acute constriction of the inferior vena cava. J. Clin. Invest. 37:1783,1958.

2. Davis, James O., Wilmot C. Ball, Jr., Robert C. Bahn and M. Jay Goodkind: Relationship of adrenocortical and anterior pituitary function to fecal excretion of sodium and potassium. Am. J. Physiol. 196:149,1959.

3. Davis, James O.: Evidence for an aldosterone stimulating hormone. Recent Progress in Hormone Research XV:298,1959.

4. Davis, James O., Nicholas A. Yankopoulos and John Holman: Chronic effects of carotid sinus denervation, cervical vagotomy and aortic depressor nerve section on aldosterone and sodium excretion. Am. J. Physiol. 197:207,1959.

5. Davis, James O., Robert C. Bahn, Nicholas A. Yankopoulos, Bernard Kliman and Ralph E. Peterson: Acute effects of hypophysectomy and diencephalic lesions on aldosterone secretion. Amer. J. Physiol. 197:380,1959.

6. Davis, James O., Robert C. Bahn and Wilmot C. Ball, Jr.: Subacute and chronic effects of hypothalamic lesions on aldosterone and sodium excretion. Amer. J. Physiol. 197:387,1959.

7. Yankopoulos, Nicholas A., James O. Davis, Bernard Kliman and Ralph E. Peterson: Evidence that a humoral agent stimulates the adrenal cortex to secrete aldosterone in experimental secondary hyperaldosteronism. J. Clin. Invest. 38:1278,1959.

In press:

8. Yankopoulos, Nicholas A., James O. Davis, John Holman and James A. McFarland: Physiological changes in dogs with congestive heart failure secondary to tricuspid insufficiency and pulmonic stenosis. Circulation Research. (In press).

9. Davis, James O., Mary Trapasso and Nicholas A. Yankopoulos: Studies of actomyosin from cardiac muscle of dogs with experimental congestive heart failure. Circulation Research. (In press).

10. Davis, James O.: Mechanisms of salt and water retention in congestive heart failure. The role of aldosterone. Amer. J. Med. (In press).

11. Davis, James O.: Hormonal control of aldosterone secretion. Hahnemann Symposium on Edema. (In press).

12. Davis, James O., Nicholas A. Yankopoulos, Ferol Lieberman, John Holman and Robert C. Bahn. The role of the anterior pituitary in the control of aldosterone secretion in experimental secondary hyperaldosteronism. J. Clin. Invest. (In press).

Serial No. NHI-237
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Direct Studies of Myocardial Contractility in Man

Principal Investigator: Robert D. Bloodwell, M. D.

Other Investigators: Leon I. Goldberg, M. D.
Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 9/12
Professional: 4/12
Other: 5/12

Patient Days (calendar year 1959): 400

Project Description:

Operations performed with total cardiopulmonary bypass provide the opportunity for the direct study of the influences of drugs and surgical procedures on the contractility of the human heart. The Walton-Brodie strain gauge arch, extensively used in animal experimentation for measuring myocardial contractile force, has been sutured to the right ventricle in 56 patients for measurement of changes in contractility following the exhibition of digitalis preparations, various sympathomimetic amines, and anesthetic agents. Monitoring with this device enables evaluation of the contractile force during extracorporeal circulation, including the use of elective anomic cardiac arrest and selective left coronary artery perfusion during open aortic valvulotomy.

Acute digitalization with acetylstrophanthidin during bypass, in 14 patients with atrial septal defects and no evidence of failure, resulted in a marked increase in contractile force, as well as a transient elevation of blood pressure indicative of vasoconstriction. This study represents the first human documentation of increased force of myocardial contraction caused by digitalis in a non-failing heart.

The comparative effects of adrenergic agents were studied in 17 patients. Both norepinephrine and epinephrine, in equivalent doses, produce nearly identical pronounced increases in cardiac contractile force. Vasoxyl, in equipressor doses, has no effect on the myocardium. Aramine and Wyamine produce a sustained increase in contractility.

Serial No. NHI-237

Part A. (continued)

Project Title: Direct Studies of Myocardial Contractility in Man

Project Description:

The absence of prolonged cardiac depression and the consistent return of contractile force to control levels, following anoxic arrest, has led us to employ this method of elective cardiac arrest.

Proposed course of project: Further studies of the inotropic effect of drugs and anesthetic agents in man and evaluation of methods of elective cardiac arrest and left coronary artery perfusion are continuing.

Part B. included Yes

Serial No. NHI-237

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Article in Periodical:

Bloodwell, R. D., Goldberg, L. I., Braunwald, E., Gilbert, J. W., Ross, J., Jr., and Morrow, A. G.: Myocardial Contractility in Man: The Acute Effects of Digitalis, Sympathomimetic Amines, and Anoxic Cardiac Arrest. Surgical Forum, American College of Surgeons - in press, 1959.

Serial No. NHI-238
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Foam Rubber Prosthetic Mitral Valve

Principal Investigator: Nina S. Braunwald, M. D.

Other Investigators: Theodore Cooper, M. D., Ph.D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 12/12
Professional: 6/12
Other: 6/12

Patient Days (calendar year 1959): 0

Project Description:

With the rapid advancement in the use of extracorporeal circulation which now permits extensive corrective surgery in patients with advanced cardiac lesions it has become apparent that there is a need for a prosthesis which can replace those valves deformed beyond repair.

A flexible foam rubber mitral valve has been developed. Preliminary tests on a pulse duplicator demonstrate the competency of the prosthesis. The valve has been inserted in 18 dogs placed on cardiopulmonary bypass making possible constant modification and improvement in the valve construction and in the technique of insertion. The technical feasibility of the operation has been demonstrated and several dogs have survived as long as 8 hours after removal of their own mitral valves. Recordings have been made of the arterial and left atrial pressures before and after insertion of the valve.

Proposed course of project: Toxicity studies are also being carried out by subcutaneous implantation of various foam plastics to find the material most suitable for valve replacement. It is felt that the open foam will provide a framework for the ingrowth of the host's own tissue resulting in a permanently viable artificial mitral valve.

Part B Included No

Serial No. NHI-239
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Effect of Acute Digitalization on the Dynamics of the Left Ventricle

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: John Ross, Jr., M. D.
Robert L. Frye, M. D.
John Oates, M. D.

Cooperating Units: Section of Experimental Therapeutics

Man Years (calendar year 1959):
Total: 7/12
Professional: 4/12
Other: 3/12

Patient Days (calendar year 1959): 200

Project Description:

The effects of acute digitalization with .75 mg. of Ouabain on left ventricular function have been studied in eight subjects. Left ventricular end-diastolic pressure is measured by means of the transseptal left heart catheterization technique and left ventricular work is measured as the product of arterial pressure and cardiac output. Patients with a variety of cardiovascular abnormalities have been studied. It has been of interest that in one patient with aortic stenosis and a markedly elevated end-diastolic pressure (25 mm.Hg) acute digitalization did not lower the end-diastolic pressure. In another patient with chronic idiopathic myocarditis, a striking fall in ventricular pressure occurred. In several patients with mitral stenosis and slight elevation of left ventricular end-diastolic pressure, no decrease of ventricular diastolic pressure or increase in left ventricular stroke work was noted. It is hoped that this technique will make it possible to determine the presence of myocardial damage in patients with valvular and other types of heart disease. It would appear that myocardial damage is present in those patients in whom a decline in filling pressure occurs or an elevation of ventricular stroke work occurs after the acute administration of digitalis glycosides.

Proposed course of project: A large number of studies similar to those already performed is contemplated.

Part B. included No

Serial No. NHI-240
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Reports
Calendar Year 1959

Part A.

Project Title: The Use of Indicator-Dilution Curves in the Study of Congenital and Acquired Heart Disease

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: M. Perryman Collins, M. D.
Robert T. L. Long, M. D.
William W. Pfaff, M. D.
Carlos R. Lombardo, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 66/12
Professional: 30/12
Other: 36/12

Patient Days (calendar year 1959):
975

Project Description:

There has been considerable interest in recent years in the use of indicator-dilution curves for the precise characterization of the circulation of patients with both congenital and acquired heart disease. During the calendar year 1959, the application of the indicator dilution method to several specific diagnostic problems has been investigated.

The paths of pulmonary venous drainage were determined in 29 patients with atrial septal defect who were subsequently operated upon by the open method. It was observed that the dilution curves following pulmonary venous injection closely resembled those following left atrial injection and were dissimilar to those resulting from right atrial injection in patients in whom the veins drained into the left atrium in normal fashion. However, when the curves closely resembled those following right atrial injection and were dissimilar to those following left atrial injection, anomalous venous drainage into the right atrium or vena cava was found to be present. This technique has been found reliable, simple to apply, and of considerable usefulness in the selection of patients for operation, as well as in the choice of surgical techniques employed.

Serial No. NHI-240

Part A. (continued)

Project Title: The Use of Indicator-Dilution Curves in the Study of Congenital and Acquired Heart Disease

Project Description:

The definitive diagnosis of pulmonic or tricuspid valvular regurgitation is often difficult by previously described clinical and laboratory means, and this diagnosis is often essential in the preoperative assessment of patients with valvular heart disease. Pulmonic valves were studied by positioning a modified double lumen catheter so that the distal lumen opened into the pulmonary artery and the proximal lumen opened into the right ventricle. When the tricuspid valve was studied the distal lumen opened into the right ventricle, and the proximal lumen into the right atrium. Cardio-green dye and radioactive krypton (Kr^{85}) were injected through the distal lumen of the catheter and were sampled from the proximal opening. In the presence of a competent valve, either no dye or Kr^{85} or only a minimal quantity could be detected in the proximal chamber immediately after injection. However, in the presence of valvular regurgitation, substantial amounts appeared in the proximal chamber immediately after injection. The competency of the pulmonic valve was examined in this fashion in 34 patients, and in ten of these significant regurgitation was considered to be present, with regurgitant fractions ranging from 12 to 72%. Competency of the tricuspid valve was tested in 25 patients in this fashion and in 14 of these significant regurgitation was demonstrated. This was estimated to range from 11 to 65% of the forward stroke volume. This technique for the detection and estimation of both tricuspid and pulmonic valvular insufficiency was found to be more sensitive than clinical means or the analysis of pressure pulses. It has been of considerable interest that many patients who were clinically believed to have pulmonary regurgitation secondary to pulmonary hypertension were found, in fact, to have aortic regurgitation by combining this technique with the methods for the detection of aortic regurgitant flow previously described from this laboratory. Thus, murmurs have been attributed to pulmonic regurgitation, perhaps unnecessarily in many instances.

In the past, indicator-dilution techniques were capable of localizing left-to-right cardiac shunts only if the injections were made in the left side of the heart or if two catheters were inserted into the heart. Both of these techniques are somewhat difficult to apply in the course of routine cardiac catheterization. A simplified indicator dilution technique for the localization of

Serial No. NHI-240

Part A. (continued)

Project Title: The Use of Indicator-Dilution Curves in the Study of Congenital and Acquired Heart Disease

Project Description:

left-to-right cardiac shunts was devised. Cardio-green dye was injected into a peripheral vein and sampled from a catheter located in the vena cava, the chambers of the right heart, or pulmonary artery. When the site of sampling was distal to the entry of the shunt the dilution curve was modified in a characteristic manner by the abnormally recirculating indicator. The accuracy of this dye dilution method was proved in a group of dogs with experimentally produced left-to-right shunts and its clinical applicability was demonstrated in 16 patients with and in 3 patients without cardiac shunts. The method proved to be both convenient and quite sensitive even in the presence of relatively small left-to-right shunts.

Proposed course of project: The usefulness of these and related techniques for the diagnostic evaluation of a large number of patients with both congenital and rheumatic heart disease will be continued.

Part B. included Yes

Serial No. NHI-240

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Individual Project Report
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Part B.

Article in Periodical:

Braunwald, E., Lombardo, C. R., and Morrow, A. G.: Determination of the Drainage Pathways of Pulmonary Veins in Patients with Atrial Septal Defect. Brit. Heart J. - in press.

Braunwald, E., Pfaff, W. W., Long, R. T. L., and Morrow, A. G.: A Simplified Indicator Dilution Technique for the Localization of Left-To-Right Circulatory Shunts. Circulation 20:875, 1959.

Collins, H. P., Braunwald, E., and Morrow, A. G.: Detection of Pulmonic and Tricuspid Valvular Regurgitation by Means of Indicator Solutions. Circulation 20:561, 1959.

Collins, H. P., Braunwald, E., and Morrow, A. G.: An Indicator Dilution Method for the Detection and Quantification of Pulmonic or Tricuspid Regurgitation. Surgical Forum - in press.

Pfaff, W. W., Braunwald, E., Long, R. T. L., and Morrow, A. G.: The Localization of Circulatory Shunts with a Simplified Indicator Dilution Technic. Surgical Forum - in press.

Collins, H. P., Braunwald, E., and Morrow, A. G.: Isolated Congenital Pulmonic Valvular Regurgitation: Diagnosis by Cardiac Catheterization and Angiocardiography. Am. J. Med. - in press.

Serial No. HNT-24(
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Experimental Studies with Digitalis

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: John A. Waldhausen, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 10/12
Professional: 5/12
Other: 5/12

Patient Days (calendar year 1959): 0

Project Description:

Forty-six dogs have been studied using complete cardiopulmonary bypass and lower aortic perfusion techniques to study the peripheral vascular effects of ouabain, acetylstrophanthidin, and lanatoside C. At constant perfusion rates a striking increase in peripheral vascular resistance has been uniformly observed. This pressor response was not eliminated by hexamethonium or adrenalectomy and was observed in the constantly perfused lower aorta. A concomitant decrease in venous return caused by pooling of blood in the portal circulation was observed. When portal vein decompression was maintained, digitalis administration resulted in increased venous return, suggesting the occurrence of venoconstriction in areas other than the hepatic veins.

