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BROOKHAVEN NATIONAL LABORATORY

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1. Alternating Gradient Synchrotron
2. Research Reactor and Hot Laboratory
3. Cosmotron
4. Cyclotron and Van de Graaff Building
5. Medical Research Center and Medical Reactor
6. Biology laboratories
7. Nuclear Engineering Building
8. Physics Building
9. High Flux Beam Research Reactor
10. Chemistry Building

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Department of Medicine

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Term expires October 1966

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Department of Physiology Medical School

University of Minnesota, Minneapolis, Minn.

Term expires October 1967

GEORGE Z. WILLIAMS, M.D.

Chief, Clinical Pathology Department

Clinical Center, Bethesda, Md.

Term expires October 1967

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Department of Radiology, University Hospitals

Western Reserve University, Cleveland, Ohio

Term expires October 1968

ELMER H. STOTZ, Ph.D.

Professor of Biochemistry, University of Rochester

School of Medicine and Dentistry, Rochester, N.Y.

Term expires October 1968

STANLEY E. BRADLEY, M.D.

Chairman, Department of Medicine

College of Physicians and Surgeons

Columbia University, New York, N.Y.

Term expires October 1969

ROBLEY D. EVANS, Ph.D.

Department of Physics

Massachusetts Institute of Technology

Cambridge, Mass.

Term expires October 1969

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Department of Health
Radiological Health Program
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Anderson, Laurens, Ph.D.
University of Wisconsin
Madison, Wis.

Aponte, Gonzalo E., M.D.
Jefferson Medical College
Philadelphia, Pa.

Archambeau, John O., M.D.
Meadowbrook Hospital
East Meadow, N.Y.

Ariel, Irving M., M.D.
Pack Medical Group
New York, N.Y.

Arnott, Struther, Ph.D.
Medical Research Council
Biophysics Unit
Kings College, London, England

Bateman, John L., M.D.*
Columbia University Radiological
Research Laboratories
New York, N.Y.

Ben-Ishay, Drori, M.D.
Hadassah Medical Organization
Jerusalem, Israel

Berman, Mervyn C., M.B., Ch.B., Ph.D.*
University of Cape Town
Cape Town, South Africa

Bessis, Marcel C., M.D.
National Center for Blood Transfusion
Paris, France

Bloom, Barry R., Ph.D.
Albert Einstein Medical College
Bronx, N.Y.

Blumberg, Baruch S., M.D., Ph.D.
National Institutes of Health
Bethesda, Md.

Brecher, George, M.D.
National Institutes of Health
Bethesda, Md.

Brown, Raymond, R., Ph.D.
University of Wisconsin
Medical School
Madison, Wis.

Burlington, Harold, Ph.D.
University of Cincinnati
College of Medicine
Cincinnati, Ohio

Carson, Paul E., M.D.
University of Chicago
Chicago, Ill.

Corpe, William A., Ph.D.
Columbia University
New York, N.Y.

Cottier, Hans, M.D.
University of Bern
Bern, Switzerland

Di Ferrante, Nicola, M.D.
Baylor University
School of Medicine
Houston, Texas

Easterday, Otho D., Ph.D.
Gulf South Research Institute
New Orleans, La.

Eisenstein, Edward M., Ph.D.
State University of New York
at Stony Brook
Stony Brook, N.Y.

Farr, Lee E., M.D.
The University of Texas
M.D. Anderson Hospital
and Tumor Institute
Houston, Texas

Feinendegen, Ludwig E., M.D.
Institut du Radium
Paris, France

Fireman, Philip, M.D.
University of Pittsburgh
School of Medicine
Childrens Hospital
Pittsburgh, Pa.

Fishman, Marvin, Ph.D.
Public Health Research Institute
of the City of New York
New York, N.Y.

Fliedner, Theodor M., M.D.
Institute of Hematology of the GSF
associated with EURATOM
Freiburg/Breisgau, West Germany

Foradori, Arnaldo C., M.D.*
Universidad Católica
Santiago, Chile

Franzblau, Carl, Ph.D.
Boston University School of Medicine
Boston, Mass.

Fraser, Alexander S., Ph.D.
University of California
Davis, California

Friedkin, Morris E., Ph.D.
Tufts University School of Medicine
Boston, Mass.

Fuller, Watson, Ph.D.
Medical Research Council
Biophysics Unit
Kings College, London, England

Furth, Jacob, M.D.
Francis Delafield Hospital
New York, N.Y.

Gandal, Charles P., D.V.M.
N.Y. Zoological Society
Bronx, N.Y.

Gitlin, David, M.D.
University of Pittsburgh
School of Medicine
Childrens Hospital
Pittsburgh, Pa.

Grady, Frank J., M.D.*
Institute of Ophthalmology
Columbia Presbyterian Medical Center
New York, N.Y.

Hale, William M., M.D.
The University of Tennessee
College of Medicine
Mountain View, Ark.

*Research Collaborator in Residence.

Hammerstrom, Richard A., Ph.D.
Shell Development Company
Modesta, Calif.

Hare, William C.D., Ph.D.
University of Pennsylvania
Philadelphia, Pa.

Hart, Hiram E., Ph.D.
City College,
City University of New York
New York, N.Y.

Hastings, A. Baird, Ph.D.
Scripps Clinic
and Research Foundation
La Jolla, Calif.

Hess, Max W., M.D.
University of Bern
Bern, Switzerland

Hughes, Edwin R., M.D.
University of Arkansas Medical Center
Little Rock, Ark.

Hughes, Walter L., Ph.D.
Tufts University School of Medicine
Boston, Mass.

Hurley, Lucille, Ph.D.
University of California
Davis, California

Jamuar, Mahendra P., Ph.D.*
Damon Runyon Memorial Fund
New York, N.Y.

Jansen, Cornelius R., M.B., Ch.B.
Atomic Energy Board
Pretoria, South Africa

Jesseph, John E., M.D.
The Ohio State University
Columbus, Ohio

Joubert, Septimus M., M.B., Ch.B.
University of Natal
Durban, South Africa

Kaplan, Neal L., D.D.S.
New York University
College of Dentistry
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Keiser, Georg A., M.D.
University of Zurich
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Krohn, David L., M.D.
Unaffiliated

Lamdin, Ezra, M.D.
University of Cincinnati
College of Medicine
Cincinnati, Ohio

Lax, Louis C., M.D.
University of California
School of Medicine
Los Angeles, Calif.

Leopold, Irving H., M.D.
The Mount Sinai Hospital
New York, N.Y.

Levine, David A., Ph.D.
Republic Aviation Corporation
Farmingdale, N.Y.

Lipkin, Martin, M.D.
Gastroenterology Laboratory
Bellevue Hospital
New York, N.Y.

Lippincott, Stuart W., M.D.
Medical College of Virginia
Richmond, Virginia

Lowenstein, Bernard E., M.D.
Unaffiliated

Lowrey, Austin, M.D.
Unaffiliated

Malis, Leonard I., M.D.
The Mount Sinai Hospital
New York, N.Y.

Malsky, Stanley J., Ph.D.
Veterans Administration Hospital
Bronx, N.Y.

Manspeizer, Sheldon, M.D.
Jacobi Hospital
Bronx, N.Y.

Marshak, Robert R., D.V.M.
University of Pennsylvania
Philadelphia, Pa.

Mathieu, Georges R., M.D.*
Fondation Curie
Paris, France

*Research Collaborator in Residence.

Maxfield, Myles, M.D., Ph.D.
University of Southern California
Los Angeles, Calif.

Mena, Ismael, M.D.
Catholic University Hospital
Santiago, Chile

Merrill, John, M.D.
Peter Bent Brigham Hospital
Boston, Mass.

Meyer, Leo M., M.D.
Queens Hospital Center
Long Island Jewish Hospital
Jamaica, N.Y.

Montjar, Monty J., Ph.D.
St. Vincent College
Latrobe, Pa.

Moon, Harley W., D.V.M., Ph.D.
University of Saskatchewan
Saskatoon, Canada

Nathan, Paul, Ph.D.
University of Cincinnati
College of Medicine
Cincinnati, Ohio

Odartchenko, Nicolas, M.D.
Swiss Institute for Cancer Research
Lausanne, Switzerland

O'Neill, John J., Ph.D.
University of Maryland
School of Medicine
Baltimore, Md.

Overweg, Norbert I.A., M.D.*
The Mount Sinai Medical
and Graduate Schools
New York, N.Y.

Pentel, Leon, D.D.S.
New York University
College of Dentistry
New York, N.Y.

Price, David C., M.D.
University of California
Berkeley, Calif.

Rahm, Laura W., Ph.D.
Unaffiliated

- Rall, Joseph E., M.D., Ph.D.**
National Institutes of Health
Bethesda, Md.
- Rasmussen, Howard, M.D., Ph.D.**
University of Pennsylvania
Philadelphia, Pa.
- Ray, Bronson S., M.D.**
The New York Hospital—
Cornell Medical Center
New York, N.Y.
- Robbins, Jacob, M.D.**
National Institutes of Health
Bethesda, Md.
- Rossi, Harald H., Ph.D.**
Columbia University Radiological
Research Laboratories
New York, N.Y.
- Schnappauf, Hanspeter, D.V.M.**
Veterinar-Physiologisches Institut
Giessen, West Germany
- Schwartz, Irving L., M.D.**
The Mount Sinai Medical
and Graduate Schools
New York, N.Y.
- Severson, Arlen, Ph.D.***
National Institutes of Health
Bethesda, Md.
- Shellabarger, Claire J., Ph.D.**
University of Michigan,
Kresge Laboratories
Ann Arbor, Mich.
- Shemin, David, Ph.D.**
Columbia University
College of Physicians and Surgeons
New York, N.Y.
- Sinex, F. Marott, Ph.D.**
Boston University School of Medicine
Boston, Mass.
- Sondhaus, Charles A., Ph.D.**
California College of Medicine
Los Angeles, Calif.
- Stickley, Elmer E., Ph.D.**
Columbia University Radiological
Research Laboratories
New York, N.Y.
- Stohlman, Frederick, M.D.**
St. Elizabeth Hospital
Brighton, Mass.
- Stone, William H., Ph.D.**
University of Wisconsin
College of Agriculture
Madison, Wis.
- Suhadolnik, Robert J., Ph.D.**
Albert Einstein Medical Center
Philadelphia, Pa.
- Sutow, Wataru W., M.D.**
The University of Texas
M.D. Anderson Hospital
and Tumor Institute
Houston, Texas
- Tashjian, Andrew J., M.D.**
Methodist Hospital
Brooklyn, N.Y.
- Teree, Thomas M., M.D.**
Western Reserve University
School of Medicine
Cleveland, Ohio
- Terres, Geronimo, Ph.D.**
Stanford University
Palo Alto, Calif.
- Theodoropoulos, Dimitrios M., Ph.D.***
The Mount Sinai Medical
and Graduate Schools
New York, N.Y.
- Thomas, E. Donnell, M.D.**
University of Washington
School of Medicine
Seattle, Wash.
- Thorp, William T.S., D.V.M.**
University of Minnesota
College of Veterinary Medicine
St. Paul, Minn.
- Tometsko, Andrew M., Ph.D.**
University of Rochester
School of Medicine
Rochester, N.Y.
- Trautman, Rodes, Ph.D.**
Plum Island Animal Disease Laboratory
U.S. Department of Agriculture
Greenport, N.Y.
- Vincent, Paul C., M.D.**
University of Melbourne
Department of Medicine
Victoria, Australia
- Walter, Roderich W., Ph.D.***
The Mount Sinai Medical
and Graduate Schools
New York, N.Y.
- Wasserman, Louis R., M.D.**
The Mount Sinai Hospital
New York, N.Y.
- Wilkins, M.H.F., Ph.D.**
Medical Research Council
Biophysics Unit
Kings College, London, England
- Wood, David, M.D.**
University of California Medical Center
San Francisco, Calif.
- Yamamoto, Y. Lucas, M.D.**
McGill University
Montreal Neurological Institute
Montreal, Canada
- Zahalsky, Arthur C., Ph.D.**
Haskins Laboratories
New York, N.Y.

*Research Collaborator in Residence.

Laboratory Objectives and Program

Brookhaven National Laboratory is a national research center in which the Laboratory staff and scientists from other institutions, especially those located in the northeastern United States, carry out fundamental and applied research in the nuclear sciences and related subjects as an integral part of the nationwide program of the Atomic Energy Commission. It was established as a cooperative venture between the Government and nine leading northeastern universities (Columbia, Cornell, Harvard, Johns Hopkins, the Massachusetts Institute of Technology, the University of Pennsylvania, Princeton, the University of Rochester, and Yale) in recognition of the need for large and expensive equipments and for concentrations of scientific manpower for the successful prosecution of nuclear research. The primary objectives of the Laboratory are:

1. To seek new knowledge in the nuclear sciences and related fields with emphasis on programs that require such large-scale research tools as nuclear reactors, accelerators, and special laboratories which are beyond the scope of most or all individual institutions.
2. To encourage appropriate use of its facilities by scientists of college, university, industrial, and other laboratories.
3. To assist the Atomic Energy Commission in the solution of specific problems by utilizing the Laboratory's unique facilities or the special talents of its staff.
4. To make use of the Laboratory as an important auxiliary in the training of scientists and engineers and otherwise to assist in the dissemination of scientific and technical knowledge.

The cooperative nature of the Brookhaven program is of paramount importance. A significant and increasing fraction of the scientists and engineers directly engaged in the scientific program is comprised of visitors from other institutions who take advantage of the special opportunities at Brookhaven to carry out specific research and to gain useful knowledge and experience.

These objectives, of pioneering in research fields requiring large and specialized equipment, of making the Laboratory's facilities available to visiting scientists, and of furthering the education and training of young scientists, exert a profound influence on the life of the Laboratory and on its planning with respect to both staff and facilities. Because of the constantly changing work and the rotation of the staff, a maximum degree of flexibility is demanded; the continuing presence of specially skilled groups and adequate and specialized laboratories and other facilities are required for the development, construction, and effective utilization of advanced scientific equipments; problems of housing, transportation, etc., are accentuated by the large number of scientists on temporary assignment, by the relative remoteness of the Laboratory from centers of population, and by the resort nature of the surrounding area. All these factors must be considered in the development of future plans.

The scientific program can be broadly divided into five general categories:

1. Fundamental studies of atomic nuclei, the particles which constitute them, and the forces involved in their structure.

2. Study and exploitation of the physical, chemical, and biological effects of nuclear radiation.

3. The use of nuclear tools such as neutrons, charged particles, gamma rays, and isotopic tracers in all branches of scientific research.