Proposed course of project: The pressor effect and volume studies have been completed. Studies are in progress to demonstrate directly the effect of digitalis on peripheral venous tone using an intermittent venous occlusion technique.

Part B Included No

Serial No. NHI-242
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Use of Ascorbic Acid and an Intra-arterial Platinum Electrode for Performing Indicator Dilution Studies in Evaluation of Intracardiac Shunts and Pulmonary and Systemic Blood Flow

Principal Investigator: William W. Pfaff, M. D.

Other Investigators: Peter Froesmer, M. D. (Laboratory of Technical Development)
E. Kent Carney, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 6/12
Professional: 3/12
Other: 3/12

Patient Days (calendar year 1959): 300

Project Description:

A standard dose of ascorbic acid solution is injected into various cardiac chambers during the course of cardiac catheterization, and the oxidation-reduction potential of the diluted bolus of injected ascorbic acid recorded by an intra-arterial platinum electrode. Numerous qualitative curves have been obtained in this manner. Quantification of the curves is now being carried out by in vitro studies in the dog.

The principle advantages of this technique are: 1) It is not necessary to remove blood to obtain a dilution curve, potentially obviating the need for replacement transfusion; 2) distortion of concentration changes by mixing in sampling tubing is avoided, notably with intracardiac sampling; 3) A physiologic compound, ascorbic acid, is injected rather than a synthetic dye.

Proposed Course of Project: Linearity of the plot of ascorbic acid concentration versus potential produced will be shown. Comparison of the curves with those obtained with cardiogreen and Evans blue will be made. Cardiac output as determined by ascorbic acid dilution studies will be compared with that determined by Fick principle, and with Evans blue. Intracardiac shunts will be created in dogs, and ascorbic acid dilution curves evaluated. An estimated 12 dogs will be required.

Part B Included No

Serial No. NHI-243
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: A Study of Starling's Law of the Heart in Patients with Mitral Valvular Disease and Atrial Fibrillation

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Robert L. Frye, M. D.
Maurice M. Aygen, M. D.
Joseph Gilbert, M. D.
Nina Braunwald, M. D.

Man Years (calendar year 1959):
Total: 7/12
Professional: 5/12
Other: 2/12

Patient Days (calendar year 1959): 250

Project Description:

In patients with mitral stenosis and atrial fibrillation there are beat to beat variations in left ventricular filling. It is possible to take advantage of these alterations in left ventricular filling and test the applicability of Starling's Law of the Heart. Studies have been done on 16 patients so far. It has been observed that the strength of contraction as determined by the arterial pulse pressure, the ventricular systolic pressure, or the deflection of the strain gauge arch of the succeeding beat is a direct function of the end-diastolic ventricular pressure of the preceding beat. In addition, in five patients so far, continuous measurements of ventricular end-diastolic fiber length have been recorded by means of the mercury filled rubber gauge designed by Rushmer. In these patients it has been found that the strength of contraction as measured with the strain gauge arch, is a function of the end-diastolic fiber length. These studies support the applicability of Starling's law to man.

Part B. included No

Serial No. NHI-244
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Digitalis-Thyroid Antagonism and Its Modification by Reserpine

Principal Investigator: Robert L. Frye, M. D.

Other Investigators: Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 5/12
Professional: 4/12
Other: 1/12

Patient Days (calendar year 1959): 400

Project Description:

It has long been a clinical impression that patients with hyperthyroidism are resistant to usual doses of digitalis. However, such a relationship has never been studied in a quantitative fashion. The relationship between digitalis and thyroid activity on a specific cardiac property, the refractory period of the A-V node, has afforded a means of studying this problem by using the ventricular rate of patients with atrial fibrillation as an index of the refractory period. Because of the work of Brewster which showed that the effects of thyroid feeding in dogs could be abolished by total sympathetic block, the effect of reserpine on the relationship between digitalis and thyroid was studied.

Five euthyroid and one myxedematous patient with atrial fibrillation have been digitalized with digoxin and their basal ventricular rate determined during a control period. After 100-250 micrograms of triiodothyronine were administered daily, the basal ventricular rate rose to an average of 137% of the control value. In three patients, in order to return the refractory period of the A-V node to the control level, the daily dose of digoxin had to be increased to an average of four times the control dose. In two patients, parenteral reserpine abolished this increased digitalis requirement. In one undigitalized myxedematous patient with atrial fibrillation 0.58 mg. of acetylstrophanthidin was required to slow the ventricular rate to 70/min. and 1.50 mg. was required after the patient had been rendered euthyroid with triiodothyronine.

Serial No. NHI-244

Part A. (continued)

Project Title: Digitalis-Thyroid Antagonism and Its Modification by Reserpine

Proposed course of project: Three patients are being studied at present by noting the effect of a single injection of 1.2-1.4 mg. of triiodothyronine intravenously in the control period and then after giving parenteral reserpine.

Part B. included No

Serial No. NHI-245
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies of Venous Volume and Distensibility

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Charles Frahm, M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 6/12
Professional: 3/12
Other: 3/12

Patient Days (calendar year 1959): 0

Project Description:

Using total cardiopulmonary bypass and level sensing electrodes to detect shifts in blood volume, the responses of intracorporeal blood volume and peripheral resistance to graded alterations of systemic flow and venous pressure are under study in dogs. In addition, venous pressure curves have been recorded during brief periods of venous occlusion at constant perfusion rates. Administration of norepinephrine caused a striking decrease in the slope of these curves, while digitalis resulted in an increased slope. The mechanism of these alterations in the venous distensibility curves has not yet been defined.

Proposed Course of Project: Additional dogs will be studied to obtain sufficient data for quantitative analysis of volume changes and slope alterations. In addition, it is planned to investigate the neurogenic regulation of venous volume by selective stimulation studies.

Part B Included No

Serial No. NHI-246
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: A Stable Sensitive Technic for Thermal Dilution Studies

Principal Investigator: Theodore Cooper, M. D., Ph.D.

Other Investigators: None

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1 /12

Patient Days (calendar year 1959): 0

Project Description:

The use of indicator dilution technics is often limited by the necessity to withdraw blood from the subject and by the necessity to introduce potentially harmful substances as indicators. The injection of cool physiologic solutions results in an indicator dilution effect if the circulation is monitored at some distant point by a sensitive, rapidly acting temperature detector. A circuit has been established which permits adequate recording of external dilution curves across the lungs of an anesthetized dog after the injection of 8 - 10 cc. of 10° C saline intravenously. The instrument is stable.

Proposed Course of Project: 1) To further increase sensitivity so that smaller quantities of injectate may be utilized at warmer temperatures; 2) to test the instrument in dogs with surgically prepared left-to-right shunts; 3) to eventually employ the technic clinically.

Part B Included No

Serial No. HHI-247
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Cardiac Denervation by a Single Stage Operation with Chronic Survival

Principal Investigator: Theodore Cooper, M. D., Ph.D.

Other Investigators: Joseph W. Gilbert, M. D.

Man Years (calendar year 1959):
Total: 9/12
Professional: 4/12
Other: 5/12

Patient Days (calendar year 1959): 0

Project Description:

Previous studies of the physiologic effects of, and the pharmacologic responses to, chronic cardiac denervation have been based on preparations which involved bilateral cervical vagotomy and bilateral thoracic sympathectomy performed in 2 or 4 stages. This operation often resulted in effects on other systems which frequently prevented long term survival. We have attacked the problem by radical mediastinal dissection of the cardiac plexuses and stripping of the aorta and pulmonary artery, venae cavae, and pulmonary veins after bilateral thoracotomy. Nine animals have been subjected to this procedure of which the last 2 have been chronic survivors.

Proposed course of project: 1) Preparation of additional animals; 2) verification of functional denervation by vagal and sympathetic stimulation; 3) the determination of the responses of these animals to transfusion and aortic constriction; 4) test of the pharmacologic responses to sympathomimetic amines and cardioactive drugs (digitalis, quinidine); 5) the neuroanatomical verification of the denervation.

Part B Included No

Serial No. NHI-240
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies of Left Ventricular Changes Following Experimental Constriction of the Ascending Aorta

Principal Investigator: Roland Folse, M. D.

Other Investigators : Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1/12

Patient Days (calendar year 1959): 0

Project Description:

The ascending aorta of dogs was constricted either by resection of a wedge of the wall or by a constricting band of nylon. The lumen was narrowed until a pressure gradient existed across the coarctation. The animals were then kept as chronic survivors for study of the degree of myocardial hypertrophy and hemodynamic changes secondary to the increased left ventricular pressure. The purpose of the study is an attempt to reproduce the functional outflow heart obstruction which occurs in the clinical syndrome of functional aortic stenosis and functional obstruction of the pulmonary conus secondary to increased right ventricular strain.

Proposed course of project: Cardiac catheterization is being performed intermittently to follow the hemodynamic alterations in the left ventricle and aorta and the animals will be sacrificed in nine months for pathological evaluation of the degree of myocardial hypertrophy.

Part B Included No

Serial No. NHI-269
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Closure of Atrial Septal Defects Utilizing General Hypothermia: Effectiveness of the technic as determined by right heart catheterization

Principal Investigator: Joseph W. Gilbert, M. D.

Other Investigators: R. Robinson Baker, M. D.
M. Perryman Collins, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 1/12
Professional: 1/12
Other: 0/12

Patient Days (calendar year 1959): 1,116

Project Description:

Thirty-six patients with atrial septal defects were subjected to corrective surgery under general hypothermia with inflow occlusion. Thirty-one of these were studied from 3 weeks to 14 months by means of right heart catheterization employing inert gas technics for the detection of residual or recurrent left-to-right shunts. In 10 instances the atria septa were found to be patent, 6 of which were hemodynamically significant. All of these lesions were either sinus venosus defects with anomalous pulmonary venous connections or very large communications of the secundum type. The period of inflow occlusion in which repair of the defects was undertaken averaged 5½ minutes, an interval too brief to permit deliberate, meticulous repair, insertion of patch prostheses, or re-direction of anomalous pulmonary veins. The manifest conclusion is that general hypothermia does not safely allow a sufficient interval for the open correction of atrial septal defect. This, with the present refinement of complete bypass technics, supports use of the pump oxygenator for this purpose.

Proposed course of project: The project has been completed.

Part B Included No

Serial No. MHI-260
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: An Evaluation of the Effect of Quinidine and Hypothermia on Myocardial Function in the Digitalized and Undigitalized Dog

Principal Investigator: E. Kent Carney, M. D.

Other Investigators: Theodore Cooper, M. D., Ph.D.
John Ross, Jr., M. D.
Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1/12

Patient Days (calendar year 1959): 0

Project Description:

The routine use of quinidine to prevent the development of ventricular arrythmias during moderate and profound hypothermia has been advocated by several investigators. The present study was undertaken to evaluate the effect of quinidine on myocardial function when this drug is utilized in conjunction with hypothermia.

Ventricular function studies are performed according to the method of Sarnoff, by measuring stroke volume with a rotameter placed in the thoracic aorta, left ventricular end diastolic pressure, and arterial pressure. The curve is determined by altering end diastolic pressure by whole blood transfusion. Curves are determined under basal conditions, after the administration of quinidine, after hypothermia is carried out, and again after rewarming.

A different method of determining similar data has been to record myocardial contractile force with a Walton-Brodie strain gauge arch sutured directly to the left ventricle. Responses under the various conditions outlined above are again determined. Alternate animals are digitalized.

Proposed course of project: Thirteen dogs have been studied, and an estimated 12 more will be required for completion of the project. In addition, the protective action of molar sodium lactate against the depressive action of quinidine will be evaluated. It is also proposed to evaluate the effects of profound hypothermia (10°-12° C) when a heat-exchanger becomes available.

Part B Included No

Serial No. NHI-251
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Production of Fungal Endocarditis in the Dog

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: L. Herman, Ph.D.
Theodore Cooper, M.D., Ph.D.

Cooperating Units: Sanitary Engineering Branch

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1/12

Patient Days (calendar year 1959): 0

Project Description:

The resistance to treatment of fungal endocarditis in cardiac patients is well known. The successful production of bacterial endocarditis in dogs with aortic insufficiency has been reported from this laboratory. The animal with aortic valvular lesions might then be also susceptible to an endocarditis caused by a fungal organism. A recent patient with endocarditis caused by Candida Guilliermondii has provided a source of organisms for injections into 12 animals with experimental aortic regurgitation. Four control animals have also been injected with the same organism. Doses have been 5 cc. of innoculum which contain from 10^6 to 10^8 organisms per cc. Five animals were given a two day course of penicillin and streptomycin. Four animals were given a seven day course of tetracycline after innoculations. To date no animals have become clinically ill. Blood cultures on these animals have been negative.

Proposed course of project: 1) To follow these animals with blood cultures and clinically; 2) sacrifice and postmortem study of heart and other viscera; 3) if dogs become ill, attempt to treat them successfully; and, 4) to introduce organisms at time of operation for production of aortic regurgitation in an attempt to produce fungal endocarditis.

Part B included No

Serial No. NHI-252
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Transseptal Left Heart Catheterization in Man

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 18/12
Professional: 4/12
Other: 14/12

Patient Days (calendar year 1959):
600

Project Description:

The practicability of transseptal left atrial puncture was established in experimental animals. It has now been applied in 90 patients without complication. Initially, a #19 gauge needle was employed; more recently, a #17 gauge needle has permitted the passage of a small plastic catheter into the left ventricle and aorta. Success in catheterizing the left ventricle has been achieved in approximately 75% of the patients. A smaller needle has also been constructed for use in children, and recently a two year old patient has been studied.

The transseptal technique has been found to have the advantages of simplicity, safety, decreased patient discomfort, and permits the use of a single venous approach for right and left heart catheterizations.

Proposed course of project: It is planned to extend the transseptal technique to include selective angiocardiography with left atrial injection. In addition, further use of the small needle in infants and children is contemplated. The technique is now employed routinely in the cardiac catheterization laboratory, and further use of the method is planned for physiologic studies of left heart hemodynamics where a steady basal state is important.

Part B. included Yes

Serial No. MHI-252

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Article in Periodical:

Ross, J., Jr., Braunwald, E., and Morrow, A. G.: Transseptal Left Atrial Pressure in Man. Am. J. Cardiol. 3:653, 1959.

Ross, J., Jr., Braunwald, E., and Morrow, A. G.: Transseptal Left Heart Catheterization. Progr. Cardiovascular Dis. - in press.

Serial No. [redacted]
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Left Ventricular Function Following Elective Cardiac Arrest

Principal Investigator: John Waldhausen, M. D.

Other Investigators: Nina S. Braunwald, M. D.
William P. Cornell, M. D.
Robert D. Bloodwell, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 13/12
Professional: 6/12
Other: 7/12

Patient Days (calendar year 1959): 0

Project Description:

Acute heart failure is occasionally observed following a period of elective cardiac arrest induced in the course of an intracardiac operation employing cardiopulmonary bypass. This complication was encountered in patients without severe pulmonary hypertension or other lesions predisposing to failure and suggested that arrest itself might have a depressant effect on myocardial contractility.

Myocardial contractility, as measured by left ventricular function curves (the relationship between ventricular stroke work and filling pressure) was determined in 44 normal dogs before periods of cardiac arrest induced by either potassium citrate, acetylcholine or aortic occlusion alone. Cardiopulmonary bypass was maintained with a rotating disc pump-oxygenator at flows of 100 cc./Kg./min. The periods of arrest varied between 10 and 30 minutes and the right heart was drained during the period of arrest and recovery. Twenty minutes following the restoration of coronary flow left ventricular function curves were again examined. Arrest with either potassium citrate or acetylcholine for 20 or 30 minutes resulted in severe depression of myocardial function in 12 of 14 animals. Uninterrupted aortic occlusion of 20 minutes caused only minimal or moderate depression of contractility in 3 of 5 dogs. Intermittent anoxia, maintained for 30 minutes did not produce severe depression in any of 5 dogs.

The studies indicate that intermittent aortic occlusion provides the advantages of a dry and quiet heart without causing subsequent impairment of myocardial function.

Part B Included Yes

Serial No. NHI-254

PHS - NIH
Individual Project Report
Calender Year 1959

Part B

Article in Periodical:

Waldhausen, J. A., Braunwald, N. S., Bloodwell, R. D., Cornell, W. P., and Morrow, A. G.: Left Ventricular Function Following Elective Cardiac Arrest. J. Thorac. Surg. In Press.

Serial No. HHI-284
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Use of Hypothyroidism as an Adjunct to Hypothermia in Increasing Survival Following Temporary Circulatory Arrest

Principal Investigator: Robert D. Bloodwell, M. D.

Other Investigators: Eugene Braunwald, M. D.
Theodore Cooper, M. D., Ph.D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1/12

Patient Days (calendar year 1959): 0

Project Description:

General body hypothermia has permitted longer periods of circulatory arrest than is possible in the normothermic individual permitting enough time for certain intracardiac procedures. Further reduction of metabolic demands to permit longer periods of circulatory arrest is desirable.

This study is planned to evaluate the addition of induced hypothyroidism to hypothermia in prolonging the permissible occlusion time by preventing ischemic central nervous system damage and perhaps rendering the heart better able to tolerate the period of inflow occlusion.

Two dogs have been rendered myxedematous by I^{131}. These myxedematous animals are subjected to periods of inflow occlusion (utilizing coronary perfusion) and evaluated as to acute and chronic tolerance of the circulatory arrest. Only one of 12 control animals has survived a 20 minute period of occlusion without cerebral damage and death; all have had extremely difficult cardiac resuscitation. Both of the two myxedematous dogs so far studied have been chronic survivors and have shown excellent cardiac function immediately after circulatory arrest.

Serial No. NHI-254

Part A. (continued)

Project Title: The Use of Hypothyroidism as an Adjunct to Hypothermia in Increasing Survival Following Temporary Circulatory Arrest

Project Description:

Proposed course of project: Further studies of the hypothyroid dogs as soon as adequate time has elapsed after I^{131} injections are planned. Evaluation of the myocardial contractile force during these procedures is planned since the hypothyroid dogs seem to maintain cardiac function better and have more easily resuscitated hearts following the occlusion than the controls.

Part B. included No

Serial No. NHI-255
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Natural History of Eisenmenger's Syndrome

Principal Investigator: Charles J. Frahm, M. D.

Other Investigators: Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 4/12
Professional: 2/12
Other: 2/12

Patient Days (calendar year 1959): 100

Project Description:

There have been many reports describing Eisenmenger's syndrome but very few well documented case reports concerning the natural history. It is hoped that a large number of patients can be studied well and followed closely over a long period of time with heart catheterizations and arterial oxygen saturations. By these studies and accurate historical appraisals it will be possible to better define the course of this disorder.

Since cardiac surgery is advancing at such a rapid pace, it is important to know the course and life expectancy of these patients.

We have included in our analysis all patients with congenital heart disease whose pulmonary artery pressures were at least 75% of the arterial pressures. So far the data on 20 patients have been analysed. Most of the patients had the onset of symptoms in infancy or shortly thereafter. Their ages at the time of analysis or last visit ranged from 1 year to 49 years. There have been three deaths, one immediately after surgery. It is possible that these patients may live for many years with moderate symptoms and yet have severely elevated right sided pressures.

Proposed course of project: These studies will require many outpatient visits with the measuring of O_2 saturations and at times admittance for catheterization.

Part B. included No

Serial No. NHI-256
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

HHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Serum Glutamic Oxaloacetic Transaminase Levels After Cardiac Surgery

Principal Investigator: Charles J. Frahm, M. D.

Other Investigators: None

Man Years (calendar year 1959):
Total: 1 /12
Professional: 1 /12
Other: 0

Patient Days (calendar year 1959): 25

Project Description:

There have been few reports on the effects of cardiac surgery on serum glutamic oxaloacetic transaminase levels. The field of open-heart surgery with the pump by-pass has not been explored in this regard. Since the transaminase level is an important adjunct in many patients for determining whether a myocardial infarct has occurred, it is important to know what levels are obtained after surgery which traumatizes heart muscle. Thus far 37 patients have been studied or are in the process of having their data completed. There have been 12 atrial septal defects, 6 mitral stenoses, 4 aortic stenoses, 4 ventricular septal defects, 1 tetralogy of Fallot, 1 ventricular aneurysm, and 1 pulmonary stenosis.

The control levels have ranged from 13 to 36 units percent and the elevated levels have ranged from 42 to 303 units percent. All patients have had some elevation after operation. The trend indicates that the higher elevations are occurring with the greatest trauma to heart muscle. The greatest elevations have been seen during the first 24 hours after surgery.

Serial No. NHI-256

Part A. (continued)

Project Title: Serum Glutamic Oxaloacetic Transaminase Levels After Cardiac Surgery

Project Description:

In general, serum glutamic oxaloacetic transaminase levels of the pump blood prior to surgery have been either normal or only slightly elevated.

Proposed course of project: It is planned to continue this study until a larger number of cardiac surgical patients can be obtained.

Part B. included No

Serial No. NHI-257
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Use of Injections of Radioactive Krypton for the Characterization of Circulatory Shunts

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Robert T. L. Long, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 10 /12
Professional: 5 /12
Other: 5 /12

Patient Days (calendar year 1959):
200

Project Description:

Following the injection of Kr^{85} in solution into the right side of the heart approximately 95% is cleared through one passage through the pulmonary circulation. Arterial blood activity is low when radioactive krypton^{85} is injected in the absence of, or distal to, the origin of a right-to-left shunt. However, when injected proximal to the origin of such a shunt, a fraction of the Kr^{85} bypasses the pulmonary capillary bed and appears in arterial blood. Thirty to 50 uc. of Kr^{85} were injected into the right heart and arterial blood was sampled during the next 15 seconds. In nine patients without right-to-left shunts the activity per ml. of arterial blood was always less than 9.0×10^{-5} and averaged 3.5×10^{-5} of the total radioactivity injected. In nine patients with proved right-to-left shunts the radioactivity per ml. of arterial blood always exceeded 8.2×10^{-5} and averaged 30.1×10^{-5} of the total activity injected. By appropriate rearrangement of the Stewart-Hamilton formula the data permits calculation of the magnitude of the right-to-left shunt.

Following injection into the left heart, proximal to the origin of the left-to-right shunt, Kr^{85} immediately appears in the expired gas and may be readily detected by means of a thin window Geiger-Mueller tube. In twenty-two such patients, Kr^{85} appeared in the expired gas in an average of 4.0 seconds. However, after injection distal to the origin of a left-to-right shunt in 15 patients

Serial No. NMI-257

Part A. (continued)

Project Title: The Use of Injections of Radioactive Krypton for the Characterization of Circulatory Shunts

Project Description:

the appearance of Kr^{85} in the expired gas was delayed to a mean of 15 seconds. The technique described was found to be convenient, simple to apply during the course of cardiac catheterization, and extremely sensitive in the detection and localization of even small cardiac shunts. It is of interest that the technique described has been found to be more sensitive than the use of indicator dilution curves.

Proposed course of project: This aspect of the program has been completely investigated. It is planned to increase the sensitivity for the detection of right-to-left cardiac shunts by drawing a continuous concentration curve of Kr^{85} in the arterial blood by means of a small continuous gas-flow tube. In addition, it may be possible to determine the appearance time of inhaled Kr^{85} in right heart blood by continuous sampling through a small continuous gas-flow tube.

It is possible to measure left ventricular output by simply utilizing injections of Kr^{85}. Experiments are now in progress to ascertain the validity of the method which consists of a single injection of a solution of known amounts of Kr^{85} into the left side of the heart and sampling continuously for 30 seconds in the peripheral artery. Determining the radioactivity of a single sample of arterial blood is all that is required for determination of cardiac output.

Part B. included Yes

Serial No. PHI-257

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Article in Periodical:

Long, R. T. L., Braunwald, E., and Morrow, A. G.: The Intracardiac Injection of Radioactive Krypton: The Clinical Applications of New Methods for the Characterization of Circulatory Shunts. Circulation - in press.

Braunwald, E., Morrow, A. G., Sanders, R. J., and Long, R. T. L.: The Characterization of Circulatory Shunts by Foreign Gas Techniques. Symposium on Congenital Heart Disease, AAAS - in press.

Serial No. NHI-258

1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Applicability of Starling's Law of the Heart to the Circulation of Intact Man

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: John Ross, Jr., M. D.
Charles J. Frahm, M. D.

Technical: Frederick Bullock
Robert Smith
Karen Krumroy

Man Years (calendar year 1959):
Total: 7/12
Professional: 5/12
Other: 2/12

Patient Days (calendar year 1959): 150

Project Description:

There has been considerable controversy regarding the applicability of Starling's law of the heart to the circulation of intact man. In four subjects to date, this has been studied by the following techniques: 1) left ventricular filling pressure is measured by means of transseptal left heart catheterization and esophageal balloon pressure. The difference between the two gives an index of effective filling pressure; 2) hypervolemia is induced by rapidly infusing 1500 cc. of blood into the subject. This blood had previously been removed, and stored in the blood bank; 3) cardiac output is measured by the indicator dilution method. This experimental approach permits a study of the relationship between left ventricular filling pressure and stroke work. To date, a consistent relationship between these two parameters has been noted, that is, as the stroke work of the left ventricle is increased by the infusion, ventricular filling pressure rose, thus supporting the concept that Starling's law applies in circulation of intact unanesthetized man.

Proposed course of project: To continue gathering more data. It is hoped that a total of ten subjects will be studied.

Part B. included No

Serial No. NHI-259
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Clinical application and Further Refinement of Kay-Cross Artificial Heart and Lung Machine

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Joseph W. Gilbert, Jr., M. D.
Nina S. Braunwald, M. D.
John Waldhausen, M. D.
John Ross, Jr., M. D.

Technical: Peter Pulman, Jr.
Fred Bullock

Cooperating Units: Instrument Shop

Man Years: (calendar year 1959):
Total: 88 /12
Professional: 48 /12
Other: 40 /12

Patient Days (calender year 1959):
2876

Project Description:

The report for the calendar year 1958 detailed the integration of a rotating disc (Kay-Cross) oxygenator with pump and control units designed and built in cooperation with the NIH Instrument Section. The evolution of this machine and its clinical application have been characterized by several significant refinements:

The constant determination of the oxygen tension of the arterialized blood by means of the Clark electrode, and of the perfusion rate by means of an electromagnetic flowmeter, have provided valuable safeguards during cardiopulmonary bypass for open heart operations. Information gained thus, and that available from the continuous record of the electroencephalogram and central venous and arterial pressures, permits instantaneous description of vital parameters during total body perfusion and provides documentation of the conduct of the procedure.