4. Research and development not necessarily of a nuclear nature but useful in atomic energy development.

5. Useful applications of nuclear power.

The research is centered on, though not confined to, the use of several large equipments and other special facilities, which include a large, graphite-moderated, air-cooled nuclear reactor with accompanying laboratories suitable for work at low radiation levels; a small nuclear reactor for medical use; a hot chemistry laboratory for work at intermediate and high radiation levels; two proton synchrotrons, the Alternating Gradient Synchrotron (AGS) and the Cosmotron, which operate at approximately 30 BeV and 3 BeV, respectively; a 60-in. cyclotron capable of accelerating deuterons, protons, helium-3 ions, tritons, and alpha particles; a 3.5-MeV positive particle Van de Graaff accelerator; and a 2-MeV electron electrostatic accelerator. Several large computers have recently been installed to process data resulting from the use of many of the Laboratory's research facilities. In mid-1963 the 80-in. Bubble Chamber was completed; it is now in full operation for high energy physics research with the AGS. The High Intensity Radiation Development Laboratory houses several million curies of gamma radiation sources for experiments designed to develop techniques in the applications of radiation energy. The High Flux Beam Research Reactor, designed to provide intense beams of neutrons for research in the physical sciences, produced its first self-sustaining chain reaction in October 1965.

The scientific work is carried on by eight departments and two divisions: The Physics, Chemistry, Biology, Medical, and Nuclear Engineering Departments, which conduct research and development in the indicated fields; the Accelerator Department, which is responsible for the design, construction, and operation of the large accelerators; the Instrumentation and Health Physics Department, which develops, constructs, and services nuclear instruments and is responsible for radiation protection throughout the Laboratory; the Reactor Division, which operates the research reactors; the Applied Mathematics Department; and the Mechanical Engineering Division.

Staffing

The Laboratory scientific staff now includes approximately 435 "regular" members, 80 postdoctoral research associates with tenure limited to two years, and 500 full- and part-time visitors from other institutions. Of the last group, approximately 300 spend a month or more at the Laboratory, an average of about 100 actually being on site at any one time. In addition, approximately 300 scientists and students participate in Brookhaven's research program during the summer.

The Medical Department Program

VICTOR P. BOND, M.D., PH.D.

Chairman, Medical Department

In developing its research program, the Medical Department has several responsibilities. It has the broad responsibility to advance medical knowledge, and the more specific responsibility to advance understanding of the effects of ionizing radiations in man. Some of the investigations are directed toward beneficial applications of these radiations and toward improvement of measures to prevent or counteract their detrimental effects. Other investigations are centered on elucidation of disease states and development of improved methods of diagnosis and therapy. Investigations in these two areas complement each other, since the study of radiation effects contributes to the understanding of diseases, and vice versa, yet the pace of progress in both is controlled by the advances in knowledge of the normal biological processes of man.

A great influence on these advances is the rapid development of new techniques for the examination of structures and mechanisms at the molecular as well as the subcellular and biochemical levels of organization. Information revealed through these techniques and with the aid of such instruments as the electron microscope, dictates re-examination of medical and biological phenomena at even finer levels of organization. Findings from such investigations present the Department with the additional responsibility to exploit them for clinical applications.

The 48-bed Hospital of the Medical Research Center, staffed and equipped to provide a high standard of clinical service to the patients, is the essential facility for those engaged in clinical investigations. Since virtually all effects produced by ionizing radiation in the living organism can be observed in pathological conditions of other etiology, information relevant to the pathogenesis, therapy, and prevention of radiation injury can be obtained from patients with a variety of diseases. Some of those currently under study include disorders of hemopoiesis, disorders of the central nervous system, disorders of metabolism, and neoplastic processes. The studies incorporate the utilization of radioactive isotopes both in the elucidation of disease conditions and in the development and use of new diagnostic possibilities.

Studies of human beings are necessarily limited and usually require preliminary and complementary studies of other mammalian species. Veterinary facilities for species ranging from mice to the bovine are available for these supplementary investigations and for the study of basic problems carried out in the laboratories. These latter, including researches on the fundamental interaction of radiation with tissues and on the pathogenesis and therapy of radiation injury in mammals, range from basic biochemical studies of the metabolism of nucleic acids, proteins, carbohydrates, enzymes, vitamins, or trace metals to those of the kinetics of cell turnover, immunological problems, and the utilization of

viable hemopoietic cell transfusion in the therapy of radiation injury by replacement or substitution. "Long-term" effects of radiation also are studied with respect to problems of carcinogenesis and phenomena frequently associated with aging.

Many special facilities are utilized in the program. The Medical Research Reactor, specifically designed for the irradiation of patients, provides also for controlled exposures of animals, irradiation of elements in tissue for activation analysis, and the manufacture of very short-lived isotopes. X rays and gamma rays are available from various conventional sources. Neutrons and accelerated charged particles are available at a Van de Graaff generator, 60-in. cyclotron, and 3-BeV proton synchrotron (the Cosmotron). Special equipment is available for handling and measuring radioactive substances with activity or energy ranging from very high to very low, including a steel room used for determining the whole-body burden in humans.

The diversified talents and extensive facilities uniquely concentrated at Brookhaven provide the Department with unusual advantages for pursuing its objectives. Its broadly based program incorporates the experience and skill not only of those devoted to research in the field of medicine but of many from the various disciplines in other Departments and from collaborating institutions. The interchange of ideas, information, facilities, and services is essential for the staff to keep abreast of the new developments that have medical implications, and within the framework of the program opportunities are limited only by the scope of the vision and interest of the individual investigator.

The scientific staff of the Medical Department is limited by policy and design to a size large enough to provoke stimulation within itself but not so large that each person may not be well acquainted with the work of his colleagues. It is large enough that necessary facets of medical and diagnostic services can be covered responsibly in the Hospital but not so large as to require organization of several services. Although the Department is administratively organized into seven divisions – the Hospital, Biochemistry, Experimental Pathology, Physiology, Microbiology, Medical Physics, and Industrial Medicine – functionally it operates as a single unit with no jurisdictional barriers impeding activities within the Department.

Since it is the policy of Associated Universities, Inc., that a large fraction of the staff shall be rotated in order that universities may benefit by having men on their faculties who have worked at Brookhaven and that Brookhaven may benefit by a constant infusion of new enthusiasm brought to it from universities and institutes, there is a general policy limiting the time a man may remain on the staff at Brookhaven in its several ranks of term appointments. On term appointments a man may, if reappointed, stay up to a maximum of nine years provided he begins his experience here as an assistant or associate scientist. A man beginning his appointment as a scientist or senior scientist is limited to three or five years depending upon his individual category. It is generally expected that most term appointees to the staff will spend approximately three to five years at Brookhaven becoming thoroughly qualified in the areas for which opportunities are provided here. It is anticipated that most appointments will be of junior men who come for education, training, and experience in the field of this Department and who will later return to university faculties.

Taking into account these policies, the scientific staff of the Medical Department is organized into three categories:

I. Full-time, Regular Staff.

- A. Tenure scientists who hold their positions under terms comparable to professional tenure in university faculties.
- B. Indefinite appointment scientists who hold their positions indefinitely for performance of a necessary function in the Department or Laboratory organization.
- C. Term scientists who hold appointments for specific terms.

II. Part-time, Temporary Staff. In a few special situations, part-time, temporary appointments are given for operational reasons to qualified persons.

III. Research Collaborator Staff.

The research collaborator staff is composed of those holding academic appointments in universities or equivalent appointments in hospitals or institutions who are given appointments to the staff of the Medical Department for terms renewable up to one year for intermittent work during those terms in collaboration, extension, or intensification of work of joint interest to the research collaborator and to a regular staff appointee in the Medical Department. Those holding fellowships from foundations or governmental agencies are generally given appointments as research collaborators in residence which permit them to be granted working privileges of the regular staff and to be integrated into the research, training, and education programs of the Department. Brookhaven does not award fellowships. On the other hand, the Department does have a limited number of one-year appointments as Research Associate renewable for an additional term of one year. These appointments are also available to persons requiring the facilities of the Medical Research Center and the Laboratory for carrying on postdoctoral investigations.

The categories of the scientific staff previously noted are further divided into four ranks: senior scientist, scientist, associate scientist, and assistant scientist.

The medical staff of the Hospital and the medical supporting staff of the Hospital are separately designated groups of the Medical Department scientific staff which include those who by training and experience are qualified to meet the specific clinical responsibilities of the Hospital. Likewise, the Hospital staff includes a separately designated group of the technical staff trained to meet specific responsibilities. Rank of a person on the medical staff of the Hospital and on the scientific staff is not always identical, being adjusted rather to specific interests, qualifications, and use. Inquiries regarding staff appointments are welcomed and should be addressed to the Chairman, Medical Department.

The technical staff of the Department is a career staff in the same manner as is the scientific staff. It is composed of technically qualified persons, individually selected for their interests and aptitudes, who bring a wide variety of skills and techniques to the Department's program. Other types of specialized professional training and skills are represented by the nursing staff, clinical services staff, and the other variously designated categories of the technical staff of the Hospital.

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The following are examples of investigative work carried out in the Medical Research Center. Most studies involve investigations in both animals and human beings, although some are limited to one or the other. Detailed coverage of the work in progress obviously has not been possible, and the reader is referred to publications of the Medical Department for additional information.

A. EFFECTS OF RADIATION

1. Extracorporeal Irradiation of the Blood and Lymph

E.P. Cronkite
H.L. Atkins
A.D. Chanana
M.L. Greenberg
D.D. Joel
J.S. Robertson
L.M. Schiffer
R.D. Stoner
P.A. Stryckmans

The major objectives in this research are the study of lymphopoietic kinetics, the study and treatment of leukemia, and the development of a method to prevent rejection of homotransplanted tissues and organs. Extracorporeal irradiation of the blood (ECIB) and lymph (ECIL) is a method designed to destroy circulating radiosensitive cells without producing radiation injury in the rest of the organism. Arterio-venous shunts are utilized in ECIB and thoracic duct-venous shunts in ECIL to route the blood or lymph through a high-intensity gamma-ray field and back into the body.

By irradiating blood in an extracorporeal circuit, advantage is taken of the dichotomy between the radiosensitivity of lymphocytes and the radioresistance of other blood elements. Blood lymphocytes recycle through the lymphoreticular organs, and the lymphocyte population of the body can be drastically depleted by ECIB and/or ECIL without injury to other tissues. ECIB selectively destroys leukemic cells in the peripheral blood without the concomitant side effects of cytotoxic drugs or external beam irradiation, and the technique is being used in the study and treatment of several different types of human leukemia.

Ten patients with acute myelocytic leukemia have been treated: one has been under treatment for 14 months and is in partial clinical remission, three others have been under treatment for 8 months, one patient has just started treatment, and five are deceased. One patient was treated during pregnancy and was delivered of a normal infant. Of four patients with chronic myelocytic leukemia that have been treated, two have had a clinical remission, together with a decrease in spleen size. Noticeable decreases in lymph node and spleen size have occurred in several of the five patients with chronic lymphocytic leukemia. In all patients there has been a decrease in peripheral blood leukemic cells (frequently spectacular), and several patients have manifested reticulocyte and platelet responses.

A. EFFECTS OF RADIATION

A major function of the lymphocytes is to initiate the immune response and thus to protect the body from foreign tissue. When an organ such as the kidney or bone marrow is transplanted from one individual to another, the rejection of the homograft is mediated by lymphocytes. By using ECIB to deplete the body of lymphocytes, the rejection time of foreign organs, including skin, can be prolonged. Three patients with rejecting kidney homotransplants have been treated with ECIB. One had a demonstrable clinical response; two responded only slightly. Although it is too early to judge the clinical effectiveness of ECIB, the results seem sufficiently impressive to warrant continuation of the study in both leukemic and renal homotransplant patients. The latter patients were sufficiently normal, hematologically, for the red cell hemolyzing effect of ECIB to be ascertained. Minor amounts of red cell destruction were found, as was noted in earlier studies on calves.

The effect of ECIB and ECIL upon skin homograft rejection was studied in calves. ECIB prolonged skin rejection time from 1 to 11 days. The administration of Imuran, with ECIB before homograft surgery, prolonged rejection time even further. Six calves were treated with ECIL or with thoracic duct drainage after homografts had been placed on the anterior and posterior parts of the animal. Anterior grafts sloughed; posterior grafts survived until the termination of the experiment. Possible mechanisms to explain this phenomenon are being investigated. ECIB also offers theoretical promise for treatment of a group of disorders, collectively known as autoimmune disease, in which lymphocytes also play a major role.

Figure 1. Three patients undergoing extracorporeal irradiation of the blood.

A. EFFECTS OF RADIATION

2. *Radiobiological Studies of Hematological Stem Cells in the Tail of the Mouse*

C.V. Robinson
J.L. Bateman
S.L. Commerford
V.P. Bond
R.G. Fairchild

If the tails of mice are shielded during exposure to x rays, colonization of the spleen occurs in excess of that found in whole-body irradiated control animals. This is true whether the measure of effect is postirradiative gain in spleen weight, uptake of 5-iodo-2'-deoxyuridine (IDU), or nodule count. The degree of colonization is a function of the length of time elapsing between the original irradiation (with tail shielded) and a subsequent, suppressive irradiation of the tail. Histological examination has shown that the spleen nodules are hemopoietic and that normal tail vertebrae contain hemopoietic tissue. A sex difference in the formation of endogenous nodules (those following whole-body irradiation) has been found: females have about twice as many nodules as do males and a much more scattered distribution.

A basic difference exists between the kinetics of spleen nodule growth in mice given whole-body irradiation and those of mice irradiated with the tail shielded. The endogenous nodules increase in number as expected post irradiation, whereas the nodules in tail-shielded animals reach a peak around 6 or 7 days post irradiation and then decrease to a lower value on day 8 or 9. The explanation for this apparent disappearance of formed nodules between days 6 and 7 and days 8 or 9 has not been found.

This system, comprising tail-shielding and nodule-counting together with test irradiations, will be used to determine whether differences in radiation dose at the bony-soft tissue interfaces of the tail vertebrae have biological significance. The test radiations will include x rays and neutrons of different energies.

3. *In Vivo Measurement of Radionuclides in Man, Kinetic Studies, and Body-Burden Measurements*

S.H. Cohn
J.S. Robertson
T. Teree
S. Bozzo
E.A. Gusmano

Knowledge of the levels and turnover rates of radionuclides is essential for the assessment of their radiation hazard to man. With the use of rate equations obtained by computer analysis of data, the time course of injected tracers of Cs^{137} , Zn^{65} , Sc^{46} , Ca^{47} , and Sr^{85} through various systems has been developed. The tracers Ca^{47} and Sr^{85} are employed in one study designed to achieve a more basic understanding of the physiological processes of mineral metabolism in various states of health and disease. A multicompartamental type of analysis is used to describe the metabolic data obtained in these tracer studies in both man and rats with the whole-body counter employed to obtain long-term retention data beyond the period during which plasma and excretion activity can be measured.

A multicompartamental model (corresponding to a first-order differential equation) was formulated to describe the kinetics of Ca^{47} and Sr^{85} in ten subjects. The solutions of the equations, performed on a high-speed digital computer, determined the sizes of the compart-

A. EFFECTS OF RADIATION

ments and the transfer constants between them and proved to be consistent mathematically and compatible with stated physiological constants. This program provides a measure of the uniqueness of fit in terms of the uncertainties of the values of the parameters, and thus offers a systematic way of comparing parameter values to improve the fit. The entire kinetic study was repeated 2 or 3 times on each patient with use of the short-lived Ca^{47} isotope to establish the reproducibility of the calculated parameters. The absolute values of the various parameters obtained with Sr^{85} were slightly lower than those obtained with Ca^{47} , but the strontium-derived values did reflect the calcium metabolism very well in terms of the assumed model when the 10-day data were used. The whole-body counter data collected over 30 days indicated differences in the whole-body turnover of strontium and calcium, which suggested that there are differences in the resorption and long-term exchange of calcium compared with strontium that are not apparent in the 10-day study.

The study of long-term turnover of Sr^{85} following both intravenous and chronic oral administration of the tracer was completed in ten adult subjects. Mathematical models are still being developed to describe the biological turnover of strontium in man, but there does not appear to be any significant difference in the biological turnover as a

result of the different modes of administration. Long-term turnover studies of Cs^{137} , Zn^{65} , and Sc^{46} in man have been completed and are being analyzed in terms of a model.

In kinetic studies of strontium metabolism in rats using both tracer techniques and neutron activation analysis of bone, the turnover of skeletally bound strontium was found to be accelerated by diets when the calcium/phosphorus ratio was ≥ 4 . In addition, supplemental magnesium increased the removal of the isotope if the dietary calcium/phosphorus ratio was ≤ 2 . Prefeeding of stable strontium was found to significantly decrease the retention of injected Sr^{85} tracer in young rats. The metabolic behavior

Figure 2. Whole-body counter used in measuring Thorotrast in patients. This counter was part of the US AEC sponsored exhibit, "Atoms at Work," in Lisbon, Portugal, in February 1965. The subject is shown being positioned under the detector in the shielded housing in the center of the counter. The electronic components of the counter can be seen in the rack in the center of the photograph.

A. EFFECTS OF RADIATION

of strontium in rats as a function of age has been studied by compartmental analysis. The same multicompartamental model formulated for man described accurately the kinetic data in rats; however, the values of the calculated parameters such as compartment size and transfer constants differ considerably in the rat as compared with man and are age dependent.

Spectrometric pulse-height distribution data have been obtained with a whole-body counter to quantify the *in vivo* levels of Cs^{137} , Zn^{65} , K^{40} , and Thorotrast. The sensitivity, precision, and accuracy of the whole-body counter permits the measurement of the long-term turnover of these elements and compounds in man, using low levels of tracers. A selected population of 10 staff members has been counted in the whole-body counter monthly for a period of 5 years now. A maximum in the Cs^{137} body burden in this population was reached in October 1964; the level has continued to fall since then. Evidence indicates that the trend shown in this small population continues to reflect the world-wide rate of fallout.

It became possible this year to study a selected population in Lisbon with a whole-body counter in order to measure their body burdens of Thorotrast. By means of a computer program the theoretical *in vivo* relationships between Th^{232} and its descendants were determined in order to calculate the radiation dose to the liver and spleen from the whole-body counting data. These estimated doses have been furnished to Dr. Horta, in Lisbon, for use in his extensive studies of Thorotrast toxicity in man.

In studies of calcium and strontium kinetics in infants and children where the use of radioactive tracers is not desirable, enriched stable isotopes Ca^{46} and Sr^{84} have been administered to a number of infants, and plasma and excreta then analyzed by means of neutron activation. The various parameter values of skeletal metabolism are being computed from these data with use of the computer program described above.

4. Antibody Responses Following Lymphocyte Depletion: Antigen-Antibody Complexes

R.D. Stoner
M. Hess
G. Terres
H. Cottier
N. Odartchenko
W. Hale

Whole-body gamma irradiation and intermittent extracorporeal gamma irradiation of circulating blood are used to compare the effects of irradiation of lymphopoietic tissues with the effects of selective irradiation of circulating lymphocytes upon repression of primary and secondary tetanus antitoxin responses. Extracorporeal gamma irradiation was used to greatly reduce the number of circulating lymphocytes of calves so as to test the capacity of the animals to respond to primary and secondary antigenic stimulation with tetanus toxoid. The depletion of circulating lymphocytes failed to significantly repress primary

antitoxin responses to adsorbed tetanus toxoid. Extracorporeal irradiation of previously immunized calves failed to significantly depress secondary antitoxin responses to fluid tetanus toxoid.

Exploratory experiments are in progress using the BNL Cosmotron to study the effects of 2.2-BeV protons on antibody formation and sensitivity to anaphylaxis in mice, and to compare the biological effectiveness of 2.2-BeV protons with gamma and x-radiation in terms of radiosensitivity of several immune mechanisms.

In a continuing study of the mechanisms involved in enhancement of primary antitoxin responses obtained in normal and irradiated mice immunized with antigen-antibody complexes composed of fluid tetanus toxoid and specific antibody, additional experiments supported the previous observation that graded doses of antigen-antibody complexes (fluid tetanus toxoid and mouse antitoxin) elicited enhanced primary antibody responses, whereas antigen alone (fluid tetanus toxoid) elicited greater secondary antibody responses in normal and irradiated mice. These findings strongly indicate that *in vivo* complexing of antigen and antibody may always occur when secondary responses are elicited with antigen-antibody systems. Efforts were made to account for the enhancement phenomenon observed with primary responses on the basis of an "adjuvant effect." When fluid tetanus toxoid was incorporated into incomplete Freund's adjuvant and administered in a single immunization, less toxoid was required to elicit primary antitoxin responses than when fluid toxoid alone was used. Approximately 100 times less toxoid (in complex with specific antibody) elicited greater primary antibody responses than toxoid in adjuvant. It is evident that increased molecular size and/or preferential selection of the complexed antigen by antibody-producing cells may be involved in the observed enhancement phenomenon.

5. Antibody Responses in Thymectomized Mice

R.D. Stoner
M. Hess
G. Terres
H. Cottier
N. Odartchenko
W. Hale

Surgical removal of the thymus of newborn and week-old mice provides an opportunity to study the influence of the thymus upon the subsequent immunological potential of the adult animal. Previous work at BNL demonstrated the capacity of thymus transplants to produce antibody. More recent work at the Laboratory tends to modify the overwhelmingly dominant role ascribed to the thymus by others in the eventual development of mature immune systems in adult animals. Total thymectomy of newborn mice is being used to study the relative importance of the thymus with regard to subsequent antibody formation to several antigens, immunological tolerance to tetanus toxoid, and rejection of skin grafts.

A. EFFECTS OF RADIATION

Mice thymectomized at birth and given a primary immunization of tetanus toxoid at the age of 4, 7, 10, or 15 wk demonstrated nearly normal primary antibody responses. Secondary antitoxin responses were repressed in 20% of these animals when tested at the age of 20 wk. Although wasting disease was not observed in thymectomized animals during the experiment, inapparent wasting and/or the lack of immunologically competent thymic cells available prior to birth could not be excluded as causes of the observed repression of secondary antibody responses. In a series of experiments conducted to test the capacity of thymectomized mice to respond to primary immunization when 10 to 15 wk of age, 164 mice were thymectomized at birth and 168 littermates were sham-operated. Primary tetanus antitoxin responses were not significantly repressed in thymectomized animals when primary immunization was accomplished during the period of previously observed repression of secondary antibody responses. Wasting disease was not encountered in thymectomized mice. Large germinal centers were observed in lymph nodes. Most of the germinal center cells incorporated H^3 -thymidine. Many plasma cells were observed in the medullary cords of lymph nodes.

6. Influence of Radiation Quality and Dose Rate on Early and Late Effects in Cell Renewal Systems in the Mouse

J.L. Bateman
H.H. Rossi
V.P. Bond
J.S. Robertson

Various cell systems in the mouse are being utilized in assays of both early and late effects following exposures to ionizing radiation under selected, graded conditions of radiation quality and dose rate. Whereas the earlier studies were concerned primarily with early effects elicited by relatively high doses of radiation, the more recent studies have focused on late changes induced by low radiation doses. The development of ocular lens opacities has been the main system employed in the latter category.

Figure 3. Lens opacification in the mouse. The curves depict the relative number of eyes, within each treatment group, that have reached or exceeded a single, selected degree of posterior subcapsular opacification. Under these conditions, a 10-fold difference in effectiveness exists between the x rays and neutrons of either 0.43- or 1.80-MeV energy.

Figure 4. Serial slit-lamp microscope examination of the mouse eye for radiation-induced opacities. The collimated light source, normally located parallel to the microscope, has been omitted for clarity.

Neutrons of 0.43 MeV in doses of 0.5 to 6.4 rads, neutrons of 14 MeV in doses of 1.0 to 60 rads, and 250-kVp x rays in doses of 6 to 48 rads are being studied for their relative biological effectiveness (RBE). Lenses are examined serially with respect to time post irradiation for minute opacities, and results are compiled and analyzed with use of a method of data analysis that circumvents certain limitations inherent in the use of a visual arbitray (although reproducible) scale of opacity size. Definite lens opacification in the posterior subcapsular region has been obtained by 10 months post irradiation from doses as low as 0.5 rads of 0.43-MeV neutrons, 5 rads of 14-MeV neutrons, or 24 rads of 250-kVp x rays. Equivalence of the incidence at each of multiple graded levels of opacity indicates an RBE of 24 for 0.43-MeV neutrons, and of 3 for 14-MeV neutrons. RBE values for the neutrons decline as the dose increases, and the RBE values above are for the degree of effect obtained at 48 rads of 250-kVp x rays. The response to 14-MeV neutrons appears to be dose-rate independent as judged by the results of a split-dose study, as has been previously noted with 0.43- and 1.80-MeV neutrons. Anterior polar opacities, slower in development, are yielding considerably lower RBT values, strongly suggestive of a major difference in the mechanism of damage.

A. EFFECTS OF RADIATION

7. Pathogenesis of the Radiation Cataract

H.A. Johnson

The sensitivity of lens epithelium to acute injury by ionizing radiation is well established, but little is known about the late changes in the lens which actually produce the opacity of the radiation cataract. To investigate the process of opacification, lenses of mice are being examined by light and electron microscopy 8 to 10 mo after low doses of whole-body x-irradiation. Opacification appears not to be a generalized deterioration of the entire lens but the result of a gradual accumulation of cells that have failed in the normal sequence of differentiation from epithelial cell to specialized lens fiber cell. The latter cell shows a high degree of ordering both at the histological level and at the molecular level, as evidenced by its strong birefringence. Failing to achieve this degree of differentiation, the young fiber undergoes cytoplasmic condensation and vacuolization with distal retraction of the cytoplasm away from the cell membrane. The resultant inhomogeneity of electron density and presumably of optical density could cause sufficient light scattering to attenuate transmitted light.

The abortive maturation of occasional lens cells months after irradiation is analogous to the dyskeratosis of individual epidermal cells in chronic radiodermatitis. That the radiation cataract and chronic radiodermatitis are histologically similar is not surprising, since the lens is closely related to the epidermis embryologically and the maturation of lens fibers is similar, chemically and structurally, to the maturation of squamous cells.

Figure 5. One of the delayed effects of exposure to ionizing radiation is the formation of cataracts. This electron micrograph of part of the lens of a mouse irradiated 30 weeks previously shows an early stage in the formation of a radiation cataract. In the center is a deteriorating lens fiber in a group of normal fibers. The gradual accumulation of these degenerate fibers results in opacity of the lens.

Figure 6. Examining the evolution and time course of the lesions on the skin of swine resulting from increasing doses of radiation.

8. Response of the Skin of Swine to Increasing Doses of High and Low Linear Energy Transfer Radiations

J.O. Archambeau
G. Mathieu
S.L. Commerford
R.G. Fairchild

In this study the skin of swine is used as an *in vivo* radiobiologic test system for determining and comparing the effects of varying doses of x-ray and other radiations. The initial index of the effect of irradiation on the skin was the radiation dose that produced a measurable and reproducible effect in 50% of the irradiated fields. Further evaluation is being made to determine whether a parameter is available that will estimate the fraction of proliferative cells of the skin that survive increasing doses of radiation.

The evolution of the gross morphological response of the skin of swine following increasing doses of x rays, thermal neutrons, fast neutrons, and the $B^{10}(n,\alpha)Li^7$ reaction was determined. The dose at which 50% of the irradiated fields are not healed at 49 days following irradiation was estimated to be 2280 rads for x rays, 1500 rads for thermal neutrons, 900 rads for fast neutrons, and 1100 rads for the $B^{10}(n,\alpha)Li^7$ reaction.

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The histological changes of the skin over a 35-day period following 2200 rads of x rays and 1100 rads from the $B^{10}(n,\alpha)Li^7$ reaction can be divided for descriptive purposes into two phases. The first is the degenerative phase which lasts about 21 days and is characterized by progressive cell loss, tissue edema, and cellular infiltration. The second phase is a regenerative one which becomes apparent around 21 days following irradiation and lasts for about 14 days. This phase consists of cellular replacement. The quantitative cytological changes in the nuclear volume of the basal (proliferative) cells and the prickle cells, the mitotic index, and the capillary diameter were obtained for the degenerative phase following doses of 2200 rads of x rays and 1100 rads from the $B^{10}(n,\alpha)Li^7$ reaction. The nuclear volume of the basal cells increased by a factor of ≈ 2.5 . The nuclear volume of the prickle cells during this period increased by a factor of 5 to 5.5. The nuclear volumes of the cells in the regenerating phase are about 1.2 to 1.5 times as large as the control volumes. The mitotic index falls to zero during the first week following irradiation in the degenerating areas, but is 5 to 6 times above control values in the regenerating areas. Capillaries show a progressive dilation between the 28th and 35th days following irradiation, with the diameter increasing by a factor of 2 to 2.5.

Since a simpler method of determining these changes was desirable, pilot studies were performed to determine the rate of loss of radioactive I^{125} -labeled iododeoxyuridine from normal nonirradiated skin. The time required for the loss of one-half the activity was 20 days. Following irradiation with 500 or 2000 rads, the corresponding time was 7 to 10 days.

9. Dosimetry of π^- Mesons

G.M. Tisljar-Lentulis
V.P. Bond
J.S. Robertson
W.H. Moore, Jr.