In order that acute shifts in the extracorporeal blood volume (incident to loss through hemorrhage or "gain" from the central circulation subsequent to cardiotomy) be avoided, a sensitive control mechanism has been developed. This is comprised of a critical

Part A (Continued)

Title: Clinical Application and Further Refinement of Kay-Cross Artificial Heart and Lung Machine

level-sensing device, an automatic pump and a reservoir. Blood is thereby either added to the oxygenator to compensate loss or withdrawn for temporary reservoir storage during cardiotomy. An inefficient excess of blood in the cylinder is thus prevented, as is the hazard of gas embolism from an inadequately filled oxygenator.

To afford the extracorporeal system maximum protection from air-borne contamination, special filter covers have been designed and installed on reservoirs, gas inlets, and vents. Special techniques have been devised in the sterilization, sterile assembly, and operation of the heart-lung machine to reduce, insofar as possible, the likelihood of perfusion bacteremia. Standard check list type discipline has been instituted in every phase of heart-lung machine preparation and operation, with prescribed bacteriologic control methods.

Protracted exposure of the aortic valve is necessary in the treatment of aortic stenosis and regurgitation and prolonged occlusion of the ascending aorta is required in the correction of certain aortic-pulmonary artery fenestrations and high arch anomalies. Provision must be made for coronary artery perfusion during such procedures. To meet this need a special cannula has been devised whereby the left coronary artery may be perfused with arterialized blood from the heart-lung machine. The efficacy of this technique has been demonstrated by periods of coronary perfusion as long as 92 minutes, while calcified stenotic valves were opened and, on one occasion, a prosthetic Ivalon cusp inserted.

The heart and lung machine, with the refinements described, has been employed in 128 patients. The repetitious performance of such open heart surgery has permitted a high degree of specialization on the many critical individual functions, and their integration into a coordinated team plan.

Proposed course of project: The unitized incorporation of the various modifications indicated above into a single compact heart and lung machine chassis is presently being carried out in cooperation with the Instrument Section of the NIH and the Pemco Company of Cleveland, Ohio. Further improvements in the apparatus, directed toward simplification, ease of assembly, and safety of operation will doubtless evolve upon its continued and widening clinical application.

Part B included Yes

Serial No. NHI-259

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B

Article in Periodical:

Waldhausen, J.A., Ross, J., Lombardo, C.R., Cooper, T., Gilbert, J. W., and Morrow, A. G.: Flow and Volume Regulation during Cardiopulmonary Bypass: The use of an electromagnetic flowmeter and a device for automatic control of oxygenator volume. Trans. Am. Soc. for Art. Int. Organs. Vol. 5, p. 172, 1959.

Serial No. HHH-260
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Hemodynamic Evaluation of Transventricular Mitral Commissurotomy

Principal Investigator: Nina S. Braunwald, M. D.

Other Investigators: Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 12/12
Professional: 7/12
Other: 5/12

Patient Days (calendar year 1959): 550

Project Description:

It has become evident in many centers that a significant number of patients with mitral stenosis are not benefited by mitral commissurotomy as most commonly performed through the left auricular appendage.

The technique of transventricular mitral valvulotomy which is currently bein utilized at the National Heart Institute has been evaluated by a comparison of the hemodynamic data in a series of 25 patients undergoing transventricular commissurotomy compared with a series of 50 control patients. The 0.1 second, end diastolic and mean left ventricular pressure gradients were measured by simultaneous punctures of the respective chambers and were used as indices of the degree of anatomical correction.

Twenty-three of 25 (92%) of patients undergoing transventricular commissurotomy had a satisfactory relief of their stenosis without the development of mitral regurgitation. In the control series, similar in all pertinent respects to the experimental series, only 34 of 50 (68%) patients benefited from operation. There was no significant increase in the operative morbidity or mortality using the transventricular route. It is thus felt that transventricular mitral commissurotomy safely provides relief of mitral stenosis in the largest number of patients undergoing corrective surgery.

Part B Included No

Serial No. NMI-261
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on in vitro Preservation of the Heart for Transplantation

Principal Investigator: Theodore Cooper, M. D., Ph.D.

Other Investigators: Joseph W. Gilbert, M. D.
Roland Folse, M. D.
William P. Cornell, M. D.

Man Years (calendar year 1959):
Total: 5/12
Professional: 2/12
Other: 3/12

Patient Days (calendar year 1959): 0

Project Description:

The successful auto- or homotransplantation of the heart will require that a heart be preserved in vitro for varying periods of time (minutes to hours) before re-establishment of its coronary circulation. To this end we have begun to study the tolerance of the heart perfused in vitro with oxygenated blood and oxygenated physiologic solutions (saline, Tyrodes, Ringers) at temperatures of 37°C. To date, in 6 preparations, maintenance of a regular, "effective contraction" have been achieved for 30-60 minutes (the longer periods having been accomplished with blood). However, intravascular clotting appears to continually reduce perfusion rate. Perfusion with non-colloidal solutions results in edema (if pressures exceed 40 - 50 cm. H_2O) and a less effective contraction.

Proposed course of project: 1) To attempt return cardiac viability by altering temperature of system; 2) altering constituents of perfusate; 3) by the addition of agents such as hypertonic glucose, insulin, ATP, digitalis, etc.; 4) to eventually use such a preserved heart in an auto- then homotransplantation.

Part B Included No

Serial No. NHI-262
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Use of Radioactive Krypton and Cardio-Green Dilution Curves in the Detection of Experimental Portal Systemic Venous Shunts

Principal Investigator: Robert T. L. Long, M. D.

Other Investigators: Carlos R. Lombardo, M. D.
Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man years (calendar year 1959):
Total: 6 /12
Professional: 3 /12
Other: 3 /12

Patient Days (calendar year 1959): 0

Project Description:

The recognition of functional communications between the portal and systemic venous system in patients with hepatic disease or with abnormalities of the portal circulation may be impossible without the application of percutaneous or operative portal venography, major and sometimes hazardous roentgenographic procedures. Following the injection of krypton^{85} and of cardio-green into the portal circulation of patients with and without esophageal varices and dogs with and without a specific degree of portal vena caval shunt, multiple dilution curves are recorded from the expired gas. The right atrium and the femoral artery. In the presence of a patent portal systemic venous shunt the curves obtained resembled those following systemic venous injection; the appearance and peak circulation times were shorter than in the absence of such a shunt. Injections of Kr^{85} were found to be more sensitive than results obtained utilizing cardio-green. In nine dogs following the injection of Kr^{85} into the splenic pulp, the appearance time in expired air ranged from 15.7 to 90.7 and averaged 35.7 seconds, where the shunt opening appearance times were only 3.2 to 9.7 and averaged 5.6 seconds. There was no overlap between the two groups. The method described is found to be technically simple and safer than contrast radiography of portal circulation. It is hoped that these techniques will be applied to the diagnosis of esophageal varices and to the postoperative evaluation of patients with portal caval anastomoses in the future.

Proposed course of project: The project has been completed.

Part B included Yes

Serial No. NHI-262

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B

Article in Periodical:

Long, R. T. L., Lombardo, C. R., and Braunwald, E.: The use of radioactive krypton and cardio-green dilution curves in the detection of experimental portal systemic venous shunts. Ann. Surg. In Press.

Lombardo, C. R., Long, R. T. L., Braunwald, E., and Morrow, A. G.: The measurement of portal systemic circulation time: A new method for detecting esophageal varices and determining the patency of a portacaval anastomosis. Surg. Forum. In Press.

Serial No. NHI-264
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Pulmonary Artery Replacement in the Dog; Insertion of Valveless Teflon Prosthesis Between the Right Ventricle and Distal Pulmonary Artery

Principal Investigator: Joseph W. Gilbert, Jr., M. D.

Other Investigators: William P. Cornell, M. D.
Theodore Cooper, M. D.
Robert T. L. Long, M. D.

Man Years (calendar year 1959):
Total: 5/12
Professional: 2/12
Other: 3/12

Patient Days (calendar year 1959): 0

Project Description:

The correction of certain congenital cardiovascular malformations (truncus arteriosus, pulmonary artery atresias, certain transpositions) may depend upon establishing communication between the right ventricle and the distal pulmonary artery. Experimental study of the technical feasibility of such an undertaking is being carried out in the dog. Twenty-one dogs have been subjected to a procedure wherein valveless teflon prostheses have been placed between the right ventricular outflow tract and the distal end of the divided right or left pulmonary artery. Thirteen animals are chronic survivors (5-60 days). Postoperative angiocardiograms, performed thus far in but two animals, have proved the patency of the grafts. Death has occurred as a result of hemorrhage at the time of operation (2), acute cardiac failure (2), delayed hemorrhage from the distal pulmonary artery suture line (1) or the right ventricular insertion (1) and postoperative pneumothorax (3).

Proposed course of project: Right heart catheterization and selective angiocardiography is planned at intervals for the survivors.

Part B included No

Serial No. NHI-265
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Surgical Treatment of Residual or Recurrent Mitral Stenosis: Clinical and Hemodynamic Observation and Operation Results.

Principal Investigator: Joseph W. Gilbert, Jr., M. D.

Other Investigator: Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 9/12
Professional: 5/12
Other: 4/12

Patient Days (calendar year 1959): 450

Project Description:

As an increasing experience accumulates in the performance of mitral commissurotomy and with the ever widening application of hemodynamic methods in the evaluation of postoperative results, an increasing number of patients will be encountered in whom reoperation must be considered. Of approximately 150 patients who have been studied at the NHI following mitral valve operations, a second (or third) such procedure has been undertaken in 12. Their preoperative findings and original operative notes have been reviewed, with the clinical and hemodynamic studies subsequently performed in the NHI. The operative findings at the time of the repeat exploration, the technical and hemodynamic results, and the clinical followup findings have been summarized.

No instance of actual re-stenosis can be substantiated. The valves were characteristically unfavorable (8 of 12) and the patients judged to be poor operative risks. One surgical death occurred. Measurements of left heart pressures before and after the second commissurotomy confirmed the improvement in each, although a deliberate compromise between mitral stenosis and insufficiency was usually imposed by the severity of the pathology encountered.

The value of hemodynamic studies, particularly left heart catheterization, is emphasized in this study. A clear physiologic indication for repeat operation must be present, without which such a procedure could not be entertained in the face of the risk and technical deterrents.

Proposed course of project: Project completed.

Part B included No.

Serial No. NHI-266
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Use of Precordial Scanning for the Detection of Left-to-Right Circulatory Shunts

Principal Investigator: William P. Cornell, M. D.

Other Investigators: Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1959):
Total: 3/12
Professional: 3/12
Other: 0

Patient Days (calendar year 1959): 200

Project Description:

Because of the marked improvement in the surgical technique of repairing intracardiac defects the detection of intracardiac shunts has become increasingly more important. Cardiac catheterization is at present the most accurate method for detecting and localizing intracardiac shunts. A simple technique for screening large numbers of patients would be very useful in order to determine which patients required cardiac catheterization. Such a technique has been developed by which the presence or absence of left-to-right circulatory shunts can be accurately detected by the intravenous injection of I^{131} labeled Diodrast and recording a time-dilution curve with a scintillation counter placed over the precordium. I^{131} Diodrast is used because of its rapid excretion and, therefore, low biological half-life. The curves obtained are usually of the double peaked type described by others. The first peak represents the isotope passing through the right ventricle and the second peak represents the appearance of isotope from the left ventricle. A prolongation of the downslope of the curve is indicative of a left-to-right shunt. In a normal curve the downslope is rapid and smooth. The procedure is very simply and quickly performed and requires a minimal of cooperation by the patient. It is routinely performed on all patients admitted to the Surgical Service of the National Heart Institute, a total of 86 patients to date. It has been shown that this method is highly accurate in detecting left-to-right shunts and there has been virtually no overlap in the two groups of patients.

Proposed course of project: The techniques will be used on all patients admitted to the Surgical Service of the NHI as a preliminary screening test. Eventually it is planned to use it on clinic patients as a screening technique and on postoperative patients to avoid repeat cardiac catheterization.

Part B included Yes

Serial No. NHI-266

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B

Article in Periodical:

Cornell, W. P., Braunwald, E., and Morrow, A. G.: External precordial scanning: A preliminary report of a simplified method for the detection of left-to-right circulatory shunts. Medical Annals of the District of Columbia. In press.

Serial No. NHI-267
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Effect of Vasopressors on the Contour of Indicator-Dilution Curves as an Aid to the Diagnosis of Hemodynamic Alterations.

Principal Investigator: William W. Pfaff, M. D.

Other Investigators: Herbert Tanenbaum, M. D.

Technical: Leander Brown

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1/12

Patient Days (calendar year 1959): 0

Project Description:

The experiment was designed to assess the alteration of dye dilution curves by the administration of pressor agents to animals in which experimental cardiac defects had been produced in hope of differentiating various cardiac anomalies by a characteristic response. In addition, alteration of relative pressure gradients would serve to alter shunt and regurgitant flow and thereby make possible diagnosis of small defects which might otherwise be overlooked in the presence of equivocal catheterization data. Ventricular septal defects, atrial septal defects, subclavian pulmonary anastomosis, and mitral insufficiency were created in a group of mongrel dogs, the total numbering 27. Arterial, left atrial, and right heart pressures were monitored during the administration of norepinephrine, and in a few instances, Vasomyl, and Arfonad. Sequential dye dilution curves were obtained in a control period, during and after administration of these drugs. Increase in the apparent left-to-right shunt was noted in all appropriate defects. The effect of levophed was less marked in mitral insufficiency. Vasomyl produced greater change in the recorded dye curves, presumably by its pressor action in the absence of a concomitant inotropic effect. It was concluded that pressor agents do not serve to provide a technique for the differentiation of atrial, ventricular, and aortico-pulmonary communications, but are valuable for exaggeration of the characteristic break and downslope prolongation of the diagnostic dye dilution curve recorded in a patient with a left-to-right shunt. This technique was applied in two patients with atrial septal defects. Enhancement of the curve was noted in one patient.