That high energy particles cause more ionization in the depth of absorbing material (Bragg peak) than on the surface is of importance for biomedical work. This project is concerned with π^- mesons, which are captured predominantly in the peak area by nuclei of the absorber material. An appreciable percentage of the pion rest-mass energy equivalent is released in the process, about one-third being carried away by protons, α particles, and heavy ions. This additional ionization effect is expected to amplify the Bragg peak of the pions. The charged particles emitted after pion capture have a relatively high linear energy transfer (LET). If the energy transferred to the absorbing material per unit path length exceeds a certain amount, the biological effectiveness rises. Furthermore, high LET helps to overcome the oxygen effect. Oxygenated, healthy cells are about three times as sensitive

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Figure 7. Block diagram of apparatus used for dosimetry of π^- mesons. The beam is measured with the counter telescope $\bar{CMS}$. Čerenkov counter $\bar{C}$ rejects μ mesons and electrons. The plastic scintillator M is kept narrow enough to accept only mesons of practically the same energy. Detector S is a thin cesium iodide crystal. It responds to high LET radiation from pion capture with pulse heights large in comparison with the pulses caused by passing particles. The pulse heights are measured by a 400-channel analyzer. The $\bar{PREDET}$ is a timing unit that switches the scalers on for the duration of the beam spill.

Figure 8. Photograph showing the counters of the telescope aligned downstream of the momentum analyzing magnet. Two sets of photomultipliers on the Čerenkov counter (C) are provided for the registration of π or μ mesons respectively. The small scintillation counter M defines the solid angle of the accepted particle trajectories. P denotes polyethylene absorber disks. The thin CsI(Tl) scintillator S serves to measure the different amplitudes of pulses caused by passing and stopping pions. The gain of this detector is stabilized by the copper cooling coil around the photomultiplier tube.

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to low LET radiation as anoxic tumor cells. However, this is not the case for high LET radiation. It appears useful, therefore, to investigate the high LET components of pion irradiations.

The equipment used at the Cosmotron was improved considerably by the introduction of a new coincidence unit with 5 input channels and 4 coincidence combinations. An X - Y plotter for the immediate evaluation of pulse-height spectra during experiments was put into operation. The detectors of the counter telescope were improved mainly with respect to lower acceptance of μ mesons and electrons and for better definition of the accepted beam trajectories. The momentum spread improved from about $\pm 5\%$ to about $\pm 2\%$. A cooling system for the star detector was installed in order to guarantee a stable gain of the photomultiplier. First tryouts with an adapted Čerenkov counter gave encouraging results. However, considerable improvement, mainly with respect to the light collection and registration efficiency, will be necessary to suppress the number of muon and electron counts below the 10% level.

For the registration of the stopping pions, very thin (1 to 5 mg/cm²) CsI(Tl), anthracene, and stilbene scintillators were tried for the first time. They showed a promising response to both high and low LET radiation. The exploitation of the registered pulse-height spectra with respect to ionization and mean LET of the beam was considerably improved and facilitated by the writing of a computer program in FORTRAN II. A preliminary idea of what the pion survival curve looks like was gained by subtraction of a carefully estimated number of background counts from the survival curve of the pion + muon + electron beam.

10. Mixed Field Dosimetry at the Medical Research Reactor

R.G. Fairchild
J.S. Robertson
E.A. Tonna

This project is concerned with the measurement of neutron and gamma-ray absorbed dose rates and neutron energy spectra at experimental ports of the Medical Research Reactor (MRR). The reaction of thermal neutrons with various neutron-capturing isotopes in tissue is also under investigation. To evaluate the results of biological experiments performed at the MRR, it is necessary to know accurately the total absorbed dose delivered by each type of radiation, as a function of depth, in an appropriate phantom.

Previous measurements showed that an advantage factor (ratio of tumor dose to maximum normal tissue dose) of 1.5 or more was obtainable at depth with an epithermal neutron beam. Figure 9 presents typical results at a depth of 4 cm. During the last 12-mo period, detailed depth dose distributions were measured in a phantom head, and isodose curves necessary for clinical evaluation were constructed for

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each type of radiation. The isodose chart from the $N^{14}(n,p)C^{14}$ reaction is shown in Figure 10. A computer code was developed that permitted the calculation of absorbed dose in a cylinder irradiated with thermal neutrons. Calculated values compared well with measured values and thus provided a check on the dosimeter system (Figure 11).

In order to evaluate the possible usefulness of the neutron capture therapy procedure, the distribution of neutron-capturing isotopes in tissue must be determined. Neutron autoradiographic procedures are being developed to determine the cellular distribution of boron compounds. In these procedures, tissue sections containing B^{10} are placed on nuclear emulsions and then irradiated with thermal neutrons. The $B^{10}(n,\alpha)Li^7$ reaction is recorded on the film, which allows the origin of the reaction (and thus the site of the boron atom in the cell) to be determined by microscopic examination. The gross distribution of B^{10} in tissue can be determined by a similar technique, with use of a solid-state radiation detector instead of film to record the relative number of reactions in various tissues when irradiated with thermal neutrons.

Figure 9. Relative dose vs relative B^{10} concentration in phantom man at a depth of 4 cm, with $35 \mu\text{g/g}$ B^{10} in tumor.

Figure 10. Isodose chart for absorbed dose from the $N^{14}(n,p)C^{14}$ reaction.

Figure 11. Comparison of absorbed dose measured in a tissue-equivalent cylinder with calculated dose.

Preliminary work with neutron autoradiographic techniques has indicated that film fogging can be reduced enough to allow the localization of physiological amounts of boron in tissue sections. Previous work in this field was done with boron in amounts that were orders of magnitude above the maximum physiological concentration of $\approx 60 \mu\text{g B}^{10}$ per g tissue, and no conclusions were drawn as to the cell distribution of boron. Poor results were attributed to unavoidable background fog. Our work has shown that with $60 \mu\text{g B}^{10}$ deposited on 1 cm^2 of film and irradiated to yield a calculated 40α tracks per cm^2 of film, the expected number of tracks were seen, with a background of ≈ 2 heavy particle tracks per field. Work with boron-loaded tissue sections prepared with use of conventional freeze-drying techniques has shown appropriate α tracks associated with the tissue sections with, again, a minimal number of tracks in sections without boron. Work is now in progress to obtain sections of sufficient quality for boron localization within the cell. This technique would also be useful for determining the cellular distribution of other elements with an appropriate neutron-charged particle cross section.

B. STRUCTURES AND FUNCTIONS OF PROTEINS, HORMONES, AND ENZYMES

1. *Synthetic and Structural Studies on Polypeptides and Proteins Possessing Biological Activity*

P.G. Katsoyannis
A. Trakatellis
A. Tometsko
M. Tilak
S. Johnson
G. Schwartz
J. Ginos
C. Zalut

These studies have been focused on the synthesis of human insulin and of analogues of both human and animal insulin in order to increase knowledge of the mechanisms of metabolism of the hormone and improve the treatment of diabetic humans. The availability of human insulin might make it possible to overcome the immunological reactions that frequently occur in human beings treated with insulin from animals.

The synthesis of the A and B chains of human insulin was accomplished, and the two were combined to yield human insulin. Initial yields, however, were small. Newer procedures have been developed by which natural A and B chains can be recombined to produce insulin in yields ranging from 45 to 55%. In addition, methods have been devised (1) for cleavage of natural insulin and isolation of its individual chains in a highly purified form and on a preparative scale, (2) for isolation of crystalline insulin produced by recombination of synthetic B chain and natural A chain, or synthetic A chain and natural B chain.

The chemical synthesis of human and sheep insulin has opened the way for the synthesis of analogues of the parent molecule and also

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for single and double labeling of insulin with radioactive elements. The availability of such analogues will make possible study of the relationship between chemical structure and biological activity, and may reveal those structural features that are essential for biological function of the insulin molecule. These studies in turn may lead to the synthesis of insulin analogues with more favorable biological properties than the parent molecule.

Another aim in these investigations is the use of insulin as an antigen in the attempt to isolate insulin antibodies, with the hope that purifications by immunological and analytical methods will eventually yield preparations of high degrees of homogeneity. A breeding colony of rabbits for use in studies on anti-insulin antibodies has been established. The rabbits, derived from a well-characterized National Institutes of Health strain, were selected because they are homologous with respect to the allotypic specificities known to exist in rabbit γ G immunoglobulin. The use of such animals circumvents a major source of heterogeneity in isolated antibodies. The first progeny from the colony are expected to be undergoing immunization soon.

Figure 12. Molecular model of human insulin.

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2. Synthetic Studies on the Active Site of Chymotrypsin

P.G. Katsoyannis
G. Schwartz

It has been established that the "active" site of many proteolytic enzymes includes a "reactive serine" residue. Specifically, the "active serine" in the chymotrypsin, trypsin, and elastase is found in the sequence -gly-asp-ser-gly-. Indirect evidence from kinetic and photo-oxidation experiments and direct evidence gained by the use of specific reagents indicate that one of the two histidine residues in chymotrypsin is also necessary for catalytic activity. Furthermore, it has been established that the two histidine residues in the molecule of chymotrypsin and trypsin are locked together by a disulfide bridge. This raised the possibility that both histidine residues in these proteolytic enzymes are required for catalytic activity. The structure of chymotrypsin has been postulated very recently and the position of the aforementioned amino acid residue has thus been revealed:

The two histidine residues are found in positions 40 and 57 (using the numbering for chymotrypsinogen). The disulfide bridge that locks together the histidine residues links cysteines found in positions 42 and 58. The "reactive serine" occupies position 194. The speculation thus arises that the folding of the chains of chymotrypsin brings the 40 and 57 histidines and the 194 serine close together to create the "active site" of this enzyme. An attempt is being made to synthesize the chymotrypsin fragment (position 38 to position 59), to establish the disulfide bridge between cysteine residues 42 and 58, and to attach to this molecule a peptide sequence that contains the "reactive serine" 194 and is of the proper length to bring the "reactive serine" in close proximity to the histidine residues. Courtauld models have indicated that attachment of a certain amino acid sequence, containing the "reactive serine," to the aforementioned dicosapeptide (22 residues) might give the desired molecule. The synthesis of such a polypeptide chain is now in progress. An amino terminal fragment and a carboxyl terminal fragment containing most of the desired sequences have been synthesized. Synthesis of the remaining fragment containing the middle sequence is under way. If such a molecule is endowed with enzymatic activity, synthesis of an "enzyme" will have been accomplished. By making changes in the structure of such an "enzyme" it will be possible

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to gain valuable information regarding the mode of action of proteolytic enzymes.

3. Mechanisms Controlling Membrane Permeability and Transport Processes at the Cellular and Molecular Levels

I.L. Schwartz
R.W. Walter
L.W. Rahm

The broad objective in these studies is the elucidation of hormonal and other mechanisms controlling membrane permeability and transport processes at the cellular and molecular levels.

One aspect of the present program is concerned with the physiologic evaluation of changes in the chemical structure of selected neurohypophyseal hormones in order to identify groupings that serve as the sites that trigger hormonal activity and other groupings that serve to bind and orient the hormone to its receptor. Another aspect of this work is concerned with study of intermediate events in hormone action and their differentiation from the primary hormone-receptor interaction and/or the final effector process.

In efforts to improve upon the Wilzbach method for random tritiation, synthetic work was initiated to develop specifically labeled oxytocin and vasopressin analogues for high-resolution radioautography of target cells and for biochemical studies on cell fractions. The synthesis of another group of neurohypophyseal analogues was initiated to re-evaluate the physiologic role of the disulfide bond of this group of hormones.

Work was continued on identification of the precise arrangement in three dimensions of a neurohypophyseal hormone through the synthesis, crystallization, and x-ray crystallographic study of selenium and tellurium analogues of oxytocin.

In the survey of the biological effects of deleting substituent groups of lysine vasopressin (LVP), studies by others were confirmed and extended. 1-*N*-acetyl-8-lysine vasopressin, an analogue of LVP in which the terminal amino group is acetylated, was found to have <1% of the mammalian antidiuretic and uterotonic activities of LVP, to inhibit competitively the pressor activity of synthetic LVP and highly purified natural arginine vasopressin (AVP), and to have reduced affinity for neurophysin, the presumptive physiologic carrier protein of vasopressin and oxytocin within the hypothalamo-neurohypophyseal system.

In sharp contrast, 1-deamino-8-lysine vasopressin (1- β -mercapto-propionic acid lysine vasopressin), an analogue of LVP in which the terminal amino group is deleted, has higher mammalian antidiuretic activity than its parent hormone, although its mammalian pressor potency and its effect on amphibian membrane permeability are less than those of LVP. Its affinity for neurophysin is decreased.

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Another striking deletion effect is observed in the case of 4-asparagine-lysine vasopressin, an analogue in which the glutamine residue of the natural hormone is replaced by an asparagine residue, which shortens the amino acid side-chain in position 4 by one methylene group. This analogue has a higher antidiuretic/pressor activity ratio than LVP or AVP; moreover, it has greater absolute antidiuretic potency than AVP, heretofore the most potent known natural or synthetic mammalian antidiuretic principle.

Thus, as a practical consideration, removal of the α -NH₂ group in position 1 or of a —CH₂— group of the side-chain of position 4 affords the therapeutic advantage of increasing antidiuretic activity relative to pressor activity. Theoretically, the effects of these deletions may shed light on fundamental determinants of hormonal potency such as the factor of molecular complementarity in contributing to the affinity of hormone for receptor.

Figure 13. Electron micrograph of an isolated nucleus obtained from striated muscle. *NU*, nucleolus; *M*, mitochondria; *B*, nuclear bleb. 8400 $\times$.

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In the study on localization and effects of insulin on muscle and adipose tissue, further improvements have been effected in the isolation and characterization of muscle cell nuclei (Figure 13).

4. *The Combinations and Function of Hydroxylysine in Collagen*

D.D. Van Slyke
R.B. Aronson
C. Franzblau
A.F. LoMonte

Further studies have been made of the chemical links of hydroxylysine in the collagen molecule and their effects on the properties of collagen. A number of different collagens were prepared and tested to determine accurately the degrees of difference in the reactivity of hydroxylysine among collagens of different species, tissues, and types of preparation, and to obtain information concerning the type of combination that causes the amino alcohol group in part of the hydroxylysine of collagen to be inert to periodate. Among the preparations were insoluble collagen of beef and rabbit bone and of rabbit skin; citrate-soluble collagen of rabbit and calf skins, rat tail tendon, and carp swim bladder; and salt-soluble collagen of rabbit skin. In each, part of the hydroxylysine was resistant to periodate, the resistant proportion varying from 50% in the two bone collagens to 10% in the preparation from carp swim bladder. The insoluble collagens tend to have a higher resistant percentage than the soluble, and the collagens from bone a higher percentage than those from other tissues. The part of the collagen hydroxylysine that reacted did so, as in free hydroxylysine, in 3 min, and no further destruction of hydroxylysine occurred when the treatment with periodate was continued for 2 hr. There was a sharp and complete difference between the reactive and nonreactive fractions, indicating the probability of a firm chemical binding of the nonreactive fraction.

Gelatin from bone collagen was digested to peptides by collagenase and to small peptides by collagenase plus trypsin. The periodate-resistant fraction of hydroxylysine remained at 50% in the digest, exactly the same as in the gelatin, which provided confirmatory evidence that the periodate-resistant hydroxylysine in the gelatin was resistant, not because of steric hindrance in the protein, but because of chemical linkage of either the NH_2 or the OH of the amino alcohol group with some other constituent of the collagen.

One possible type of such linkage was combination of the OH of the hydroxylysine with an acid group to form an ester. Evidence has been published of ester linkages in collagen. Ester links are broken by treatment with hydroxylamine. Such treatment was applied to bone gelatin. The proportion of collagen hydroxylysine that was resistant to periodate remained at 50%, exactly the same as before treatment with hydroxylamine. This result indicates that the linkage of the periodate-

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resistant amino alcohol group of hydroxylysine in collagen is in some form other than that of an ester.

5. Mechanism of Formation of Hydroxylysine in Collagen and Metabolism of Mucopolysaccharides

E.A. Popenoe
D.D. Van Slyke
R.B. Aronson
N. Di Ferrante

In animals, hydroxylysine is found only in collagen, a protein that constitutes one-third of the total mammalian body protein. Hydroxylysine formation is essential to normal development and function of the collagen. Studies in this laboratory are directed toward an understanding of the normal biochemical mechanism by which one H atom on carbon 5 of lysine

1 2 3 4 5 6

is replaced by OH to form hydroxylysine. With an understanding of the normal mechanism, it will be possible to seek defects in hydroxylysine formation in disease states.

Lysine-6- H^3 was prepared by two methods, one biosynthetic (in yeast) and the other chemical, on a scale sufficient to provide material for biochemical studies. Chemical degradation showed that 100% of the tritium was on carbon 6 in the biosynthetic lysine. In the lysine prepared by catalytic reduction with tritium of a suitable nitrile, 75% of the tritium was found on carbon 6 and 25% on carbons 4 and 5. Studies in rat tissues in which these lysine preparations served as precursors of collagen by hydroxylysine showed that the hydroxylation proceeds without loss of tritium from lysine carbon 6. This finding considered along with earlier results indicates that hydroxylation probably proceeds by a direct uptake of oxygen at one position on carbon 5.

Figure 14. In the study of hydroxylysine biosynthesis, tritium from labeled samples is transferred in this apparatus to tubes for counting.

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In another phase of research on connective tissue components, methods of labeling various mucopolysaccharides are being developed and their metabolism is being studied. Heparin has been labeled with tritium by two different methods, the better being that of exposure to tritium gas under electric discharge. This tritium-labeled product was further labeled chemically with S^{35} in the sulfamic acid groups to give a product with full biological potency. By using this doubly labeled heparin in experiments in intact rabbits and in isolated, perfused organs, it was shown that (1) there is considerable retention of heparin in the organism after it has disappeared from the blood; (2) one of the first steps in degradation of heparin is removal of sulfamic acid groups; and (3) the kidney, but not the liver, carries out the desulfamation.

6. Bioenergetic Mechanisms Involving Free Radicals

D.C. Borg

This investigation seeks to elucidate the roles of free radicals as reactive intermediates in normal and pathological metabolism. Electron paramagnetic resonance (EPR) spectroscopy can identify and describe free radicals with greatest sensitivity and specificity, and in this work EPR is used to establish the existence and properties of free radical forms of biologically important substances, with initial emphasis on hormones and drugs. Other procedures support and develop EPR evidence, particularly methods defining oxidation-reduction properties (e.g., polarography, potentiometric titrations, and amperometry).

Chemical studies have been completed of the formation of free radicals from the iodothyronine group of natural and synthetic thyroid hormones. From each of the hormonally active triiodo- and tetraiodothyronine derivatives more than one free radical was identified by EPR. Analysis of the EPR data and supporting chromatographic studies of reaction products pointed to the existence of labile, fully iodinated free radical forms, followed by more stable radicals that were at least partially deiodinated. In general, a pattern of oxidative deiodination was encountered in the free radical reactions, and this finding is consistent with the paths of thyroid hormone metabolism *in vivo* and *in vitro*. In addition to the recently improved continuous flow apparatus for EPR spectroscopy, new facilities and special techniques were developed to support this work, including (a) an apparatus of the Bray type for "freezing-in" at low temperatures products of rapid chemical reactions within 10 msec of their formation; (b) a facility for obtaining EPR spectra at 35 Gc from quick-frozen samples whose temperature must not be allowed to reach the annealing point at any time;

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and (c) a thin-layer chromatography procedure for separation and identification of several different thyronines, iodothyronines, substituted tyrosines, and iodide ions.

By using the $\text{Ti}^{+3}/\text{H}_2\text{O}_2$ reaction as a probable $\text{OH}\cdot$ -generating system, other reactions were investigated as models for certain radiochemical effects and as models for microsomal hydroxylating reactions for which free radical mechanisms may be postulated. Free radical-forming reactions on L-lysine may have produced 5-hydroxylysine. Preliminary amino acid analyses indicated a small yield of a product eluting at the position of authentic 5-hydroxylysine with at least two solvent systems at different temperatures.

A dual EPR cavity was calibrated for accurate quantitation of both stable and unstable paramagnetic species. Standardization was effected by combining data from four separate primary spin standards, two paramagnetic metal ions (Mn^{++} , assayed by radioactivation analysis, and Cu^{++} , assayed iodometrically and polarographically) and two stable free radicals (2-2-diphenyl-1-picrylhydrazyl in benzene, assayed spectrophotometrically, and peroxyamine disulfonate, assayed potentiometrically and spectrophotometrically). The system was then checked by comparing a calculated dismutation constant of a free radical with published values. Finally, the dual cavity quantitation method was applied to calculate the kinetics of reactions previously shown to produce very short-lived free radicals from tyrosine, insulin, and casein with the aim of assessing the initial yields of free radicals in the overall reactions. Disappointingly, the reaction kinetics were so rapid that extrapolation of steady state-free radical concentrations to zero time could not be carried out with meaningful accuracy.

Figure 15. Left: An improved reaction-mixer and EPR aqueous solution cell for use in conjunction with a liquid flow apparatus. The cell is fabricated of quartz, and its integrated construction minimizes dead time for flow from the mixer (bottom) to the detection region (flat portion), which facilitates study of short-lived free radical reaction products.

Center: The cell in place within a microwave resonance cavity between the poles of the electromagnet of an EPR spectrometer.

Right: A general view of the EPR spectrometer. The pressurized containers for the reactant solutions and the liquid valving system of the flow apparatus are at the right. The oscilloscope recording equipment is not shown.

Successful development and operation of a 35-Gc continuous flow system were secured, with linear flow rates through a 0.3-mm-i.d. capillary of >10 m/sec and a dead time from reaction to detection of the order of ≤ 1 msec. This exceeds the performance of the highly successful BNL-type mix-and-flow cells used for 9.5-Gc EPR, yet the volume flow rate of reactants is $<5\%$ as great for the 35-Gc microflow apparatus. This conservation of material can be of critical importance in flow experiments designed to study transient free radicals from biological or biochemical materials of extremely restricted availability, or when expensive bulk solvents, such as D_2O , are to be used. With the 35-Gc microwave bridge now available, the signal-to-noise ratios of EPR spectra from aqueous reactions studied in the new flow system have not yet exceeded one-half of those obtained from the same reactions examined with the 9.5-Gc apparatus.

C. STRUCTURE AND FUNCTION OF DNA AND RNA

1. *The Fine Structure and Function of Nucleic Acids*

L.D. Hamilton
M.H.F. Wilkins
S. Arnott
W. Fuller
H. Lyman
S.L. Commerford
N. Delihias
P. Reilly

Definition of the fine structure of nucleic acids is the aim of this research. The interaction of these macromolecules with small molecules may provide information on the mechanism of mutagenesis and carcinogenesis and add to knowledge of how inheritance and protein synthesis work. Different salts of deoxyribonucleic acid (DNA) and different ribonucleic acids (RNA's) are prepared from a variety of sources, both nuclear and cytoplasmic. Suitable preparations are characterized by several physical-chemical and analytical techniques; these are studied by x-ray diffraction in collaboration with Dr. Wilkins and colleagues, Kings College, London. The interaction of various chemicals, e.g., antibiotics and antimetabolites, with DNA and RNA are studied, and the structures of these complexes can then be inferred by molecular model-building and comparison of proposed structures with the x-ray and other physical chemical data.

The x-ray diffraction patterns of fibers of many specimens of DNA change reversibly with change of water content of the fibers. A relative humidity (r.h.) of 75% was optimum for crystallization of the *A* form, whereas at 92% r.h. and above, the semicrystalline *B* form was observed. Some specimens, however, remained in the *B* form at all humidities – an anomalous behavior attributed to impurities in the DNA. It was noted that NaCl contents of $\approx 10\%$ could have this effect (salt contents are given as percentage weight of salt to dry weight of DNA).

C. STRUCTURE AND FUNCTION OF DNA AND RNA

The *A-B* reversible conformational change in the 75 to 92% r.h. range was thought to be a property of pure sodium DNA. Recently, however, many samples of sodium DNA have been found which retained the *A* form at 92% r.h. or even at 98%. DNA samples taken from preparations made over a period of many years were examined and the NaCl content estimated by chloride analysis. Samples giving diffraction patterns of the *B* form at both 75 and 92% r.h. had NaCl contents $\geq 9\%$, and all samples giving the *A-B* transition contained some salt.

Further studies showed that up to 5% NaCl could be added to a salt-free *A* DNA without producing the *B* pattern at 92% r.h., but above this concentration (6 to 7%) the normal *A-B* transition was obtained. With this salt content there is considerable diffraction from the NaCl crystals at 75% r.h. However, about half the samples exhibiting the transition contained less NaCl (0.6 to 4%) and did not show salt diffraction. This indicated that additional factors were involved in inducing the *B* form. The following factors had no effect: (1) long periods of humidity equilibration; (2) presence of protein, polysaccharide, or EDTA (up to 0.3%); presence of divalent metal ions; and (4) presence of polyamines or xylene sulfonate (up to 2%).

When sodium acetate alone is added to a salt-free sample of sodium DNA, the same effect is obtained as with NaCl; with approximately the same percentage by weight the sample gave the normal *A-B* transition. With mixtures of salts the transition to *B* at 92% was obtained with no salt diffraction at 75% r.h. Thus, it seems clear that the change in structure from the *A* to *B* form in sodium DNA depends on the presence of salts as well as on an increase of relative humidity and that a salt-free sodium DNA does not change its structure with increasing relative humidity.

Figure 16. Large transformed cells after 5-day culture of sensitized cells exposed to *Trichinella spiralis* antigen. 400 $\times$.

C. STRUCTURE AND FUNCTION OF DNA AND RNA

Daunomycin – an antibiotic isolated from *Streptomyces Str. peucetius* – consists of a pigmented tetracyclic group bound in glycosidic form to an aminosugar. Daunomycin is bacteriostatic and kills mammalian cells in tissue culture; it inhibits RNA and DNA synthesis. The inhibition of RNA synthesis correlates with inhibition of DNA-dependent RNA polymerase activity. In contrast to actinomycin, daunomycin affects DNA replication at nearly the same concentrations active on RNA synthesis. X-ray diffraction patterns from fibers of the complex of daunomycin and DNA are less well defined than those from DNA but allow an approximate value for the pitch of the helix to be determined. When the content of the drug in the fiber was 1 molecule per 8 pairs, the pitch of the DNA helix was 41 rather than its normal 34 Å; for 1 molecule of daunomycin per 4 or 5 pairs, the pitch became 48 Å. These observations can be explained if it is assumed that the planar tetracyclic ring of the daunomycin intercalates between adjacent base pairs, stretching the DNA molecule by 3.3 Å per insertion of 1 molecule of drug and uncoiling the helix at the point of insertion by about 24°. The radius of the molecule complexed with daunomycin was slightly less than that of native DNA. Preliminary model-building studies indicate that the tetracyclic ring of daunomycin can be accommodated in the extended DNA molecule. The point of attachment of the aminosugar to the tetracyclic group has not yet been established. However, it could probably be accommodated in either the large or small groove of DNA.

2. Cellular Aspects of the Transfer of Delayed Hypersensitivity

L.D. Hamilton
C.W. Kim
H. Savel

One biological expression of information storage and transfer in mammals is that carried by lymphocytes. How this information is transmitted is being investigated by study of the transfer of delayed hypersensitivity. The effects of phytohemagglutinin and specific antigens, e.g., *Trichinella* and picryl chloride, on human and guinea pig lymphocytes *in vitro* are being studied to clarify the mechanism of antigenic stimulation and the nature of the response of immunologically competent cells. The biological activity of lymphocytes is being measured by their ability to transfer delayed hypersensitivity in guinea pigs in order to pinpoint the cells responsible for the transfer of information.

Phytohemagglutinin transforms small lymphocytes into large pyrinophilic blast cells; the transformation is accompanied metabolically by synthesis of RNA and protein and eventually by DNA synthesis. Approximately 90% of guinea pig lymphocytes from lymph nodes are susceptible to transformation by phytohemagglutinin. Spontaneous transformation of guinea pig lymphocytes into large blast

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pyrinophilic cells was not seen, however, in 5- and 6-day cultures in which only the minimal essential medium and horse serum were used. Fetal calf serum caused transformation, although to a much lesser extent than phytohemagglutinin. Thus published results describing spontaneous transformation may be due to stimulation of lymphocytes by foreign protein.

Clumping is an important factor in ensuring transformation when using phytohemagglutinin with guinea pig lymph node cell cultures. If the guinea pig lymph node cells are prevented from clumping after exposure to phytohemagglutinin, they do not transform; i.e., clumping is a necessary part of the transformation process. On the other hand, neuraminidase (sialidase) clumped guinea pig lymphocytes but did not transform them as did phytohemagglutinin. Sensitized guinea pig lymphocytes are also transformed by specific antigens, e.g., *Trichinella*. Clumping is a necessary part of this transformation response also. Non-sensitized lymphocytes that did not transform also failed to clump in response to the specific antigen *Trichinella*. Therefore, clumping of lymphocytes by itself does not appear to be sufficient to initiate blast transformation but does appear to be a necessary part of the transforming process.

In lymphocyte transformation resulting from use of phytohemagglutinin the effect is independent of previous exposure of cells to phytohemagglutinin. In contrast, after exposure to a specific antigen only 5 to 15% of lymphocytes are transformed *in vitro*, and this only in a population of cells previously specifically sensitized to the antigen tested. In the absence of previous specific sensitization, there is no transformation by antigen. Preliminary evidence suggests that these transformed cells are responsible for the ability to transfer delayed hypersensitivity to the same antigen from sensitized donors to normal recipients.

3. The Preparation and Structural Analysis of Ribonucleic Acids

N. Delihias

Ribonucleic acids play a crucial part in protein synthesis, and knowledge of their structure would be of great importance to the understanding of cellular protein synthesis. To study nucleotide sequences, some effort has first been concentrated on the preparation and analysis of RNA, since it is of fundamental importance to have purified preparations of RNA for structural studies. Whole-cell RNA and fractionated RNA's such as sRNA and ribosomal RNA from mammalian and avian liver are purified and analyzed. Nucleotide sequence analyses are then performed on purified RNA preparations.