Proposed course of project: It is hoped this experience will be further applied in clinical usage in the diagnosis and differentiation of congenital and acquired cardiac disease.

Part B included NO

Serial No. NHI-268
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Myocardial Necrosis Associated with Selective Potassium Asystole

Principal Investigator: Joseph W. Gilbert, Jr., M. D.

Other Investigators: James A. McFarland, M. D.
Louis B. Thomas, M. D.
William P. Cornell, M. D.

Cooperating Units: Pathological Anatomy Department, Clinical Center

Man Years (calendar year 1959):
Total: 5/12
Professional: 3/12
Other: 2/12

Patient Days (calendar year 1959): 0

Project Description:

Focal areas of myocardial destruction have been demonstrated at postmortem study in a substantial number of patients expiring following open heart procedures in which elective arrest was effected by injection of 2.5% potassium citrate into the root of the aorta. The occurrence of similar lesions was sought in the heart of the dog, as an explanation for the depressed ventricular function encountered clinically and experimentally. Twenty dogs were subjected to potassium citrate arrest of 15-30 minutes duration during cardiopulmonary bypass. Fifteen minutes after resuscitation of cardiac action and termination of bypass, the animals were sacrificed and the heart preserved in 10% formalin solution.

Proposed course of project: Blocks taken from serial sections of all four heart chambers are to be examined microscopically. This study is in progress.

Part B included Yes

Serial No. NHI-268

PHS - NIH
Individual Project Reports
Calendar Year 1959

Part B

Article in Periodical:

McFarland, J. A., Thomas, L. B., Gilbert, J. W., Jr., and Morrow, A. G.: Myocardial necrosis following elective cardiac arrest induced with potassium citrate. J. Thorac. and Cardiovascular Surg. In Press.

Serial No. NHI-268

PHS - NIH
Individual Project Reports
Calendar Year 1959

Part B

Article in Periodical:

McFarland, J. A., Thomas, L. B., Gilbert, J. W., Jr., and Morrow, A. G.: Myocardial necrosis following elective cardiac arrest induced with potassium citrate. J. Thorac. and Cardiovascular Surg. In Press.

Serial No. NHI-269
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Relationship Between Ventricular Filling Pressure and Fiber Length

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Robert F. Frye, M. D.
John Ross, Jr., M. D.

Technical: Mr. Robert Lewis

Man Years (calendar year 1959):
Total: 2/11
Professional: 1/11
Other: 1/12

Patient Days (calendar year 1959):
0

Project Description:

For the past year the influence of a variety of physiologic, pathologic, and pharmacologic interventions on the relationship between end-diastolic left ventricular pressure and end-diastolic left ventricular circumference has been determined in the dogs. Thirty-two experiments have been performed in the open chest dog in which left ventricular circumference was measured with a delicate rubber gauge of the type described by Buchner. Blood infusions were given to alter the left ventricular filling pressure and circumference.

It has been found 1) the operative distensibility of the left ventricle is decreased progressively with tachycardia at rates above 150-175/mn. At any given end-diastolic circumference the filling pressure is greater, the more rapid the heart rate, above this critical rate; 2) at a constant heart rate hypothermia displaces the curve in the same direction. It is believed that the shorter duration of ventricular filling is responsible for the shift in the curve in both hypothermia and tachycardia; 3) deterioration of the preparation, that is depression of the ventricular function curve, results in an opposite shift of the curve, and the end-diastolic circumference is greater for any given end-diastolic filling pressure; 4) acute digitalization which will elevate the external work of the heart performed with any given end-diastolic circumference will not modify the relationship between filling pressure and circumference which was previously altered by heart failure; 5) alterations in the manner

Serial No. MHI-269

Part A. (continued)

Project Title: The Relationship Between Ventricular Filling Pressure and Fiber Length

Project Description:

in which external work of the heart is performed, by modifying either aortic pressure or stroke volume does not shift the relationship between end-diastolic filling pressure and circumference. In several experiments it has been observed that the tension time index, which in previous experiments in collaboration with Dr. Sarnoff had been found to be very closely related to myocardial oxygen consumption, is not directly correlated with ventricular dimensions. This last finding would imply that myocardial oxygen consumption is not primarily dependent on ventricular fiber length and this data would then not be consonant with the classic views of Starling and Visscher.

Proposed course of project: It is planned to complete this project by performing another five to ten experiments in order to confirm the findings listed above. It is then hoped to study these relationships in the closed chest dog.

Part B. included Yes

Serial No. NHI-269

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B

Article in Periodical:

Braunwald, E., Frye, R. L., and Ross, J., Jr.,: Effect of heart rate on end-diastolic ventricular pressure-volume relationships, Clin. Res. 7:228, 1959.

Serial No. NHI-270
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Use of Oral Wyamine in the Treatment of Congestive Heart Failure

Principal Investigator: Robert L. Frye, M. D.

Other Investigators: Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 4/12
Professional: 3/12
Other: 1/12

Patient Days (calendar year 1959): 400

Project Description:

Wyamine (Mephentermine Sulfate) has been shown to have a striking effect in stimulating the heart of experimental animals in the presence of severe failure. Because of this potent inotropic action it was thought to be of importance to evaluate an oral preparation of the drug as a possible means of therapy in patients with congestive heart failure.

Five patients have been studied. All have had rheumatic heart disease and required digitalis, salt restriction, and diuretics for maintenance therapy. Congestive heart failure has been induced by discontinuing diuretics and adding extra salt to the diet as needed to result in significant fluid retention. Sodium excretion, weight of patient, urine output, and venous pressure have been followed daily. After reaching a stable period of moderate congestive heart failure, Wyamine has been administered in doses up to 300 mg. q.d. and maintained for several days. None of the five patients have shown any significant benefit from oral Wyamine. Intramuscular Wyamine in doses of 225 mg./day has also been ineffective.

Proposed course of project: It is planned to study several more patients in a similar manner, and include patients with arteriosclerotic heart disease with failure.

Part B. included No

Serial No. NHI-271
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Effect of Morphine on Central Blood Volume in Normal Subjects

Principal Investigator: Robert L. Frye, M. D.

Other Investigators: Eugene Braunwald, M. D.
Charles J. Frahm, M. D.

Man Years (calendar year 1959):
Total: 2/12
Professional: 1/12
Other: 1/12

Patient Days (calendar year 1959): 25

Project Description:

The mechanism of action for the beneficial effect of morphine in patients with pulmonary edema has never been explained. Previous studies in man have revealed a potent vasodilatory effect of morphine particularly in the upright position. We postulate that the venodilatory action of morphine results in a tourniquet-like action with a shift of blood from the lungs to the peripheral vascular bed.

Cardiac output and central blood volume will be determined by indicator-dilution techniques. After obtaining control observations, 10-15 mg. morphine (depending on size of subject) will be given intravenously. Fifteen minutes and 30 minutes following the injection, repeat determinations of central blood volume will be made.

Two subjects have been studied thus far with one exhibiting a 43% decrease in central blood volume, and the other showing a 39% decrease in central blood volume.

Proposed course of project: Study of more subjects in a similar manner is anticipated.

Part B. included No

Serial No. NHI-272
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Circulatory Response to Hypervolemia and Its Modification by Ganglionic Blockade

Principal Investigator: Robert L. Fryc, M. D.

Other Investigators: Eugene Braunwald, M. D.

Man Years (calendar year 1959):
Total: 9/12
Professional: 5/12
Other: 4/12

Patient Days (calendar year 1959): 281

Project Description:

The validity of Starling's Law of the Heart as applied to the intact circulation of man has been the subject of great controversy. The response of cardiac output to infusion of intravenous fluids and its relationship to filling pressure has been the subject of numerous studies with conflicting results. The present study was devised to attempt an explanation for the differences in response to infusion with an intact circulation and when neuro-circulatory reflexes were modified by ganglionic blockade.

Seven subjects have been studied by performing three phlebotomies over a period of ten days and storing the blood until the day of study. On the day of the study the 1500 ml. of whole blood was infused at a rate of 19 ml./min. with measurement of cardiac output, arterial and venous pressure, and heart rate before infusion, immediately and 15 minutes following the infusion. After a repeat series of phlebotomies the procedure was repeated in an exactly similar manner except for the presence of ganglionic blockade induced by Arfonad. The Arfonad was given at a rate ranging from 1-10.4 mg./min. sufficient to result in an average decrease in systolic pressure of 40 mm.Hg.

The infusion resulted in a mean change of 10 ml. in central blood volume in the control study as compared to a mean increase in central blood volume of 350 ml. in the presence of ganglionic blockade. The mean change in cardiac output in the control study

Serial No. NHI-272

Part A. (continued)

Project Title: The Circulatory Response to Hypervolemia and Its Modification by Ganglionic Blockade

Project Description:

was +500 ml./min. as compared to +2.4 L./min. in the presence of ganglionic blockade. The mean change in stroke volume following infusion in the control study was -1.2 ml./m^2 as compared to +17.75 ml./m^2 in the presence of ganglionic blockade. Mean arterial pressure showed a mean increase in the control studies of 18 mm.Hg as compared to 30 mm.Hg in the presence of ganglionic blockade. The mean change in minute work of the left ventricle was +1.31 Kg.M/min./m^2 during control study while in the presence of ganglionic blockade the mean change was 3.03 Kg.M/min./m^2. The mean change in stroke work of the left ventricle in the control study was 12.6 Gm.M/m^2 as compared with a mean increase of 39.16 Gm.M/m^2 in the presence of ganglionic blockade. The difference in response to infusion between the control study and during ganglionic blockade is statistically significant as regards all the parameters mentioned above.

Proposed course of project: Project completed.

Part B. included No

Serial No. NHI-273
1. Clinic of Surgery
2. Section of Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Effect of Exercise on Central Blood Volume in Man

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Eugene R. Kelly, M. D.

Man Years (calendar year 1959):
Total: 11/12
Professional: 6 /12
Other: 5 /12

Patient Days (calendar year 1959): 75

Project Description:

There has been some controversy about the effect of exercise on possible redistribution of circulating blood volume. In particular some previous reports have indicated that central blood volume falls with exercise. In this study the effect on central blood volume of ten minutes of moderately heavy leg exercise in the supine position was studied in ten normal subjects. Central blood volume was calculated by the Stewart-Hamilton formula from arterial dye-dilution curves following superior vena caval or right atrial injection. It was observed that during exercise the mean oxygen consumption rose from 144 ml./m^2 body surface area to 1,011 ml./m^2 body surface area. The cardiac index rose from 3.42 L./min./m^2 to 7.99 L./min./m^2. Central blood volume increased by 141 to 745 ml. in eight subjects. In the entire group of ten subjects the increase in central blood volume averaged 285 ml. During 20 minutes of recovery the central blood volume declined in all ten subjects by an average of 375 ml. with a fall ranging from 127 to 782 ml.

Proposed course of project: This particular project has been completed, but it is one phase of a long range investigation on the determinants of the distribution of circulating blood volume.

Part B. included Yes

Serial No. NHI-273

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B.

Article in Periodical:

Braunwald, E. and Kelly, E. R.: The Effects of Exercise on Central Blood Volume in Man. J. Clin. Invest. - in press.

Serial No. NMI-274
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of a Probabilistic Model for Growth

Principal Investigator: Murray Eden

Cooperating Units: None

Man Years (calendar year 1959) Patient Days - None
Total: .2
Professional: .2

Project Description:

Progress During Past Year: (Project begun June, 1955)

A program has been prepared for the generation of suitable samples of large size by Monte Carlo methods using TX-0 computer available at MIT. Preliminary runs indicate that the program is essentially correct.

Direction of Current Research:

The results of the Monte Carlo sample will be studied in terms of possible pertinent parameters of morphology of such objects: e. g., density, eccentricity or ellipticity, moments of second and higher orders. In addition, a program will be prepared to utilize TX-2 a much larger computer than TX-0, which is housed in the Lincoln Laboratories of MIT at Lexington, Massachusetts.

Part B included - No

Serial No. NHI-275
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of a Procedure for Machine Recognition of Handwriting

Principal Investigator: Murray Eden

Cooperating Units: Professor Morris Halle,
Department of Modern Languages,
MIT,
Cambridge, Massachusetts

Man Years (calendar year 1959):
Total: .4
Professional: .4

Project Description

Progress During Past Year:

The study of handwriting can be justified from several points of view. It is a problem requiring the non-numerical use of digital computers and as such can suggest programming procedures in handling other non-numerical problems. It also is a typical problem in pattern perception and recognition. A problem of pattern recognition that has been discussed in recent months and that can be handled in a fairly simple way is the problem of medical diagnosis by computer techniques. This is a much more difficult problem but some of its aspects may well be elucidated by working on simpler problems that have similar methodological features.

A general scheme has been proposed for the analysis of reasonably legible handwriting, in terms of a small set of strokes and a set of operations defined on these strokes. It has been possible to make certain semi-quantitative predictions regarding the readibility of handwritten texts in terms of this model. It has also been possible to predict with a fair amount of success, the kinds of degeneracy that will arise in an individual handwriting as the speed of writing increases.

Direction of Current Research:

Attempts will be made to program the TX-0 computer to "write" handwriting in accordance with the model mentioned above. It should be possible to write the program so that various kinds of distortion

can be introduced to the computers "handwriting" (which will be displayed on the face of an oscilloscope tube). If this generative program is reasonably successful an attempt will be made to write a program for reading handwriting. It is anticipated that this task will be considerably more difficult. However, it is believed that it should be possible to program a digital computer to recognize carefully written handwriting. It has been shown that the problem of reading more degenerate handwritings (which are the kind ordinarily used in correspondence) can not be solved by reading letter by letter but a stored dictionary will be required.

Part B included - No.

Serial No. NHI-276
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Research
Calendar Year 1959

Part A.

Project Title: Development of an Ultramicroanalytic Method for Sodium and Potassium Determination in Micropuncture Samples

Principal Investigator: Robert L. Bowman

Other Investigators: Christopher Eve
David Townsend (summer employee)

Cooperating Units: Dr. John R. Jaenike (LKEM)

Man Years (Calendar year 1959) Patient Days: None
Total:
Professional: 0.1
Others 0.1

Project Description:

Progress During Past Year:

Dr. John R. Jaenike's evaluation of the method showed that sodium levels could be measured in the range of 10^{-12} moles to only about ± 20%. This evaluation pointed to the need for control of the volatilization of the sample and more control of the RF glow intensity, focus and optical efficiency. After a period of inactivity, due to lack of personnel, the system has been reassembled with several improvements.

The single straight wire has been replaced by a .004" platinum "U" that can be heated electrically to volatilize the sample. Standard taper fittings orient the sample holder at the optical focal point.

As preliminary work also showed that organic and other inorganic residues present in blood and urine produced some changes in the emission, and since we wished to evaluate the possibilities of analyzing for other cations and anions, a series of spectra were taken of the emission from several salts and organic compounds.

Spectrographic plates showed the presence of the expected alkali metal lines but the chloride bands have not yet been identified due to the complexity of the spectra in the region of the chloride lines. The complex spectrum is produced by traces of N_2 in the helium, so several methods of purification of the helium were tried to eliminate the nitrogen spectrum. Purification with hot calcium and activated charcoal failed to reduce the nitrogen level enough to eliminate the nitrogen spectrum.

During the survey of the spectra the same equipment was used to examine the emission spectra of volatile organic compounds when excited by the helium glow. It was found that volatile organic materials generally produced broad blue bands of no structure. Some notable exceptions were CCl_4 and other highly halogenated compounds where the spectra consisted of many orders of complex bands throughout the ultraviolet blue and green regions of the spectra.

These spectra are only partially analyzed at the present time except for a few special cases the spectrum above 550 was free of emission that would interfere with the determination of the alkali metals. More thorough analysis of the plates will be made to determine utility of the method as a means of characterizing organic materials as they appear in the effluent of the gas chromatographic column. The high efficiency of helium in ionizing trace gases venders the spectra non-specific and puts extreme requirements on purity of the gas vapor pressure of the stationary phase and freedom from air leaks.

The analysis equipment has now been provided with easily replaced capillary pipettes, regulated metered RF source, standard taper electrode holders, facilities for volatilizing the sample by heating the sample filament, electrometer amplifier, regulated supply for phototube, X-Y recorder for integrated or peak measurements and other conveniences to make definitive testing easier.

Line intensity changes caused by variation in pressure, other trace materials, and temperature of the filament appear to be correctable by changes in the RF supply voltage to correct the RF supply current to present level.

The sensitivity of the method as it now stands is such that 10^{-11} mole samples overload the system.

Direction of Current Research:

The rare gas discharge activation of alkali metal emission will be studied further to establish its ultimate sensitivity and dependability with an ultimate aim of an instrument capable of analyzing these materials in a range of quantity available by micro-puncture techniques.

Part B included - No

Serial No. WAI-377
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Coriolis Flowmeter

Principal Investigators: Robert L. Bowman
Peter L. Frommer

Other Investigators: Charles Van Way (summer student)

Cooperating Units: None

Man Years: (calendar year 1959) Patient Days: None
Total: 0.33
Professional: 0.08
Other: 0.25

Project Description:

Progress During Past Year: (Project began June 1959)

There is no simple accurate, continuously operating device for metering blood flow, even in tubing. Standard metering devices fail because of properties peculiar to blood and because of peculiarities of the system in which it flows; the specially developed electronic system are inherently complex and they have limited accuracy.

One can rotate a tube about an axis perpendicular to its longitudinal axis. If a liquid is made to flow from the axis of rotation outward along the tube, the liquid will acquire a kinetic energy in the direction of rotation at the expense of the rotating tube, not at the expense of its own pump, and thus will have a retarding force on the rotating tube. By appropriate arrangement of tubing, and axes of rotation and free movement, one can acquire the additive effects of the retarding force exerted by the liquid moving outward and the accelerating force on another tube in which the fluid moves back to the axis of rotation. For a system at constant rate of rotation, this force is linearly proportional to the mass of fluid moving through it per unit time; it is a true

mass flow meter independent of such variable properties of liquids (and of different blood specimens) as homogeniety, viscosity, specific heat, speed of sound propagation, or density and independent of the pressure and temperature of the system. The system was tried initially on various glass and metal assemblies rotated or swung as pendula with the Coriolis force measured by properly placed strain gages, but with equivocal results.

Flowmeters based on mass-kinetic principles (e. g., "On Coriolis force") have been described within the past decade and their application to blood flow metering is a logical development, using the following method:

Now another instrument has been built as outlined above. The path of rotation has a 1 foot diameter, rotation is at 120 rpm, the Coriolis force is developed along a torsion bar which is essentially free from other forces, and the deflection is detected by a mirror and optical lever. The scale is linear; full deflection is 100 gm./sec.; accuracy is consistently within ± 5%; there are no pressure effects. The instrument is in a crude form and all tests were on water flow but refinement with higher accuracy and for blood flow is expected.

Direction of Current Research:

A more precisely constructed instrument will be built embodying refinements in design to produce greater sensitivity, greater accuracy, wider range, and compatibility for blood flow measurement.

Consideration will be given to building a differential flowmeter by putting two flowpaths on a single yoke in such a way that their individual Coriolis forces oppose each other.

Part B included - No

Serial No. NHI-278
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of Methodology for the Effective Application of Gas Chromatography to Biochemical and Medical Research Problems

Principal Investigator: Arthur Karmen

Other Investigators: R. L. Bowman, P. L. Frommer, H. Tritch(LCPM) L. Giuffrida, E. Kilbourne(summer employee), J. Bradgon (LCPM), E.Shafrir (LCPM)

Man Years (calendar year 1959): Patient days: None
Total: 2.35
Professional: 1
Other: 1.35

Project Description:

I. Development of Radio Frequency Glow Discharge Detector: A. Karmen, R. L. Bowman and L. Giuffrida

Progress During Past Year:

By the use of radio frequency voltages, it has been found possible to excite a stable glow discharge in helium at atmospheric pressure. Passage of an organic vapor in low concentration in the helium through the discharge tends to quench the discharge and causes a change in most of its electrical characteristics. The direct current resulting from the rectifying action of this discharge reflects passage of extremely small concentrations of organic vapor by decreasing in direct proportion to the concentration of vapor. Progress during 1959 in the development of this aspect of the discharge as a detector for gas chromatography can be summarized as follows:

1. The detector was modified for use with recently developed high resolution capillary gas chromatography columns, fulfilling the requirement for microscopic effective volume combined with fast response time, and high sensitivity. Several capillary columns were constructed; their applicability for fatty acid research evaluated. The ability to detect 10^{-14} moles of fatty acid vapor with the RF detector was demonstrated, using these columns.

The possibility of further improvement in the resolving power of these columns through the use of still smaller samples was demonstrated, showing the need for still greater sensitivity.

2. Sensitivity of one part in 10^9 mole ratio of fatty acids in helium was confirmed, using the electronic equipment constructed previously. By means of better control of operating parameters and elimination of variables of mechanical construction, the sensitivity was carried to the limit of the stability of the radio power sources used to excite the discharge. Although the sensitivity thus obtained compares quite favorably with other recently developed high sensitivity detectors, the inherently greater efficiency and sensitivity of this method of detection encouraged development of more elegant, regulated power sources, which have demonstrated encouraging increases in stability and therefore in sensitivity in early tests.

II. Evaluation of Argon Ionization Detector:
A. Karmen and L. Giuffrida

To evaluate the possibility of utilizing the efficiency of the RF discharge as an ionization source, and to gain better understanding of the electrical behavior of the rare gases, a direct current argon ionization detector, as described by Lovelock, was constructed and evaluated. This device is based on the observation that, in the presence of ionizing radiation, the electrical conductivity of argon at high voltages increases with the addition of small quantities of organic vapor.

Experiments revealed:

(1) The sensitivity of this device was found to approach within an order of magnitude of that of the RF detector.

(2) Records obtained with this detector show comparative, apparent great stability because changes in operating parameters such as temperature are reflected as changes in sensitivity with this device, rather than changes in baseline current, as is the case with the RF detector.

(3) It is desirable to have constant sensitivity for accurate quantitation. The conductivity of argon, however, is a complex exponential rather than linear function of vapor concentration. At low vapor concentrations the conductivity increases exponentially with increases in vapor concentrations: At higher concentrations of vapor the conductivity decreases with increasing vapor concentration.

(4) Since sensitivity is also an exponential function of the applied voltage a high resistance placed in series with the chamber was predicted to compensate for increasing sensitivity with increases of vapor concentration.

An extensive calibration procedure was evolved for the choice of series resistance. An approximation of constant sensitivity over a narrow range of concentration of vapor was obtained. The resistor, however, is specific for the ion chamber, the column, and the temperature of operation.

III. Development of a Modified Argon Ionization Detector: A. Karmen, P. L. Frommer and L. Giuffrida

To explore the use of the D. C. argon ionization detector at maximal sensitivity without sacrifice of quantitative accuracy, a device was constructed to measure the conductivity of argon by determining the voltage necessary to produce an electronically regulated, constant, predetermined current. Preliminary experiments have revealed a promising increase in useful sensitivity, as well as increased reliability of result. Final evaluation of this approach is pending.

IV. Investigation of a Detector Based on Measurement of Ions in the Vicinity of a Discharge Excited by Radiofrequency Voltage: A. Karmen, L. Giuffrida

Use of the RF discharge as a source of ions, in an ionization detector was investigated. The ionization current away from the center of the discharge was found to undergo larger relative change than the parameters of the discharge itself, suggesting greater sensitivity, and the possibility of controlled constant sensitivity. The feasibility of use of a detector based on this idea was demonstrated through the use of argon in preliminary experiments. The possibility of constant sensitivity, using this scheme, was shown, in that by proper choice of RF voltage, the RF discharge could be made to increase, remain constant, or decrease with passage of organic vapor. Evaluation of a working detector based on this scheme is pending.

V. Development of Methodology for the Radioassay of Carbon-14 Labeled Fatty Acids by Gas Chromatography:
A. Karmen, H. R. Tritch, L. Giuffrida

The object of this study was to provide methodology for the radioassay of components analyzed by gas chromatography which would (1) have sufficiently high sensitivity to make biological studies feasible and (2) accomplish this without sacrificing any of the resolving power of the gas chromatograph. The methods of radioassay were surveyed, and it was concluded: 1. that any method employing a radiation detector through which the column effluent flowed could not possess sufficient sensitivity without excessively large, resolution destroying, detector volume; 2. ultimately high sensitivity could be attained by collecting the effluent materials into aliquots for subsequent radioassay; resolution of this method would be determined by the number of fractions taken; 4. continuous monitoring of the total radioactivity collected, as a method of assay, would maintain the high resolution of the gas chromatograph with minimal loss in sensitivity.

Experiments were therefore carried out to determine an efficient method of trapping or recovering components following analysis.

1. A short section of gas chromatography column operated at room temperature was found effective in collecting the effluent materials quantitatively from a high temperature chromatograph.

2. The liquid phase coating in this column was found essential for effective trapping. A survey revealed several liquid phases that did not interfere with the measurement of radioactivity by the liquid scintillation counting technique.

3. Incorporation of a solid scintillator, anthracene crystals, as supporting material for the liquid phase in the short trapping column permitted direct radioassay of the sample collected by the "liquid" scintillation technique.

4. By continuously monitoring the total collected radiation during a gas chromatographic analysis, integrated records of the activity in emerging radioactive components were obtained that paralleled the simultaneous conventional quantitative analytic record.

Existing commercial liquid scintillation equipment was adapted to this technique of continuous measurement.

An automatic fraction collector was designed, utilizing this method of trapping, to permit collection of low activity components for long periods of counting.

Preliminary Report: A. Karmen and H. R. Tritch, in press.

Final manuscript in preparation.

VI. Separation of Lipids by Paper Chromatography:
A. Karmen, L. Giuffrida

With the object to provide a high resolution, convenient, micro preparative method for lipid analysis prior to analysis of constituent fatty acids by gas chromatography, development of the silicic acid impregnated paper technique described in 1958 annual report was continued. Through trial of a number of different solvent systems, improvement in resolution was obtained, to the degree of resolution now obtained by more cumbersome silicic acid column chromatography. Experimentation remains to be done to define the possibility of further separation of the lipids within the larger classes.

Manuscript in preparation.

VII. Applications of Gas Chromatographic Techniqus

1. Chylomicron Composition after Fat Investion:
J. Bragdon (LCPM), A. Karmen

Several different fats were fed to rats. The chyle and serum chylomicrons after feeding these fats were found to have fatty acid compositions similar to that of the fed fat, suggesting ingested fat as a major source of the chylomicron fatty acids. Analysis of human serum chylomicrons after a fat meal suggested that human chylomicrons are derived similarly.

A manuscript has been submitted for publication.

2. Study of binding of unesterified fatty acids to various serum proteins:
E. Shafrir (LCPM), A. Karmen

Analysis of unesterified fatty acids bound to serum proteins separated by ultracentrifugation, following equilibration of these proteins with known amounts of fatty acids revealed selective affinity

of the various proteins for different fatty acids, to be confirmed by and interpreted in the light of data obtained by study of the binding equilibria done by other methods.