Standard methods for the large-scale preparation of yeast sRNA, involving purification by DEAE-cellulose chromatography, were found

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Figure 17. Chromatography of 3 different sRNA preparations with Sephadex G-200. (a) Purified rat liver sRNA, 3.6 mg added to column; (b) General Biochemicals yeast sRNA, 4.4 mg added to column; (c) General Biochemicals rat liver sRNA, 4.6 mg added to column.

Figure 18. Sephadex G-200 column analysis of 1 M NaCl precipitate RNA.

unsuitable when applied to mammalian tissue. Approximately one-fourth of the nucleic acid could be accounted for by ribosomal RNA and DNA contamination. A method for the large-scale preparation of sRNA from animal sources was devised in which contamination with other RNA's and DNA had been minimized. Final purification was achieved with Sephadex G-200 chromatography by using a 7-liter column and eluting with potassium acetate. The preparation has twice the amino acid acceptor activity of yeast sRNA and appears homogeneous when analyzed with the ultracentrifuge, by gel electrophoresis, and chromatographically. Liver sRNA purified by this method is at least as pure as yeast sRNA prepared by standard methods (Figure 17).

Gel filtration with Sephadex G-200 has been developed as a convenient and rapid method for observing the quality of an RNA preparation. By using long, thin Sephadex columns, the purity of an RNA preparation can easily be determined within a short period of time with a minimum of effort. Figures 17 and 18 represent examples of RNA purity studies. (Most of the above work was performed while the author was on leave of absence from BNL and in collaboration with M. Staehelin at CIBA, Basel, Switzerland.)

D. METABOLIC STUDIES

1. *Studies of Extrapyramidal Diseases*

G.C. Cotzias
M.H. Van Woert
A. Foradori
I. Mena

Current studies in this program are aimed at clinical control of extrapyramidal diseases and at definition of homeostatic mechanisms controlling trace mineral metabolism. The attempt reported earlier to treat patients with Parkinson's disease with melanophore-stimulating hormone (β MSH) indicated that by the time the injected hormone had been raised to 30 mg a day, the manifestations of the disease became worse in all cases studied. The aggravation was compatible with the possibility that the peripheral melanophores were sequestering melanin precursors away from the brain, and this was investigated by means of melanin precursors and β MSH antagonists. Initial studies on two patients who were given the β MSH antagonist melatonin showed that the disease might be affected beneficially by this agent. When the melatonin was discontinued slowly, the basal clinical state was slowly reached. When the melatonin was stopped abruptly after one of these trials, a dramatic self-limited episode followed.

In an effort to replete the precursor pools for melanin synthesis, DL Dopa was administered to 11 individuals with various forms of Parkinsonism. The striking response in two instances, sustained for the several weeks of DL Dopa administration, showed that some individuals who have long suffered from tremor, rigidity, and dyskinesia may be restored almost to normal provided that a sufficiently large daily oral dose is administered. The effective daily dose, which varied between 4 and 14 g DL Dopa, did not seem to induce the mental aberrations characteristic of agents used earlier. A significant drop in blood manganese, a drop in plasma iron, and a possible rise in blood magnesium during the administration of DL Dopa were observed. Some adverse clinical effects were noted. One patient suffered a drop in circulating white cells, affecting primarily the granulocytes, accompanied by a severe but reversible vacuolization of the bone marrow cells, but no clinical consequences were observed. Atypical lymphocytes were also found following heavy doses of DL Dopa for extended periods.

The binding of Mn^{54} by plasma proteins was studied further, and recent resolution of the β globulins into β_1 and β_2 showed that the β_1 globulins are the principal binding sites among the globulins.

The manganese-containing heme of human erythrocytes was investigated further. Serial neutron activation analyses for manganese were performed on the blood of two patients, one of whom was fed large amounts of the metal, the other being a Chilean miner who inhaled manganese ores. In both, the high levels of blood manganese

declined slowly with an approximate half-life of 145 days, which suggested that the metal might be very firmly bound in the blood. In one patient fed large amounts of manganese sulfate, the blood manganese reached an elevated plateau after about 140 days. The entire blood manganese was accounted for by the manganese in heme. In two cases of Parkinsonism treated with DL Dopa, the decline of blood manganese stopped after about 120 to 140 days on DL Dopa (approximately one erythrocyte life-span). These data suggest that manganese is incorporated into red cell heme and is released only on death of the cell.

In the collaborative study with the Catholic University of Santiago, Chile, on chronic manganese poisoning in Chilean miners, the total-body loss of Mn^{54} was found to be highest in the "healthy" miners, whereas the values for the chronically ill patients approximated those of the normals. This indicates that "healthy" miners have high body burdens of manganese, whereas patients do not. Evidence based on neutron activation analysis for manganese in various tissues, plasma clearance of Mn^{54} , and regional counting for the same tracer corroborates these findings and tends to dissociate high tissue burdens of this metal from neurotoxicity.

2. Effect of Radiation on Metabolism of Trace Metals

G.C. Cotzias
K.N. Prasad
M.H. Van Woert
H.A. Johnson

Radiation of animals having abundant visceral melanocytes is being used to study the interrelationships between melanins, trace metals, and free radicals. During the past year it was shown that manganese accumulated in melanin granules of *Amphiuma* liver to an equal or larger extent than in mitochondria; in addition, the melanin granules do not constitute independent cytoplasmic organelles but are subunits of larger cytoplasmic inclusions that vary in appearance. In lightly pigmented melanocytes they appear round, are bounded by a membrane, and contain few melanin granules in addition to onion-shaped, membranous subunits. In darkly pigmented cells, these inclusions are often dense, contain dense granules, are sometimes shrunken, and sometimes ruptured. Following segmental irradiation of the amphiumas across the liver, hepatocytes showed the customary radioresistance observed in mammals, but the melanocytes of these livers showed selective radiosensitivity. Following doses of 8000 R the melanocytes diminished in number at the expense of the lightly pigmented ones. This fall was reversible, since basal levels were reached a month after irradiation. In similar studies with *Necturus*, in which the liver displays primarily dense melanocytes, no radiosensitivity was displayed by either the melanocytes or the animals even with segmental irradiation at doses approximating 150,000 R. Since some other amphibians (as

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well as one fish and one reptile) show in their liver melanocytes still different cytoplasmic organelles, further study is planned to determine whether the different organelles simply represent different stages of maturation.

In further studies on the enzyme monoamine oxidase it was found that the enzyme becomes inhibited by excesses of some of its substrates as well as by the anions that form an integral part of these substrates. Application of this knowledge to the intercomparison between melanin-containing organelles and mitochondria indicated that monoamine oxidase activity represents an additional functional similarity between them. Furthermore, both mitochondria and melanin organelles contained cytochrome oxidase in similar yields, but their succinic dehydrogenase was of similar activity in only some of the experiments. Oxidative phosphorylation was lower in these novel bodies than in the mitochondria from the same livers. Hence for the moment it must be concluded that, although several functional similarities exist between mitochondria and these structurally different organelles, not all functions tested were present in both to the same extent.

3. *Interrelationship of Sodium and Genetics With Hypertension*

L.K. Dahl
K. Knudsen
D. Ben-Ishay
L. Tobian
N. Miller
G. Wolf
M. Kuschner

There is considerable circumstantial evidence suggesting that chronic excess salt ingestion can play a causal role in human hypertension. Direct and unequivocal evidence has established that hypertension can be induced in rats by salt-feeding, and this experimental hypertension is very similar to the human variety. There is also considerable evidence suggesting that genetic (or at least *familial*) factors are involved in human hypertension. Two unique strains of rats having opposite and striking differences in predisposition to hypertension have been developed by selective inbreeding, and the differences appear to have a genetic basis. These animals aid in elucidating basic mechanisms involved in the hypertensive process by enabling simultaneous study of animals that are known to have an innate propensity for, or resistance to, developing hypertension.

Studies have been directed toward understanding the genetic characteristics and the influence of various factors (kidney, adrenal, dietary potassium and sodium, stress, age, sex, and vascular reactivity) on the development of hypertension, and some results are indicated below.

The biological half-life ($T_{1/2}$) of Na^{22} has been redetermined in 13 patients with hypertension and 13 without. The $T_{1/2}$ was not significantly longer in patients with hypertension than in the controls. This is in contrast to an earlier study on a much smaller series of patients in

which it was found that the $T_{1/2}$ for Na^{22} was longer for hypertensives than for normotensives. The current study is in agreement with earlier studies at BNL, which showed that hypertensives did not have more sodium in their tissues.

Among rats from the strain predisposed to hypertension (the *S* strain), if extra dietary NaCl is started at 12 weeks of age rather than at weaning, the severity of the resulting hypertension is markedly lessened. If this is true for man, it suggests that a high salt diet in early life may be much more damaging than later. Among rats from the *S* strain placed on high NaCl diets at weaning, but changed to low NaCl diets at intervals thereafter, it was found that many animals developed permanent hypertension after only 6 weeks on salt. If this is true for man, it suggests that even a transient high salt diet in early life may be influential in leading to hypertension in later life.

In efforts to determine the genetic characteristics of the two strains, the first and second generations (f_1 and f_2) after crossing the strains have been studied. Among members of the f_1 generation, those with fathers from the *S* strain had significantly more hypertension than those with mothers from the *S* strain. Among members of the f_2 generation, those with *both* grandfathers from the *S* strain had more hypertension ($p < 0.01$) than did those from the other three possible grandparental crosses. Initial analysis suggests that several loci, rather than only one, may be involved. The genetic difference between the two strains of rats originally unmasked by salt-feeding was similarly shown to be true for four other "varieties" of experimental hypertension. If these experimental models are relevant to man, they suggest that hy-

Figure 19. Apparatus for study of renal function in rats with experimental hypertension from salt. Animals have chronically indwelling catheters in jugular veins, through which a solution of inulin C^{14} is being infused.

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pertension may result from one or more nongenetic factors (salt ingestion, kidney disease, "stress," hormonal influences) interacting with the appropriate genetic factors. There is clinical evidence to support such a hypothesis.

Innate appetite for saline was found to differ in the two strains. Given free choice of water or saline, animals from the *S* strain had an aversion for saline as compared with those from the *R* strain (genetically resistant to developing hypertension). Other than differences in blood pressure, this is the first physiological difference observed in these two strains and suggests that other, possibly more subtle, biochemical or biophysical differences may be present as well. Renal function studies including measurements of glomerular filtration rate (using inulin C^{14}), renal plasma flow (using paraaminohippurate), and urine osmolality failed to suggest that renal function was impaired prior to or during the early development of hypertension. These observations suggest that the impairment of kidney function so often observed in human hypertension is a result of the hypertension, rather than a cause.

4. Mechanisms of Action of Hormones, Drugs, and Vitamins on Carbohydrate and Fat Metabolism

W.W. Shreeve
N. Oji
E. Lamdin
A.J. Tashjian
S.M. Joubert
J.J. O'Neill

The objective of this study is the investigation, largely with use of radioisotopes, of the sites and mechanisms of action of hormones, drugs, and vitamins. It is hoped that such studies will lead to the use of radioisotopes for better diagnostic criteria and provide basic understanding of biochemistry and physiology to guide the development of drugs and other appropriate treatment of disease. Diabetes, obesity, and hyperadrenal cortical function are the metabolic disorders under study. C^{14} and H^3 are used as tracers of the metabolism of carbohydrates and fats, and the functions of synthesis, storage, energy utilization, and specific metabolic pathways are evaluated.

Previous studies of the rate of oxidation of glucose-1- C^{14} and glucose-1- H^3 by obese patients after intravenous administration were extended to studies in which the labeled compounds were added to an oral glucose load with and without prior administration of cortisone. The latter procedures compared directly the metabolism of the labeled glucose with disappearance from the blood of total glucose during a test (oral cortisone-glucose tolerance test) which is at present considered the most sensitive for detection of the diabetic trait. At 1 hr after administration of the glucose load containing glucose-1- C^{14} , when preceded by administration of cortisone, the rate of expiration of CO_2^{14} in the breath was 175% higher in normal than in obese subjects. At the same time the blood glucose concentration (present criterion of difference in the test) was only 70% higher for obese than for control

Figure 20. Collection of breath from patient for analysis of CO_2^{14} during study of metabolism of C^{14} -labeled carbohydrate.

subjects. Intravenous glucose tolerance tests showed 120% difference between the two groups in rate of glucose disappearance. Thus the breath CO_2^{14} difference may be superior to either of the methods based on blood glucose analysis. Furthermore, the CO_2^{14} test after oral glucose load avoids the discomfort of venipunctures for the patient.

Studies were started in diabetic and obese patients with glycerol-1,3- C^{14} , glycerol-2- H^3 , and dihydroxyacetone-U- C^{14} to investigate abnormalities of oxidation, gluconeogenesis, and lipogenesis from these intermediate carbohydrates. Compared with normal subjects the patients oxidized these C^{14} -labeled compounds to CO_2^{14} only $\frac{1}{2}$ to $\frac{1}{3}$ as fast. More of the C^{14} was converted to glucose by obese than by normal subjects. In one diabetic and two obese subjects, prior infusion of insulin and glucose increased the CO_2^{14} production at least 100% and in the diabetic markedly decreased formation of blood glucose from glycerol. These studies further indicate the effects of insulin on the metabolism of intermediate carbohydrates as well as glucose. The

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Figure 21. Section of a 12-in. gun barrel used as shielding for low-level anticoincidence C^{14} counting. Samples were taken from patients given labeled tryptophan metabolites.

conversion of glycerol-2- H^3 to body water was not affected by diabetes, obesity, or insulin, but insulin decreased the amount of tritium converted to glucose.

Further studies in rats of conversion of H^3 - and C^{14} -labeled intermediate carbohydrates to blood glucose and liver glycogen indicate that cortisone (administered for 5 days) increases the transfer of tritium from lactate and malate to glucose 4- and 5-fold but has no effect on transfer from H^3 -labeled succinate or glycerol. Transfers of C^{14} from alanine and lactate were increased 2-fold, but there was no significant change with C^{14} -labeled succinate and glycerol. These findings indicate some selectivity of effect of cortisone on oxidation of substrates or subsequent metabolism of the labeled derivatives, with some suggestion that only those metabolized *outside* the mitochondrion are affected by cortisone. The tritium label may be a particularly good indicator of the increase in gluconeogenesis caused by cortisone.

Studies of synthesis of fatty acids (f.a.) of liver and carcass of obese mice have been continued with the aid of H^3 - and C^{14} -labeled lactate, malate, succinate, and glucose. Another doubly labeled compound, glycerol-1,3- C^{14} -2- H^3 , also shows a relatively high degree of transfer of tritium. Since oxidation of glycerol utilizes primarily the coenzyme NAD^+ , this finding further emphasizes the participation of NAD^+ in the transfer of hydrogen for f.a. synthesis. Preliminary study suggests that refeeding after starvation markedly increases f.a. synthesis in lean mice but has little effect in obese siblings. Upon refeeding, the extent of C^{14} and H^3 incorporation into f.a. of lean mice approaches or exceeds that observed in obese mice.

Study of f.a. synthesis in liver slices indicates that transfer to f.a. of tritium relative to C^{14} is much reduced compared with the situation *in vivo*. However, the H^3/C^{14} ratio is still higher for succinate than for malate, which supports the view that succinate is metabolically different from malate and is superior to it as a source of reductive hydrogen for synthesis of hepatic f.a.

Further studies of the effect of ethanol load on intermediary metabolism and particularly gluconeogenesis have explored the means for disposal of reductive hydrogen from ethanol by tracing ethanol-1- H^3 into the glycogen of rat liver *in vivo*. The finding of a position-labeling ratio of 2:1 between carbon 4 and carbon 6 of liver glycogen suggests transfer of hydrogen through lactate (which labels similarly) or a cofactor pool shared commonly with lactate in extramitochondrial oxidation.

The studies relating to carbohydrate metabolism in brain slices have been extended to analyze the concentrations of glycolytic inter-

mediates (by the ultrasensitive method of fluorometry) and their C^{14} concentrations after metabolism of glucose- C^{14} . The Pasteur effect has been further elucidated by observations with the above techniques of inhibition of glycolysis by pyruvate and citrate at the level of phosphofructokinase. Electrical stimulation of the brain cortical slices appears to overcome this inhibition.

5. Metabolism of C^{14} -Labeled Tryptophan and Its Urinary Metabolites in Animals and Man With Specific Reference to Cancer

L.V. Hanks
R.R. Brown

In recent years tryptophan, an essential dietary amino acid, has been shown to produce several metabolic products which can cause the formation of cancer cells in animal urine bladders. The objectives of this project are to label tryptophan and its carcinogenic urinary metabolites in different positions of their structure with C^{14} , and then, after the administration of these labeled components to animals and, subsequently, man, to isolate various urinary metabolites of these compounds and determine the position of the C^{14} label in the structure of these urinary metabolites.

Synthetic procedures for labeling tryptophan metabolites, such as anthranilic acid, kynurenine, hydroxykynurenine, hydroxyanthranilic acid, and quinolinic acid, have been or are being developed. As these C^{14} -labeled tryptophan metabolites become available their metabolism will be studied in animals first, and then in humans with scleroderma, multiple myeloma, bladder tumors, or anemias.

DL-Kynurenine-keto- C^{14} , previously synthesized, was resolved into the D and L isomers on powdered paper columns. Pairs of rats were given intraperitoneal tracer doses of either isomer; the respiratory CO_2 and urine were assayed for C^{14} content; and the urinary metabolites, nicotinic acid and quinolinic acid, were assayed and isolated. From the D and L isomers, respectively, 78 to 85% and 97 to 98% of the dose were recovered. Urine contained 77 to 84% of the isotope from the D isomer compared with 8.9 to 10.7% from the L isomer. Only 1.2 to 1.4% of the D isomer appeared in the expired CO_2 , while 87 to 90% of the L isomer was present. About ten times as much L isomer was converted into urinary quinolinic acid, compared with the D isomer. The urinary nicotinic acid accounted for equivalent amounts of the D and L isomers.

The B-complex vitamins, such as vitamin B_6 , play an important role in tryptophan metabolism. Their influence on the metabolism of these C^{14} -labeled compounds will be studied.

A study in humans showed that, as vitamin B_6 was depleted, the quinolinic acid levels rose in the urine. When the vitamin B_6 level in the diet was restored, the urinary quinolinic acid levels returned to

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normal. This elevation of quinolinic acid levels, in conjunction with normal levels of the nicotinic acid metabolites during the same period, suggested that vitamin B₆ coenzymes were necessary in the further metabolism of quinolinic acid.

6. *The Source of Cyanide Carbon in a Chromobacterium*

L.V. Hanks
W.A. Corpe

In many enzyme systems, cyanide plays an important role. A chromobacterium was found which produced large quantities of cyanide from the amino acid glycine, which is a product of tryptophan metabolism. C¹⁴-labeling studies were initiated to determine the source of the cyanide carbon.

Cultures of *Chromobacterium violaceum* cells incubated in a chemically defined medium containing methionine and C¹⁴-labeled glycine produced C¹⁴-labeled cyanide. The cyanide carbon produced by the cells was derived from the methyl carbon of glycine. The carboxyl carbon of glycine and the methyl carbon of methionine are apparently only indirectly involved in the biogenesis of cyanide. The greatest portion of the carbons of glycine fixed in the cells appeared in the "protein fraction." The fraction of the glycine carboxyl carbon present in the involved carbon dioxide remained almost constant for 2 to 6 hr of incubation and was almost equivalent to the cyanide arising from the methyl carbon of glycine. The mechanism for the formation of the CO₂ from the carboxyl group of glycine and cyanide from the methyl carbon has not been defined, although theoretical mechanisms have been suggested which involve the formation of cyanuric acid and cyanofornic acid.

7. *Metabolism, Turnover Rate, and Kinetics of Radiovitamin B₁₂ and Radioiron*

E.P. Cronkite
L.M. Schiffer

Vitamin B₁₂ is an essential substance in the metabolism of all cells of the body, and its deficiency can be noted in the red blood cells of circulating blood and in bone marrow. The B₁₂ molecule contains a cobalt atom capable of being made radioactive. Thus, with appropriate electronic detection equipment, tracer studies and investigations of absorption, excretion, daily requirement, and whole-body turnover of vitamin B₁₂ are made possible. Of particular interest in these studies has been the relationship of protein-bound and nonprotein-bound B₁₂ substances which have different modes of transport to the sites of their utilization and storage. Whole-body counting of patients already carrying isotopic vitamin B₁₂ was continued for the third year. The same nonlinear disappearance rate of B₁₂ as previously reported has been noticed in the recent counts, which indicates a very slow turnover of a multicompartimented body pool of vitamin B₁₂.

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Fe^{59} was used as a tracer for erythropoiesis in determining the sites of active bone marrow production in adult dogs. This had not been performed previously in the dog and is of considerable importance for radiobiological and stem cell studies. In addition, this study also combined a technique to measure colloidal Tc^{99m} uptake in the reticulo-endothelial compartment of the bone marrow. The two isotopes proved to be in almost exact relative concentration in all bones of the body, which indicates similar bone marrow distribution of erythropoietic and reticuloendothelial activity.

Fe^{59} was also used in experiments in which an erythropoietic depressor factor was found in the blood of animals "brought down from high altitudes" in a pressure chamber.

8. Radiochemical and Instrumental Methods in Neutron Activation Analysis

G.C. Cotzias
D.C. Borg

Neutron activation analysis is of interest in chemical studies of trace metals since it has high sensitivity and inherently permits avoidance of contamination. Analysis of radioactivities induced in samples can be achieved either by chemical separation of a given nuclide from the radioactive sample or by instrumental differentiation of the radio-

active emissions from the total sample. The procedure involving chemical separation is sensitive and precise but represents a bottleneck because of its slowness (8 single analyses per day per person). The instrumental method is fast and readily applicable to simultaneous multi-element analysis, but is much less sensitive.

Figure 22. Neutron activation analysis. A radiochemical separation is being performed.

A previous report indicated that, when a method developed here for chemical separation of radioactive elements was used, analytical results of human serum yielded considerably and persistently lower values than were obtained at most other laboratories, including those using neutron activation analysis. Continuing studies during the past year have shown (1) that established methods duplicated earlier results if the samples were handled similarly, (2) that contamination (and not loss) seemed to be the main pitfall in ultramicroanalysis, and (3) that awareness of this problem leads to recognition of many additive sources of contamination, even where samples are handled minimally.

The availability of larger Ge(Li) solid-state radiation detectors and the resulting gain in their gamma-ray counting efficiencies led to their application in instrumental analyses of neutron-irradiated tissues. Although NaI(Tl) scintillation detectors are more efficient detectors of monoenergetic gamma rays, the much greater spectral resolution of Ge(Li) crystal detectors permits more sensitive identification of many simultaneous radioemissions from one sample. In testing the applicability of instrumental analysis of tissues, accurate quantitation of the manganese concentration in rat liver was readily achieved. Neither brain nor plasma samples, in which there is much more sodium per unit manganese, could be thus analyzed by this method. Nonetheless, it is clear that routine instrumental analysis can be achieved for most samples of medical interest.

E. CELL PROLIFERATION KINETICS

1. Studies of the Histo-Cytophysiology of Cells of the Skeleton and Teeth

E.A. Tonna
E.P. Cronkite
W.S. Hwang
S. Manspeizer
A. Severson
L. Pentel
G. Hatzel
C.P. Gandal
M. Pavelec

The over-all aim in these widely varied studies is to obtain information concerning the potentialities, normal behavior, and response of the cells constituting the skeletal and dentinal systems. During the past year, the distribution and quantity of osteocytes in cortical bone from birth to old age have been studied in the mouse. Results showed that up to 1 yr of age cortical bone consisted of osteocyte-filled lacunae. However, by 2 yr of age 31% of the osteocytes had disappeared. With the help of H^3 -proline autoradiography the time required for conversion of osteoblasts to osteocytes was shown to be a minimum of 6 days. Many cells exhibited a maximum time equivalent to the entire animal's life-span, which indicates that osteoblasts exist which are never transformed into osteocytes. Differences in cellular kinetics have been observed between rodents and other mammals, including man.

E. CELL PROLIFERATION KINETICS

It was learned from a study of the origin of osteoblasts in normal and traumatized animals of different ages that in old mice trauma can stimulate old skeletal cells to generate osteoblasts that have a functional capacity equal to that of young animals. In a 2-yr study, recently completed, morphologic and autoradiographic evidence was obtained to show that osteoclasts which appear in old animals undergo aging. Other studies showed that older animals do not produce osteoclasts except following trauma, in which case these cells are formed from fusion of the progeny produced by proliferation of old skeletal progenitor cells. The analyses of H^3 -proline turnover data revealed similarities between amino acid utilization and the RNA activity of skeletal cells from birth to old age. Studies in young mice on the cell kinetics of incisor tooth formation using H^3 -thymidine revealed that it takes a minimum of 57 hr for preameloblasts to complete their maturation. The cellular kinetics of the incisors of old animals were generally similar.

The automatic, cell scanning, microscope stage and accompanying optical and recording setup were tested with respect to the sensitivity of the instrument complex in characterizing intracellular components with the use of extremely small apertures having an equivalent of 0.2μ in diameter. Salivary gland chromosomes, muscle fibers, and a large variety of plant and animal cells, including human cells, were scanned with extremely satisfying results.

2. The Use of Radioactively Labeled DNA in Determining Long-Term Cell Survival in Vivo

S.L. Commerford

Mammalian cells given I^{125} -labeled 5-iodo-2' deoxyuridine (IDU) incorporate it into their DNA. The labeled DNA remains with the cell for the rest of its life and can be easily detected without disturbing the cell or the organism containing it. Because of these properties, the effect of ionizing radiation and toxic drugs on the survival of labeled cells can be determined. Since DNA labeled with I^{125} -IDU is easily detected and measured, labeling also aids the development of procedures for isolating and fractionating DNA.

Previous work had shown that about 85% of I^{125} -labeled IDU given to 5-day-old mice is either incorporated into cells with a life span of <5 days or is immediately degraded, the label being excreted as iodide; about 10% is incorporated into cells with a biological half-life of 9 to 11 days; and about 5% is incorporated into cells with a biological half-life of >1 yr. This year the distribution of the latter two components among several organs was measured. The skin, small intestine, liver, kidneys, lungs, stomach, spleen, heart, and thymus contained 29, 15, 2, 1, 2, 3, 1, 0.2, and 3%, respectively, of the activity in cells with the shorter life-span, and 11, 10, 5, 5, 4, 4, 1, 1, and 0.2%, respectively, of the activity in cells with a longer life-span.

Figure 23. Autoradiographs made from the periosteum of a 1-yr-old mouse whose left femur had been fractured 6 days earlier, with administration of H^3 -glycine on the 5th day. (a) The uptake at the nonfractured right femur; (b) (a comparable site) the thickened osteogenic layer of the periosteum following stimulation of cell proliferation and the deposition of a layer of new bone matrix. Note that the initial deposition of label is scanty (farthest point away from periosteum), and within a short time an intense band is formed. This is probably due to the rapidity with which new bone was laid down. Under normal conditions of growth in 5-wk-old mice it would have taken about 4 to 5 days to produce what appears in 1-yr-old mice within 1 day following fracture. The periosteal site selected in (b) is a considerable distance away from the fracture callus. These studies showed that old skeletal cells cannot participate in bone matrix production directly, but, as a result of stimulated cell proliferation, extensive bone matrix formation occurs through the activity of their young progeny. The organism is thus supplied with a mechanism capable of responding to emergency needs throughout the life of the individual and long after skeletal growth has ceased. Hematoxylin stained. 750 $\times$.

E. CELL PROLIFERATION KINETICS

Figure 24. Effect of thiotepa on the viability of cells. Each point represents the average I^{125} activity retained by 5 mice given 15 μ g thiotepa at 4 different times (indicated by arrows) relative to the activity retained by 5 littermates injected with saline.

The effect of certain cytotoxic agents on the viability of labeled cells with a long life-span also was measured. Four doses of thiotepa, equivalent on a weight basis to that used to treat human carcinomas and given at 8-day intervals, killed $<2\%$ of the labeled cells over a 3-mo period. The effect of ionizing radiation on these cells depends on the interval between labeling and exposure. Exposure to 300 R of x rays 2, 5, 8, 16, and 24 hr after labeling killed 45, 45, 40, 12, and 10% of the cells. The life-span of cells surviving 1 mo was the same as that of unirradiated littermates. X rays given 1 mo or more after labeling had no immediate effect, but, if given 6 mo after labeling, a single exposure to 400 rads Co^{60} reduced the life-span of cells to 85% that of unirradiated littermates; 3 exposures to 400 rads Co^{60} reduced the life-span to 75%.

Labeling of cells with I^{125} -IDU not only introduces into DNA a radioactive isotope but also substitutes an unnatural nucleotide for thymidine. To determine whether either factor decreases the viability of labeled cells, mice given 20 μ Ci I^{125} -IDU were compared with littermates given 1 μ Ci, and mice given I^{125} -IDU plus 2 μ M carrier IDU were compared with littermates given I^{125} -IDU plus $<10^{-4}$ μ M carrier IDU. No decrease in the viability of labeled cells was observed in either instance.