3. Analysis of Respiratory Gases by Gas Chromatography:
A. Karmen, E. Kilbourne (summer high school teacher)

a. Columns

Oxygen and nitrogen can be conveniently analyzed by "molecular sieve" zeolite gas chromatography columns, which, however retain carbon dioxide strongly. Methods employing a combination of columns: silica gel columns, which separate carbon dioxide from the other two gases, in conjunction with molecular sieve columns, for the separation of oxygen, nitrogen, and carbon dioxide have been described. The sensitivity of this method was explored and found feasible for application to the determination of blood gases. Attempts to improve the accuracy of the determination by utilizing a single column rather than a combination of two were aimed at chemical modification of the molecular sieve column to release carbon dioxide at room temperature. Each method found successful in doing this destroyed the ability of the column to separate oxygen from nitrogen.

b. Detector

The RF discharge in helium was used to excite the spectra of oxygen and nitrogen analyzed by a molecular sieve gas chromatography. column. Attempts to quantitate the amount of these gases by measurement of the increase in light at the specific wavelengths for each gas were only partially successful, since the presence of these gases in the discharge cell tended to diminish the intensity of the discharge. At the same time, these experiments revealed a promising increase in sensitivity for the detection of these gases. Construction of an electronically regulated RF power source to maintain the discharge constant in intensity despite changes in gas composition is projected. It is felt that measurement of the emitted light from a discharge held constant in this way would increase the quantitative accuracy, as well as the sensitivity of the determination.

Part B included - Yes

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Part B: Honors, Awards, and Publications

Karmen, A. and Bowman, R. L.: A Radio Frequency Glow Detector for Gas Chromatography, Ann. N. Y. Academy of Sciences 72, Art. 13, p. 714-719, 3/20/59.

Karmen, A. and Bowman, R. L.: A Radio Frequency Discharge Detector for Gas Chromatography, 1959 2nd Bi-Annual International Gas Chromatography Symposium; Instrument Society of America, June 10-12, 1959.

Scientific Exhibit: Society for Clinical Investigation; Atlantic City, New Jersey, May 1959.

Karmen, A.: Electronic Detection Systems for Gas Chromatography, NIH Instrument Symposium, September 1959.

Eden, M., Karmen, A., Stephenson, J.: Use of Katharometers in Gas Chromatography, Nature 183; 1322, May 1959.

Serial No. NHI-279
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Gas Chromatograph Detector Based on Measurement of Sound Velocity

Principal Investigator: Frank W. Noble

Other Investigators: Theodore Goldsmith (summer employee)
Alexander McInnis

Cooperating Units: None

Man Years (Calendar Year 1959): Patient Days: None
Total: 1
Professional: 0.5
Other: 0.5

Project Description:

Progress During Past Year:

The effluent from a gas chromatographic column varies in its density when components of the sample are emerging. Since the velocity of sound is affected by the density of the medium through which it travels, a device which measures the sound velocity can be used as a detector for gas chromatography.

For ideal gases it can be shown that if a minute amount of a foreign substance having a molecular weight much larger than the carrier gas appears in the effluent, the phase change will be

$$\Delta\phi = \frac{180\, f\, s\, a\, M_2}{\sqrt{R\, T\, \gamma_1\, M_1}} \quad \text{degrees}$$

where: f = sound frequency
s = sound path length
a = mole fraction of sample gas
M_2 = molecular weight of sample gas
R = universal gas constant
T = absolute temperature
γ = ratio of specific heats for the carrier gas
M_1 = molecular weight of the carrier gas

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The three models which have been constructed all measure the electrical phase delay between the voltage driving a sound transmitter located at one end of a tube containing the effluent gas and the voltage generated by a sound receiver located at the opposite end. The first model operates at 1000 KC with a cell volume of 35 cc. Since this volume is larger than desired, particularly for use with capillary columns, the second model operating at 4000 KC with a cell volume of 0.08 cc was constructed. The third model uses the same frequency and cell volume as the second model, but uses two cells, one containing carrier gas only, the second containing carrier plus sample gas. A measurement is made of the phase difference between the two receivers. This procedure reduces the sensitivity to pressure, temperature, and frequency variations. The sensitivity of the device can be increased theoretically without limit by repeated frequency multiplication and conversion of the two received signals. A sensitivity increase of nine times by this means is included in the third model.

The sonic detector has been used with a Linde 13X molecular sieve to chromatograph air samples in a helium carrier. The relative areas of the oxygen and nitrogen peaks agree within about 2% of the known relative weights of these constituents in air. The detector has also been used in obtaining simultaneous chromatograms with the Burrell Thermal Conductivity detector. The extrapolated sensitivity of the sound detector is roughly 700 times that of the Burrell on the basis of the simultaneous chromatography of methyl pelargonate.

Direction of Current Research:

The detector is being tested for sensitivity to known gas mixtures to determine its measured response as compared with the theoretical values. It is predicted that in the case of heavy molecular weight samples the area under the peaks is proportional to the weight of the sample component.

Part B included - yes

Serial No. NHI-279

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

A brief paper describing the instrument will be presented on November 11th at the 12th Annual Conference on Electrical Techniques in Medicine and Biology in Philadelphia, Pennsylvania. The instrument will be on exhibit throughout the conference.

Serial No. NHI-280
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Hydraulic Pressure Generator

Principal Investigator: Frank W. Noble

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 0.10
Professional: 0.10

Project Description:

Progress During Past Year:

In order to determine the dynamic performance of cardiac manometers it is necessary to have a generator of hydraulic pressure waves of known intensity, frequency, and contour. The object of this project is to provide such a generator.

A final model of the pressure generator has been built and tested. This model will generate any pressure waveform having frequency components in the range from zero to 100 c.p.s. and having peak-to-peak amplitude of up to 50 m. m. Hg.

Direction of Current Research:

The generator is being used for testing and correcting the dynamic response of cardiac catheters (See report "Catheter Compensating Amplifier, F. Noble).

Part B included - yes.

Serial No. NHI-280

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

The pressure generator will be published in the November issue of the Journal of Laboratory and Clinical Medicine under the title, "A Device for Testing the Dynamic Performance of Blood-pressure Manometers".

Serial No. NHI-281
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: An Analog Computer for the Analysis of Overlapping Absorption Spectra

Principal Investigators: Frank W. Noble
Joseph E. Hayes, Jr.
Murray Eden

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 0.20
Professional: 0.20

Project Description:

Progress During Past Year:

A ten channel instrument of improved design was completed by the NIH Instrument Section. The new machine was installed in the Laboratory of Technical Development for use by Dr. J.E.Hayes,Jr. in the analysis of optical absorption spectra.

A series of pairs of overlapping distributions was prepared and analyzed by the new model for the purpose of determining the conditions for the detection of a second peak. These conditions were noted and included in the publication.

Direction of Current Research:

The engineering phase of this project is now complete.

Part B included: Yes

Serial No. NNI-281

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

A description of the computer will appear in the November issue of the Proceedings of the Institute of Radio Engineers under the title "A Repetitive Analog Computer for Analysis of Sums of Distribution Functions".

Serial No. NHI-282
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Catheter Compensating Amplifier

Principal Investigators: Frank W. Noble
Guy Barnett

Other Investigators: None

Cooperating Units: General Medicine and Experimental Therapeutics Branch

Man Years (Calendar year 1959) Patient Days - None
Total: 0.20
Professional: 0.20

Project Description:

Progress During Past Year:

The dynamic performance of cardiac catheters as they are commonly used leaves much to be desired. The amplitude response exhibits a sharp peak of several hundred percent at the first system resonance and the phase response is far from linear. A compensating amplifier having proper amplitude and phase characteristics may be connected between the pressure gauge and the recorder so as to improve the response of the overall system.

A compensating amplifier has been constructed and tested with Statham p-23D gauge and Cournand No. 8 catheter. The response of the uncompensated catheter system shows a resonant peak of 385% at 48 c.p.s. and very poor phase linearity. When the compensating amplifier is connected, this same system is flat within 5% to 55 c.p.s. and has very good phase linearity.

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Direction of Current Research:

An amplifier having variable controls for resonant frequency, sharpness of resonance (Q), and low frequency gain has been built. This unit is being tested on catheters of different dimensions to determine the quality of correction which can be obtained.

Part B included - No

Serial No. NHI-283
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of Nucelar Magnetic Apparatus for Blood Flow Measurement

Principal Investigators: Vsevolod Kudravcev
Robert L. Bowman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959) Patient Days: None
Total: 1.15
Professional: 1.15

Project Description:

Progress During Past Year:

The continuing project is to produce an efficient method for measuring blood flow, utilizing N.M.R. of proton nuclei in the blood stream. The basic considerations for such a flowmeter are: absence of electrical or mechanical coupling to the blood circulatory system which could influence the system's functioning, and relative freedom from electrical noises present under surgical conditions.

During the past year, new sensitive and stable experimental N.M.R. apparatus has been designed, and some additional equipment necessary to make the physical measurement of flow has been developed.

The overall performance of the apparatus is such that a strong and relatively stable N.M.R. signal is obtained over the range of 1 cc. per minute to 500 cc. per minute of liquid flow. The basic N.M.R. signal detector was designed with a special flexible circuit in mind to make measurement of the N.M.R. flow signal possible by several different methods: absorption, induction, spin nutation, spin generator (self excitation), and others. The detection device was made relatively complex in order to make choice of the best method possible for flow signal detection and measurement.

Utilizing the experimental apparatus, many different methods of N.M.R. signal excitation in flow have been investigated. As a result, nuclear induction by pulse excitation has been found more promising even with a relatively small magnetic field (2000-1500 gauss) of low homogeneity. Although somewhat different, the method is quite similar to the superregenerative method of N.M.R. signal excitation and detection.

To avoid interference from modulation pickup, and to obtain better signal/noise ratio, various methods have been devised to balance out spurious signals and background noises. On the basis of experiments with different filter networks, it was found that the best results were obtained with an LC filter inserted between the N.M.R. probe and the oscillator grid. Also, application of a negative feedback has been found to benefit the signal/noise ratio. To maximize the N.M.R. signal detectibility and measurement in small tubes, a transistorized D. C. meter amplifier has been constructed. Also, a phase detector method has been utilized, and a Q multiplier circuit introduced in the detection device.

In order to simplify the detection apparatus, semiconductor techniques have been employed, and some small transistorized devices have been designed, and tested. The devices promise to eliminate bulky power supplies, save space, and minimize weight and cost of the apparatus. However, they still need considerable experimental work to obtain performance equal to that of present vacuum tube devices.

An improved circuit utilizing semiconductor elements is now under investigation. Also, different arrangements and sizes of magnets have been constructed and investigated in order to obtain the smallest possible arrangement without sacrificing the sensitivity of the signal. To investigate the modulation effect on the signal in flowing liquid, a special flexible modulation exciter amplifier was designed and constructed; optimal modulation rate was determined experimentally.

Flow probes of different size and form have been constructed and investigated. Experimental flow curves were obtained for slow and fast flow rates of various liquids. Using the apparatus, considerable practical experience of the N.M.R. technical application to flowing liquid has been obtained, which is providing a basis for the development of a practical N.M.R. flowmeter.

Direction of Current Research:

To continue to test various combinations of conditions and techniques until the optimal arrangement for biological applications is determined. In addition to the application of N.M.R. techniques to flow measurement, consideration will be given to its analytical capabilities to measure content of a particular element in an intact expermental animal or part thereof.

Part B included - Yes

Serial No. NHI-283

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Bowman, R. L.; Kudravcev, V: "Blood Flowmeter Utilizing Nuclear Magnetic Resonance", accepted for publication in IRE Transactions on Medical Electronics.

Serial No. NHI-284
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Biochemical Effects of Ultrasonic Waves

Principal Investigator: Alfred Weissler

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959): Patient Days: None
Total: 1.0
Professional: 1.0
Other: None

Project Description:

Progress During Past Year: (Began July, 1957)

The continuing study of the effects of ultrasound on biochemical systems has concentrated mainly on hemoglobin in dilute aqueous solution. This substance was chosen because of both its function in the circulatory system and the completeness with which its various derivatives have been characterized by, for example, spectrophotometry.

It was found that oxyhemoglobin in solution, when irradiated with ultrasound, undergoes a rapid change in absorption spectrum, with the Soret peak shifting from 4150 A to 4050 A and also increasing in height. The new spectrum is characteristic of methemoglobin, in which the ferrous iron of oxyhemoglobin has undergone oxidation to the ferric state. Continued irradiation of the solution causes gradual destruction of the methemoglobin, as shown by the progressive disappearance of the absorption peak at 4050 A.

Although others have reported that sonochemical oxidation effects are suppressed by the addition of a little ether or acetone to the solution, it was found that, in the presence of ether, oxyhemoglobin does not remain unchanged by ultrasound, nor is it converted into methemoglobin. Instead, the absorption peak shifts to 4200 A, which is characteristic of carboxyhemoglobin. The carbon monoxide is formed

presumably by cavitational disruption of the ether molecule. Upon prolonged irradiation the carboxyhemoglobin suffers some destruction, but it is more resistant than methemoglobin.

Inasmuch as heme is an iron-porphyrin complex, the effect of ultrasound on a dilute solution of hematoporphyrin hydrochloride was investigated. Spectrophotometry showed that the porphyrin suffered a partial breakdown in a manner similar to that of hemoglobin. This destruction was confirmed by measurement of the porhyrin's characteristic red fluorescence on a spectrophotofluorometer.

Ultracentrifuge studies showed that lengthy irradiation of oxyhemoglobin in more concentrated solution causes also some splitting off of the heme from the heavy protein portion of the molecule.

Irradiations were performed in various chemical environments (buffer, sulfhydryl protective agent, or replacement of dissolved air by argon, nitrogen, or oxygen). The results obtained suggest that sonochemically-produced nitrous and nitric acids (rather than hydroxyl radicals or hydrogen peroxide) are the agents mainly responsible for the formation and destruction of methemoglobin by ultrasound.

Direction of Current Research:

On the basis of the hemoglobin results, solutions of sulfhydryl and other enzymes will be subjected to ultrasonic treatment under conditions which may modify the amount of activity loss. Another subject of interest is the way in which ultrasonic irradiation causes cell damage and killing; attempts will be made to assess the relative importance of chemical versus mechanical mechanisms in the damage produced by ultrasound in a protozoan, Paramecium caudatum. Also under consideration is a study of ultrasonic absorption spectra and velocity dispersion in biochemical materials.

Part B included - yes

Serial No. NHI-284

PHS - NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Award, and Publications

Weissler, A.: "Formation of Hydrogen Peroxide by Ultrasonic Waves: Free Radicals", J. Am. Chem. Soc., 81, 1077-1081 (1959).

Serial No. MHI-285
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Machine Analysis of Absorption Spectra

Principal Investigator: Joseph E. Hayes, Jr.

Cooperating Units:
Spectra are determined in a Cary Model 11 Spectrophotometer in the Laboratory of Natural Products, National Heart Institute.

Man years (calendar year 1959): Patient days - none
Total: .1
Professional: .1
Other:

Project Description:

Progress During Past Year: (Began 1958)

Time spent on this project during the past year has been devoted to further extending the series of compounds whose spectra, obtained in various solvents, are to be analyzed; and to further purification of some of them.

Beginnings have been made toward a cooperative project with Dr. H. Fales of LCNP, the object of which is an analysis of the infra-red spectra of a selected series of hydrocarbons with a view toward increased precision of band assignments in such spectra. It is hoped that data of this kind can increase the usefulness of analysis of infrared spectra as a tool in differentiating closely related chemical structures.

Direction of Current Research:

The spectra of these compounds, in nonaqueous, acid aqueous, and alkaline aqueous solution in the case of the compounds whose UV-visible spectra are to be studied and in trimethylpentane solution for the infrared series, should permit assessment of whether the correlations found are useful. If so, they will be extended to compounds of greater intrinsic interest.

Part B Included - NO

Serial No. NHI-286
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Phosphorescence in Aqueous Media

Principal Investigator: Joseph E. Hayes, Jr.

Cooperating Units:

Man years (calendar year 1959): Patient Days - None
Total: .5
Professional: .5
Other:

Project Description:

Progress During Past Year: (Began 1959)

The Aminco-Keirs Spectrophosphorimeter which has become available offers a possibility of applications of the type of study in which the Spectrophotofluorimeter has been so valuable to certain compounds which do not fluoresce. It may be generally stated that while it is highly unlikely that in any given case phosphorescence would be as intense as fluorescence, numerous non-fluorescent substances are phosphorescent and thus measurable though at a sacrifice in sensitivity from that enjoyed in the fluorimetric methods. Also, for characterization purposes, an additional parameter is measurable: the mean lifetime of the excited state.

Now the basic requirements for a suitable phosphorimetric solvent are that it have low absorption in the activating region of the spectrum and that at suitably low temperatures it set to a rigid glass. Crystallization upon freezing vastly complicates the system and renders measurements of emission intensity unreproducible. All solvents heretofore used have been organic mixtures with liquid nitrogen as coolant. In these mixtures most compounds of biological interest are difficultly soluble and many quite insolubls.

We have not been successful in finding a basically aqueous system which does not crystallize at -196°C., the temperature of liquid nitrogen. However, the use as a coolant of liquid nitrous oxide which boils at -89.5°C. permits

Progress During Past Year (continued)

an aqueous solution to which has been added an equal volume of propylene glycol to form a perfectly clear and suitable glass. The only real disadvantage is that at this higher temperature the lifetimes of excited states are generally somewhat shorter. Using this technique frozen glasses containing, for example, 2 mg./ml. of hemoglobin have been prepared. Thus a system has been developed which should be of quite general applicability to examination of the phosphorescence of biological substances.

A simple example of a phosphorescent but not fluorescent substance is benzoic acid. A series of the three position isomers of each of ten monosubstituted benzoic acids has been collected and is currently being studied to learn the effect of structure in this sense on phosphorescence. These measurements are actively under way and on the basis of those completed at present two conclusions may be drawn:

1) In the dry crystalline state, activated at 366 mu, light emission becomes less likely with increasing electronegativity of the substituent.

2) In the glass at low temperature, at least for substituents more electropositive than hydrogen, the meta isomers emit with much lower intensity and much shorter lifetime than the corresponding ortho and para isomers.

Direction of Current Research:

Current work is directed toward completion of measurements on the benzoic acid series as the undissociated acids and as benzoate ions in the aqueous system described and also as the acids in a solvent known as "EPA", a 5:5:2 mixture of ether, isopentane, and ethanol in which the great majority of published phosphorimetric measurements have been made. This is to be followed by a survey of materials of biological and pharmacological importance.

Part B Included - NO

Serial No. NHI-237
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Physics of Ultra-Rapid Freezing of Water, Colloidal Solutions and Protoplasm.

Principal Investigator: John L. Stephenson

Other Investigators: Artrice Valentine

Cooperating Units: National Cancer Institute, Pathologic Anatomy Branch.

Laboratory of Kidney Electrolyte Metabolism.

Man Years (calendar year 1959) Patient Days - None
Total: 1.5
Professional: .5
Other: 1.0

Project Description:

Progress During Past Year: (Began 1954)

The general purpose of this project remains: (1) To investigate the basic physics of the rapid freezing process in water, colloidal systems and protoplasm. (2) To apply this information to the analysis of hydration phenomena in protoplasm. (3) To extend the range of application of freezing and drying as a method of fixation and preservation of biological material.

During the past year we have begun an experimental investigation of nucleation in colloidal systems. One of the principal problems in the solidification of any system is the rate at which freezing "nuclei" appear as the system is cooled below its equilibrium freezing temperature. (The other main problem is the subsequent growth of these nuclei.) Extensive studies on this problem have been carried out for pure water because of its importance to rain and snow formation. However, very little work has been done on colloidal aqueous systems. We have recently begun work on this problem. The first step has been to design a

suitable cold stage for microscopic observations of the freezing of small drops. Using this stage, we have been able to observe the super-cooling of small water droplets to about -40°C, in agreement with the observations of others. This work will now be extended to various colloidal systems.

Theoretical work has continued on a variety of heat transfer problems which have arisen in connection with the analysis of cooling curves recorded from micro-thermocouples. The problem of heat evolution from a growing ice-crystal has also been studied. This is a very interesting problem with general application to solidification of melts. It also is analogous to a variety of precipitation problems with moving phase boundary. Unfortunately these problems have been solved for only plane-parallel geometries and then only for very restricted cases. Some computer work is beginning to be done in this area. We have obtained what appear to be some useful approximate solutions and are considering the feasibility of some machine computation.

We have cooperated with Dr. Bray in his study of comparative melting temperatures of different localities of the rat kidney, doing some routine histology for him. In addition some of theory of nucleation and crystal growth appear to have some bearing on the interpretation of the results of melting point determination of small crystals.

The facilities of the electron microscopic set up have been made available to the pathology section in their study of kidney biopsy material. So far this material has been fixed by conventional osmic acid methods.

Two papers describing current and earlier work have been prepared for publication. One "Fundamental Physical Problems in Freezing and Drying of Biological Materials" will appear in Recent Advances in Freezing and Drying, now in press. The other was presented at N.Y. Acad. Sciences symposium and should ultimately appear in Annals N. Y. Acad. Sciences.

Direction of current research: Work is being continued on the current problems described above.

Serial No. NHI-288
Laboratory of Technical Development
Bethesda 14, Maryland

PHS - NIH
Individual Project Report
Calendar Year, 1959

Part A.

Project Title: Mathematical Investigation of Biological Transport Problems

Principal Investigator: John L. Stephenson

Other Investigators: Arnold Jones

Cooperating Units: Dr. Donald Fredrickson, Laboratory of Cellular Physiology and Metabolism

Man Years (calendar year 1959)
Total: 1.25
Professional: .5
Other: .75

Patient Days - None

Project Description:

Progress During Past Year: Began 1957

The general purpose of this research has remained to develop a general theory of transport phenomena in linear biological systems and to apply it to particular biological problems. The work on multicompartment systems and the general problems of data analysis and model construction have been extended. Two papers: "Theory of Transport in Linear Biological Systems, Part I. Fundamental Integral Equation", and "Theory of Transport in Linear Biological Systems, Part II. Multiflux Problems", have been put in final form and have been accepted for publication in the Bull. Math. Biophys.

Work on the analysis of tracer data on fatty acid metabolism in collaboration with Dr. Donald Fredrickson has been continued. Mr. Arnold Jones has completed the programming for the IBM 650 and some data has been run on the machine.

Direction of Current Research:

There are two phases of the present program. One is to apply the general theory to particular problems in tracer analysis. Here, work is being continued on the fatty acid problem and on capillary exchange problems. It also may be possible to consider other particular problems.

The other phase is extension of the general theory. Here the obvious extension is to consider the general theory as a problem in linear analysis.

Part B included - yes.

Serial No. NHI-288

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Stephenson, J. L.: "Theory of Measurement of Blood Flow by Dye Dilution Technique", IRE TRANSACTIONS ON MEDICAL ELECTRONICS, Volume PGME-12, pp. 82-88, December 1958.

Serial No. MHI-289
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Quantitation of Mitral Valve Regurgitation

Principal Investigators: Peter L. Frommer
Eugene Braunwald

Other Investigators: None

Cooperating Units: The project is a joint undertaking of the Laboratory of Technical Development and of the Cardiology Section of the Surgery Branch, both of the National Heart Institute. The physical facilities of the Diagnostic X-ray Department of the Clinical Center and the assistance of its technical staff has been made available.

Man Years (calendar year 1959)
Total: 0.37
Professional: 0.33
Other: 0.04

Patient days: None

Project Description:

Progress During Past Year: (Began July 1959)

The overall purpose of this investigation is the establishment of a technique for accurately quantitating valvular regurgitation. The existing methods were reviewed - pressure curves and indicator dilution methods - and it was felt that the inherent limitations of these techniques require a brand new approach, rather than attempts at refinements on the present methods.

We could not envision any method of direct measurement of the regurgitant flow across the valve. Calculating regurgitant flow as the difference between change in ventricular volume during systole and the forward stroke volume was rejected because the existing methods of determining intra-ventricular volume are too crude and no promising method could be envisioned.

It was felt that the most promising approach would be the optical density quantitation of a left heart angiogram.

Radiopaque dye would be injected into the left ventricle, rapid serial X-rays would be taken, and the optical density increase over the left atrium would be quantitated in terms of dye regurgitated.

While other investigators have used left heart angiograms for diagnosis and crude evaluation of the degree of regurgitation (0 to 4 plus), there have been no attempts at closely controlling the conditions of the test let alone at quantitating the results.

The investigation was begun with a series of tests to determine favorable conditions of film exposure for quantitation of densities. This was followed by in-vitro studies still in progress in which the amount of radiopaque dye added to a pool of water is quantitated by the change in density produced in "before and after" films. A step wedge filled with known depths and concentration of the radiopaque dye is in one corner of each film to serve as a means of calibrating for minor variations in exposure and development. In a point by point scan of the pool of water, pre-existing optical densities are expressed as equivalent milligrams of the radiopaque dye and are subtracted from the densities obtained after the addition of dye. Studies to date suggest that in-vitro accuracies of 10 to 20% are feasible.

As a by-product of the above investigation a novel mathematical analysis of dye dilution curves for systems with or without regurgitation was developed. A very simple electrical analogue for this was built and in this analogue the effects of incomplete mixing and of gradual injection have been simulated. While the analog system precisely reproduces the mathematical analysis and the mode of incomplete mixing assumed, these are but qualitative approximations of the dye dilution process and the incomplete mixing as they occur in the heart. Therefore the results serve for qualitative evaluation of dye curves and they reveal further the pitfalls of reliance on quantitative data from such curves.

Direction of current research:

The dye dilution analysis and its electrical analogue are almost ready for publication. Thereafter, in-vitro quantitations of dye will be resumed with further efforts to minimize effects of non-uniform X-ray beam, intensifiers, development, and secondary scatterings. Modification of the existing rapid X-ray film changer will be considered so that it would be synchronized with components of the electrocardiogram pattern. Because the

procedures contemplated on the patient are essentially standard and proven safe, after brief trials in the dog lab the technique can be applied to patients undergoing left heart catheterization and compared with the accepted dye dilution and pressure gradient methods. If the results continue to be satisfactory to this point, methods of automatic scanning and quantitating of the film densities will be considered.

If satisfactory results are not obtained from this technique, consideration will be given to another method of quantitating regurgitation in which radiopaque particles 1 or 2 mm. in size would be injected into the ventricle and the number of particles over the atrial shadow would be compared to that in the ventricle and in the aorta and to that injected. Studies with intravascular radiopaque particles (Urokon-dextrose pellets) have been restricted to observing streaming and hydrodynamic phenomena in dogs. While it has been observed that such pellets dissolve completely within a fraction of a minute and the dogs have shown no sign of embolization, very extensive fundamental tests would have to be introduced before clinical use is contemplated.

Part B Included - NO