3. Study of the Use of Tritiated Pyrimidines in the Treatment of Human Malignancies

E.P. Cronkite
V.P. Bond
A.D. Chanana
M.L. Greenberg
J.S. Robertson
L.M. Schiffer
P.A. Stryckmans
E. Adamik
C. Sipe

The selective incorporation into the cell nucleus of tritiated thymidine provides the most reliable approach to characterization of blood cell generative cycles in normal and abnormal states. Techniques of autoradiography and cytology are being used in *in vivo* and *in vitro* studies designed to elucidate the origin, rate of division, and fate and function of hemopoietic cells. These studies provide information on the nature of malignancies, necessary for the rational development of chemotherapeutic drugs. Studies on individuals with apparently normal hemopoiesis have indicated that in the human being the DNA synthesis times of erythrocytic and myelocytic precursors are markedly longer than in the dog or lower mammals. In studies on hemopoietic cell kinetics before and during extracorporeal irradiation of the blood

E. CELL PROLIFERATION KINETICS

in three leukemic patients, preliminary results indicate no difference in the output of leukemic cells from the bone marrow. Lymphocytes from patients with chronic lymphocytic leukemia, irradiated, labeled, and returned *in vivo*, manifest markedly shortened life-spans.

During the course of the studies it was noted that large amounts of tritiated thymidine caused favorable decreases in the leukemic cell concentration of blood and bone marrow in two patients with acute leukemia. Concomitant investigations have begun on a transmittable acute leukemia of rats to aid in determining the potential usefulness of tritiated thymidine (and other tritiated pyrimidines) in the treatment of leukemia and other malignancies.

One patient with acute lymphocytic and another with acute myelocytic leukemia received some therapeutic benefit from large amounts of tritiated thymidine of high specific activity. Objective clinical measurements, as well as subsequent studies of their blood and bone marrow cells, indicated an extensive "kill" of leukemic cells. In one patient with reticulum cell sarcoma who received 420 μCi tritiated thymidine, regression of skin lesions was noted. Definite prolongation of life has been obtained in rats with transmittable leukemia treated with large doses of tritiated thymidine. Additional patterns of dose administration are being tried in an effort to improve the results. Autoradiographic studies on tissues of the treated rats are being carried out to assess the results at the cellular level.

4. Use of Radioactive Nucleosides for the Investigation of Specific Events Occurring in Thymidine-Synchronized Mammalian Cell Cultures

R.M. Drew
S.L. Commerford

A high degree of synchrony can be established in mammalian cell cultures by the excess thymidine blocking technique. The purpose of this study is to determine the variables that influence and, possibly, limit the use of thymidine-synchronized cultures for specific metabolic studies; e.g., is the metabolic competence impaired by the procedure? The ability of synchronized HeLa S3 cells to utilize H^3 -thymidine, H^3 -deoxycytidine, and I^{125} -deoxyuridine is being determined. Chemical extraction of nucleic acid precursors and nucleic acids and estimation of the radioactivity in the various fractions provide quantitative information about the capacity of these cells for synthesizing essential genetic material. Autoradiograms are being used to determine what influence excess thymidine may have on the various phases of the life cycle.

Pulse-labeling experiments with H^3 -thymidine showed that the DNA synthetic activity of the cells was markedly depressed in the first part of the S phase and that about $2\frac{1}{2}$ hr were required, following excess thymidine treatment, to establish full competence. H^3 -deoxy-

Figure 25. Marshallese patient being examined during annual medical survey.

cytidine incorporation studies demonstrated that the excess thymidine treatment used to induce synchrony caused a deficiency of one of the precursors of DNA.

The synchronized cultures are being used to determine quantitatively the biological effects of intranuclear tritium incorporated as H^3TDR , with use of mitotic delay as the criterion of biological effect. Dose effect curves have been obtained from which it will be possible to evaluate the relative effectiveness of radiation from intranuclear tritium compared with that from 250-kVp x rays.

F. SPECIAL STUDIES

1. Medical Studies of the People of the Marshall Islands Accidentally Exposed to Fallout

R.A. Conard
J.E. Jesseph
L.M. Meyer
W.W. Sutow
J.E. Rall
M. Eicher
A. Hicking
J.B. Deisher
I. Lanwi

Annual medical surveys are carried out on the people of the Marshall Islands who were accidentally exposed to fallout in 1954. The surveys are carried on under the auspices of the Division of Biology and Medicine of the U.S. Atomic Energy Commission, under the direction of the Medical Department of Brookhaven National Laboratory, with the assistance of medical and technical personnel from other institutions and from the Trust Territory of the Pacific Islands. Some 239 Marshallese on Rongelap and Utirik Atolls who were involved in the accident are examined, and some 200 unexposed Marshallese relatives of the Rongelap people are included in the examinations for comparison purposes. The annual physical examinations include complete medical histories and physical examinations, examinations of skin, hematological studies, and evaluation of body burden of radionuclides by radiochemical analyses or by whole-body gamma spectroscopy. Complete reports of these surveys, including addenda of raw data, are published annually at BNL.

F. SPECIAL STUDIES

Current studies are concerned with the residual or late effects of exposure. The most important finding has been the development of definite nodules of the thyroid gland in 11 exposed people, minimal changes in 5 others, and hypothyroidism in 2 cases. All but one case occurred in the more heavily exposed people (55 living of the original 64), the one exception being that of a woman in the smaller Rongelap group (16 living of 18) that had received less than half the exposure of the other group. In the more heavily exposed group, abnormality of the thyroid developed in 34% of the children <20 years of age when exposed, and in 7% of the adults. Results of surgery in 6 cases revealed nonmalignant nodules in 5 children and a malignant nodule in 1 adult. These thyroid lesions are thought to be a result of internal radiation from radioiodines at the time of fallout and of external radiation. The previous observation of slight retardation of growth and development in boys <5 yr of age when exposed appears to be related to thyroid effects, since hypothyroidism has recently developed in two boys showing the most growth retardation. Growth response to thyroid hormone will test this hypothesis. No other findings related to possible late effects of radiation were noted during the past year. In September 1965 the exposed people were started on treatment with thyroid hormone in an attempt to prevent the development of further nodules and possibly induce regression of existing nodules.

2. *Computer Application in the Medical Research Program*

J.S. Robertson
S.H. Cohn
R. Straub
E. Gusmano

The use of computers in the medical research program is extensive and increasing. Some of the applications during the past year include the processing of clinical data, dose calculation for extracorporeal irradiation of the blood and for special isotopic distributions, and inert gas equilibration during diving descent and ascent.

A system using the IBM 7094 for data storage and retrieval has been put into use for processing clinical laboratory data. A number of source documents that serve both as laboratory request forms and as laboratory report forms have been developed for use in clinical chemistry, clinical hematology, and medical records. In this system a request originating at one of the nursing stations in the Hospital of the Medical Research Center is completed by the appropriate laboratory and the source document is read by an IBM 1232 optical mark reader which automatically prepares a set of punched cards. On a daily basis these data are added to the tape serving as the memory, and the cumulative data for the preceding three months plus the new data are printed out as a report to the nursing station, with an individual report for each patient. At the same time a report organized by procedures

is prepared for the laboratory. Similarly, data on patient admissions and discharge, diagnosis, treatment, etc., are reported to the medical records office.

A computer program developed for calculating the dose distribution in extracorporeal irradiation of the blood has been modified for use with intermittent irradiation such as is used in the treatment of leukemia. For this application it is necessary to take into account the normal finite lifetimes of the formed elements in the blood and the effects of radiation on these lifetimes.

In collaboration with Dr. G. Moeller at the Submarine Medical Center of the U.S. Naval Submarine Base, New London, Conn., work has been done on applying the principles of gas exchange physiology to the improvement of the Navy's diving tables. On paper, schedules have been developed that will permit continuous ascent from depths of 200 ft or more, rather than by steps, as in the schedule currently in use. A problem with the stepped schedule is that while the rapid turnover tissues are being desaturated, the slower turnover tissues may actually be taking up more gas, which increases the length of time required for the next stage. The continuous ascent method uses a critical tissue to determine the maximum safe rate of ascent at any time; in this way the problem of continued gas uptake during ascent is minimized and the total ascent time is shortened.

3. *Methods of Extending and Improving Radiographic and Isotopic Diagnostic Techniques*

H.L. Atkins
R.G. Fairchild
L. Schiffer
P. Richards

These studies are concerned with the extension of the scanning techniques that use neutrons, transmission scanning methods, and short-lived, low-energy emitters, primarily Tc^{99m} .

The properties of Tc^{99m} make it close to an ideal scanning agent. Large amounts of activity can be administered, which enhances statistical accuracy and speeds up scanning, particularly of large body areas. In addition, several compounds that extend its utility to a number of organs have been produced. A highly efficient collimation system has been developed for use with Tc^{99m} . Sensitivity and resolution are much better than in commercially available collimators, and the system, when used with a data-blending presentation technique, has produced excellent photoscans. The system has proved particularly useful for thyroidal scanning where the elimination of the high background activity has allowed clearer visualization of the organ.

A technetium-sulfur colloid was used extensively for bone marrow scanning and as an objective measurement of spleen size in leukemic patients undergoing extracorporeal irradiation of the blood. A study has been initiated to investigate phagocytic activity by determining

Figure 26. Technetium-iron-ascorbate being used for renal scanning.

blood disappearance half-times. Initial studies with a technetium-iron-ascorbic acid complex have been undertaken in an effort to elucidate the physiology and dosimetry and to evaluate the usefulness of the complex in renal scanning. Uptake in the thyroid as determined by the scan is being correlated with I^{131} uptake and protein-bound iodine levels.

Transmission scanning is useful in a number of ways. It can be used as a method of anatomical mapping of isotope concentration when combined with isotope scanning. The use of monoenergetic beams near absorption edges can give a semiquantitative estimate of specific elements naturally present or administered to a patient. For example, because of its natural iodine content the thyroid can be visualized, or the liver, following the administration of a heavy metal colloid.

Transmission scanning, carried out with an I^{125} source, provided very fine detail with phantoms, but its use in patients was limited by the low transmission through most portions of the body. Am^{241} , a gamma source, was used for films of the chest and neck in conjunction with isotope scans of these regions. Poor detail was obtained because of the rather large (2-mm) beam required. The strength of the source is limited by self-absorption. Transmission scanning may possibly be useful in neutron radiography. Its possible advantages are the use of very low doses of radiation and avoidance of scatter by use of a narrow beam.

Neutron radiography has been investigated mainly from the standpoint of its use in nondestructive testing. Biological use of this

method has proved difficult because of the scatter caused by hydrogen in living tissue and because of problems in collimation.

G. RESEARCH HOSPITAL AND INDUSTRIAL MEDICINE CLINIC OPERATIONS

The 48-bed Hospital of the Medical Research Center supports the clinical aspects of the medical research program. Each physician on the scientific staff of the Medical Department participates to some degree in the Hospital's activities, even though his primary investigative endeavors are in nonclinical areas. The proximity of the laboratories of the Center to the Hospital facilitates the participation of the entire scientific staff in furthering clinical investigations.

Except for emergencies requiring the use of its facilities, admission to the Hospital is limited to patients who it is believed will be benefited by their hospital stay and whose observation or treatment will advance the studies under way. The disease states under investigation may vary; typical during the past year were Parkinson's disease, leukemia and breast cancer, diabetes mellitus, and essential hypertension.

Patients are admitted only upon referral by their physicians or clinics and are returned to the care of their physician at the end of their hospitalization. The great majority of patients are residents of Suffolk and Nassau Counties on Long Island, but many are referred from New York City and other parts of New York State as well as from various states throughout the nation. The active medical staff of the Hospital is limited to physicians holding appointments to the scientific staff of Brookhaven National Laboratory. Other specialists from surrounding communities are called upon as needed to assure the finest possible care for each patient.

As part of the Laboratory's occupational health program, an Industrial Medicine Clinic is maintained in a wing of the Medical Research Center by members of the medical staff. The facilities of the Hospital are made available when emergencies arise or as dictated by the interests of the Laboratory or the Atomic Energy Commission. Approximately 16,000 employee visits are handled per year, including those intended to prevent loss of time due to illness or injury and visits for counseling. The special equipment of the Medical Research Center is available to the Industrial Medicine Clinic. For example, the whole-body counter is used in an investigative program that involves the monitoring of persons working with radioactive material.

The circular design of each of the four nursing pavilions enables nurses at the central station to see each patient's bed.

Employees receiving medical care in the Industrial Medicine Clinic.

Review of patient's lymphangiogram after extracorporeal irradiation of the blood.

The Medical Research Center

The Medical Research Center is comprised of the Hospital, Industrial Medicine Clinic, Research Laboratory Wing, and Medical Research Reactor in one main building, and additional Veterinary Service Facilities in two nearby small buildings.

The Hospital has a capacity of 48 beds in 4 identical circular nursing units, or pavilions. These consist of 12 individual patient rooms on the periphery of a 72-ft circle, in the center of which is the nurses' station from which each bed is visible. The work of the Hospital is devoted entirely to the investigational activities of the physicians who are members of the scientific staff of the Department; routine care of employees or their dependents is not provided.

The Industrial Medicine Clinic serves the medical staff of the Laboratory's employee health program. It is designed for handling pre-employment, termination, and annual physical examinations of employees, the typical daily industrial medicine visits of employees, and emergency first aid. This wing also serves the Hospital Staff for out-patient visits by research patients.

The Research Laboratory section of the Medical Research Center provides an area of 58,000 sq ft for laboratories, offices, and special service facilities to accommodate the full-time staff and a number of visiting scientists and research collaborators. Available for scheduled use by each scientist are many departmental facilities such as controlled temperature rooms; humidity-controlled balance and instrumentation rooms; veterinary service rooms; areas for special equipment such as ultracentrifuges, electrophoresis apparatus, and time-lapse photography; x-ray and gamma source areas; glass-blowing rooms; sample and *in vivo* counting rooms; dark rooms, special rooms for chromatographic work and tissue culture; and special areas for receiving, processing, and using radioactive materials not permitted in the general laboratory area. The building also includes a seminar room with a seating capacity of 130 and a medical library which now contains 4751 books and 4717 bound volumes of journals.

The Medical Research Reactor (MRR) was constructed for the purpose of exploring the possible applications of nuclear reactors in the study of the diseases of man. The MRR operates at a maximum power of 5 megawatts and provides 3 areas, appropriately shielded, for exposure to a thermal neutron beam. Shutters are provided in two of these areas for more precise control and limitation of radiation fields. Two isotope production tubes provide for the instant use of short-lived radioactive materials *in vivo* or for activation analysis of biological materials.

Nearing completion is a low-background counting facility that will be an integral part of the main building adjacent to the hospital. Special whole-body counting and scanning spectrometer systems are being designed to supplement existing instrumentation for clinical and laboratory studies in these facilities.

Two relatively new, concrete-block veterinary service buildings supplement the facilities of the main building. One provides temporary holding facilities for observation of newly acquired animals as a safeguard against the introduction of infectious diseases. The other, designed to house large animals such as cattle, is used primarily for animals undergoing extracorporeal irradiation of their circulating blood. Funds have been appropriated for a 21,000-sq-ft addition to the main building for use in the care of animals.

Floor plan of the Medical Research Center, showing block, corridor, and room numbers.

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